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Calcium Selective Channel TRPV6: Structure, Function, and Implications in Health and Disease

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Abstract

Calcium-selective channel TRPV6 (Transient Receptor Potential channel family, Vanilloid subfamily member 6) belongs to the TRP family of cation channels and plays critical roles in transcellular calcium (Ca^{2+}) transport, reuptake of Ca^{2+} into cells, and maintaining a local low Ca^{2+} environment for certain biological processes. Recent crystal and cryo-electron microscopy-based structures of TRPV6 have revealed mechanistic insights on how the protein achieves Ca^{2+} selectivity, permeation, and inactivation by calmodulin. The TRPV6 protein is expressed in a range of epithelial tissues such as the intestine, kidney, placenta, epididymis, and exocrine glands such as the pancreas, prostate and salivary, sweat, and mammary glands. The TRPV6 gene is a direct transcriptional target of the active form of vitamin D and is efficiently regulated to meet the body's need for Ca^{2+} demand. In addition, TRPV6 is also regulated by the level of dietary Ca^{2+} and under physiological conditions such as pregnancy and lactation. Genetic models of loss of function in TRPV6 display hypercalciuria, decreased bone marrow density, deficient weight gain, reduced fertility, and in some cases alopecia. The models also reveal that the channel plays an indispensable role in maintaining maternal-fetal Ca^{2+} transport and low Ca^{2+} environment in the epididymal lumen that is critical for male fertility. Most recently, loss of function mutations in TRPV6 gene is linked to transient neonatal hyperparathyroidism and early onset chronic pancreatitis. TRPV6 is overexpressed in a wide range of human malignancies and its upregulation is strongly correlated to tumor aggressiveness, metastasis, and poor survival in selected cancers. This review summarizes the current state of knowledge on the expression, structure, biophysical properties, function, polymorphisms, and regulation of TRPV6. The aberrant expression, polymorphisms, and dysfunction of this protein linked to human diseases are also discussed.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Keywords

TRPV6; TRPV5; calcium channel; calcium absorption; transcellular pathway; gating mechanism; calmodulin; maternal-fetal transport; transient neonatal hyperparathyroidism; chronic pancreatitis; kidney stones; cancer

1 Classification and Identification

1.1 Classification

The TRPV6 protein is a highly selective epithelial calcium (Ca^{2+}) channel belonging to the transient receptor potential family (TRP) of proteins (Venkatachalam and Montell, 2007). TRP channels were first identified in the *trp* mutant strain of fruit fly *Drosophila*, a strain that exhibits transient elevation of receptor potential in response to photogenic stimuli instead of a sustained receptor potential in wild-type *Drosophila* (Minke et al., 1975). The TRP family is now a widely recognized group of cation channel proteins that are known to detect a diverse range of chemicals and physical stimuli such as light, temperature, osmotic pressure, olfaction, taste, and mechanical forces (Nilius and Owsianik, 2011). Mutations and dysregulations in the TRP family of proteins are linked to multiple cancers as well as several skeletal, neurodegenerative, and kidney disorders; connections that highlight the importance of basic and applied research in this realm (Nilius, 2007; Brinkmeier, 2011; Yang and Kim, 2020).

The current classification of TRP channels based on sequence, topological differences, and the evolutionary organization was formalized and published in 2002 (Montell et al., 2002; Venkatachalam and Montell, 2007). According to this scheme of classification, TRP superfamily includes two broad groups and six subfamilies. The group 1 TRP channels bear a strong sequence homology to *Drosophila* TRP and share considerable sequence homology within the six-transmembrane (TM) domain which is common among the five structurally-related subfamilies in this group that include: TRPC ('Canonical'), TRPM ('Melastatin'), TRPV ('Vanilloid'), TRPA ('Ankyrin') families found in mammals; and an additional TRPN ('NO-mechano-potential C') family found in non-mammals. The name "Vanilloid" comes from the original name of the first member of the family Vanilloid Receptor 1 (VR1, now TRPV1). In contrast to the first four members of the TRPV family which are primarily involved in nociception, thermo-sensing, and osmosensing; the TRPV5 and TRPV6 are extremely selective Ca^{2+} ion channels that play pivotal roles in Ca^{2+} reabsorption in the kidney and intestine (Nijenhuis et al., 2003b). The group 2 TRP channels bear a large loop that separates the first two TM domains, exhibit significant sequence homology in the TM segments, and are known for pathological phenotypes caused by mutations in the founding member of the subfamily (Venkatachalam and Montell, 2007). This group includes TRPML ('Mucolipins') and TRPP ('Polycystin') subfamilies, which are associated with polycystic kidney disease (ADPKD) and mucopolipidosis type IV (MLIV) respectively. The number of TRP channels in each group and subfamily varies by species. In humans, 28 TRP channels are currently recognized which are critical for ionic homeostasis and perception of various physical and chemical stimuli (Montell et al., 2002; Montell, 2005; Venkatachalam and Montell, 2007; Pan et al., 2011).

1.2 Identification

The transient receptor potential (TRP) channel, subfamily vanilloid, member 6 (TRPV6) was first identified in 1999 from rat duodenum (Peng et al., 1999). To facilitate the cloning of TRPV6, Peng and colleagues fed rats Ca^{2+} -deficient diet for 2 weeks to boost the expression Ca^{2+} -absorptive channels and proteins and restricted mRNA isolation primarily from duodenum and cecum as these intestinal sites show a high level of absorption. Based on the moderate abilities of mRNA from duodenum and cecum to induce $^{45}\text{Ca}^{2+}$ -radiotracer uptake upon injection in *Xenopus* oocytes, the investigators successfully identified a channel by expression cloning using *Xenopus* oocytes. The primary structure of the protein suggested its role as a prototypical ion channel; however, it exhibited saturation kinetics of $^{45}\text{Ca}^{2+}$ uptake typically observed in transporters. Now widely recognized as TRPV6, this protein was initially termed Ca^{2+} transport protein (CaT1) at the time of its discovery and was believed to play a critical role in intestinal uptake of Ca^{2+} . The CaT1 protein was found to have a 75% amino acid sequence identity to the epithelial Ca^{2+} channel (ECaC) (Hoenderop et al., 1999). For this reason, the name ECaC2 has also been used in the past to describe TRPV6.

Soon after, Wissenbach et al described the cloning of human TRPV6 which was termed CaT-like (CaT-L) due to its high structural similarity to CaT1 protein; though it was unclear at that time whether it represents the human ortholog (Wissenbach et al., 2001). The discoveries with CaT-L, CaT1, ECaC, and related orthologues or homologs happened in quick succession in a relatively short period. Therefore, there was some initial ambiguity in the field about the nomenclature of these channels in different species. For instance, it was found that human CaT-L shared 90% and 77% to amino acid sequence identity to rat CaT1 and human ECaC (then recently discovered renal human renal epithelial Ca^{2+} channel). However, unlike CaT1, it was undetectable in the small intestine and colon, prompting investigators to conclude that CaT-L does not represent the human version of CaT1. Indeed, the CaT-L transcript was found to be identical to a human cDNA sequence of 446 bp deposited to the GenBank™ database (accession number [AJ277909](#)) which was postulated to represent part of human CaT1, but there was no functional data to support this hypothesis. Moreover, this same study by Wissenbach et al also described a second gene product shared 92% sequence identity with human CaT-L, 95% with human ECaC, and 81% with rat CaT1 sequences.

Soon after the discovery of TRPV6 in rat intestine (Peng et al., 1999), its mouse (Suzuki et al., 2000; Weber et al., 2001) and human homologs (Peng et al., 2000; Hoenderop et al., 2001; Wissenbach et al., 2001) were discovered within two years. These initial studies indicated the role of TRPV6 as a pivotal channel for Ca^{2+} entry into enterocytes. Since its initial discovery over three decades ago, subsequent studies have provided additional insights into regulatory mechanisms and functions of Ca^{2+} -selective channel TRPV6. In this review, we provide a narrative review of this protein describing the current state of knowledge on expression, structure, function, polymorphisms, and regulation of TRPV6. We also describe how aberrant expression, polymorphisms, and dysfunction of this protein are involved in diseases such as transient neonatal hyperparathyroidism, chronic

pancreatitis, hypercalciuria, renal stone formation, bone disorders, skin abnormalities, skeletal deformities, male sterility, and cancer.

2 Gene structure, Phylogeny, and Chromosomal location

Genomic and structural studies have revealed several interesting and noteworthy characteristics in the *TRPV6* gene and TRPV6 protein. In this section, we summarize some key findings related to the gene structure, phylogenetic aspects, and chromosomal location TRPV6 (Hirnet et al., 2003; Fecher-Trost et al., 2013; Fecher-Trost et al., 2014).

The human *TRPV6* gene consists of 15 exons (Peng et al., 2001a) and is located on chromosomal 7q33-34 (Peng et al., 2000). The human *TRPV6* gene is 14,543 bases long spanning from nucleotide position 142,568,956 to 142,583,507 (OMIM Entry – 606680, Gene ID – 55503, GenBank Accession – [NM_018646.6](#), Table 1). The *TRPV6* gene lies in close proximity to its homolog *TRPV5* (located on 7q35) (Muller et al., 2000; Fecher-Trost et al., 2014). *TRPV6* and *TRPV5* genes are completely conserved in exon size in the coding region. They also share similar intron–exon organization with other members of the TRPV family (Peng et al., 2000a). The rat and mouse versions of the *Trpv6* gene are located on chromosome 4 (Fecher-Trost et al., 2014) and chromosome 6 (Weber et al., 2001), respectively.

It is speculated that *Trpv5* and *Trpv6* genes evolved from a single ancestral gene through gene duplication events (Qiu and Hogstrand, 2004; Flores-Aldama et al., 2020). The evidence for this phylogenetic event comes from the observation that some organisms such as pufferfish *Takifugu rubripes* have only one gene that bears a higher degree of similarity to *Trpv6* in comparison to *Trpv5* (Qiu and Hogstrand, 2004). This hypothesis was recently confirmed in the phylogenetic analysis that discovered the paralogs of TRPV6 found in mammals, sauropsids, amphibians, and Chondrichthyes as products of independent duplication events in the ancestor of each group (Flores-Aldama et al., 2020).

It has been hypothesized that gene duplication that resulted in these two specialized Ca^{2+} -selective *Trpv* homologs was an adaptation to achieve a greater degree of functional specialization for navigating complex renal challenges of terrestrial animals (Qiu and Hogstrand, 2004; Fecher-Trost et al., 2014; Flores-Aldama et al., 2020). Recent transcriptome analysis by Flores-Aldama et al has shown a change in tissue expression from gills, in marine vertebrates to kidneys in terrestrial vertebrates (Flores-Aldama et al., 2020). The investigators identified a three amino acid signature in amniotes that are found in the amino-terminal intracellular region which correlates with gene duplication events and the phenotype of fast inactivation observed in mammalian TRPV6 channel. Electrophysiological recordings suggest that the signature sequence modulates the phenotype of fast inactivation in all clades of vertebrates but reptiles (Flores-Aldama et al., 2020).

Another comparative analysis of reindeer (*Rangifer tarandus*) in Northern Eurasia has shown has the *Trpv6* gene may have been important in conferring evolutionary advantage in terms of improving vitamin D metabolism (Weldenegodguad et al., 2020). Although no genes similar to *Trpv5/6* genes have been identified in Prokaryotes or Saccharomyces, *Trpv5/6*

like genes have been identified in choanoflagellates *Monosiga brevicollis* (King et al., 2008). Moreover, the occurrence of *Trpv5/6*-like like genes in green algae *Chlymadomonas reinhardtii* and *Volvox carteri* suggest potential horizontal gene transfer events that may have occurred during the course of evolution (Merchant et al., 2007).

In humans, the *TRPV6* gene encodes for 2906 bp-long mRNA which is translated *in vivo* into a 765-aa-long protein through a process that is characterized by non-AUG-codon-mediated reading (Fecher-Trost et al., 2013). The translation of TRPV6 initiates at the codon ACG which is located upstream of the annotated AUG. The ACG codon is nevertheless decoded as a methionine. In mouse, the *Trpv6* gene extends over a region of 15.7 kb and spans 15 exons (interspersed with 14 introns and a 5'-/3'- non-coding region) coding for a 767-aa-long protein. The length of TRPV6 protein in different species ranges from 703 aa to 767 aa. Notably, the full-length TRPV6 protein *in vivo* bears a 40-aa-long N-terminal extension in comparison to the annotated version of the protein that has been utilized in most studies (Fecher-Trost et al., 2013). To be consistent with these original studies, we use the amino-acid numbering based on the annotated version of TRPV6 in most sections of this review.

In humans, two alleles of the *TRPV6* gene have been identified (originally called CaT-La and CaT-Lb) (Wissenbach et al., 2001). These alleles are a result of coupled polymorphisms that generate two versions of the gene called the “ancestral variant” and the “derived variant” that differ in five bases leading to three amino acid substitutions (Wissenbach et al., 2001; Akey et al., 2006). The ancestral allele encodes for the residue C157, M378, and M681 whereas the derived allele encodes for R157, V378, and T681. The ancestral variant is well-conserved across mammals and found both in humans and fish. In contrast, the derived variant is detectable only in humans (Wissenbach et al., 2001; Akey et al., 2006).

The frequency of the ancestral *TRPV6* allele is subject to ethnic variations (Akey et al., 2006; Hughes et al., 2008; Soejima et al., 2009). The frequency of the ancestral variant decreases with increasing distance from the African continent, with allele frequency decreasing from 50% in African-Americans to 5% in Caucasians (Soejima et al., 2009). The selection pressures accounting for such differences in *TRPV6* alleles across continents could be not entirely clear and has invited many interesting hypotheses (Akey et al., 2004; Stajich and Hahn, 2005; Akey et al., 2006; Hughes et al., 2008; Soejima et al., 2009). It is speculated that some factors that influenced selection pressures altering *TRPV6* allele frequencies across various demographics could include a) changes in patterns of milk consumption influenced by the domestication of animals, b) reduction in ultraviolet light exposure due to trans-equatorial migration, c) genomic adaptations conferring an innate immune advantage to brush-bordered-localized TRPV6 protein that may encounter new pathogens in digested food during the course of migration and evolution (Akey et al., 2004; Stajich and Hahn, 2005; Akey et al., 2006; Hughes et al., 2008; Soejima et al., 2009).

Overall, these coupled polymorphisms could represent adaptations in the *TRPV6* gene that conferred resistance to pathogens and ensured adequate Ca²⁺ and vitamin D supply (Akey et al., 2004; Stajich and Hahn, 2005; Akey et al., 2006; Hughes et al., 2008; Soejima et al., 2009). Nevertheless, the physiological relevance behind phylogenetic divergence of the

TRPV6 allele is hard to dissect since both ancestral and derived alleles display similar in their biophysical properties (Hughes et al., 2008). Some evidence suggests that this allelic divergence plays a role in the differential susceptibility of demographic groups towards nephrolithiasis (Suzuki et al., 2008d). The variations in TRPV6 alleles across different demographics remains an intriguing puzzle that continues to baffle experts in the field and invite novel hypothesis (Akey et al., 2004; Stajich and Hahn, 2005; Akey et al., 2006; Hughes et al., 2008; Soejima et al., 2009).

3 Tissue Distribution

In comparison to TRPV5, which is specifically expressed in the kidney, TRPV6 has a broader expression profile. TRPV6 is expressed in epithelial tissues such as the intestine, kidney, placenta, epididymis, and exocrine glands such as the pancreas, prostate and salivary, sweat, and mammary glands (see Table 2). Several investigators have examined TRPV6 mRNA expression profile using Northern Blotting, RT-PCR, RNA *in-situ* hybridization, and more recently by RNA-seq based methods (see Table 2). TRPV6 protein level has been examined utilizing immunodetection methods (e. g. IHC, ICC, Western); although disagreement within results is fairly common due to lack of a rigorously validated antibody (Zhuang et al., 2002; Lehen'kyi et al., 2012; Stewart, 2020). It can be suspected that differences in antibody characteristics, physiological conditions at the time of the experiment, and other assay-dependent variables have a substantial impact on TRPV6 immunodetection (Lehen'kyi et al., 2012; Stewart, 2020). Despite its relatively early discovery and the existence of multiple studies examining TRPV6 expression in several species, multiple discrepancies and knowledge gaps confound our understanding of the TRPV6 expression profile (den Dekker et al., 2003; Lehen'kyi et al., 2012; Fecher-Trost et al., 2014; Fecher-Trost et al., 2017; Stewart, 2020). This is primarily because TRPV6 expression varies significantly not only across various species and tissues; but is also impacted by method of detection, age, gender, Ca²⁺, and vitamin D levels in food, hormonal status, location within the tissue, cellular location, reproductive status, and weaning status (see Section 6).

A simplified view of TRPV6 distribution is presented in Table 2 summarizes the methods through which expression in different tissues has been reported in mice, rats, and humans. Notwithstanding some early disagreements regarding TRPV6 expression assessed by hybridization-based methods (e.g. differences in detection from northern blots vs. dot blots vs. qRT-PCR); northern blot-based expression of this gene has been consistently reported TRPV6 transcripts in the placenta, pancreas, prostate cancer, and duodenum in humans; in the duodenum, caecum, small intestine, and colon in rats; and in the placenta, pancreas, prostate, and epididymis in the mouse (Peng et al., 1999; Peng et al., 2000; Hoenderop et al., 2001; Peng et al., 2001b; Wissenbach et al., 2001; Hirnet et al., 2003; Fecher-Trost et al., 2014). However, one of the most confounding variables appears to be the method used for measuring TRPV6 expression (Lehen'kyi et al., 2012; Stewart, 2020). For instance, no TRPV6 transcripts could be detected by Northern blot analysis in murine brain, heart, and intestine by Hirnet et al; an observation that contradicts the initial discovery of the gene in duodenum and observations made by Nijenhuis et al who reported significant expression of this gene in the all these organs by qRT-PCR (Peng et al., 1999; Hirnet et

al., 2003; Nijenhuis et al., 2003c). In fact, method-dependent variations in the expression of TRPV6 transcripts in mouse, human, and rat tissues could be fairly common, especially in the light of anomalous detection of closely related TRPV5 orthologue (Lehen'kyi et al., 2012; Stewart, 2020). Similarly, early inter-study inconsistencies in human and murine TRPV6 expression in the small intestine have been previously reported, presumably due to differences in the region of the intestine used for analysis and differences in hybridization probes and/or primer design (Hoenderop et al., 2001; Wissenbach et al., 2001; den Dekker et al., 2003; Lehen'kyi et al., 2012). For instance, in murine duodenal tissues; TRPV6 is undetectable at P1 and increases 6-fold as mice age to P14 to 1 month whereas in jejunum it increases from P1 to P14, becomes weak at 1-month age, and is undetectable in older mice (Beggs et al., 2019). Although TRPV6 mRNA is barely detectable in the ileum of mice and rats, immunodetection of TRPV6 has shown the presence of protein in the ileum (Peng et al., 1999; Zhuang et al., 2002). In sheep, the jejunum displays a higher abundance of TRPV6 mRNA and protein in comparison to the duodenum (Wilkens et al., 2009). Expression of TRPV6 mRNA in horses is considerably higher in proximal jejunum in comparison to duodenum; suggesting that jejunum, rather than duodenum, could be important for Ca^{2+} absorption in equines (Rourke et al., 2010). TRPV6 mRNA and protein are weakly expressed in ovine rumen although sheep and goats partake active intestinal Ca^{2+} absorption (Wilkens et al., 2009; Schroder et al., 2015).

TRPV6 expression profile has been investigated both in non-pathological and pathological settings and several reviews have succinctly cataloged this information (den Dekker et al., 2003; Lehen'kyi et al., 2012; Fecher-Trost et al., 2014; Fecher-Trost et al., 2017; Stewart, 2020). Examination of these reviews suggests that in contrast to consolidated hybridization-based studies measuring TRPV6 expression across multiple human tissues, a rigorous and unified real-time qPCR-based expression profiling of TRPV6 in multiple human tissues is wanting. This is because the results with less-abundant transcripts seem to vary based on primer design, assay characteristics, and possibly other physiological conditions prevalent at the time of the experiment. An early report by Hoenderop et al have presented a comparative analysis of TRPV5 and TRPV6 expression pattern in a panel of human tissues by semi-quantitative PCR (Hoenderop et al., 2001; den Dekker et al., 2003). This report showed that TRPV6 is expressed in the duodenum, jejunum, placenta, pancreas, prostate, testis, kidney, brain, and colon. Although expression of TRPV6 mRNA in murine kidneys is difficult to capture and often barely detectable by qRT-PCR- and Northern blot-based methods; a fair amount of protein is detected in the apical domain of the distal convoluted tubules (DCT2), connecting tubules (CNT), and cortical and medullary collecting ducts (CD) (Nijenhuis et al., 2003c). The expression of TRPV6 transcripts using RT-PCR has been reported in medullary thick ascending limb in microdissected kidney tubules (Diepens et al., 2004). Unpublished observations from our lab suggest that TRPV6 is high in the outer medulla and low in the cortex, opposite to that of TRPV5. In mice and rat kidneys, TRPV5 mRNA is more than 10-to-20 folds higher in comparison to TRPV6 (Song et al., 2003b). In humans, renal TRPV6 mRNA expression is much higher in comparison to TRPV5 (Peng et al., 2001a). Similarly, in horse kidneys, TRPV6 is more abundant in comparison to TRPV5 (Rourke et al., 2010). A recent study comparing vitamin D responsive genes in ovine, canine and, equine kidney using quantitative real-time PCR (RT-qPCR) indicated that TRPV6,

calD_{9k}/calD_{28k}, and PMCA appear to be the main pathways involved in transcellular Ca²⁺ transport in the kidney of sheep, dogs, and horses (Azarpeykan et al., 2016). Furthermore, expression of all Ca²⁺ transporting transcripts including TRPV6 was highly correlated with VDR in the equine kidney, but not in sheep and dogs (Azarpeykan et al., 2016). Overall, these studies suggest that in large mammals TRPV6 could play a bigger role in Ca²⁺ reabsorption than previously envisioned in rodents (Peng, 2011).

A recent review has observed that Transcript Per Million (TPM) values of TRPV6 in a collection of 91 human tissues across 296 subjects from 4 studies varied dramatically when measured by RNA-Sequencing (Stewart, 2020). Overall, this analysis suggested that TRPV6 mRNA expression is low in most human tissues except for the pancreas and prostate which also exhibited considerable variation in TPM values (Stewart, 2020). According to the TPM counts from the now-retired Expressed Sequence Tags (EST) database, TRPV6 expression was detected in the human placenta, prostate, bladder, cervix, trachea, blood, eye, intestine, mammary, testis, pancreas, brain, and lung (Na and Peng, 2014). Observations regarding RNA-Seq-based expression profiles by Stewart et al are consistent with TRPV6 expression profile reported on the Human Protein Atlas which abstracts normalized expression (NX) for 74 human tissues based on transcriptomics data from three sources: Human Protein Atlas (HPA), Genotype-Tissue Expression Database (GTEx), and Functional Annotation of the Mammalian Genome (FANTOM5) [(Uhlén et al., 2015; Stewart, 2020) and [ENSG00000165125\(Hs\)](#)]. As observed in the consolidated analysis of with RNA-Seq studies, TRPV6 mRNA is extremely low to barely detectable in most tissues except for the placenta, salivary gland, pancreas, and prostate (normalized expression (NX) values ranging from 12.6 to 65.1). In this database, the ranking of TRPV6 mRNA expressing human tissues as per their NX values (in parentheses) was as follows: duodenum (4.1) > gall bladder (4.1) > skin (3.7) > kidney (2.5) > stomach (2.4) > breast (2.1) > spinal cord (2.0) > esophagus (1.7) > colon (1.2) > small intestine (1.0) > tonsil (1.0). Most other tissues expressed very low to no TRPV6 expression (NX values ranging from 0 to 1) (Uhlén et al., 2015; Stewart, 2020). These findings with more sensitive genomic detection methods explain some early observations where detection utilizing northern blots or dot blots reported failure or negligible expression of TRPV6 transcripts in the tissues such as the trachea, mammary glands, kidney, esophagus, and lungs (Peng et al., 2000; Hoenderop et al., 2001; Wissenbach et al., 2001). Overall, these studies highlight the need for rigorous hybridization probes, primer design, and careful assay control.

In mice, an early study by Nijenhuis et al have measured TRPV6 expression in multiple murine tissues. In this study, murine tissue was ranked on basis of TRPV6 expression as follows: prostate > stomach, brain > lung > duodenum, cecum, heart, kidney, bone > colon > skeletal muscle > pancreas (Nijenhuis et al., 2003c). Zhuang et al have demonstrated immunofluorescence-based TRPV6 protein expression in the murine esophagus, stomach, duodenum, ileum, cecum, colon, pancreas, kidneys, and duodenum (Zhuang et al., 2002). TRPV6 is also expressed in the apical domain of murine osteoclasts of cortical bone (van der Eerden et al., 2012; Chen et al., 2014a). However, cortical and trabecular osteocytes have no TRPV6 expression, and osteoblasts show weak expression (Little et al., 2011).

Notwithstanding some discrepancies in the genomic expression of TRPV6, the immunohistochemistry-based expression of TRPV6 protein in humans has been demonstrated in the esophagus, stomach, small intestine, colon, pancreas, mammary glands, ovary, thyroid, colon, and prostate (Zhuang et al., 2002). Immune-based detection has provided insights into the relative location of various tissues. TRPV6 expression is confined mainly to the apical epithelial cell surface. The protein is expressed on the apical brush-border membrane of the intestinal enterocyte and superficial layers of stratified esophageal epithelia. Mammary glands display TRPV6 staining primarily on the inner surface of ductal epithelial cells whereas weak signals are detected on the basolateral membrane. Sweat gland epithelial cells exhibit similar staining patterns although the difference in signal intensity is observed between the inner duct and basolateral surfaces. In the pancreas IHC-staining of TRPV6 was found to be restricted to exocrine acinar cells with signals predominantly displaying granular staining within the secretory pole on the apical membrane (Zhuang et al., 2002). Functional and pathological implications of TRPV6 expression in the intestine, kidney, placenta, ear, bone, epididymis, uterus is described in Section 5.

TRPV6 is overexpressed in colon, breast, prostate, parathyroid, pancreatic, and thyroid cancer (Zhuang et al., 2002). In contrast, TRPV6 expression is downregulated in esophageal cancer (Sun et al., 2016), non-small cell lung cancer (Fan et al., 2014), and renal cancer (Wu et al., 2011). TRPV6 is expressed in breast adenocarcinoma tissues (Bolanz et al., 2008; Dhennin-Duthille et al., 2011) and breast cancer cell lines MDA-MB-231, MDA-MB-468, T47D, SKBR3, ZR-75-1, BT-483, MCF-7 (Peters et al., 2012). Prostate cancer cell lines LNCaP and PC-3 overexpress TRPV6 relative to benign epithelial cells PrEC and BPH-1 whereas studies have indicated both presence and absence of TRPV6 in androgen-insensitive lines such as DU-145 (Peng et al., 2001b; Peng et al., 2003; Lehen'kyi et al., 2007b; Lehen'kyi et al., 2011a; Kim et al., 2014; Jiang et al., 2016). The expression profile of TRPV6 and its implications on disease progression and aggressiveness are discussed in Section 7.7. TRPV6 channel expression has been reported in T cells and it appears that expression is switched off in quiescent lymphocytes and turns on only after mitogen stimulation (Vasil'eva et al., 2008; Vassilieva et al., 2013). No information is available on expression pattern on specific immune cell subtypes (e.g. changes in expression profile in CD4 vs. CD8 vs. Tregs or M1 vs. M2 macrophages etc.) and could open up an exciting avenue for the role of TRPV6 in immune functions.

4 Structure

Although the initial identification of TRPV6 was accompanied by a description of primary structure as well as important secondary structure elements (Peng et al., 1999; Peng et al., 2000; Wissenbach et al., 2001), its tertiary and quaternary structures have only been elucidated over the last half of this decade (Saotome et al., 2016; Singh et al., 2017; McGoldrick et al., 2018; Sakipov et al., 2018; Singh et al., 2018a; Singh et al., 2018b). This has resulted in a barrage of data gathered from crystallographic (Saotome et al., 2016; Singh et al., 2017; Singh et al., 2018b) and Cryo-EM-based (McGoldrick et al., 2018; Singh et al., 2018a) structures as well as cognate molecular dynamics simulations (Sakipov et al., 2018) to reveal critical insights that elegantly connect structural architecture of the protein

to its biophysical properties and functions (recently summarized in an elegant review by Sobolevsky's group)(Yelshanskaya et al., 2020).

4.1 Primary and Secondary Structure

In contrast to naturally occurring TRPV6, which is 765-aa-long, the annotated human TRPV6 used for structural studies is 725 amino acids long (Fecher-Trost et al., 2014; McGoldrick et al., 2018; Singh et al., 2018a). Similarly, In contrast to naturally occurring rat TRPV6 which is 767 amino acids long, the annotated version of this protein used in crystallization studies is 727 amino acids long (Fecher-Trost et al., 2014; Saotome et al., 2018). Unless otherwise stated, amino acid numbers used to describe the structural details of TRPV6 hereon correspond to the annotated (shorter) version of TRPV6 that has been utilized in structural characterization.

Overall, four subunits of TRPV6 arrange to form a tetrameric channel displaying a four-fold symmetry (three-dimensional structure described in Section 4.2) (Saotome et al., 2016). The secondary structure elements presented in each TRPV6 monomer are schematically illustrated in Figure 1A, 1B. Starting from N-terminus, each TRPV6 polypeptide contains the following secondary structure elements: an N-terminal helix, an ankyrin repeat domain (ARD) with six ankyrin repeats (ANK1 through ANK6), a linker domain comprised of a β -hairpin (β 1 and β 2) and two linker helices (LH1 and LH2). A pre-S1 helix connects the linker domain and the transmembrane (TM) domain that comprises of six TM helices (S1 through S6) and a pore helix (P-helix) connecting S5 and S6. The S6 helix is followed by an amphipathic TRP helix, which is the defining feature of all TRP channels. The TRP helix is followed by a six-residue β -strand (β 3) that forms a β -sheet with β 1 and β 2 in the linker domain. Two helices located in the C-terminus of TRPV6 can bind CaM and they are named C-terminal interacting helices (CIH1 and CIH2). The organization of these secondary structural elements into tertiary structure and higher level homotetrameric structure of TRPV6 is described in the next section.

4.2 Tertiary Structure and Domain Organization

The crystal structure of rat TRPV6 containing both ankyrin repeat domain and the transmembrane domain was first described by Saotome et al at 3.25 Å resolution (Saotome et al., 2016). This was followed by additional discoveries by Sobolevsky's group who discovered the cryo-electron microscopy (Cryo-EM) structures of rat (Singh et al., 2018a; Singh et al., 2018b) and human TRPV6 (McGoldrick et al., 2018; Singh et al., 2018a; Singh et al., 2018b) and also presented domain-swapped correction (Singh et al., 2017) of the initial TRPV6 crystal structure (Saotome et al., 2016). These elucidations collectively revealed the structural insights into TRPV6 pore opening (McGoldrick et al., 2018), interactions with calmodulin (CaM) (Singh et al., 2018a), and inhibition of Ca^{2+} -uptake by the compound 2-APB (Singh et al., 2018b). These descriptions were further refined by molecular dynamics simulations that utilized the crystallographic structure of TRPV6 as templates for mining additional insights that link the structural architecture of TRPV6 to its biophysical properties (Sakipov et al., 2018).

The TRPV6 protein is a four-fold symmetrical structure that contains two main compartments (Figure 1C, 1D): a) transmembrane domains with a central ion channel pore, and b) an intracellular skirt formed by ankyrin repeat domains of TRPV6 that act as the walls enclosing a wide cavity underneath the ion channel (Saotome et al., 2016). The intracellular skirt in the TRPV6 protein is ~70 Å-tall and ~110 Å-wide and encloses a 50 Å × 50 Å cavity. The S1-S4 domain comprises a transmembrane helical bundle which is inserted almost perpendicularly to the plane of the plasma membrane (Saotome et al., 2016; Yelshanskaya et al., 2020). The 30 Å-tall TM domain of TRPV6 resembles the general architecture of voltage-gated K⁺ and Na⁺ channels (Long et al., 2007; Payandeh et al., 2011). However, in contrast to other TRP channels, where S1-S4 domains act as voltage sensors and display movements within positively-charged arginine and lysine residues (located on S4) that shift relative to the plane of plasma membrane; the S4 helix in TRPV6 exhibits no such activity. The S1-S4 helical bundle in TRPV6 maintains its rigidity through hydrophobic interactions occurring between aromatic side chains in S1-S4 and is relatively static during gating (Long et al., 2007; Payandeh et al., 2011; Saotome et al., 2016). The pore module elements S5, S6, and the pore helix in TM domains of the neighboring subunits participate in intersubunit interactions to generate the central ion pore. The pore-forming elements of each TRPV6 subunit interact with S1-S4 domains of the adjacent subunit in a characteristic domain-swapped arrangement observed in TRP channels (Singh et al., 2017), a conformation which can be remarkably reversed by single residue L495Q mutation (Saotome et al., 2016; Singh et al., 2017). Extracellular inter-subunit interactions also occur between S1-S2, S5-P, and S6-P loops of the neighboring TRPV6 subunits (Saotome et al., 2016). The conserved N-linked glycosylation site (N358) on the S1-S2 loop is required for the Klotho-mediated activation (Lu et al., 2008). The three-stranded β-sheets along with the N-terminal helix and loop between the TRP domain and CIH1 participates in inter-subunit interactions with the ARDs which cements the elements of the intracellular skirt together. The N-terminal helix is positioned as a pillar along the corner of the intracellular skirt and is critical for inter-subunit interactions. The TRP domain, a shared feature among channels from the C, V, and M subfamilies, comprises of a 25-amino-acid sequence (Steinberg et al., 2014). The amphipathic TRP domain in TRPV6 runs parallel to the plane of the membrane. This orientation of the TRP helix creates a hub for simultaneous hydrophobic interactions from the TM domain and the hydrophilic interactions in the intracellular skirt. The exact function of TRP helix is not clearly known, although it is suspected that it is involved in mediating interactions with the lipid PIP₂ (Steinberg et al., 2014).

The intracellular skirt portion of the TRPV6 protein is mainly composed of the ankyrin repeat domains (Saotome et al., 2016; Yelshanskaya et al., 2020). Most notably, deletion or mutations of amino acid residues in the third ankyrin repeat of TRPV6 abolishes tetramer formation and renders the channel nonfunctional (Erler et al., 2004). A 1.7 Å resolution crystal structure of the TRPV6-ARD deduced by Phelps et al. has revealed conserved structural elements unique to the ARDs of TRPV proteins (Phelps et al., 2008). These ankyrin repeats are characterized by anti-parallel inner- and outer-α-helices, with the helical layers linked together by finger loops. The finger loops in TRPV6 are longer in comparison to corresponding regions in canonical ankyrin repeats. Finger loop 3 is the longest among all loops and displays a high degree of conformational variability in residues 153-159.

TRPV6-ARD structure exhibits a large twist between ANK4 and ANK5 that results from displacement of inner helix 5 from the regular packing of hydrophobic side chains observed between canonical ankyrin repeats (Phelps et al., 2008).

4.3 Pore Architecture

The architecture of the ion-conducting pore is remarkably conserved across the family of tetrameric ion channels (Owsianik et al., 2006; Saotome et al., 2016; van Goor et al., 2017). Starting from the extracellular side and continuing to the intracellular side, four main elements can be used to describe TRPV6 pore architecture. These include the extracellular vestibule, a selectivity filter, a hydrophobic cavity, and a lower gate. The extracellular loops connecting the P-loop helix to S5 and S6 helices form the outer extracellular segment of the TRPV6 channel. The S6 helices of TRPV6 form the intracellular segment and line the ion conduction pathway. Eight acidic residues are found in the region connecting S5 and S6 in each subunit. Four of these residues face the ion conduction pathway and generate a highly electronegative vestibule facing the extracellular side (Saotome et al., 2016).

This electronegative extracellular vestibule is followed by a four-residue selectivity filter (rat TRPV6 residues ⁵³⁸TIID⁵⁴¹) (Voets et al., 2004; Saotome et al., 2016). The side chains from four highly-conserved D541 residues (D542 in hTRPV6), one from each protomer, form a negatively charged ring or selectivity filter facing the central lumen of the pore. This selectivity filter is critical for Ca²⁺ selectivity, permeation (Voets et al., 2004; Owsianik et al., 2006), and voltage-dependent Mg²⁺ block (Voets et al., 2003). The defining feature of TRPV6 pore is its high Ca²⁺-selectivity with Ca²⁺ to Na⁺ permeability ratio (P_{Ca}/P_{Na}) > 100 in comparison to other TRP channels with P_{Ca}/P_{Na} ranging from 1-10 (Yue et al., 2001). The selectivity filter discriminates ions based on the size as well as charge and is believed to generate a pore diameter ranging from 4.6 Å to 5.4 Å (Voets et al., 2004; Owsianik et al., 2006). In fact, the substitution of the critical pore-forming residue D541 of mouse TRPV6 renders the Ca²⁺-selective nonfunctional and blocks Ca²⁺ uptake (Nilius et al., 2001; Woudenberg-Vrenken et al., 2012). This has been widely used to generate TRPV6 loss of function models to examine the consequences of TRPV6 dysfunction on animal physiology (Weissgerber et al., 2011; Woudenberg-Vrenken et al., 2012; Masamune et al., 2020).

Below the selectivity filter, the pore diameter widens considerably and the area encloses a large 13 Å-wide hydrophobic cavity (Saotome et al., 2016). The lateral pore portal in this area is thought to provide access to small molecules and lipids, akin to voltage-gated Na⁺ channels (Payandeh et al., 2011). The hydrophobic cavity can accommodate a hydrated Ca²⁺ ion, which has an effective diameter of 8-10 Å. The lower gate is formed by the intracellular portions of S6 helices. Of the residues in the lower gate, the residues M577 of rat TRPV6 (M578 in hTRPV6) form a narrow constriction point with a diameter of 5 Å. This narrow constriction creates a hydrophobic seal that occludes the pore and keeps it in a closed state (Saotome et al., 2016; Singh et al., 2017). Notably, residues in the lower gate facing the center of the pore change under open (N572 and I575) and closed state (M578 and L574) as observed in the Cryo-EM structures of human TRPV6 (McGoldrick et al., 2018).

5 Biophysical Properties

5.1 Mechanism of Ion Permeation

Biophysical properties of TRPV6 have been dissected from natural and reconstituted cellular systems by employing Ca^{2+} -sensing dyes (e.g. Fura 2), radiotracer $^{45}\text{Ca}^{2+}$ uptake, two-electrode voltage-clamp studies, and patch-clamp approaches (Peng et al., 2018). The TRPV6 protein is exquisitely sensitive to intracellular Ca^{2+} ion concentration $[\text{Ca}^{2+}]$ (Bodding and Flockerzi, 2004). Lowering the intracellular $[\text{Ca}^{2+}]$ promotes Ca^{2+} influx with the amplitude of Ca^{2+} current correlating with intracellular EGTA or BAPTA (Ca^{2+} chelators) concentration (Bodding, 2005). TRPV6 conducts divalent cations with the following preference - $\text{Ca}^{2+} > \text{Ba}^{2+} > \text{Sr}^{2+} > \text{Mn}^{2+}$, however, the channel does not conduct Mg^{2+} and is weakly permeable to trivalent cations La^{3+} and Gd^{3+} (Yue et al., 2001; Voets et al., 2003; Kovacs et al., 2011). Furthermore, the intracellular or extracellular presence of Mg^{2+} inhibits TRPV6 and contributes to the strong inward rectification of the channel (Voets et al., 2003). TRPV6 activity is inhibited by cations in the following order $\text{Pb}^{2+} = \text{Cu}^{2+} = \text{Gd}^{3+} > \text{Cd}^{2+} > \text{Zn}^{2+} > \text{La}^{3+} > \text{Co}^{2+} > \text{Fe}^{2+} \gg \text{Fe}^{3+}$, with IC_{50} values ranging between 1 to 10 μM (Kovacs et al., 2011). The TRPV6 protein on cellular membranes is constitutively active displaying a single-channel conductance of 42-58 ps (Yue et al., 2001; McGoldrick et al., 2018).

The elucidation of the crystal structure of TRPV6 was accompanied by a description of the locations of the cation binding sites (Saotome et al., 2016; Yelshanskaya et al., 2020). Four different types of cation binding sites are thought to exist in the TRPV6 channel (Figure 4). The evidence for such sites is gathered from scrutinizing differences in anomalous peaks that are collected as part of X-ray-diffraction studies on distinct TRPV6 crystals bound to different types of cations such as Ca^{2+} , Ba^{2+} , and Gd^{3+} (Kovacs et al., 2011). Site 1, which is thought to be the main cation binding site, is located in the central pore and shares the same plane that is occupied by the key selective residues D541 in rat TRPV6. The predicted interatomic distance is 2.4 Å between carboxylate oxygens of D541 and partially dehydrated Ca^{2+} . This data corroborates very well with previous studies on Ca^{2+} -binding proteins (Yang et al., 2002). Site 2 is thought to be present about 6-8 Å below Site 1 between backbone carbonyls and side-chain hydroxyl groups of residues T538. The third site is believed to be located in the central pore axis about 6.8 Å below Site 2. This site is thought to occupy the same plane as the residue M569. Site 2 and 3 are thought to interact with partially-hydrated to equatorially-hydrated Ca^{2+} ions. In contrast to the first three sites that are located in the permeation pathway, Site 4 contains four symmetric cation binding sites in the extracellular vestibule, which are involved in the recruitment of cations towards the extracellular vestibule of TRPV6 and are thus referred to as recruitment sites (Saotome et al., 2016).

These insights from the cation binding sites provide clues into the ion permeation mechanism of TRPV6. This was followed by a molecular dynamics study that expands the description of the “knock-off” mechanism responsible for the ion permeation mechanism (Sakipov et al., 2018). The study showed that at low $[\text{Ca}^{2+}]$, a single Ca^{2+} ion binds with D541 at the narrow constriction point of the selectivity filter. At high $[\text{Ca}^{2+}]$, Ca^{2+} permeates the pore following the knock-off mechanism that mainly has two processes: i) an incoming

Ca²⁺ ion approaches Site 1a from the extracellular vestibule, and ii) the resident Ca²⁺ ion from Site 1b departs to the central cavity. The transition state for this permeation process involves three ions occupying two binding sites (Sites 1a and 1b) formed by D541 residues (Sakipov et al., 2018).

5.2 Channel Gating Mechanism

Unlike the crystal structure of rat TRPV6 that captured structural information of closed TRPV6, the cryo-EM structure of human TRPV6 revealed both the closed and open conformations of the channel (Saotome et al., 2016; McGoldrick et al., 2018). The ion permeation pathway is lined by the side chains of residues D542, T539, N572, I575, D580, and W583 and the backbone carbonyl oxygens of I540, I541, and G579 (McGoldrick et al., 2018). The selectivity filter of human TRPV6 is formed by four D542 side chains (one from each subunit of TRPV6) protruding in the center of the ion path. Remarkably, the lower gate of human TRPV6 can be switched from open to the closed state by the introduction of a single R470E mutation at the binding site for natural lipid (lipid density 2) similar to the vanilloid binding site in TRPV1.

The opening of the channel is characterized by a local transition in the S6 helix, which goes from adopting an α -helical conformation in the closed state to a π -helix in the open state (Figure 2) (McGoldrick et al., 2018). The α -to- π helical transition occurring in the middle of S6; forces the lower portion of the helix that is gating the pore to twist by 100 degrees and curve away from the pore axis by 11 degrees. This conformational change widens the pore size and alters the residues facing the pore axis. For instance, the residues L574 and M578 facing the pore axis in the closed state, are swapped by residue N572 and I575 in the open state. The lower gate opening also triggers the formation of a salt bridge between residues Q473 (in the S4-S5 elbow) and R589 (in TRP helix) and a hydrogen bond between residues D489 (in S5) and T581 (in S6). These electrostatic interactions are believed to offset the high energetic cost of unfavorable α -to- π helical transition. Since the energetic cost between closed and open conformation is similar, the lower gate can easily switch the closed/open states by different physiological stimuli. The conformational changes during lower gate opening originate at residue A566 in S6. The A566 residue acts as a hinge that allows local conformational changes in the S6 bundle without affecting the conformation of the selectivity filter (McGoldrick et al., 2018).

5.3 Regulation by Phosphatidylinositol 4,5-bisphosphate (PIP₂) and CaM

Cellular regulation of TRPV6 channel activity involves interactions with Ca²⁺-CaM and phospholipid PIP₂ (Figure 3). The influx of Ca²⁺ inside the cell triggers two negative feedback mechanisms to tone down TRPV6 activity in order to protect cells from Ca²⁺ overload (Peng et al., 2018). These mechanisms include PIP₂-depletion-induced and CaM-binding-mediated inactivation. Most TRP channels, including TRPV5 and TRPV6, require PIP₂ for activation (Nilius et al., 2002; Thyagarajan et al., 2009; Zakharian et al., 2011). The first evidence for PIP₂-mediated regulation of TRPV6 was provided by Thyagarajan *et al.* (Thyagarajan et al., 2009). The investigators showed that TRPV6 undergoes Ca²⁺-induced inactivation through a PIP₂-depletion-dependent mechanism. Ca²⁺ influx in TRPV6-expressing cells activates phospholipase C (PLC) which in turn hydrolyzes PIP₂ to

produce IP₃. The ensuing PIP₂ depletion due to this conversion leads to a decline in channel activity (Thyagarajan et al., 2009; Cao et al., 2013). The PIP₂-mediated regulation of TRPV6 could have implications in the pathogenesis of Cystic fibrosis, Lowe syndrome, and Dent's disease (Wu et al., 2012; Vachel et al., 2015). In comparison to bronchial epithelial cells from non-CF individuals, cells derived from individuals suffering from cystic fibrosis express low levels of PLC- δ 1 – a phospholipase that hydrolyzes and consequently depletes PIP₂. Therefore, a decrease in PLC- δ 1 is thought to diminish the negative regulation of TRPV6 activity brought about by a reduction in PIP₂. The concomitant increase in TRPV6 activity correlates with an increase in constitutive Ca²⁺ influx and is thought to exacerbate CF inflammation possibly through an increase in cytokine secretion. Similarly, the activity of TRPV6 is also inhibited by the protein oculocerebrorenal syndrome of Lowe (OCRL), which is a PIP₂ 5'-phosphatase involved in pathogenesis of Lowe syndrome and Dent disease (Wu et al., 2012). The protein OCRL regulates TRPV6 activity in part by regulating the PIP₂ level required for the TRPV6 function. When TRPV6 is co-expressed with Dent disease-causing mutants of OCRL, the Ca²⁺ uptake activity of TRPV6 is inversely related to the PIP₂ 5-phosphatase activity of the OCRL mutants. These observations suggest that the pathogenesis of these diseases could be influenced by the dysregulation of PIP₂-mediated TRPV6 activity. The Mg-ATP complex provides the substrate for Type III phosphatidylinositol 4 kinases which allows the resynthesis of PIP₂ and subsequent activation of TRPV6 (Zakharian et al., 2011).

However, the exact location of PIP₂ binding sites in TRPV6 is debatable and is conjectured based on insights from PIP₂-bound-TRPV5 and lipid densities observed in the crystal structure of TRPV6 (Velisetty et al., 2016; Hughes et al., 2018b; McGoldrick et al., 2018). The presence of four lipid-like densities around each subunit of human TRPV6 in Cryo-EM reconstruction supports the hypothesis for protein-lipid interactions (Saotome et al., 2016). These observations suggest the location for such a site between the linker domain (R302 and R305), S4-S5 linker (K484), and the S6 helix (R584) of the channel. The presence of positively charged residues R470 and K484 and polar residues T479, Q483, and Q596 around the lipid density 2 in human TRPV6 also presents an environment favorable for interactions with the polar head group of PIP₂. The lipid-like densities 5 and 6 are also considered putative candidates for PIP₂ interaction, with ambiguous evidence for this hypothesis coming from the open-state structure of PIP₂-bound TRPV5 (Velisetty et al., 2016; Hughes et al., 2018b; McGoldrick et al., 2018; Yelshanskaya et al., 2020). A positively charged arginine residue located between S4 and S5 in TRPV1 is important for high-affinity binding to PIP₂ (Velisetty et al., 2016). The introduction of this arginine residue in place of a glycine residue at position 488 produces a TRPV6 that displays high-affinity binding to PIP₂ and abolishes Ca²⁺-dependent inactivation of TRPV6 (Velisetty et al., 2016). More recently, Cai et al. identified an autoinhibition mechanism involving intramolecular interactions among TRPV6 termini and the S4-S5 linker; PIP₂ suppresses the autoinhibition and thereby activates TRPV6 (Cai et al., 2020).

A CaM binding site was first identified in the C-terminal region of TRPV6 that is involved in the Ca²⁺-dependent inactivation mechanism of TRPV6 (Niemeyer et al., 2001). Cao et al utilized inside-out membrane patches to show that Ca²⁺-CaM complex inhibits TRPV6 by binding to the distal C-terminal region of TRPV6 (Cao et al., 2013). The interaction

of TRPV6 and CaM increases dynamically with increasing intracellular $[Ca^{2+}]$ (Derler et al., 2006). The addition of PIP_2 overrides Ca^{2+} -CaM mediated inhibition of TRPV6 in a dose-dependent manner (Cao et al., 2013). TRPV6 inactivation by CaM is orchestrated by a balance of intracellular Ca^{2+} and PIP_2 concentration (Cao et al., 2013). However, the structural basis of Ca^{2+} -CaM mediated inhibition has only recently been resolved through the elucidation of TRPV5 and TRPV6 structures (Hughes et al., 2018a; Singh et al., 2018a; Dang et al., 2019). Insights from these structures show that the mechanism of CaM-mediated inactivation of TRPV5 and TRPV6 is not different. Examination of hTRPV6 - CaM and rTRPV6 - CaM complexes reveal 1:1 stoichiometry wherein the TRPV6 tetramer binds to one two-lobe CaM molecule (Singh et al., 2018a; Singh et al., 2018b). CaM sits in a $50 \text{ \AA} \times 50 \text{ \AA}$ wide cavity below the channel that is enclosed by a 110 \AA wide intracellular skirt. Most surprisingly, CaM adopts a peculiar head-to-tail arrangement for its N- and C-terminal lobes, which has not been previously observed within other CaM-TRP interactions (Singh et al., 2018a; Singh et al., 2018b).

The lysine 115 (K115) residue protruding from the loop between helices $h6$ and hi in the C-terminal lobe of CaM, interacts with all four TRPV6 subunits by extending its side chain into the intracellular crevice of the ion channel pore formed by four tryptophans (W582 in rTRPV6, W583 in hTRPV6), one from each TRPV6 subunit at the S6 bundle crossing (Singh et al., 2018a; Singh et al., 2018b). In this arrangement traps, K115 residues of CaM are sequestered in a tight square cage formed by four tryptophan residues. This in turn facilitates cation- π interaction between the π - system of four tryptophan indole rings and the positively charged ϵ - amino group of lysine. The transition of TRPV6 from open to the closed state involves tilting of the lower portion of S6 helices which pivot around the gating residue A566. This movement orients the S6 bundle towards the central axis of the pore resulting in the closure of the pore. In this conformational maneuver, the cation- π interactions between K115 and tetra-tryptophan cage are believed to offset the energetic cost of lost R589-Q473 salt bridges.

How is Ca^{2+} influx linked to channel CaM-mediated inhibition of the TRPV6 channel? The C-terminal and N-terminus of CaM display differential affinity to Ca^{2+} (Bate et al., 2018; Singh et al., 2018a; Singh et al., 2018b). Under basal conditions, it is suspected that the C-terminal lobe is perpetually bound to Ca^{2+} and thereby to the distal C-terminus of TRPV6. TRPV6 channel opening is thought to increase local $[Ca^{2+}]$ and saturate both the N-terminal and C-terminal lobe of CaM. A CaM fully saturated with Ca^{2+} ions on both N- and C-terminal then adopts a distinct head-to-tail arrangement described earlier wherein the C - terminal lobe points towards the pore's intracellular entrance and the K115 side chain plug into the tetra - tryptophan cage. This conformation securely fits the CaM protein right underneath the cavity and blocks the ion pore entrance (Bate et al., 2018).

5.4 TRPV6-Interacting Proteins

Studies utilizing yeast two-hybrid, pull-down assays, and antibody-based affinity purification have revealed several interesting TRPV6 protein interactors (van de Graaf et al., 2006b). Many studies have also charted out interacting important domains within TRPV6 that participate in protein-protein interactions (Fecher-Trost et al., 2014). Selected

studies have also identified the regulatory implications of some interactions and important insights have been summarized on the manually curated TRIP database (Shin et al., 2010; Peng, 2011). Among 20 TRPV6 interactors cataloged on this database, the functional consequences of CaM and Glucuronidase Klotho have been most extensively characterized (Niemeyer et al., 2001; Lambers et al., 2004; Derler et al., 2006; Cao et al., 2013). The database is not all-inclusive. For example, Nedd4-2, which was shown to interact with TRPV6 is not included in the table. The interaction with the ubiquitin E3 ligase Nedd4-2 may be involved in the degradation of TRPV6 (Zhang et al., 2010), even though it is unclear whether this is the main pathway. Some proteins, although may regulate TRPV6 activity, trafficking, or degradation, a direct physical association with TRPV6 may not be involved. For example, serine/threonine-protein kinase With No Lysine (K) 3 (WNK3) increases both TRPV5- and TRPV6-mediate Ca^{2+} uptake activity *in vitro*, the mechanism involves the kinase activity of WNK3; however, a direct WNK3-TRPV6 interaction may not be involved (Zhang et al., 2008).

As described in the previous section, CaM inactivates TRPV6 activity as a part of the Ca^{2+} -dependent negative feedback mechanism. At least 7 CaM-binding sites have been reported on TRPV6 (Yelshanskaya et al., 2020). The action of CaM is antagonized by protein kinase C which phosphorylates the CaM-binding site at threonine residue 702 (Niemeyer et al., 2001). The glucuronidase Klotho hydrolyzes N-linked asparagine 357 (N358 in hTRPV6) and entraps the channel protein in the plasma membrane and thereby increases channel activity (Chang et al., 2005; Lu et al., 2008). Important aspects related to screening, *in vitro* validation, *in vivo* validation, and functional consequences of TRP interactions are available in a manually curated TRIP database (TRPV6 relevant details summarized in Table 3)(Shin et al., 2010).

6 Physiological Functions

Ca^{2+} ions are essential for many biological processes such as intracellular signaling processes, bone mineralization, muscle contraction, neurotransmission, and blood coagulation (Suzuki et al., 2008b; Brini M., 2013). For this reason, the $[\text{Ca}^{2+}]$ in the extracellular fluid is maintained at a narrow range through the action of the Ca^{2+} -sensing receptor in the parathyroid gland (Brown, 2013). Ca^{2+} homeostasis is achieved mainly by intestinal absorption and renal reabsorption of Ca^{2+} , and calcium deposition into and resorption from the bone (Shaker and Deftos, 2000; Diaz de Barboza et al., 2015; Beggs and Alexander, 2017). TRPV6 plays a role in both intestinal absorption and renal reabsorption of Ca^{2+} ; it is also involved in bone formation/mineralization. Although the $[\text{Ca}^{2+}]$ in the extracellular fluid is maintained at around 1 mM, in some local environments, such as in the inner ear and epididymis, a much lower $[\text{Ca}^{2+}]$ is required for their physiological function. As a selective Ca^{2+} channel, TRPV6 contributes to both systemic Ca^{2+} homeostasis and local Ca^{2+} regulation required for physiological function.

The most well-characterized physiological role of TRPV6 is serving as the apical Ca^{2+} entry channel in Ca^{2+} transporting epithelia (Peng, 2011; Na and Peng, 2014; van Goor et al., 2017). The high-level expression of TRPV6 in exocrine tissues also indicates its likely role in exocrine function—possibly by replenishing Ca^{2+} in the secretory vesicles

after a discharge of secretory cargo. Figure 5 shows some physiological roles of TRPV6. These include roles in intestinal Ca^{2+} absorption, in developing a low luminal $[\text{Ca}^{2+}]$ in the epididymis for sperm fertilization capacity (Weissgerber et al., 2011; Weissgerber et al., 2012), in maternal-fetal Ca^{2+} transport (Bond et al., 2008; Suzuki et al., 2008a; Suzuki et al., 2018; Yamashita et al., 2019), and exocrine function of pancreas. Although characterized in lesser details TRPV6 is also believed to play a role in keratinocyte differentiation (Lehen'kyi et al., 2007a; Lehen'kyi et al., 2011b), ion regulation in the endolymphatic system of the vestibular system (Yamauchi et al., 2005; Yamauchi et al., 2010; Bachinger et al., 2019), maintaining skeletal and cartilage integrity (Lieben et al., 2010; Lieben and Carmeliet, 2012; Song et al., 2017; Suzuki Y, 2018; Fecher-Trost et al., 2019; Mobasheri et al., 2019; Yamashita et al., 2019), and possibly in viable embryo implantation and pregnancy (Moreau et al., 2002b; Bernucci et al., 2006; Suzuki et al., 2008a; Hache et al., 2011; Yang et al., 2013b). We will discuss the roles of TRPV6 in the following sections. In addition, we will concurrently describe the disease implications corresponding to aberrant expression, mutation, and dysfunction of TRPV6.

6.1 Ca^{2+} Absorption in the Intestine

Intestinal Ca^{2+} absorption takes place via paracellular and transcellular routes. The paracellular route involves passive diffusion of Ca^{2+} via paracellular channel protein claudins in the tight junctions. The transcellular route which entails three steps: (1) luminal Ca^{2+} enters across the brush border membrane via Ca^{2+} channels down an approximately 1,000 to 10,000 fold of $[\text{Ca}^{2+}]$ gradient; (2) Ca^{2+} ions diffuse from apical to basolateral side, a process that is facilitated by binding of Ca^{2+} to calbindin- D_{9k} ; and (3) energy-dependent basolateral release mainly through plasma membrane Ca^{2+} ATPase (Bronner, 2003; Diaz de Barboza et al., 2015; Peng et al., 2018) (Figure 6). The paracellular pathway works predominantly when the dietary Ca^{2+} level is high so that there is a favorable $[\text{Ca}^{2+}]$ gradient across the intestinal epithelium; however, if the luminal $[\text{Ca}^{2+}]$ is lower than that in the interstitium, only the transcellular pathway will function. The transcellular pathway is subject to regulations, among which it is the major target of the active form of vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25(\text{OH})_2\text{D}_3$) (Bronner, 2003).

Lines of evidence indicate that TRPV6 plays an important role as the Ca^{2+} entry channel in the plasma membrane. TRPV6 was identified as a result of the search of Ca^{2+} -transporting proteins in the intestine (Peng et al., 1999; Wissenbach et al., 2001). In fact, many early studies demonstrated a strong expression of TRPV6 transcripts in rodent and human duodenal tissue (Peng et al., 1999; Peng et al., 2000; Barley et al., 2001; Hoenderop et al., 2001; Zhuang et al., 2002). The apical localization of the protein on epithelial cells in human and mouse-specific GI tissues was confirmed by Zhuang et al. Subsequent characterization of TRPV6 deficient mice revealed significant defects in intestinal Ca^{2+} absorption (Bianco et al., 2007; Lieben et al., 2010). Furthermore, as discussed in Section 6, duodenal TRPV6 expression is highly regulated by vitamin D (Van Cromphaut et al., 2001). A growing body of work has resulted in a significant understanding of mechanisms governing Ca^{2+} absorption and the role of TRPV6 in transcellular Ca^{2+} transport has been recognized in several interesting reviews (Bronner, 2003; Suzuki et al., 2008b; Diaz de Barboza et al., 2015; Moor and Bonny, 2016).

The paracellular route of absorption is predominant when a high level of Ca^{2+} is available in the diet (Bronner, 2003) (Figure 6, lower panel). The transcellular route, though active under such conditions, contributes only to a minor fraction of Ca^{2+} reabsorption. A high level of Ca^{2+} in the diet generates an ion gradient favoring paracellular transport of Ca^{2+} across the epithelium of the intestine. Under such conditions, the Ca^{2+} in the lumen of the intestine is absorbed via the paracellular route with the ion passively meandering between the tight junctions between epithelial cells formed in part by claudins. Indeed, a high level of Ca^{2+} in the diet and sufficient $[\text{Ca}^{2+}]$ in the plasma will decrease the synthesis of $1,25(\text{OH})_2\text{D}_3$ and in turn, downregulates *TRPV6* transcription. The calbindin- D_{9k} expression is not directly regulated by vitamin $1,25(\text{OH})_2\text{D}_3$ but is very much dependent on TRPV6 expression (Cui et al., 2012). The decrease in TRPV6 expression will also cause a reduction in calbindin- D_{9k} , thus the transcellular pathway of Ca^{2+} absorption will be downregulated when the dietary calcium level is sufficient. More recently the role of Ca^{2+} -sensing receptor (CaSR) in the regulation of TRPV6-dependent intestinal Ca^{2+} absorption has come into the picture (Lee et al., 2019b). CaSR in the basolateral membrane of the intestine directly attenuates local Ca^{2+} absorption via TRPV6 to prevent hypercalcemia. When the interstitial $[\text{Ca}^{2+}]$ level is elevated due to high paracellular Ca^{2+} absorption, CaSR will be activated to inhibit Ca^{2+} fluxes through TRPV6 as observed using *Xenopus* oocytes. Thus, high dietary Ca^{2+} may downregulate TRPV6-mediated transcellular Ca^{2+} absorption through CaSR through reducing $1,25(\text{OH})_2\text{D}_3$ synthesis thus TRPV6 transcription systemically and through inhibiting TRPV6 activity locally (Figure 6).

However, when the $[\text{Ca}^{2+}]$ in the lumen of the intestine is lower in comparison to that in the plasma, the paracellular pathway is not functional and the transcellular pathway is required for Ca^{2+} absorption (Figure 6, upper panel). The slight reduction in $[\text{Ca}^{2+}]$ extracellular fluid will initiate parathyroid hormone (PTH) secretion through CaSR and in turn stimulate $1,25(\text{OH})_2\text{D}_3$ synthesis. The increased $1,25(\text{OH})_2\text{D}_3$ stimulates intestinal Ca^{2+} absorption through various mechanisms, among which are transcriptional upregulation of TRPV6 and calbindin- D_{9k} (see Figure 6, upper panel). Aspects related to vitamin D-mediated regulation of TRPV6 and its implications on intestinal reabsorption of Ca^{2+} will be revisited in Section 6.1.

Most studies indicate TRPV6 as the major player in $1,25(\text{OH})_2\text{D}_3$ -mediated intestinal Ca^{2+} absorption, however, other reports have challenged this notion and proposed redundancy of TRPV6 in this process (Hoenderop et al., 1999; Peng et al., 2003; Walters et al., 2006; Kutuzova et al., 2008; Fleet and Schoch, 2010; Christakos et al., 2011). The transgenic expression of TRPV6 under the villin promoter in the intestine rescues the Ca^{2+} deficiency in vitamin D receptor (VDR) knockout mice, strongly supports the role of TRPV6 in mediating vitamin D-regulated intestinal Ca^{2+} absorption (Cui et al., 2012). This does not exclude the possibility that alternative Ca^{2+} channels may also be involved in vitamin D-regulated Ca^{2+} absorption (Kutuzova et al., 2008; Fleet and Schoch, 2010; Christakos et al., 2011). In addition to vitamin D and level of Ca^{2+} in the diet, physiological factors such as age, gender, pregnancy, lactation, the level of physical activity, and hormonal influences from parathyroid hormone, estrogens, and progesterone strongly influence TRPV6 functions (Peng, 2011). We will return to these aspects of TRPV6 regulation in later sections of this review where we describe the regulatory mechanisms governing TRPV6 expression.

6.2 Ca²⁺ Reabsorption in the Kidney

The kinetics of Ca²⁺ reabsorption in nephrons is dictated by distinct segment-specific mechanisms (Blaine et al., 2015; Moor and Bonny, 2016; Peng et al., 2018). The majority of Ca²⁺ is reabsorbed through the paracellular pathways in the proximal tubule and the loop of Henle; however, active Ca²⁺ reabsorption through the transcellular pathway does occur in these tubule segments even though the detailed mechanisms are less clear. On the other hand, about 5-10% of Ca²⁺ reabsorption occurs in the distal tubules entirely by the transcellular route, and this pathway is thought to be a regulatory mechanism for fine-tuning Ca²⁺ homeostasis in the body. The steps of Ca²⁺ absorption by the transcellular route are similar in the intestine and kidneys. In contrast to the rodent intestine where TRPV6 is the major player in the reabsorption of Ca²⁺, the transcellular reabsorption of this ion in the kidney occurs through TRPV5 on the apical membrane of epithelial cells lining the distal convoluted tubule and connecting tubule (Hoenderop et al., 2003a; Na and Peng, 2014). This is followed by the escort of the ion to the basolateral side of epithelial cells by Calbindin-D_{28K}. The final step in this pathway entails the basolateral exit of Ca²⁺ via PMCA and Na⁺/Ca²⁺ exchanger NCX1 (Blaine et al., 2015; Moor and Bonny, 2016; Peng et al., 2018).

TRPV6 mRNA level in mouse fetal kidney is comparable to that of TRPV5; a significant decrease in renal TRPV6 occurs at the time of weaning and lasts to the adulthood when renal TRPV6 mRNA is about 5 to 10% that of TRPV5 (Song et al., 2003b). Even though expressed at a low level in the kidney, TRPV6 plays a role in Ca²⁺ reabsorption because TRPV6 KO mice exhibit hypercalciuria (Bianco et al., 2007). More importantly, in larger animals examined, such as horses, the renal mRNA level of TRPV6 appears to be higher than that of TRPV5 (Rourke et al., 2010). A recent study with quantitative real-time PCR indicated that TRPV6 is the main Ca²⁺ channel in transcellular Ca²⁺ transport in the kidney of sheep, dogs, and horses (Azarpeykan et al., 2016). In humans, TRPV6 mRNA expression is much higher in comparison to TRPV5 in the human kidney (Peng et al., 2001a). Wu et al. showed that it is unlikely TRPV5 that is expressed exclusively in the distal tubule, TRPV6 is also abundantly expressed in the proximal tubule in the human kidney by IHC (Wu et al., 2011). Consistent with this, we observed that TRPV6 mRNA is more abundant in the cortex than in the medulla in the human kidney, and TRPV5 shows an opposite pattern (unpublished result). Thus, TRPV6 could play a bigger role in Ca²⁺ reabsorption in large mammals including humans than previously envisioned in rodents (Peng, 2011).

6.3 Maternal-Fetal Ca²⁺ Transport

The placenta plays an indispensable role in supporting the nutritional and mineral requirements of the fetus (Lager and Powell, 2012; Brett et al., 2014; Ohata et al., 2016). A higher [Ca²⁺] in fetal blood in comparison to maternal blood necessitates an active transcellular transport of this ion during late pregnancy when fetal bone mineralization is at its peak (Kovacs and Kronenberg, 1997; Stulc, 1997; Kumar and Kaur, 2017). Placental transport of Ca²⁺ is critical for overall infant bone health and defects in maternal-fetal transport are linked to Ca²⁺ deficiency syndromes and intrauterine growth restrictions (Goodfellow et al., 2011; Sharma et al., 2016). The molecular underpinnings of Ca²⁺ transporters linked to this process were unknown until pioneering studies by Suzuki *et al*

demonstrated the role of TRPV6 in this process. More recently the pathological implications on fetal skeletal architecture have become apparent with high throughput exome studies which are described in the next section.

TRPV5 and TRPV6 expression have been observed in the placenta in multiple studies, however, TRPV6 expression is ~1000-fold higher in comparison to TRPV5 (Moreau et al., 2002a; Bernucci et al., 2006; Stumpf et al., 2008; Suzuki et al., 2008a; Lee et al., 2009; Hache et al., 2011; Yang et al., 2013b). TRPV6 is abundantly expressed in trophoblasts and syncytiotrophoblasts of the human placenta with both apical and basolateral expression being reported for the latter cell type (Wissenbach et al., 2001; Bernucci et al., 2006). In mice, TRPV6 mRNA and protein are mainly found in the intraplacental yolk sac and the visceral layer of the extraplacental yolk sac (Suzuki et al., 2008a). More importantly, TRPV6 expression increases by almost 14-fold during the last 4 days of the murine gestational period, coinciding with the peak phase of fetal bone mineralization (Suzuki et al., 2008a).

The first evidence of the role of TRPV6 in maternal-fetal transport was reported by Suzuki *et al* who demonstrated a 40% reduction in $^{45}\text{Ca}^{2+}$ transport activity in TRPV6 KO fetuses (Suzuki et al., 2008a). The TRPV6 KO mice display a dramatic decrease in the ash weight of the fetus indicating the critical role of TRPV6 in this physiological process (Suzuki et al., 2008a). TRPV6 expression correlates with the Ca^{2+} uptake potential of human trophoblasts. Furthermore, fluid shear stress (FSS) triggers a TRPV6-mediated Ca^{2+} influx in human trophoblasts (Miura et al., 2015). This process in turn induces microvilli formation through the functional activation of Ezrin via Ca^{2+} -dependent Akt phosphorylation (Miura et al., 2015). Another study has shown that Cyclophilin B, a member of the immunophilin family, associates with TRPV6 in syncytiotrophoblasts and increases its activity *in vitro*. These studies suggest that role of maternal-fetal Ca^{2+} transport is broadly conserved across mammals (Stumpf et al., 2008).

6.4 Epididymal Ca^{2+} Regulation and Implications on Male Fertility

The precise regulation of ionic milieu in the epididymal lumen is critical for sperm maturation and motility (Turner, 2002). The influx of Ca^{2+} in spermatozoa plays significant roles in hyperactivation, chemotaxis, acrosome reactions, transport to the oocyte, and fertilization (Rahman et al., 2014). Most importantly, the regulation of $[\text{Ca}^{2+}]$ in the epididymal lumen is indispensable for proper sperm motility (Ecroyd et al., 2004). Weissgerber *et al* have shown that male fertility in mice is crucially dependent on TRPV6-mediated reduction of luminal $[\text{Ca}^{2+}]$ in the epididymis (Weissgerber et al., 2011). The investigators conducted elegant experiments utilizing a mouse model homozygous for *Trpv6*^{D541A} mutation. This substitution in the critical pore-forming residue of TRPV6 eliminates Ca^{2+} selectivity and blocks Ca^{2+} uptake. Male mice harboring this nonfunctional TRPV6 on the epididymal epithelium were found to have severely impaired fertility. Closer examination revealed that in comparison to WT mice, mice harboring nonfunctional TRPV6 exhibited 10 times higher $[\text{Ca}^{2+}]$ in the epididymal lumen, and $^{45}\text{Ca}^{2+}$ uptake from was concomitantly reduced by 7-to-8 folds. This increase in epididymal luminal $[\text{Ca}^{2+}]$ in cauda epididymis leads to significant defects in motility, fertilization capacity, and viability of

sperms in *Trpv6*^{D541A} mice. Not surprisingly, the observed male fertility defects observed in *Trpv6*^{D541A} homozygous mice were mirrored in *Trpv6*^{-/-} mice (Weissgerber et al., 2012).

A recent study suggests that TRPV6 works in a concerted fashion with chloride channel transmembrane member 16 A (TMEM16A) to reduce the [Ca²⁺] in the epididymal lumen (Gao da et al., 2016). The study showed that the Ca²⁺ influx in epididymal principal cells through constitutively open TRPV6 channels induces a local rise in intracellular Ca²⁺ levels. This subsequently depolarizes the apical membrane of the epididymis and triggers Ca²⁺-activated chloride conductance (CaCC) channel TMEM16A which acts to bring membrane potential back to normal; thereby facilitating the subsequent reduction of [Ca²⁺] in the epididymal lumen (Gao da et al., 2016). Proteomic analysis by Lin et al have shown that TRPV6 is markedly reduced in the seminal extracellular vesicles and ejaculated spermatozoa of asthenozoospermic patients, suggesting that the protein could be contributing to multiple aspects of male fertility (Lin et al., 2019).

6.5 Bone and Cartilage Health

The bone relies on Ca²⁺ absorption from the intestine and Ca²⁺ reabsorption in the kidney to maintain an adequate supply of Ca²⁺ required to maintain skeletal integrity (Lieben and Carmeliet, 2012). Indeed, 99% of the body's Ca²⁺ is stored in the bone. Since the majority of Ca²⁺ absorption in the intestine occurs through the paracellular route, the loss of TRPV6 and the accompanying defect in Ca²⁺ transcellular transport is not severe enough to induce bone loss or cause architectural damage when Ca²⁺ is sufficiently high in the diet (Lieben et al., 2010; Lieben and Carmeliet, 2012). However, when dietary Ca²⁺ is insufficient, normal serum Ca²⁺ levels in TRPV6 KO mice are maintained at the expense of bone (Lieben et al., 2010; Lieben and Carmeliet, 2012).

TRPV6 is expressed at low levels in osteoblasts and osteoclasts (Lieben et al., 2010; Little et al., 2011; Lieben and Carmeliet, 2012) whereas TRPV5 is present only on osteoclasts (van der Eerden et al., 2005). Evidence regarding the role of TRPV6 in the differentiation and function of these cell types is confounding. The prevailing body of literature suggests that TRPV6 plays an important role in osteoclasts but not in osteoblasts (Lieben et al., 2010; Little et al., 2011; Lieben and Carmeliet, 2012). Three different studies have demonstrated that TRPV6 does not play a role in Ca²⁺ uptake or mineralization in osteoblasts (Little et al., 2011; van der Eerden et al., 2012; Munson et al., 2019). Chen *et al* have shown that TRPV6 depletion results in increased osteoclasts differentiation (Chen et al., 2014a) whereas TRPV5 is essential for proper osteoclastic bone resorption (van der Eerden et al., 2005).

Although the role of TRPV4 in chondrocyte function has been established, the role of TRPV6 in this context has only recently come to light (Song et al., 2017; Mobasheri et al., 2019). Experiments using a rat osteoarthritis (OA) model have shown that TRPV6 plays a pleiotropic role in chondrocyte biology (Song et al., 2017). TRPV6 significantly affects chondrocyte cell proliferation and apoptosis. TRPV6 contributes significantly to extracellular matrix secretion and the release of matrix-degrading enzymes from rat chondrocytes.

6.6 Embryo-Implantation and Maintenance of Pregnancy

Given the continuum of the placenta as a specialized organ that develops within the uterus, the expression of TRPV6 in uterine and endometrial tissues is not surprising. Uterine and endometrial expression of TRPV6 has been observed in birds (Yang et al., 2013c), mice (Lee and Jeung, 2007; De Clercq et al., 2017), rats (Kim et al., 2006), dogs (Kim et al., 2011), pigs (Choi et al., 2009), cows (Sprekeler et al., 2012), and humans (Yang et al., 2011). TRPV6 mRNA in the rodent is expressed in placenta-unattached areas of the uterus and the labyrinth and spongy zone of the placenta (Lee et al., 2009). Uterine TRPV6 immunoreactivity has been seen in luminal and glandular epithelial cells. TRPV6 expression in uterus peaks at pregnancy day (P) 0.5, P5.5, and P13.5 while placental expression increases until mid-gestation. The expression of TRPV6 in the uterus is thought to be hormonally regulated by 17β -estradiol and progesterone in rodents (Lee et al., 2009).

Overall, four lines of evidence suggest the involvement of TRPV6 in embryo-implantation and maintenance of pregnancy in mammals: a) juxtaposition of uterine and placental expression, b) pregnancy-stage-dependent cyclical changes in expression, c) the contribution of the estrous cycle in the modulation of TRPV6 in mammals, and d) stringent regulation of channel expression by sex hormone (described in section 8.2) (Kim et al., 2006; Choi et al., 2009; Lee et al., 2009; Yang et al., 2011; Tran et al., 2018). Notably, downregulation of TRPV6 expression and a concomitant decline in Ca^{2+} transport in the mammalian syncytiotrophoblasts is thought to alter the oxygen perfusion of placental tissues; a perturbation which in turn has been linked to the development of preeclampsia (Hache et al., 2011; Yang et al., 2013a; Yang et al., 2013b; Yang et al., 2015).

6.7 Keratinocyte Differentiation

The epidermis has a Ca^{2+} gradient, with the lowest concentrations in the stratum basal and the highest in the granulosum stratum (Floriana et al., 2014). The Ca^{2+} gradient within the epidermis promotes sequential differentiation of keratinocytes as they cross the multiple layers of the epidermis to form stratum corneum (Bikle et al., 2012). Keratinocytes cultured in low $[Ca^{2+}]$ remain proliferative but fail to differentiate unless they are moved to more conducive conditions with elevated $[Ca^{2+}]$ (Bikle et al., 2012; Floriana et al., 2014). Therefore, keratinocytes differentiation requires a Ca^{2+} switch, a process that triggers an influx of Ca^{2+} in keratinocyte and triggers transcriptional changes and signaling cascade necessary for processes such as desmosome formation, stratification, and cornification. These observations prompted investigations into membrane mechanisms that can regulate Ca^{2+} entry and trigger keratinocyte differentiation (Bikle et al., 2012; Floriana et al., 2014). The role of TRPV6 in the skin was alluded by earlier studies by Hediger and coworkers who reported that TRPV6 KO mice had thinner layers of stratum corneum (Bianco et al., 2007). The mice displayed a decreased total Ca^{2+} content and loss of the normal Ca^{2+} gradient. Furthermore, alopecia and dermatitis were observed in more than 20% of all TRPV6 KO mice (Bianco et al., 2007).

Despite these early observations, a mechanistic basis of these phenotypes was not appreciated until studies by Lehen'kyi *et al* demonstrated the critical role of TRPV6 in Ca^{2+} -influx-mediated keratinocyte differentiation (Lehen'kyi et al., 2007a). Lehen'kyi

et al found that TRPV6 knockdown impairs Ca^{2+} -mediated differentiation of human primary keratinocytes and downregulates differentiation markers such as involucrin, transglutaminase-1, and cytokeratin-10. Mechanistic experiments indicated that 1,25-dihydroxyvitamin-D₃-induced upregulation of TRPV6 in keratinocytes is the critical event for triggering a Ca^{2+} influx and orchestrating differentiation-specific pathways (Lehen'kyi et al., 2007a). The authors also reported in a separate report that Avene Thermal Spring Water (TSW), a formulation suspected to provide benefits in dermatological diseases, induces a constitutive influx of Ca^{2+} and concomitantly accelerates differentiation of human keratinocytes (Lehen'kyi et al., 2011b). A compromised ability to regulate intracellular $[\text{Ca}^{2+}]$ due to TRPV6 downregulation in keratinocytes has been linked to the development of psoriatic epidermis (Cubillos and Norgauer, 2016).

6.8 Role in the Inner Ear

Modulation of $[\text{Ca}^{2+}]$ in the vestibular labyrinth is critical for normal hearing and balance (Ceriani and Mammano, 2012). The low $[\text{Ca}^{2+}]$ in the lumen of mammalian endolymph in the inner ear is required for normal hearing and balance (Yamauchi et al., 2010). Studies by Marcus *et al* suggest that both TRPV5 and TRPV6 play a role in the function of the inner ear by modulating Ca^{2+} gradient in the inner ear (Yamauchi et al., 2005; Nakaya et al., 2007; Wangemann et al., 2007; Yamauchi et al., 2010). Several expression studies have demonstrated the expression of TRPV5 and TRPV6 in several regions of the inner ear (Yamauchi et al., 2005; Nakaya et al., 2007; Wangemann et al., 2007; Yamauchi et al., 2010). TRPV5 and TRPV6 are expressed in primary cultures of (SCCD) epithelial cells from neonatal rats (Yamauchi et al., 2010). TRPV5 mRNA but not protein is upregulated following treatment with 1,25(OH)₂D₃ (Yamauchi et al., 2005). In rats, TRPV5/6 and Ca^{2+} transport proteins are expressed in multiple regions of the vestibular system including native SCCD, inner sulcus cells of the cochlea, cochlear lateral wall, and stria vascularis (TRPV5 only) (Yamauchi et al., 2005; Nakaya et al., 2007; Wangemann et al., 2007; Yamauchi et al., 2010). TRPV5 protein is believed to be localized on the apical membrane of stria marginal cells (Yamauchi et al., 2010). Age-dependent decline in TRPV5 and TRPV6 expression in mouse inner ear has been verified by immunostaining (Takumida et al., 2009).

7 Role in Human Diseases

7.1 Nephrolithiasis

Hypercalciuria is one of the most recognized risk factors for stone formation. Two important types of calciuria which are important in the context of Ca^{2+} -selective channels TRPV5/6 are absorptive hypercalciuria and renal-leak hypercalciuria (Worcester and Coe, 2008). In contrast to renal-leak hypercalciuria caused by defects in reabsorption of Ca^{2+} in kidneys, absorptive hypercalciuria is caused by excessive Ca^{2+} absorption in the intestine. Since TRPV5 is considered the gatekeeper of transcellular Ca^{2+} reabsorption in kidneys whereas TRPV6 acts the key Ca^{2+} -transporter in the intestine; the TRPV5 and TRPV6 channels are potentially linked to renal-leak hypercalciuria and absorptive hypercalciuria, respectively. Recently, an L530R variation (rs757494578) in TRPV5 was found to be associated with recurrent kidney stones in 2636 individuals from a founder population in Iceland (Oddsson et al., 2015), and the L530R is dysfunctional when expressed *Xenopus* oocytes (Wang et

al., 2017). This suggests that the dysfunction of TRPV5 likely contributes to kidney stone formation. Given the fact that TRPV6 KO mice display hypercalciuria and TRPV6 appear to play a more significant role in the reabsorption of Ca^{2+} in larger mammals including humans, it is possible that TRPV6 also contributes to stone formation directly if its function is impaired.

The support for the role of TRPV6 in renal stone formation comes from sequencing studies conducted on a cohort of 170 patients in Switzerland (Suzuki et al., 2008d). The studies revealed that the frequency of TRPV6 gain-of-function haplotype is significantly higher in Ca^{2+} -stone formers. These results coupled with observed hypercalciuria phenotypes from animal studies suggest correlations between derived TRPV6 haplotype as a potential risk factor for absorptive hypercalciuria. Interestingly TRPV6 mRNA is higher than TRPV5 in human kidneys (Peng et al., 2001a). Furthermore, proximal tubule and distal tubule in human kidneys express high levels of TRPV6 protein (Wu et al., 2011). The lower incidence of kidney stone diseases in African Americans coupled with the relatively higher prevalence of ancestral haplotype suggests a theory under which the haplotype confers an advantage of increased Ca^{2+} reabsorption in this demographic. In fact, evidence from two studies suggests that three SNPs on TRPV6; namely C157R, M378V, and M681T were positively selected during human evolution and confer a selective advantage to specific demographics (Suzuki et al., 2008d). The nature of evolutionary advantage remains speculative but biophysical characterization of “divergent TRPV6” revealed enhancement in Ca^{2+} -dependent inactivation – a property that would translate to increased Ca^{2+} uptake and could, in theory, lead to hypercalciuria. Despite these hints from biophysical studies, the reason for the positive selection of these SNPs remains enigmatic (Fecher-Trost et al., 2014).

7.2 Fetal Skeletal Integrity and Transient Neonatal Hyperparathyroidism

The indispensability of TRPV6 in the maternal-fetal Ca^{2+} transport by the placenta was recently corroborated by a recent study by Fecher-Trost *et al* (Fecher-Trost et al., 2019). The investigators showed that deficiency of TRPV6 in the placenta compromises Ca^{2+} transport across trophoblast and leads to reduced embryo growth, induces bone calcification, and impairs bone development in mice (Fecher-Trost et al., 2019). Similarly, a loss-of-function TRPV6 mutation in zebrafish called “matt-und-schlapp” leads to a more than 65% reduction in Ca^{2+} content and results in bone mineral defects induced by a reduction of Ca^{2+} uptake by the yolk sac and gills (Vanoevelen et al., 2011).

Recent exome sequencing studies have revealed that skeletal defects in transient neonatal hyperparathyroidism (TNHP) are a result of insufficient maternal-fetal transport caused by pathogenic genomic variants of *TRPV6* which presumably alter the plasma membrane localization of the protein (Yamashita et al., 2019) (Table 4). Similarly, a genomic analysis of an infant with severe antenatal onset thoracic insufficiency displaying dramatic skeletal abnormalities indicated the critical importance of TRPV6 in maternal-fetal transport in maintaining the skeletal architecture of the fetus (Burren et al., 2018). The study showed that compound heterozygous *TRPV6* variants lead to undermineralization and dysplasia of the fetal skeleton. Bioinformatic studies revealed that phenotype was a result of maternally inherited missense variant, c.1978G > C p. (G660R), and paternally inherited

nonsense variant, c.1528C > Tp. (R510Ter). These variants are hypothesized to cause steric clashes between important residues in the protein and alter TRPV6 tetramer stability. The investigators also reported the presence of abnormal bone architecture in a post-mortem histology report of this case (Mason et al., 2020).

Six variations have been reportedly linked to transient neonatal hyperparathyroidism: C212Y, I223T, R425Q, G428R, G451E, and R483W (Suzuki Y, 2018) (Table 4). Based on their positions in TRPV6 protein and their effects on the function of TRPV6 (Table 4), the variations can be divided into three groups. Group 1 contains R425Q, G428R, and R483W, which are located in transmembrane (TM) helices (R425Q and G428R in TM2, and R483W in TM3). These variations impair the ability of TRPV6 protein to reach the plasma membrane. Group 2 contains C212Y and I223T, which lie in the fourth ankyrin (ANK) repeat domain. These variations decrease the function of TRPV6 in the plasma membrane, thus the possibility that these variations may affect the localization of TRPV6 to the plasma membrane cannot be excluded. However, since ankyrin repeats have been reported to be critical for protein folding, these variations in ANK4 more likely lead to the abnormal structure of TRPV6 and result in the degradation of TRPV6 as a consequence of cellular protein quality-control mechanisms. These two groups of variations reduce the function of TRPV6, which ultimately reduces the maternal-fetal Ca^{2+} transport. Group 3 contains variation G451E, which is located in the intracellular loop between TM2 and TM3. In contrast to the other variations, the G451E variation increases the intracellular $[\text{Ca}^{2+}]$, which causes the overload of intracellular Ca^{2+} and cell death.

7.3 Chronic Pancreatitis

TRPV6 is highly expressed in exocrine organs such as the pancreas, prostate, mammary glands, sweat glands, and salivary glands (Zhuang et al., 2002; Peng, 2011; Fecher-Trost et al., 2014). It is plausible that this expression highlights a mechanism for replenishing Ca^{2+} stores of exocrine cells that release a copious amount of fluids along with secretory vesicles (Peng, 2011). Despite the early characterization of high expression of TRPV6 in exocrine organs and the elucidation of its role as a Ca^{2+} -selective channel, the functional relevance of TRPV6 expression in exocrine organs, particularly the pancreas remains speculative (Zhuang et al., 2002; Peng, 2011; Fecher-Trost et al., 2014). However, accumulating evidence in the recent past indicates that naturally occurring TRPV6 loss of function variants predisposes certain demographics to chronic pancreatitis (CP) by impairing calcium homeostasis in the pancreatic cells (Table 4) (Masamune et al., 2020; Zou et al., 2020).

To assess if changes in pancreatic calcium levels are involved in the development of chronic pancreatitis, Masamune et al initiated their studies by systematically investigating the association of early-onset CP in non-alcoholic patients aged ≤ 20 (Masamune et al., 2020). Whole-exome sequencing-based comparison of DNA data from index patients with idiopathic CP and their parental controls led to the identification of 2 variants: a *de novo* heterozygous c.970G>A (p.D324N) variant and a rare maternally-inherited nonsynonymous variant c.629C>T (p.A210V). The investigators then conducted confirmatory sequencing studies in three independent cohorts that included patients and controls from Japan (CP, N=300; controls, N=1070), France (CP, N=470; controls, N=570), and Germany (CP,

N=410; control N=750). The analysis of sequencing data from Japanese patients revealed the presence of 33 missense and 2 nonsense variants spread across 4.3% (13/300) of CP patients in comparison to a frequency of 0.1% (1/1070) in controls ([OR], 48.4; 95% CI: 6.3-371.7; $P=2.4 \times 10^{-8}$). In this cohort, 9.7% of patients with early-onset CP had at least 1 TRPV6 defective function variant (Masamune et al., 2020).

Similarly, TRPV6 dysfunctional variants were also overrepresented in German and French CP patients in comparison to controls in their respective groups (2.2% vs 0.0%; $P=.0001$ and 1.9% vs 0.0%; $P=.00075$, respectively) (Masamune et al., 2020). Forced expression of functionally defective TRPV6 in HEK293 showed that Ca^{2+} uptake activity and/or protein expression was severely compromised in many mutants. As an *in vivo* validation to this hypothesis, the investigators also demonstrated that Cerulein-Induced Pancreatitis was exacerbated in *TRPV6^{mut/mut}* mice. The studies suggested that p.L299Q and p.D324N mutations constitutively suppress TRPV6 activity potentially by blocking the channel pore (Masamune et al., 2020). More recently, another study identified a set of 25 TRPV6 variants that are associated with CP in Chinese patients (Zou et al., 2020). In agreement with studies by Masamune et al, the study showed that loss of function TRPV6 variants in this set of identified variants predispose the Chinese population to CP. Overall, these studies by showing a strong global association early-onset nonalcoholic CP and TRPV6 dysfunction open up a new avenue of investigation. These studies harbinger a deeper mechanistic understanding of Ca^{2+} uptake in ductal cells as well as in acinar cells of the pancreas, an area which could be promising for developing personalized medicine strategies for therapeutic management of CP in patients harboring TRPV6 loss of function mutations (Masamune et al., 2020).

7.4 Bone Diseases

The importance of TRPV6 in fetal bone mineralization by its Ca^{2+} -absorptive role in the placenta is well established and has been described previously in this review (Suzuki et al., 2008a; Suzuki et al., 2018). Given the critical importance of Ca^{2+} in maintenance and regulation of bone mineral density, previously suggested links between Ca^{2+} -selective channels TRPV5/6 and osteoporosis in the literature are not surprising (Suzuki et al., 2008b; Tai et al., 2015). Notably, Ca^{2+} and vitamin D supplementation is a common strategy for the management of osteoporosis (Sunyecz, 2008). The hormone estrogen which is known to contribute significantly to pathogenesis of osteoporosis also regulates the expression of TRPV5 and TRPV6 (Van Cromphaut et al., 2003; Chen et al., 2014b; Levin et al., 2018; Li et al., 2019). Estrogen deficiency in postmenopausal women is linked to the development and progression of osteoporosis (Levin et al., 2018). In this regard, the lower Ca^{2+} absorption seen in older postmenopausal women is attributed to reduced TRPV6 and VDR expression (Walters et al., 2006). Furthermore, the loss of TRPV5 abrogates the inhibitory effects of estrogen on osteoclasts differentiation and bone resorption activity (Chen et al., 2014b).

Importantly, TRPV6 KO mice display osteoporosis-like symptoms such as hypercalciuria and reduced bone mineral density (Bianco et al., 2007; Suzuki et al., 2008b; Lieben et al., 2010; Lieben and Carmeliet, 2012). Eldecacitol, a vitamin D analog approved for treatment

for osteoporosis in Japan is known to upregulate TRPV6 expression in the intestine (Saito and Harada, 2014). Insights from rodent experiments hypothesize that restoration of Ca^{2+} reabsorption through the anti-depressant-mediated rescue of TRPV6 expression could be a potential strategy for reducing the severity of stress-induced osteoporosis (Charoenphandhu et al., 2012). Perhaps the most compelling evidence regarding the role of TRPV6 in osteoporosis comes from preclinical observations demonstrating the efficacy of the C-terminal portion of Soricidin in the treatment of bone resorptive diseases (Stewart, 2020). Despite multiple lines of evidence suggesting links between TRPV6 and osteoporosis, a compelling mechanistic understanding of this process is lacking and is an exciting unexplored avenue of research in the field.

Hereditary vitamin D-resistant rickets (HVDRR, or VDR-resistant rickets type II) is characterized by rickets, hypophosphatemia, hypocalcemia, and secondary hyperparathyroidism. Alopecia is frequently observed in early-onset HVDRR (Suzuki et al., 2008b). Mutations in the VDR gene are thought to be the primary cause of this disorder, although in select instances the etiology of the diseases has remained elusive. For instance, in 200 HVDRR-affected children in Colombia and for a patient identified in England, no VDR-associated mutations could be identified. These etiological gaps and the high degree of similarity between HVDRR disease symptoms and observed phenotypes in TRPV6 KO and TRPV5/1- α -OHase double-KO mice have led experts in the field to hypothesize a possible pathological connection between the disease and TRPV6. Interestingly HVDRR patients treated with high-dose of Ca^{2+} -rich diet exhibit partial recovery which supports further exploration of this hypothesis (Suzuki et al., 2008b).

Recent observations indicate that perturbing the chondroprotective role of TRPV6 may be linked with Osteoarthritis (OA) pathogenesis (Song et al., 2017). The most compelling evidence for this hypothesis comes from the observation that TRPV6 knockout mice display multiple OA phenotypes such as cartilage fibrillation, eburnation, and loss of proteoglycans.

7.5 Reproductive Diseases

The well-established role of TRPV6 in regulation of Ca^{2+} in the epididymal in murine models suggests that protein is critical for male fertility in mammals, although there is no evidence for such a connection in humans (Weissgerber et al., 2011). Nevertheless, proteomic analysis by Lin et al have shown that TRPV6 is markedly reduced in the seminal extracellular vesicles and ejaculated spermatozoa of asthenozoospermic patients, suggesting that the protein could be contributing to multiple aspects of male fertility (Lin et al., 2019). The role of TRPV6 in maternal-fetal Ca^{2+} transport and its abundant expression in reproductive organs has prompted investigators to examine the role of this channel in Preeclampsia. Notably, downregulation of TRPV6 expression and a concomitant decline in Ca^{2+} transport in the mammalian syncytiotrophoblasts is thought to alter the oxygen perfusion of placental tissues; a perturbation which in turn has been linked to the development of preeclampsia (Hache et al., 2011; Yang et al., 2013a; Yang et al., 2013b; Yang et al., 2015).

7.6 Pendred Syndrome

TRPV5 and TRPV6 dysfunction have been linked to Pendred syndrome – a genetic disorder which causes one of the most common syndromic deafness in children. The disease is caused by mutations in gene *Slc26a4* which compromise the function of the encoded protein pendrin - an anion $\text{Cl}^-/\text{HCO}_3^-$ exchanger expressed in the inner ear, the thyroid, and the kidney. This loss-of-function and the accompanying reduction in pH of mammalian endolymph is believed to inhibit Ca^{2+} absorption through acid-sensitive channels TRPV5 and TRPV6. The luminal acidification of vestibular endolymph and the concomitant increase in endolymphatic $[\text{Ca}^{2+}]$ is believed to compromise hearing abilities in two ways; a) by inhibiting sensory transduction necessary for hearing and b) promoting the degeneration of the sensory hair cells. In support of this hypothesis, Dror *et al* have speculated that inhibition of TRPV5 and TRPV6 due to luminal acidification of vestibular endolymph could also contribute to Ca^{2+} oxalate stone formation in the inner ear (Dror et al., 2011). On the other hand, a recent contradictory report has demonstrated that TRPV6 is not involved in hair cell mechanotransduction of mouse cochlea (Morgan et al., 2018).

7.7 Cancer Progression

The role of TRPV6 in cancer progression has been widely studied and has been recently summarized in an independent review by J.M. Stewart and previously by Lehen'kyi and co-investigators (Lehen'kyi et al., 2012; Stewart, 2020). TRPV6 overexpression in human malignancies is now well established (see Table 5). Accumulating evidence has confirmed that TRPV6 overexpression occurs both at the level of mRNA and protein (see Table 5). TRPV6 is intimately linked to tumor aggressiveness and its expression is elevated in prostate, breast, thyroid, colon, and ovarian carcinomas relative to normal tissues (Peng et al., 2001b; Zhuang et al., 2002).

More than 90% of ovarian, prostate, and pancreatic cancers consistently exhibit TRPV6 overexpression (Cerami et al., 2012; Gao et al., 2013; Stewart, 2020). Not surprisingly, the TRPV6 gene has been long recognized as a conventional oncogene and the channel itself is considered an oncochannel (Lehen'kyi and Prevarskaya, 2011; Huber, 2013; Stewart, 2020). Mechanistic insights on the exact role of TRPV6 in cancer progression are not entirely clear, although, it has been shown that TRPV6 mediates constitutive Ca^{2+} influx into epithelial cells to continuously suppress IGF1 receptor-mediated Akt-mTOR and Erk signaling to maintain cell quiescence (Xin et al., 2019). This finding is important with a mechanistic viewpoint since more than 90% of human cancers arise in epithelial tissues and aberrant cellular proliferation resulting from cell-cycle reactivation of quiescent cells is a long-recognized hallmark of cancer. Transfection of TRPV6 induces Ca^{2+} -dependent cell proliferation in HEK293 cells (Xin et al., 2019). It is suspected that TRPV6 mediates cancer progression by triggering Ca^{2+} -entry induced aberrations in molecular drivers regulating processes such as cell cycle, apoptosis, and migration thereby conferring proliferative and survival advantages (Lehen'kyi and Prevarskaya, 2011). In this section, we examine the implications of TRPV6 overexpression and dysfunction in cancer progression.

7.7.1 Prostate Cancer—In prostate cancers, TRPV6 expression correlates strongly with pathological stage, tumor grade, extra-prostatic invasion, lymph node metastasis, and

resistance to androgen-targeted therapies (Peng et al., 2001b; Wissenbach et al., 2001; Zhuang et al., 2002; Fixemer et al., 2003). In contrast, TRPV6 is low and sometimes undetectable in benign prostate tissue and benign prostate hypertrophy samples, prompting investigators to classify it as a prognostic marker for advanced prostate cancer (Peng et al., 2001b; Fixemer et al., 2003). Similarly, TRPV6 mRNA is increased in prostate cancer cell lines (LNCaP and PC-3) in comparison to normal prostate epithelial cells PrEC and BPH (Peng et al., 2001b).

Knockdown of TRPV6 in LNCaP cells decreases proliferation rate, S-phase accumulation, and expression of tumor marker proliferating cell nuclear antigen (PCNA) expression (PCNA) (Lehen'kyi et al., 2007b). Early attempts to identify store-operated Ca^{2+} channels (SOCs) that mediate Ca^{2+} entry in prostate cancer cells indicated TRPV6, TRPC1, TRPC3 as potential candidates for this process (Pigozzi et al., 2006). Later it was confirmed that Ca^{2+} -uptake in prostate cancer line LNCaP and neuroendocrine tumor (NET) cells is mediated by TRPV6 and is accompanied by downstream activation of the nuclear factor of activated T cells (NFAT) (Lehen'kyi et al., 2007b; Skrzypski et al., 2016). However, contrasting evidence questions the SOC-mediated mechanism of TRPV6 in the context of cancer progression (Bodding et al., 2003). It is possible that TRPV6 acts as a SOC but cooperates with other related channels to fuel cancer progression through pleiotropic mechanisms. For instance, the SOCs Orai1 promotes TRPV6 trafficking (among other TRP channels) to the plasma membrane, and concomitantly increases cell proliferation, reduces apoptosis, and induces chemotherapeutic resistance in prostate cancer cells (Raphael et al., 2014). Furthermore, increased expression of TRPV5, TRPV6, Orai2, and some other TRPC channels is correlated to a lower risk of systemic recurrence after radical prostatectomy, independently of the prostate-specific antigen (PSA) level, percentage of positive biopsies, and surgical margin status. These findings highlight the potential of TRPV6 as a potential cancer biomarker when measured in conjunction with other biomarkers (Raphael et al., 2014; Perrouin-Verbe et al., 2019).

Store-operated Ca^{2+} current is downregulated during differentiation of LNCaP cells into an androgen insensitive and apoptotic-resistant neuroendocrine phenotype (Fixemer et al., 2003). Furthermore, the preferential expression of intermediate-conductance- Ca^{2+} -activated K^{+} channels is thought to hyperpolarize cancer and promote TRPV6-mediated Ca^{2+} entry and proliferation of cancer cells (Lallet-Daher et al., 2009). Indeed, a reduction in cytosolic Ca^{2+} entry through blockade of the K^{+} channel blunts the proliferation of prostate cancer cells (Lallet-Daher et al., 2009). Another report by Kim et al has indicated that TRPV6 interactions with the tumor suppressors Numb and PTEN are an important determinant of cytosolic Ca^{2+} influx in prostate cancer cells (Kim et al., 2014).

The role of TRPV6 in androgen-insensitivity was implicated based on early observations showing an absence of TRPV6 expression in prostate cancer cell lines DU-145 and PC-3 (androgen-insensitive) in contrast to its high expression in LNCaP (androgen-sensitive) (Peng et al., 2001b; Fixemer et al., 2003). Similarly, TRPV6 is expressed in androgen-insensitive prostatic lesions whereas its expression is almost undetectable in healthy prostate tissue and benign prostatic hyperplasia (Wissenbach et al., 2001). Androgen receptor (AR) antagonist bicalutamide increases TRPV6 expression whereas AR agonist

dihydrotestosterone inhibits TRPV6 expression (Bodding et al., 2003; Vanden Abeele et al., 2003). Paradoxically, AR knockdown reduces TRPV6 mRNA and TRPV6 protein levels (Lehen'kyi et al., 2007b). Despite evidence suggesting TRPV6 involvement in androgen insensitivity, no androgen response elements (ARE) have been mapped on the TRPV6 gene promoter, although the presence of a partial ARE on position -13,232 of the TRPV6 gene has been recently proposed (Wilson et al., 2016; Stewart, 2020).

The existence of coupled polymorphisms in the *TRPV6* gene and the observed higher risk of prostate cancer in African-Americans prompted Kessler et al to investigate if differences in frequencies of ancestral and derived alleles contribute to prostate cancer risk. The study found that although ethnic differences in allele frequency exist between Caucasians and African-Americans, no correlations exist between allele frequencies and prostate cancer aggressiveness (Kessler et al., 2009). However, the sample size from African descendants is small in that study, further investigations are needed to confirm this result.

7.7.2 Breast Cancer—TRPV6 role has been implicated in breast cancer progression and therapeutic resistance to endocrine-targeted therapies (So et al., 2020; Stewart, 2020). Among all TRPV channels, TRPV6 has the most pronounced and varied overexpression in different breast cancer subtypes with Basal-like and HER2-enriched subtypes displaying the highest TRPV6 expression (So et al., 2020). TRPV6 is aberrantly overexpressed in breast adenocarcinomas and ductal adenocarcinoma in comparison to normal breast tissue (Bolanz et al., 2008; Dhennin-Duthille et al., 2011). TRPV6 mRNA is upregulated 2-to-15 folds in breast cancer tissue in comparison to normal breast tissue (Bolanz et al., 2008). TRPV6 expression is upregulated by estrogen, progesterone, and estradiol in breast cancer cell line T47D. In agreement with these observations, the estrogen receptor antagonist Tamoxifen reduces TRPV6 expression in T47D cells (Bolanz et al., 2008).

Interestingly, Tamoxifen also inhibits Ca²⁺ transport activity of TRPV6 in both ER-positive breast cancer cell line MCF-7 as well as in ER-negative line MDA-MB-231 (Bolanz et al., 2009). Estrogen receptor antagonist, ICI 182,720 does not rescue TRPV6 inhibition and downstream defects in Ca²⁺ transport activity induced by Tamoxifen. On the other hand, activation of protein kinase C blocks Tamoxifen-induced inhibition of TRPV6 activity and Ca²⁺-uptake defects. These observations highlight TRPV6 as a potential target for both ER+ and ER- breast cancers (Bolanz et al., 2009). Indeed, RNAi mediated knockdown of TRPV6 inhibits migration and invasion of MDA-MB-231 cells (Dhennin-Duthille et al., 2011). In contrast, the gain of function mutation R532Q in TRPV6 increases migration and invasion of both MCF-7 and MDA-MB-231 cells (Cai et al., 2021). More recent studies shown that ZFH3 appears to play important roles in intracellular Ca²⁺ homeostasis in untransformed mammary epithelial cells, at least in part, by regulating TRPV6 (Zhao et al., 2019). Adequate TRPV6 expression is necessary to maintain mammary epithelial integrity and the level of epithelial mesenchymal transition markers, suggesting the involvement of TRPV6 in mesenchymal invasion of breast cancer cells (Kärki et al., 2020). It remains to be seen if dysfunction in this aspect of TRPV6 regulation is linked with the neoplastic transformation of mammary epithelial cells.

7.7.3 Colorectal Cancer—TRPV6 overexpression is associated with early-stage colon cancer (Peleg et al., 2010). The knockdown of TRPV6 in colon cancer inhibits cancer cell proliferation and induces apoptosis (Peleg et al., 2010). In a *Citrobacter rodentium*-induced transmissible murine colonic hyperplasia (TMCH) model, a 10-to-20-fold increase in TRPV6 was observed following the induction of colonic hyperplasia (Peleg et al., 2010). TRPV6 overexpression and colonic hyperplasia in this model were abrogated by a high-Ca²⁺ diet, hinting towards a potential chemo-preventive role of TRPV6 colon cancer progression (Peleg et al., 2010). Although TRPV6 overexpression correlates to tumor aggressiveness in multiple cancers, high dietary Ca²⁺ intake by itself is thought to play a chemopreventive role in colon cancer (Zhang and Giovannucci, 2011). In this regard, curcumin, an established chemopreventive agent is known to induce an increase in TRPV6 expression through the VDR-dependent mechanism hinting that part of curcumin chemopreventive action in colon cancer could be through Ca²⁺influx (Bartik et al., 2010).

Until recently, the mechanism dictating the role of TRPV6 in colon cancer was unknown. However, recent studies have shed some light on the possible role of TRPV6 in colon cancer. It appears that mutations in CaM-binding domains of TRPV6 channels confer invasive properties to colon adenocarcinoma cells (Arbabian et al., 2020). Another study utilizing the colorectal cell line SW480 has suggested that p38 α and GADD45 α enhance vitamin D signaling to upregulate TRPV6 expression in colon cancer cells (Ishizawa et al., 2017). In this regard, TRPV6-dependent amplification of IGF-induced PI3K-PDK1-Akt signaling in human colon cancer has been put forth as another mechanism that mediated colon cancer development (Dai et al., 2014).

7.7.4 Pancreatic Cancer—TRPV6 is up-regulated in primary cancer tissues from pancreatic cancer patients, although a recent study has also documented a decrease in TRPV6 expression in a 3D-model of pancreatic ductal adenocarcinoma (PDAC) (Song et al., 2018; Tawfik et al., 2020). TRPV6 is expressed in pancreatic neuroendocrine tumors and regulates cellular proliferation through Ca²⁺- and NFAT-dependent mechanisms (Skrzypski et al., 2016). TRPV6 knockdown induces apoptosis and cell cycle arrest in pancreatic cancer cells and inhibits invasion, proliferation, and migration (Song et al., 2018). The observed phenotypes were consistent with molecular changes observed upon TRPV6 knockdown; namely, downregulation of B-cell lymphoma 2 (Bcl-2, an anti-apoptotic protein), Matrix metalloprotease 9 (MMP9, ECM protein and a marker for invasion), and Proliferating cell nuclear antigen (PCNA, a marker for DNA synthesis/proliferation) and upregulation of Bax (a pro-apoptotic Ca²⁺ channel) and E-Cadherin (a marker for epithelial-to-mesenchymal transition) (Song et al., 2018). These studies highlight that TRPV6 can influence cancer progression through pleiotropic mechanisms.

7.7.5 Rapidly Emerging Implications in Multiple Cancer Subtypes—Emerging data from studies conducted over the last decade has brought an increasing appreciation for the TRPV6 in multiple cancer subtypes. Increased expression of TRPV6 in gastric cancer cells increases their sensitivity to capsaicin-induced apoptosis whereas the siRNA mediated knockdown of the channel suppresses this sensitivity (Chow et al., 2007). Another report has shown that capsaicin induces apoptosis in human small cell lung cancer through a

TRPV6 and Calpain-dependent mechanism (Lau et al., 2014). TRPV6 is downregulated in esophageal carcinoma has been emerging as a novel cancer biomarker for predicting disease-specific survival in patients suffering from esophageal cancer (Zhang et al., 2016a). Low TRPV5 and TRPV6 co-expression have been recently touted as an independent predictor of poor recurrence-free survival in non-small cell lung cancer (Fan et al., 2014).

7.7.6 Mutation Frequency and Expression: Insights from Cancer Genomic Databases—Despite rapidly emerging evidence linking TRPV6 overexpression to tumor aggressiveness, mechanistic understanding of factors fueling TRPV6 upregulation in cancer is lacking (Stewart, 2020). One of the most evident gaps in the fields is the paucity of information regarding TRPV6 genomic aberrations in different cancer subtypes. This is evident from the observation that despite its validated role in cancer progression, no cancer driver mutations have been identified in the TRPV6 gene. Recent advent in high throughput technologies and democratization of OMICS data has enabled intuitive visualization of cancer genomics databases such as The Cancer Genome Atlas (TCGA), The Catalogue of Somatic Mutations (COSMIC), Oncomine, and UALCAN. To our knowledge, a Pan-Cancer description of TRPV6 mutation distribution is lacking (Creighton, 2018).

Figure 7 summarizes an analysis of TRPV6 Copy Number Alteration frequency in 32 cancer subtypes analyzed on the cBioPortal pipeline. The cBioPortal Cancer Genomics database offers intuitive tools to visualization, analysis, and download of large-scale cancer genomics data sets (Cerami et al., 2012). The graph represents one of the outputs from a typical cBioPortal-powered analysis of the TRPV6 mutation profile from 10953 patients distributed across 32 TCGA datasets. Preliminary examination of the output reveals that Melanoma (cumulative CNA frequency 9.91 % across 444 cases) has the highest TRPV6 CNA frequency whereas clear cell renal cell carcinoma (ccRCC) has the lowest CNA frequency (0.59% across 511 cases) [No of cases not shown for simplicity].

In terms of the type of genomic aberration, most tumors exhibit the greatest frequency of TRPV6 mutations (substitutions, denoted in green) followed by amplification (red) and deep deletions (dark blue). The portal mined 192 missense mutations and 22 truncations across the length of the protein from patient data (output not shown). Some other visible trends become apparent on more careful examination. For instance, type 2 endometrial carcinoma (uterine carcinosarcoma) and adenoid cystic carcinoma display only amplifications whereas testicular germ cell tumors, cervical cancers, and colorectal cancer exclusively harbor substitutions and do not exhibit any other type of alteration. Cholangiocarcinoma, diffuse large B-cell lymphoma (DLBC), chromophobe RCC, mesothelioma, pheochromocytoma/ paraganglioma (PCPG), thyroid Cancers, and thymoma did not display any CNAs.

7.7.7 Pharmacological Targeting—Given the critical role of TRPV6 in cancer progression, several attempts have been made to inhibit this Ca^{2+} -channel for therapeutic intervention (see Table 6). Some important examples targeting TRPV6 include TH-1117, 2-APB, 2-APB derivative 22b, Econazole, Miconazole, Piperazine derivative Cis-22a, Capsaicin, 9 -tetrahydrocannabivarin, Xestospongine C, Lidocaine, gold-caged nanoparticle (PTX-PP@Au NPs) and SOR-C13 synthetic peptide (see Table 6 and references listed therein). A recent structural study shows that while universal Ca^{2+} channel ruthenium red

plugs the pore of TRPV6, Econazole binds to an allosteric lipid-binding site (Neuberger et al., 2021). This results in a conformational change leading to the closure of the gate of TRPV6. Among different strategies that have been attempted to target TRPV6, the 13-amino acid peptide SOR-C13 has shown the most promise. This lead agent reduces growth in cell and animal models and recently has completed Phase I clinical safety trial that had enrolled 23 patients with advanced solid tumors of epithelial origin non-responsive to all standard-of-care treatments (Bowen et al., 2013; Fu et al., 2017; Xue et al., 2018). The pharmacological strategies targeting TRPV6 have been summarized in Table 6.

8 Regulation of TRPV6

TRPV6 is regulated by a wide variety of physiological, hormonal, and genetic factors. In this regard, the regulation of TRPV6 by hormones has been most extensively studied. In addition to the level of Ca^{2+} in the diet, which plays a key role in the regulation of TRPV5/6 channels, other important physiological factors that contribute to the regulation of these Ca^{2+} -selective channels include pregnancy, lactation, sex hormones, exercise, age, and gender (Peng, 2011).

Regulation of TRPV6 in response to glucocorticoids, immunosuppressive drugs, and diuretics has also been examined in selected studies, uncovering additional insights into pharmacological aspects of TRPV6 regulation (Jeon, 2008). In this section, we will discuss the key aspects of TRPV6 regulation; focusing first on hormonal aspects (vitamin D, sex hormones, and glucocorticoids) of channel regulation and then describe the influence of physiological factors (diet, age, pregnancy, lactation, exercise) and pharmacological factors (diuretics) on this process.

8.1 Vitamin D

Kinetic and dose-response experiments evaluating the responsiveness of TRPV6 mRNA to the active form of vitamin D (also referred to as $1,25(\text{OH})_2\text{D}_3$) have demonstrated that the gene is strongly regulated by this steroid hormone. Wood and colleagues discovered that TRPV6 mRNA was found to be upregulated within 2 hours of treatment with $0.1 \mu\text{M}$ of $1,25(\text{OH})_2\text{D}_3$ in Caco-2 cells (Wood et al., 2001). In fact, the response of TRPV6 to $1,25(\text{OH})_2\text{D}_3$ is significantly more rapid and robust in comparison to calbindin- D_{9k} , which is considered to be a conventional vitamin D-responsive Ca^{2+} -binding protein (Wood et al., 2001). In fact, the calbindin- D_{9k} gene promoter is not very responsive to $1,25(\text{OH})_2\text{D}_3$, calbindin- D_{9k} gene transcription it is upregulated as Caco-2 cells differentiate over time (Wang et al., 2004).

The robust upregulation of calbindin- D_{9k} transcripts in the small intestine of TRPV6 transgenic mice bearing a global deletion of vitamin D receptor (VDR) suggests that induction of calbindin- D_{9k} to Vitamin D could be occurring secondary to TRPV6 (Cui et al., 2012). Therefore, the vitamin D-responsiveness of the transcellular pathway of Ca^{2+} absorption is mostly due to that of the TRPV6 gene. In this sense, it is fair to say that TRPV6 is the gatekeeper of vitamin D-regulated transcellular pathway of Ca^{2+} absorption.

VDR knockout mice exhibit a 90% reduction in the duodenal TRPV6 mRNA level (Van Cromphaut et al., 2001). A single injection of 1,25(OH)₂D₃ in vitamin D deficient mice induces a 10-fold increase in TRPV6 expression in duodenal tissues and a 4-fold increase in renal tissues within 6 and 12 hours of treatment, respectively, suggesting differential regulation of the gene influenced by tissue-specific mechanisms (Song et al., 2003b). Interestingly, initial studies suggested that TRPV6 transcripts in humans do not correlate to vitamin D levels in humans (Barley et al., 2001). However, contrasting studies by Walter *et al* suggested that correlation of TRPV6 transcripts to vitamin D in humans is dependent upon gender, age, and inheritance of VDR variants (Walters et al., 2006). In some studies, TRPV6 expression in human duodenal tissues was particularly responsive to 25-hydroxyvitamin D (25(OH)D), potentially due to elevated activity of 25(OH)D 1 α -hydroxylase which converts 25-(OH)-D to 1,25(OH)₂D₃ (Taparia et al., 2006; Balesaria et al., 2009).

Chromatin Immunoprecipitation (ChIP) scanning of the human *TRPV6* gene promoter by Pike et al have revealed the presence of five vitamin D response elements (VDREs) at positions -1.2, -2.1, -3.5, -4.3, and -5.5 kb relative to transcriptional start (TSS) site (Meyer et al., 2006; Meyer et al., 2007). The studies showed that VDREs at positions -1.2, -2.1, and -4.3 kb are significantly more responsive to 1,25-(OH)₂D₃ in comparison to VDREs located at -3.5 and -5.5 kb which do not contribute substantially to vitamin D mediated transcriptional regulation in the intestine. Further analysis showed that among all VDREs, the VDRE at position -2.1- and -4.3-kb position contribute to 85% and 15% of transcriptional activity in response to vitamin D (Meyer et al., 2006; Meyer et al., 2007).

The molecular details of vitamin D-induced transcriptional regulation have been examined in considerable detail for multiple genes (Pike and Christakos, 2017; Gil et al., 2018). ChIP-based studies on TRPV6 promoter and insights from the classical model of 1 α ,25-(OH)₂-D₃-induced transcriptional regulation has revealed some important steps important in TRPV6 transcriptional activation (Pike et al., 2007a; Pike et al., 2007b; Pike and Christakos, 2017; Gil et al., 2018). These include binding of Vitamin D on its cognate vitamin D receptor (VDR), the translocation of vitamin D receptor (VDR)-retinoid X receptor heterodimer complex in the nucleus followed by its subsequent binding on the TRPV6 promoter, recruitment of steroid receptor coactivator 1 and RNA polymerase II on the promoter, and finally, transcriptional activation mediated through histone H4 acetylation events (Pike et al., 2007a; Pike et al., 2007b; Pike and Christakos, 2017; Gil et al., 2018). The 1 α ,25-(OH)₂-D₃-induced upregulation of TRPV6 is thought to be an important step that promotes transcellular absorption of Ca²⁺ from the intestine (Figure 6). However, a non-genomic action of TRPV6 activation has been put forth by Khanal et al (Khanal et al., 2008). This study showed that 1,25(OH)₂D₃ upregulates Ca²⁺ uptake by stimulating the PKA pathway which subsequently results in the release beta-glucuronidase and concomitantly activates TRPV6 (Khanal et al., 2008).

8.2 Sex hormones

Sex hormones play an instrumental role in the regulation of TRPV5 and TRPV6. Estrogen treatment upregulates TRPV6 mRNA by 4-fold in ovariectomized mice (Song et al., 2003a).

This upregulation is even more pronounced in ovariectomized VDR KO mice which display a more than 8-fold increase in TRPV6 following estrogen treatment (Song et al., 2003a). Estrogen receptor α KO mice exhibit a greater than 50% reduction in TRPV6 mRNA, although this phenotype was not observed in estrogen receptor β KO mice (van Abel et al., 2006). Keeping in line with this observation, a recent study by Nie *et al* has shown that pharmacologic or genetic suppression of ER α , but not ER β , inhibits 17 β -estradiol treatment-induced TRPV6 expression increase in SCBN cells (Nie et al., 2020). The study showed that estrogen modulates Ca²⁺ absorption in duodenum through differential effects of ER α and ER β on TRPV6 and PMCA1b expression, respectively (Nie et al., 2020). Similarly, 17 β -estradiol is known to enhance TRPV6 activity in human colonic cell line T84 (Irnaten et al., 2008). Not surprisingly, anti-estrogen agent ICI 182,780 and anti-progesterone agent RU486 block TRPV6 expression in rodents by their antagonist action on estrogen and progesterone receptors, respectively (Lee et al., 2009).

Female mice show a higher responsivity to 1,25(OH)₂D₃, exhibiting a 2-fold higher increase in duodenal expression of TRPV6 mRNA in comparison to males (Song and Fleet, 2004). Interestingly, this phenomenon was independent of VDR expression across genders (Song and Fleet, 2004). Aromatase deficient KO mice, which are frequently used as models for mimicking estrogen deficiency, display hypercalciuria as well as a decline in renal expression of TRPV5/6 (Oz et al., 2007). Sex hormone-associated differential regulation of TRPV6 in females could have important implications for relative risk to osteoporosis – a disease that frequently affects older postmenopausal women. The evidence for this theory comes from a study by Walter *et al* who showed that reduced TRPV6 and VDR expression are correlated to lower Ca²⁺ absorption in older postmenopausal women (Walters et al., 2006).

Estrogen, progesterone, and dexamethasone upregulate TRPV6 expression in the cerebral cortex and hypothalamus suggesting a potential involvement of TRPV6 in Ca²⁺ absorption in the brain (Park et al., 2020). In line with this recent observation, previous studies seem to suggest that circulating levels of estradiol during the estrous cycle may differentially regulate the activity of all TRPV ion channels in the brain (Kumar et al., 2018). The estrogen-mediated regulation of TRPV6 could have important functional implications in embryo-implantation, maintenance of pregnancy, and development and progression of breast cancer. These implications are discussed in Sections 6.5 and 7.7.2 respectively.

In contrast to our understanding of the role of female sex hormones on TRPV6, much less is known about the role of male sex hormones. It has been shown previously that TRPV6 expression is negatively regulated by androgens in LNCaP cells (Peng et al., 2001b). Male mice display higher urinary Ca²⁺ excretion and lower renal TRPV5 expression in comparison to females, although it is not known if this phenotype associated with TRPV6 (Hsu et al., 2010). Orchidectomy of mice increases TRPV5 expression and decreases Ca²⁺ excretion in the urine. It is not known if this surgical ablation affects murine TRPV6 expression (Hsu et al., 2010).

8.3 Glucocorticoids

Glucocorticoids are a class of hormones that are widely used as immunosuppressive and anti-inflammatory agents (Timmermans et al., 2019). One of the most common side effects of this class of drugs is osteoporosis – a condition that frequently develops due to deficiencies of Ca^{2+} homeostasis (Timmermans et al., 2019). Furthermore, reduced intestinal absorption and renal reabsorption of Ca^{2+} are believed to be important factors that contribute to glucocorticoid-induced osteoporosis (Kim et al., 2009a). In fact, dexamethasone induces both intestinal and renal expression of TRPV6 in mice within 24 hours of subcutaneous administration (Kim et al., 2009a). The regulation of TRPV5/6 in response to glucocorticoids occurs both at the level of expression and at the level of channel activity, the latter aspects of which are controlled by glucocorticoid-inducible protein kinases (SGKs) (Palmada et al., 2005; Huybers et al., 2007; Kim et al., 2009a; Kim et al., 2009b; Sopjani et al., 2010; Kim et al., 2011; Diaz de Barboza et al., 2015).

Oral application of prednisolone reduces intestinal Ca^{2+} absorption and leads to a reduction in TRPV6 and calbindin- D_{9k} mRNA levels (Huybers et al., 2007). Dexamethasone blocks the compensatory increase in duodenal expression of TRPV6 that is observed in calbindin- D_{9k} and calbindin- D_{28k} mice (Kim et al., 2009b). Dexamethasone also results in a time-dependent decrease in the renal expression of TRPV6 in calbindin- D_{28k} KO mice (Kim et al., 2009a; Kim et al., 2011). In these studies, the intestinal regulation of TRPV6 in response to glucocorticoids appears to be VDR-dependent (Kim et al., 2009a; Kim et al., 2009b). A recent study has shown that dexamethasone exerts an inhibitory effect on mucin secretion in the lung by modulating the expression of TRPV6 in the tracheal epithelium (Lee et al., 2019a). Alendronate and aqueous extract of pomegranate seed (AE-PS) reverse the decrease in protein expression of TRPV6 and other Ca^{2+} transport proteins in a mouse model of glucocorticoid-induced osteoporosis (GIOP) (Zhang et al., 2016b).

TRPV6 is upregulated by SGK1, SGK3, and PKB/Akt (Sopjani et al., 2010). The constitutively active version of the SGK1 increase TRPV6 protein abundance on the cell membrane. SGK1-mediated increase in TRPV6 is enhanced through phosphatidylinositol-3-phosphate-5-kinase PIKfyve (PIP5K3), a kinase that is critical for the generation of secondary messenger phosphatidylinositol 3,5-bisphosphate [PIP_2]. However, $\text{S}^{318\text{A}}\text{PIKfyve}$, a version of the kinase that lacks the SGK1 phosphorylation site failed to enhance SGK-mediated increase in TRPV6 showing that PIKfyve requires its phosphorylation by SGK1 to act on TRPV6 (Sopjani et al., 2010).

The role of vitamin D in modulating Ca^{2+} absorption by TRPV6 in the intestine has been described in Sections 6.1 and 5.1 of this review. Multiple physiological factors influence this process. Some important factors influencing vitamin D-mediated Ca^{2+} reabsorption through TRPV6 include diet [e.g. levels of Ca^{2+} , vitamin D, and composition] (Brown et al., 2005), age (van Abel et al., 2006; Walters et al., 2007), gender and associated contributions from sex hormones (Walters et al., 2006; Jung et al., 2011), level of physical activity (Teerapornpantakit et al., 2009), pregnancy (Van Cromphaut et al., 2003), and lactation (Charoenphandhu et al., 2009). We describe the role of these physiological factors on TRPV6 and their regulatory interactions on vitamin D induced Ca^{2+} absorption through this Ca^{2+} -selective channel.

8.4 Diet

TRPV6 is highly responsive to the body's Ca^{2+} requirement and its expression is modulated to fine-tune Ca^{2+} absorption from the diet. $1,25\text{-(OH)}_2\text{D}_3$ and Ca^{2+} levels in the diet play an instrumental role in the expression of this channel (Song et al., 2003b; van de Graaf et al., 2004; Brown et al., 2005; Replogle et al., 2014). The relatively higher abundance of TRPV6 in duodenum and jejunum is believed to correlate with the high capacity of these intestinal regions for transcellular absorption of Ca^{2+} from the diet (Emkey and Emkey, 2012). Restriction of Ca^{2+} in diet results in a dramatic upregulation of duodenal, and to some extent renal expression, of TRPV6 in rodents (Song et al., 2003b; Brown et al., 2005; Van Cromphaut et al., 2007; Woudenberg-Vrenken et al., 2012).

As alluded earlier in Section 6.1, when dietary Ca^{2+} is insufficient, normal serum Ca^{2+} levels in TRPV6 KO mice are maintained at the expense of bone (Lieben et al., 2010; Lieben and Carmeliet, 2012; Replogle et al., 2014). A gene-by-diet interaction study conducted across 11 inbred lines of mice showed that genetic variations in TRPV6, calbindin- D_{9k} , PMCA1b mRNA influenced intestinal Ca^{2+} absorption and its impact on bone marrow density (BMD) (Replogle et al., 2014). Insights from Leuven and Tokyo VDR KO mice suggest that Ca^{2+} influx from the diet and its subsequent binding to calbindin D_{9k} is the rate-limiting step that modulates vitamin D-dependent regulation TRPV6 (Bouillon et al., 2003). High Ca^{2+} diet abrogates TRPV6 overexpression induced in a transmissible murine colonic hyperplasia (TMCH) model, suggesting suppression of this channel by high Ca^{2+} diet could mediate some chemopreventive actions in the colon (Peleg et al., 2010).

However, low- Ca^{2+} -induced upregulation of TRPV6 can also occur independently of VDR (Song et al., 2003a). Ca^{2+} absorption in response to low-dietary Ca^{2+} occurs even in calbindin- D_{9k} /TRPV6 double knockouts, suggesting the presence of alternative channels that compensate for the loss of TRPV6 (Benn et al., 2008). In this regard, the channels $\text{Ca}_v1.3$, TRPM6, and TRPM7 have received considerable attention (Beggs et al., 2019). Interestingly, the dietary composition is also known to influence TRPV6 expression (Fukushima et al., 2009; Beggs et al., 2019). Notably, change in diet from breast milk to solid food modulates the switch in Ca^{2+} absorption by TRPV6 or $\text{Ca}_v1.3$ in jejunum and ileum in early-life rodents (Beggs et al., 2019). In this regard, short-chain fatty acids and indigestible oligosaccharides are known upregulate TRPV6 expression (Fukushima et al., 2009).

8.5 Pregnancy and Lactation

Duodenal expression of TRPV6 transcripts is significantly increased in WT and VDR KO mice during pregnancy and lactation (Van Cromphaut et al., 2003). Notably, prolactin upregulates TRPV6 transcription and promotes an increase in intestinal Ca^{2+} absorption in lactating and pregnant rats (Charoenphandhu et al., 2009). This increase in TRPV6 is believed to be an adaptation that occurs in pregnant rodents to upregulate intestinal Ca^{2+} absorption as a compensatory mechanism for overcoming the loss in bone mineralization content during lactation. However, no studies have confirmed the role of TRPV6 in non-genomic mechanisms that influence the increase in Ca^{2+} absorption after suckling-induced prolactin surge (Charoenphandhu et al., 2009). The impact of female sex hormones such as

progesterone and estrogen that ebb and surge dramatically during pregnancy and lactation play a key role in the regulation of TRPV6. The impact of these hormones on TRPV6 regulation is examined in Section 6.2.

8.6 Exercise

The increased Ca^{2+} absorption induced during endurance swimming has been associated with an increased level of TRPV6, VDR, and other critical genes that are important in transcellular and paracellular transport (Teerapornpuntakit et al., 2009). In agreement with this notion, TRPV6 and $1,25(\text{OH})_2\text{D}_3$ levels are decreased in immobilized rats and subsequently result in a reduction of Ca^{2+} absorption (Sato et al., 2006; Charoenphandhu et al., 2012). Overall, these studies hint that level of physical activity contributes to Ca^{2+} absorption in vitamin D-dependent manner.

8.7 Aging

Several pre-clinical and clinical reports have shown that aging is associated with decreased Ca^{2+} absorption and hypercalciuria (Veldurthy et al., 2016). Age-associated impairments in Ca^{2+} homeostasis predisposes elderly adults to hyperparathyroidism and osteoporosis (Veldurthy et al., 2016). Younger rats (2-months old) exhibit more than 2-fold higher expression of TRPV6 in comparison to their older counterparts (12-months old) (Brown et al., 2005). In both WT and VDR mice, age-associated decrease in duodenal expression of TRPV6 [and decrease in renal expression of TRPV5] correlates with a decline in intestinal absorption and renal reabsorption (van Abel et al., 2006). The intestinal expression of TRPV6 in mice varies dramatically by age and relative tissue location (Beggs et al., 2019). TRPV6 expression in duodenum is undetectable at P1 in mouse duodenum and increases 6-fold as mice age to P14 to 1 month. In Jejunum on the other hand TRPV6 expression increases from PI to P14, becomes weak at 1-month age and is undetectable in older mice (Beggs et al., 2019).

8.8 Diuretics

Thiazide diuretics, which block $\text{Na}^+\text{-Cl}^-$ cotransporters (NCC), are known to induce hypocalciuria (Friedman and Bushinsky, 1999; Lee et al., 2004). Evidence regarding the possible mechanisms for this phenomenon is controversial. Thiazide diuretics can modulate the expression of Ca^{2+} transport proteins such as TRPV5, calbindin- $\text{D}_{28\text{k}}$, and NCX in distal convoluted tubule (DCT), although the evidence regarding their effect on TRPV6 is nebulous (Nijenhuis et al., 2003a; Lee et al., 2004; Nijenhuis et al., 2005; Jeon, 2008; Lee et al., 2016). Hydrochlorothiazide (HCTZ) and chlorothiazide (CTZ) can modulate TRPV5 expression in rats and mice, respectively (Nijenhuis et al., 2003a; Lee et al., 2004). The effects of CTZ on TRPV5 in mice is influenced by dose, timing, and volume contractions in response to salt supplementation. The effect of diuretics on closely-related homolog TRPV6 is not clear, although a combination of long-term chlorothiazide and salt supplementation is known to significantly increase the expression of both TRPV5 and TRPV6 channels. The emerging evidence regarding coordinated regulation of Ca^{2+} reabsorption in various segments of nephron compels a re-examination of the effect of diuretics on TRPV6 regulation (Nijenhuis et al., 2003a; Lee et al., 2004).

In contrast to thiazide diuretics, loop diuretics such as furosemide induce urinary hypercalciuria (Friedman and Bushinsky, 1999). Acute and chronic treatment with furosemide increases TRPV6 expression (Lee et al., 2007; Lee et al., 2016). Similarly, hypercalciuria induced by gentamicin and streptozotocin-induced diabetes mellitus is accompanied by an upregulation in TRPV5 and TRPV6 (Lee et al., 2006; Lee et al., 2012).

9 Concluding Remarks and Future Outlook

A great deal of progress has been made in understanding how TRPV6 works and the roles it plays under normal and disease conditions since it was identified over two decades ago. Specifically, we have a much better understanding of the structure, activation, and inactivation of TRPV6 due to the elegant work in the crystal and cryo-EM structures of TRPV6. In addition, human diseases, including some forms of transient neonatal hyperparathyroidism and early-onset chronic pancreatitis have been linked to genetic defects in *TRPV6* gene. Since TRPV6 exhibits the highest expression level in the placenta and pancreas in humans, it is not surprising that genetic defects in the *TRPV6* gene would significantly affect the function of these organs, especially when both alleles of *TRPV6* gene are defective. Although speculative, some of the defective TRPV6 proteins may serve as a dominant-negative inhibitor of TRPV5, which may compensate for the loss of function of TRPV6 even though it is expressed at a much lower level if at all in these organs. The two diseases exemplify the main physiological roles of TRPV6: Ca²⁺ transport in absorption and exocrine function, although we have much less understanding of the process TRPV6 involved in the latter. It is anticipated that much progress will be made in the roles of TRPV6 in exocrine organs such as the pancreas, prostate, mammary, salivary, and sweat glands in the next decade. TRPV6 proves to be a critical player in biological processes including vitamin D-regulated Ca²⁺ absorption, in maintaining a local low Ca²⁺ environment in the epididymis and the inner ear. Lastly, aberrant TRPV6 expression, copy number variation, and mutations are often observed in cancers of epithelial origin such as prostate and breast cancers. A deeper understanding of how these abnormalities affect the progression of individual cancers yet to be achieved. A novel understanding of the TRPV6 structure is likely to spur renewed interest and optimism towards identification of reagents that modulate TRPV6 activity. Progress in this area in the future may lead to a new treatment for diseases involving dysfunction of TRPV6, such as chronic pancreatitis and certain cancers.

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Commonly Used Abbreviations

1,25(OH) ₂ D ₃	1,25-dihydroxyvitamin D ₃
AA	Amino Acid

ACG	Adenine-Cytosine-Guanine
ANK	Ankyrin Repeat
AR	Androgen Receptor
ARD	Ankyrin Repeat Domain
ARE	Androgen Response Element
ATP	Adenosine Triphosphate
AUG	Adenine-Uracil-Guanine
BAPTA	1,2-Bis(O-Aminophenoxy) Ethane-N,N,N',N'-Tetraacetic Acid
BPH	Basal Prostatic Hypertrophy
CaBPD	Calcium Binding Protein D
CaSR	Calcium-Sensing Receptor
CB	Cannabinoid Receptor
CD	Collecting Ducts
CaM	Calmodulin
CNA	Copy Number Alteration
CNT	Connecting Tubules
CP	Chronic Pancreatitis
Cryo-EM	Cryogenic Electron Microscopy
CT	Calcitonin
CTZ	Chlorothiazide
DCT	Distal Convolute Tubule
ECaC	Epithelial Calcium Channel
ECM	Extracellular Matrix
EGTA	Ethylene Glycol-Bis (B-Aminoethyl Ether)-N, N,N',N'-Tetraacetic Acid
EM	Electron Microscopy
ER	Estrogen Receptor
FL	Full Length
GADD	Growth Arrest And DNA-Damage-Inducible Protein

GI	Gastrointestinal
GIOP	Glucocorticoid-Induced Osteoporosis
HCO₃⁻	Bicarbonate Ion
HCTZ	Hydrochlorothiazide
HEK	Human Embryonic Kidney Cells
HER	Human Epidermal Growth Factor Receptor 2
HVDRR	Hereditary Vitamin D-Resistant Rickets (HVDRR)
IC₅₀	Half maximal inhibitory concentration
ICC	Immunocytochemistry
ICI 182,720	Commercially Used Estrogen Receptor Antagonist Used In in vitro and in vivo studies
IF	Immunofluorescence
IGF	Insulin-Like Growth Factor 1
IHC	Immunohistochemistry
IP	Immunoprecipitation
ISH	In Situ Hybridization
IV	Intravenous
KO	Knock-Out
MAPK	Mitogen-Activated Protein Kinase
MMP	Matrix Metalloprotease
MS	Mass Spectrometry
mTOR	Mammalian Target of Rapamycin
NCC	Sodium Chloride Cotransporter
NCX	Sodium-Calcium Exchanger
NET	Neuroendocrine Tumor
NF	Nuclear Factor
NFAT	Nuclear Factor of Activated T Cells
NSCLC	Non-Small Cell Lung Carcinoma
NX	Normalized Expression

OA	Osteoarthritis
OH	Hydroxyl Group (Short-Hand)
OMICS	Shorthand For "Omics", e.g. Genomics, Proteomics, Transcriptomics, etc.
PCNA	Proliferating Cell Nuclear Antigen
P_{Ca}	Conductivity of Ca
P_{Na}	Conductivity of Na
PCR	Polymerase Chain Reaction
PDAC	Pancreatic Ductal Adenocarcinoma
PDK	3-Phosphoinositide-Dependent Protein Kinase-1
PI3K	Phosphoinositide 3-Kinase
PIKfyve	Phosphatidylinositol-3-Phosphate-5-Kinase
PIP₂	Phosphatidylinositol 4,5-Bisphosphate
PKA	Protein Kinase A
PKB/Akt	Protein Kinase B
PLC	Phospholipase C
PMCA	Plasma Membrane Ca ²⁺ ATPase
PSA	Prostate-Specific Antigen
PTEN	Phosphatase and Tensin Homolog
PTH	Parathyroid Hormone
PTM	Post-Translational Modification
PTP	Protein Tyrosine Phosphatase
RANK	Receptor Activator of Nuclear Factor K B
RANKL	Receptor Activator of Nuclear Factor Kappa-B Ligand
RCC	Renal Cell Carcinoma
RNA	Ribonucleic Acid
RT	Reverse Transcriptase or Real-Time
RYR1	Ryanodine Receptor 1
SCBN	Canine Epithelial Cell Line

SCCD	Semicircular Canal Duct
SCLC	Small Cell Lung Cancer
SGK	Serum- And Glucocorticoid-Inducible Protein Kinase,
SNP	Single Nucleotide Polymorphisms
SOC	Store-Operated Ca ²⁺ Channels
SOCE	Store-Operated Calcium Entry
TCGA	The Cancer Genome Atlas
THCV	Tetrahydrocannabivarin
TM	Transmembrane
TMCH	Transmissible Murine Colonic Hyperplasia
TNHP	Transient Neonatal Parathyroidism
TPM	Transcripts Per Million
TRIP	Transient Receptor Potential Channel-Interacting Protein
TRP	Transient Receptor Potential
TRPV	Transient Receptor Potential Vanilloid Subfamily
UALCAN	Comprehensive, User-Friendly, And Interactive Web Resource For <u>Cancer</u> Genomics resource for analyzing Cancer OMICS Data Developed at <u>University Of Alabama</u> At Birmingham
ZRBV3	Zinc Finger Homeobox 3

12 References

- Akey JM, Eberle MA, Rieder MJ, Carlson CS, Shriver MD, Nickerson DA and Kruglyak L, 2004. Population history and natural selection shape patterns of genetic variation in 132 genes. *PLoS Biol* 2, e286. [PubMed: 15361935]
- Akey JM, Swanson WJ, Madeoy J, Eberle M and Shriver MD, 2006. TRPV6 exhibits unusual patterns of polymorphism and divergence in worldwide populations. *Hum Mol Genet* 15, 2106–13. [PubMed: 16717058]
- Almidani E, Elsidawi W, Almohamedi A, Bin Ahmed I and Alfadhel A, 2020. Case Report of Transient Neonatal Hyperparathyroidism: Medically Free Mother. *Cureus* 12, e7000. [PubMed: 32206464]
- Arbaban A, Iftinca M, Altier C, Singh PP, Isambert H and Coscoy S, 2020. Mutations in calmodulin-binding domains of TRPV4/6 channels confer invasive properties to colon adenocarcinoma cells. *Channels (Austin)* 14, 101–109. [PubMed: 32186440]
- Azarpeykan S, Dittmer KE, Marshall JC, Perera KC, Gee EK, Acke E and Thompson KG, 2016. Evaluation and Comparison of Vitamin D Responsive Gene Expression in Ovine, Canine and Equine Kidney. *PLoS One* 11, e0162598. [PubMed: 27632366]

- Bachinger D, Egli H, Goosmann MM, Monge Naldi A and Eckhard AH, 2019. Immunolocalization of calcium sensing and transport proteins in the murine endolymphatic sac indicates calciostatic functions within the inner ear. *Cell Tissue Res* 378, 163–173. [PubMed: 31338584]
- Balesaria S, Sangha S and Walters JR, 2009. Human duodenum responses to vitamin D metabolites of TRPV6 and other genes involved in calcium absorption. *Am J Physiol Gastrointest Liver Physiol* 297, G1193–7. [PubMed: 19779013]
- Barley NF, Howard A, O'Callaghan D, Legon S and Walters JR, 2001. Epithelial calcium transporter expression in human duodenum. *Am J Physiol Gastrointest Liver Physiol* 280, G285–90. [PubMed: 11208552]
- Bartik L, Whitfield GK, Kaczmarek M, Lowmiller CL, Moffet EW, Furmick JK, Hernandez Z, Haussler CA, Haussler MR and Jurutka PW, 2010. Curcumin: a novel nutritionally derived ligand of the vitamin D receptor with implications for colon cancer chemoprevention. *J Nutr Biochem* 21, 1153–61. [PubMed: 20153625]
- Bate N, Caves RE, Skinner SP, Goult BT, Basran J, Mitcheson JS and Vuister GW, 2018. A Novel Mechanism for Calmodulin-Dependent Inactivation of Transient Receptor Potential Vanilloid 6. *Biochemistry* 57, 2611–2622. [PubMed: 29505720]
- Beggs MR and Alexander RT, 2017. Intestinal absorption and renal reabsorption of calcium throughout postnatal development. *Exp Biol Med (Maywood)* 242, 840–849. [PubMed: 28346014]
- Beggs MR, Lee JJ, Busch K, Raza A, Dimke H, Weissgerber P, Engel J, Flockerzi V and Alexander RT, 2019. TRPV6 and Cav1.3 Mediate Distal Small Intestine Calcium Absorption Before Weaning. *Cell Mol Gastroenterol Hepatol* 8, 625–642. [PubMed: 31398491]
- Benn BS, Ajibade D, Porta A, Dhawan P, Hediger M, Peng JB, Jiang Y, Oh GT, Jeung EB, Lieben L, Bouillon R, Carmeliet G and Christakos S, 2008. Active intestinal calcium transport in the absence of transient receptor potential vanilloid type 6 and calbindin-D9k. *Endocrinology* 149, 3196–205. [PubMed: 18325990]
- Bernucci L, Henriquez M, Diaz P and Riquelme G, 2006. Diverse calcium channel types are present in the human placental syncytiotrophoblast basal membrane. *Placenta* 27, 1082–95. [PubMed: 16564089]
- Bianco SD, Peng JB, Takanaga H, Suzuki Y, Crescenzi A, Kos CH, Zhuang L, Freeman MR, Gouveia CH, Wu J, Luo H, Mauro T, Brown EM and Hediger MA, 2007. Marked disturbance of calcium homeostasis in mice with targeted disruption of the *Trpv6* calcium channel gene. *J Bone Miner Res* 22, 274–85. [PubMed: 17129178]
- Bikle DD, Xie Z and Tu C-L, 2012. Calcium regulation of keratinocyte differentiation. Expert review of endocrinology & metabolism 7, 461–472. [PubMed: 23144648]
- Blaine J, Chonchol M and Levi M, 2015. Renal control of calcium, phosphate, and magnesium homeostasis. *Clin J Am Soc Nephrol* 10, 1257–72. [PubMed: 25287933]
- Bodding M, 2005. Voltage-dependent changes of TRPV6-mediated Ca²⁺ currents. *J Biol Chem* 280, 7022–9. [PubMed: 15582993]
- Bodding M, Fecher-Trost C and Flockerzi V, 2003. Store-operated Ca²⁺ current and TRPV6 channels in lymph node prostate cancer cells. *J Biol Chem* 278, 50872–9. [PubMed: 14534305]
- Bodding M and Flockerzi V, 2004. Ca²⁺ dependence of the Ca²⁺-selective TRPV6 channel. *J Biol Chem* 279, 36546–52. [PubMed: 15184369]
- Bolanz KA, Hediger MA and Landowski CP, 2008. The role of TRPV6 in breast carcinogenesis. *Mol Cancer Ther* 7, 271–9. [PubMed: 18245667]
- Bolanz KA, Kovacs GG, Landowski CP and Hediger MA, 2009. Tamoxifen inhibits TRPV6 activity via estrogen receptor-independent pathways in TRPV6-expressing MCF-7 breast cancer cells. *Mol Cancer Res* 7, 2000–10. [PubMed: 19996302]
- Bond H, Dilworth MR, Baker B, Cowley E, Requena Jimenez A, Boyd RD, Husain SM, Ward BS, Sibley CP and Glazier JD, 2008. Increased maternofetal calcium flux in parathyroid hormone-related protein-null mice. *J Physiol* 586, 2015–25. [PubMed: 18258656]
- Borthwick LA, Neal A, Hobson L, Gerke V, Robson L and Muimo R, 2008. The annexin 2-S100A10 complex and its association with TRPV6 is regulated by cAMP/PKA/CnA in airway and gut epithelia. *Cell Calcium* 44, 147–57. [PubMed: 18187190]

- Bouillon R, Van Cromphaut S and Carmeliet G, 2003. Intestinal calcium absorption: Molecular vitamin D mediated mechanisms. *J Cell Biochem* 88, 332–9. [PubMed: 12520535]
- Bowen CV, DeBay D, Ewart HS, Gallant P, Gormley S, Ilenchuk TT, Iqbal U, Lutes T, Martina M, Mealing G, Merkley N, Sperker S, Moreno MJ, Rice C, Syvitski RT and Stewart JM, 2013. In vivo detection of human TRPV6-rich tumors with anti-cancer peptides derived from soricidin. *PLoS One* 8, e58866. [PubMed: 23554944]
- Brett KE, Ferraro ZM, Yockell-Lelievre J, Gruslin A and Adamo KB, 2014. Maternal-fetal nutrient transport in pregnancy pathologies: the role of the placenta. *International journal of molecular sciences* 15, 16153–16185. [PubMed: 25222554]
- Brini M, O.D., Cali T, Carafoli E, 2013. Calcium in Health and Disease., in: Sigel A, Sigel Helmut, Sigel Roland K. O. (Eds.) (Ed.), *Interrelations between Essential Metal Ions and Human Disease*. Springer, Dordrecht, Switzerland, pp. 81–137.
- Brinkmeier H, 2011. TRP Channels in Skeletal Muscle: Gene Expression, Function and Implications for Disease, in: Islam MS (Ed.), *Transient Receptor Potential Channels*. Springer Netherlands, Dordrecht, pp. 749–758.
- Bronner F, 2003. Mechanisms of intestinal calcium absorption. *J Cell Biochem* 88, 387–93. [PubMed: 12520541]
- Brown AJ, Krits I and Armbrecht HJ, 2005. Effect of age, vitamin D, and calcium on the regulation of rat intestinal epithelial calcium channels. *Arch Biochem Biophys* 437, 51–8. [PubMed: 15820216]
- Brown EM, 2013. Role of the calcium-sensing receptor in extracellular calcium homeostasis. *Best Pract Res Clin Endocrinol Metab* 27, 333–43. [PubMed: 23856263]
- Burren CP, Caswell R, Castle B, Welch CR, Hilliard TN, Smithson SF and Ellard S, 2018. TRPV6 compound heterozygous variants result in impaired placental calcium transport and severe undermineralization and dysplasia of the fetal skeleton. *Am J Med Genet A* 176, 1950–1955. [PubMed: 30144375]
- Cai R, Liu X, Zhang R, Hofmann L, Zheng W, Amin MR, Wang L, Hu Q, Peng J-B, Michalak M, Flockerzi V, Ali DW, Chen X-Z, Tang J, 2020. Autoinhibition of TRPV6 Channel and Regulation by PIP2. *iScience*. 23:101444. [PubMed: 32829285]
- Cai R, Wang L, Liu X, Michalak M, Tang J, Peng J-B, Chen X-Z, 2021. Auto-inhibitory intramolecular S5/S6 interaction in the TRPV6 channel regulates breast cancer cell migration and invasion. *Commun Biol*. 4:990. [PubMed: 34413465]
- Cao C, Zakharian E, Borbiri I and Rohacs T, 2013. Interplay between calmodulin and phosphatidylinositol 4,5-bisphosphate in Ca²⁺-induced inactivation of transient receptor potential vanilloid 6 channels. *J Biol Chem* 288, 5278–90. [PubMed: 23300090]
- Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C and Schultz N, 2012. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2, 401–4. [PubMed: 22588877]
- Ceriani F and Mammano F, 2012. Calcium signaling in the cochlea – Molecular mechanisms and physiopathological implications. *Cell Communication and Signaling* 10, 20. [PubMed: 22788415]
- Chang Q, Hoefs S, van der Kemp AW, Topala CN, Bindels RJ and Hoenderop JG, 2005. The beta-glucuronidase klotho hydrolyzes and activates the TRPV5 channel. *Science* 310, 490–3. [PubMed: 16239475]
- Charoenphandhu N, Nakkrasae L.-i., Kraidith K, Teerapornpantakit J, Thongchote K, Thongon N and Krishnamra N, 2009. Two-step stimulation of intestinal Ca²⁺ absorption during lactation by long-term prolactin exposure and suckling-induced prolactin surge. *American Journal of Physiology-Endocrinology and Metabolism* 297, E609–E619. [PubMed: 19567804]
- Charoenphandhu N, Teerapornpantakit J, Lapmanee S, Krishnamra N and Charoenphandhu J, 2012. Duodenal calcium transporter mRNA expression in stressed male rats treated with diazepam, fluoxetine, reboxetine, or venlafaxine. *Mol Cell Biochem* 369, 87–94. [PubMed: 22766765]
- Chen F, Ni B, Yang YO, Ye T and Chen A, 2014a. Knockout of TRPV6 causes osteopenia in mice by increasing osteoclastic differentiation and activity. *Cell Physiol Biochem* 33, 796–809. [PubMed: 24686448]

- Chen F, Ouyang Y, Ye T, Ni B and Chen A, 2014b. Estrogen inhibits RANKL-induced osteoclastic differentiation by increasing the expression of TRPV5 channel. *J Cell Biochem* 115, 651–8. [PubMed: 24150765]
- Choi Y, Seo H, Kim M and Ka H, 2009. Dynamic expression of calcium-regulatory molecules, TRPV6 and S100G, in the uterine endometrium during pregnancy in pigs. *Biol Reprod* 81, 1122–30. [PubMed: 19641180]
- Chow J, Norng M, Zhang J and Chai J, 2007. TRPV6 mediates capsaicin-induced apoptosis in gastric cancer cells--Mechanisms behind a possible new "hot" cancer treatment. *Biochim Biophys Acta* 1773, 565–76. [PubMed: 17292493]
- Christakos S, Dhawan P, Porta A, Mady LJ and Seth T, 2011. Vitamin D and intestinal calcium absorption. *Molecular and cellular endocrinology* 347, 25–29. [PubMed: 21664413]
- Creighton CJ, 2018. Making Use of Cancer Genomic Databases. *Current protocols in molecular biology* 121, 19.14.1–19.14.13. [PubMed: 29337373]
- Cubillos S and Norgauer J, 2016. Low vitamin D-modulated calcium-regulating proteins in psoriasis vulgaris plaques: S100A7 overexpression depends on joint involvement. *Int J Mol Med* 38, 1083–92. [PubMed: 27573000]
- Cui J, Bian JS, Kagan A and McDonald TV, 2002. CaT1 contributes to the stores-operated calcium current in Jurkat T-lymphocytes. *J Biol Chem* 277, 47175–83. [PubMed: 12361955]
- Cui M, Li Q, Johnson R and Fleet JC, 2012. Villin promoter-mediated transgenic expression of transient receptor potential cation channel, subfamily V, member 6 (TRPV6) increases intestinal calcium absorption in wild-type and vitamin D receptor knockout mice. *J Bone Miner Res* 27, 2097–107. [PubMed: 22589201]
- Dai W, Bai Y, Hebda L, Zhong X, Liu J, Kao J and Duan C, 2014. Calcium deficiency-induced and TRP channel-regulated IGF1R-PI3K-Akt signaling regulates abnormal epithelial cell proliferation. *Cell Death Differ* 21, 568–81. [PubMed: 24336047]
- Dang S, van Goor MK, Asarnow D, Wang Y, Julius D, Cheng Y and van der Wijst J, 2019. Structural insight into TRPV5 channel function and modulation. *Proc Natl Acad Sci U S A* 116, 8869–8878. [PubMed: 30975749]
- De Clercq K, Van den Eynde C, Hennes A, Van Bree R, Voets T and Vriens J, 2017. The functional expression of transient receptor potential channels in the mouse endometrium. *Hum Reprod* 32, 615–630. [PubMed: 28077439]
- den Dekker E, Hoenderop JG, Nilius B and Bindels RJ, 2003. The epithelial calcium channels, TRPV5 & TRPV6: from identification towards regulation. *Cell Calcium* 33, 497–507. [PubMed: 12765695]
- Derler I, Hofbauer M, Kahr H, Fritsch R, Muik M, Kepplinger K, Hack ME, Moritz S, Schindl R, Groschner K and Romanin C, 2006. Dynamic but not constitutive association of calmodulin with rat TRPV6 channels enables fine tuning of Ca²⁺-dependent inactivation. *J Physiol* 577, 31–44. [PubMed: 16959851]
- Dhennin-Duthille I, Gautier M, Faouzi M, Guilbert A, Brevet M, Vaudry D, Ahidouch A, Sevestre H and Ouadid-Ahidouch H, 2011. High expression of transient receptor potential channels in human breast cancer epithelial cells and tissues: correlation with pathological parameters. *Cell Physiol Biochem* 28, 813–22. [PubMed: 22178934]
- Diaz de Barboza G, Guizzardi S and Tolosa de Talamoni N, 2015. Molecular aspects of intestinal calcium absorption. *World J Gastroenterol* 21, 7142–54. [PubMed: 26109800]
- Diepens RJ, den Dekker E, Bens M, Weidema AF, Vandewalle A, Bindels RJ and Hoenderop JG, 2004. Characterization of a murine renal distal convoluted tubule cell line for the study of transcellular calcium transport. *Am J Physiol Renal Physiol* 286, F483–9. [PubMed: 14625201]
- Dror AA, Brownstein Z and Avraham KB, 2011. Integration of human and mouse genetics reveals pendrin function in hearing and deafness. *Cell Physiol Biochem* 28, 535–44. [PubMed: 22116368]
- Ecroyd H, Asquith KL, Jones RC and Aitken RJ, 2004. The development of signal transduction pathways during epididymal maturation is calcium dependent. *Developmental Biology* 268, 53–63. [PubMed: 15031104]
- Emkey RD and Emkey GR, 2012. Calcium metabolism and correcting calcium deficiencies. *Endocrinol Metab Clin North Am* 41, 527–56. [PubMed: 22877428]

- Erler I, Hirnet D, Wissenbach U, Flockerzi V and Niemeyer BA, 2004. Ca²⁺-selective transient receptor potential V channel architecture and function require a specific ankyrin repeat. *J Biol Chem* 279, 34456–63. [PubMed: 15192090]
- Fan H, Shen YX and Yuan YF, 2014. Expression and prognostic roles of TRPV5 and TRPV6 in non-small cell lung cancer after curative resection. *Asian Pac J Cancer Prev* 15, 2559–63. [PubMed: 24761864]
- Fecher-Trost C, Lux F, Busch KM, Raza A, Winter M, Hielscher F, Belkacemi T, van der Eerden B, Boehm U, Freichel M and Weissgerber P, 2019. Maternal Transient Receptor Potential Vanilloid 6 (Trpv6) Is Involved In Offspring Bone Development. *J Bone Miner Res* 34, 699–710. [PubMed: 30786075]
- Fecher-Trost C, Weissgerber P and Wissenbach U, 2014. TRPV6 channels. *Handb Exp Pharmacol* 222, 359–84. [PubMed: 24756713]
- Fecher-Trost C, Wissenbach U, Beck A, Schalkowsky P, Stoerger C, Doerr J, Dembek A, Simon-Thomas M, Weber A, Wollenberg P, Ruppert T, Middendorff R, Maurer HH and Flockerzi V, 2013. The in vivo TRPV6 protein starts at a non-AUG triplet, decoded as methionine, upstream of canonical initiation at AUG. *J Biol Chem* 288, 16629–44. [PubMed: 23612980]
- Fecher-Trost C, Wissenbach U and Weissgerber P, 2017. TRPV6: From identification to function. *Cell Calcium* 67, 116–122. [PubMed: 28501141]
- Fixemer T, Wissenbach U, Flockerzi V and Bonkhoff H, 2003. Expression of the Ca²⁺-selective cation channel TRPV6 in human prostate cancer: a novel prognostic marker for tumor progression. *Oncogene* 22, 7858–61. [PubMed: 14586412]
- Fleet JC, Eksir F, Hance KW and Wood RJ, 2002. Vitamin D-inducible calcium transport and gene expression in three Caco-2 cell lines. *Am J Physiol Gastrointest Liver Physiol* 283, G618–25. [PubMed: 12181175]
- Fleet JC and Schoch RD, 2010. Molecular mechanisms for regulation of intestinal calcium absorption by vitamin D and other factors. *Critical reviews in clinical laboratory sciences* 47, 181–195. [PubMed: 21182397]
- Flores-Aldama L, Vandeweghe MW, Zavala K, Colenso CK, Gonzalez W, Brauchi SE and Opazo JC, 2020. Evolutionary analyses reveal independent origins of gene repertoires and structural motifs associated to fast inactivation in calcium-selective TRPV channels. *Sci Rep* 10, 8684. [PubMed: 32457384]
- Floriana E, Christian H, Walter M and Kristina F, 2014. Calcium - a central regulator of keratinocyte differentiation in health and disease. *European Journal of Dermatology* 24, 650–661. [PubMed: 25514792]
- Friedman PA and Bushinsky DA, 1999. Diuretic effects on calcium metabolism. *Semin Nephrol* 19, 551–6. [PubMed: 10598542]
- Fu S, Hirte H, Welch S, Ilenchuk TT, Lutes T, Rice C, Fields N, Nemet A, Dugourd D, Piha-Paul S, Subbiah V, Liu L, Gong J, Hong D and Stewart JM, 2017. First-in-human phase I study of SOR-C13, a TRPV6 calcium channel inhibitor, in patients with advanced solid tumors. *Invest New Drugs* 35, 324–333. [PubMed: 28150073]
- Fukushima A, Aizaki Y and Sakuma K, 2009. Short-chain fatty acids induce intestinal transient receptor potential vanilloid type 6 expression in rats and Caco-2 cells. *J Nutr* 139, 20–5. [PubMed: 19056662]
- Gao da Y, Zhang BL, Leung MC, Au SC, Wong PY and Shum WW, 2016. Coupling of TRPV6 and TMEM16A in epithelial principal cells of the rat epididymis. *J Gen Physiol* 148, 161–82. [PubMed: 27481714]
- Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C and Schultz N, 2013. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 6, p11. [PubMed: 23550210]
- Gil Á, Plaza-Díaz J and Mesa M, 2018. Vitamin D: Classic and Novel Actions. *Annals of Nutrition and Metabolism* 72.
- Goodfellow LR, Cooper C and Harvey NC, 2011. Regulation of placental calcium transport and offspring bone health. *Frontiers in endocrinology* 2, 3–3. [PubMed: 22649358]

- Guo Z, Grimm C, Becker L, Ricci AJ and Heller S, 2013. A novel ion channel formed by interaction of TRPML3 with TRPV5. *PLoS One* 8, e58174. [PubMed: 23469151]
- Hache S, Takser L, LeBellego F, Weiler H, Leduc L, Forest JC, Giguere Y, Masse A, Barbeau B and Lafond J, 2011. Alteration of calcium homeostasis in primary preeclamptic syncytiotrophoblasts: effect on calcium exchange in placenta. *J Cell Mol Med* 15, 654–67. [PubMed: 20178461]
- Heise N, Palme D, Misovic M, Koka S, Rudner J, Lang F, Salih HR, Huber SM and Henke G, 2010. Non-selective cation channel-mediated Ca²⁺-entry and activation of Ca²⁺/calmodulin-dependent kinase II contribute to G2/M cell cycle arrest and survival of irradiated leukemia cells. *Cell Physiol Biochem* 26, 597–608. [PubMed: 21063097]
- Hellwig N, Albrecht N, Harteneck C, Schultz G and Schaefer M, 2005. Homo- and heteromeric assembly of TRPV channel subunits. *J Cell Sci* 118, 917–28. [PubMed: 15713749]
- Hirnet D, Olausson J, Fecher-Trost C, Bodding M, Nastainczyk W, Wissenbach U, Flockerzi V and Freichel M, 2003. The TRPV6 gene, cDNA and protein. *Cell Calcium* 33, 509–18. [PubMed: 12765696]
- Hoenderop JG, van der Kemp AW, Hartog A, van de Graaf SF, van Os CH, Willems PH and Bindels RJ, 1999. Molecular identification of the apical Ca²⁺ channel in 1,25-dihydroxyvitamin D₃-responsive epithelia. *J Biol Chem* 274, 8375–8. [PubMed: 10085067]
- Hoenderop JG, van Leeuwen JP, van der Eerden BC, Kersten FF, van der Kemp AW, Merillat AM, Waarsing JH, Rossier BC, Vallon V, Hummler E and Bindels RJ, 2003a. Renal Ca²⁺ wasting, hyperabsorption, and reduced bone thickness in mice lacking TRPV5. *J Clin Invest* 112, 1906–14. [PubMed: 14679186]
- Hoenderop JG, Vennekens R, Muller D, Prenen J, Droogmans G, Bindels RJ and Nilius B, 2001. Function and expression of the epithelial Ca(2+) channel family: comparison of mammalian ECaC1 and 2. *J Physiol* 537, 747–61. [PubMed: 11744752]
- Hoenderop JG, Voets T, Hoefs S, Weidema F, Prenen J, Nilius B and Bindels RJ, 2003b. Homo- and heterotetrameric architecture of the epithelial Ca²⁺ channels TRPV5 and TRPV6. *EMBO J* 22, 776–85. [PubMed: 12574114]
- Hofer A, Kovacs G, Zappatini A, Leuener M, Hediger MA and Lochner M, 2013. Design, synthesis and pharmacological characterization of analogs of 2-aminoethyl diphenylborinate (2-APB), a known store-operated calcium channel blocker, for inhibition of TRPV6-mediated calcium transport. *Bioorg Med Chem* 21, 3202–13. [PubMed: 23602525]
- Hsu YJ, Dimke H, Schoeber JP, Hsu SC, Lin SH, Chu P, Hoenderop JG and Bindels RJ, 2010. Testosterone increases urinary calcium excretion and inhibits expression of renal calcium transport proteins. *Kidney Int* 77, 601–8. [PubMed: 20090667]
- Huber SM, 2013. Oncochannels. *Cell Calcium* 53, 241–255. [PubMed: 23357407]
- Hughes DA, Tang K, Strotmann R, Schoneberg T, Prenen J, Nilius B and Stoneking M, 2008. Parallel selection on TRPV6 in human populations. *PLoS One* 3, e1686. [PubMed: 18301763]
- Hughes TET, Lodowski DT, Huynh KW, Yazici A, Del Rosario J, Kapoor A, Basak S, Samanta A, Han X, Chakrapani S, Zhou ZH, Filizola M, Rohacs T, Han S and Moiseenkova-Bell VY, 2018a. Structural basis of TRPV5 channel inhibition by econazole revealed by cryo-EM. *Nat Struct Mol Biol* 25, 53–60. [PubMed: 29323279]
- Hughes TET, Pumroy RA, Yazici AT, Kasimova MA, Fluck EC, Huynh KW, Samanta A, Molugu SK, Zhou ZH, Carnevale V, Rohacs T and Moiseenkova-Bell VY, 2018b. Structural insights on TRPV5 gating by endogenous modulators. *Nat Commun* 9, 4198. [PubMed: 30305626]
- Huybers S, Naber TH, Bindels RJ and Hoenderop JG, 2007. Prednisolone-induced Ca²⁺ malabsorption is caused by diminished expression of the epithelial Ca²⁺ channel TRPV6. *Am J Physiol Gastrointest Liver Physiol* 292, G92–7. [PubMed: 16901990]
- Imaten M, Blanchard-Gutton N and Harvey BJ, 2008. Rapid effects of 17beta-estradiol on epithelial TRPV6 Ca²⁺ channel in human T84 colonic cells. *Cell Calcium* 44, 441–52. [PubMed: 18395250]
- Ishizawa M, Akagi D, Yamamoto J and Makishima M, 2017. 1alpha,25-Dihydroxyvitamin D₃ enhances TRPV6 transcription through p38 MAPK activation and GADD45 expression. *J Steroid Biochem Mol Biol* 172, 55–61. [PubMed: 28578001]

- Janssens A, Silvestri C, Martella A, Vanoevelen JM, Di Marzo V and Voets T, 2018. Delta(9)-tetrahydrocannabinol impairs epithelial calcium transport through inhibition of TRPV5 and TRPV6. *Pharmacol Res* 136, 83–89. [PubMed: 30170189]
- Jeon US, 2008. Kidney and calcium homeostasis. *Electrolyte & blood pressure : E & BP* 6, 68–76. [PubMed: 24459525]
- Jiang Y, Gou H, Zhu J, Tian S and Yu L, 2016. Lidocaine inhibits the invasion and migration of TRPV6-expressing cancer cells by TRPV6 downregulation. *Oncol Lett* 12, 1164–1170. [PubMed: 27446413]
- Jung EM, Kim JH, Yang H, Hyun SH, Choi KC and Jeung EB, 2011. Duodenal and renal transient receptor potential vanilloid 6 is regulated by sex steroid hormones, estrogen and progesterone, in immature rats. *J Vet Med Sci* 73, 711–6. [PubMed: 21228508]
- Kessler T, Wissenbach U, Grobholz R and Flockerzi V, 2009. TRPV6 alleles do not influence prostate cancer progression. *BMC Cancer* 9, 380. [PubMed: 19857260]
- Kärki T, Rajakylä EK, Acheva A, Tojkander S, 2020. TRPV6 calcium channel directs homeostasis of the mammary epithelial sheets and controls epithelial mesenchymal transition. *Sci Rep*. 10:14683. [PubMed: 32895467]
- Kever L, Cherezova A, Zenin V, Negulyaev Y, Komissarchik Y and Semenova S, 2019. Downregulation of TRPV6 channel activity by cholesterol depletion in Jurkat T cell line. *Cell Biol Int* 43, 965–975. [PubMed: 31141273]
- Khanal RC, Peters TM, Smith NM and Nemere I, 2008. Membrane receptor-initiated signaling in 1,25(OH)2D3-stimulated calcium uptake in intestinal epithelial cells. *J Cell Biochem* 105, 1109–16. [PubMed: 18773429]
- Kim HJ, Lee GS, Ji YK, Choi KC and Jeung EB, 2006. Differential expression of uterine calcium transporter 1 and plasma membrane Ca²⁺ ATPase 1b during rat estrous cycle. *Am J Physiol Endocrinol Metab* 291, E234–41. [PubMed: 16825604]
- Kim HJ, Yang DK and So I, 2007. PDZ domain-containing protein as a physiological modulator of TRPV6. *Biochem Biophys Res Commun* 361, 433–8. [PubMed: 17645868]
- Kim JA, Yang H, Hwang I, Jung EM, Choi KC and Jeung EB, 2011. Expression patterns and potential action of the calcium transport genes *Trpv5*, *Trpv6*, *Ncx1* and *Pmca1b* in the canine duodenum, kidney and uterus. *In Vivo* 25, 773–80. [PubMed: 21753133]
- Kim MH, Lee GS, Jung EM, Choi KC and Jeung EB, 2009a. The negative effect of dexamethasone on calcium-processing gene expressions is associated with a glucocorticoid-induced calcium-absorbing disorder. *Life Sci* 85, 146–52. [PubMed: 19490920]
- Kim MH, Lee GS, Jung EM, Choi KC, Oh GT and Jeung EB, 2009b. Dexamethasone differentially regulates renal and duodenal calcium-processing genes in calbindin-D9k and -D28k knockout mice. *Exp Physiol* 94, 138–51. [PubMed: 18931045]
- Kim SY, Hong C, Wie J, Kim E, Kim BJ, Ha K, Cho NH, Kim IG, Jeon JH and So I, 2014. Reciprocal positive regulation between TRPV6 and NUMB in PTEN-deficient prostate cancer cells. *Biochem Biophys Res Commun* 447, 192–6. [PubMed: 24704446]
- Kim SY, Yang D, Myeong J, Ha K, Kim SH, Park EJ, Kim IG, Cho NH, Lee KP, Jeon JH and So I, 2013. Regulation of calcium influx and signaling pathway in cancer cells via TRPV6-Numb1 interaction. *Cell Calcium* 53, 102–11. [PubMed: 23140583]
- King N, Westbrook MJ, Young SL, Kuo A, Abedin M, Chapman J, Fairclough S, Hellsten U, Isogai Y, Letunic I, Marr M, Pincus D, Putnam N, Rokas A, Wright KJ, Zuzow R, Dirks W, Good M, Goodstein D, Lemons D, Li W, Lyons JB, Morris A, Nichols S, Richter DJ, Salamov A, Sequencing JG, Bork P, Lim WA, Manning G, Miller WT, McGinnis W, Shapiro H, Tjian R, Grigoriev IV and Rokhsar D, 2008. The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. *Nature* 451, 783–8. [PubMed: 18273011]
- Kovacs CS and Kronenberg HM, 1997. Maternal-Fetal Calcium and Bone Metabolism During Pregnancy, Puerperium, and Lactation*. *Endocrine Reviews* 18, 832–872. [PubMed: 9408745]
- Kovacs G, Danko T, Bergeron MJ, Balazs B, Suzuki Y, Zsembery A and Hediger MA, 2011. Heavy metal cations permeate the TRPV6 epithelial cation channel. *Cell Calcium* 49, 43–55. [PubMed: 21146870]

- Kovacs G, Montalbetti N, Simonin A, Danko T, Balazs B, Zsembery A and Hediger MA, 2012. Inhibition of the human epithelial calcium channel TRPV6 by 2-aminoethoxydiphenyl borate (2-APB). *Cell Calcium* 52, 468–80. [PubMed: 23040501]
- Kumar A and Kaur S, 2017. Calcium: A Nutrient in Pregnancy. *Journal of obstetrics and gynaecology of India* 67, 313–318. [PubMed: 28867880]
- Kumar S, Singh O, Singh U, Goswami C and Singru PS, 2018. Transient receptor potential vanilloid 1-6 (Trpv1-6) gene expression in the mouse brain during estrous cycle. *Brain Res* 1701, 161–170. [PubMed: 30194920]
- Kutuzova GD, Sundersingh F, Vaughan J, Tadi BP, Ansay SE, Christakos S and Deluca HF, 2008. TRPV6 is not required for 1 α ,25-dihydroxyvitamin D₃-induced intestinal calcium absorption in vivo. *Proc Natl Acad Sci U S A* 105, 19655–9. [PubMed: 19073913]
- Lager S and Powell TL, 2012. Regulation of Nutrient Transport across the Placenta. *Journal of Pregnancy* 2012, 179827. [PubMed: 23304511]
- Lallet-Daher H, Roudbaraki M, Bavencoffe A, Mariot P, Gackière F, Bidaux G, Urbain R, Gosset P, Delcourt P, Fleurisse L, Slomianny C, Dewailly E, Mauroy B, Bonnal JL, Skryma R and Prevarskaya N, 2009. Intermediate-conductance Ca²⁺-activated K⁺ channels (IKCa1) regulate human prostate cancer cell proliferation through a close control of calcium entry. *Oncogene* 28, 1792–1806. [PubMed: 19270724]
- Lambers TT, Mahieu F, Oancea E, Hoofd L, de Lange F, Mensenkamp AR, Voets T, Nilius B, Clapham DE, Hoenderop JG and Bindels RJ, 2006. Calbindin-D28K dynamically controls TRPV5-mediated Ca²⁺ transport. *EMBO J* 25, 2978–88. [PubMed: 16763551]
- Lambers TT, Weidema AF, Nilius B, Hoenderop JG and Bindels RJ, 2004. Regulation of the mouse epithelial Ca²⁺(+) channel TRPV6 by the Ca(2+)-sensor calmodulin. *J Biol Chem* 279, 28855–61. [PubMed: 15123711]
- Landowski CP, Bolanz KA, Suzuki Y and Hediger MA, 2011. Chemical inhibitors of the calcium entry channel TRPV6. *Pharm Res* 28, 322–30. [PubMed: 21057859]
- Lau JK, Brown KC, Dom AM, Witte TR, Thornhill BA, Crabtree CM, Perry HE, Brown JM, Ball JG, Creel RG, Damron CL, Rollyson WD, Stevenson CD, Hardman WE, Valentovic MA, Carpenter AB and Dasgupta P, 2014. Capsaicin induces apoptosis in human small cell lung cancer via the TRPV6 receptor and the calpain pathway. *Apoptosis* 19, 1190–201. [PubMed: 24878626]
- Lee B, Ahn C, Jeon BH, Jung EM, Yoo YM and Jeung EB, 2019a. Regulatory effect of dexamethasone on tracheal calcium processing proteins and mucosal secretion. *J Physiol Pharmacol* 70.
- Lee BM, Lee GS, Jung EM, Choi KC and Jeung EB, 2009. Uterine and placental expression of TRPV6 gene is regulated via progesterone receptor- or estrogen receptor-mediated pathways during pregnancy in rodents. *Reprod Biol Endocrinol* 7, 49. [PubMed: 19457270]
- Lee CT, Chen HC, Lai LW, Yong KC and Lien YH, 2007. Effects of furosemide on renal calcium handling. *Am J Physiol Renal Physiol* 293, F1231–7. [PubMed: 17652376]
- Lee CT, Chen HC, Ng HY, Lai LW and Lien YH, 2012. Renal adaptation to gentamicin-induced mineral loss. *Am J Nephrol* 35, 279–86. [PubMed: 22378246]
- Lee CT, Lien YH, Lai LW, Chen JB, Lin CR and Chen HC, 2006. Increased renal calcium and magnesium transporter abundance in streptozotocin-induced diabetes mellitus. *Kidney Int* 69, 1786–91. [PubMed: 16557223]
- Lee CT, Ng HY, Lee YT, Lai LW and Lien YH, 2016. The role of calbindin-D28k on renal calcium and magnesium handling during treatment with loop and thiazide diuretics. *Am J Physiol Renal Physiol* 310, F230–6. [PubMed: 26582761]
- Lee CT, Shang S, Lai LW, Yong KC and Lien YH, 2004. Effect of thiazide on renal gene expression of apical calcium channels and calbindins. *Am J Physiol Renal Physiol* 287, F1164–70. [PubMed: 15265769]
- Lee GS and Jeung EB, 2007. Uterine TRPV6 expression during the estrous cycle and pregnancy in a mouse model. *Am J Physiol Endocrinol Metab* 293, E132–8. [PubMed: 17374692]
- Lee JJ, Liu X, O'Neill D, Beggs MR, Weissgerber P, Flockerzi V, Chen XZ, Dimke H and Alexander RT, 2019b. Activation of the calcium sensing receptor attenuates TRPV6-dependent intestinal calcium absorption. *JCI Insight* 5.

- Lehen'kyi V, Beck B, Polakowska R, Charveron M, Bordat P, Skryma R and Prevarskaya N, 2007a. TRPV6 is a Ca²⁺ entry channel essential for Ca²⁺-induced differentiation of human keratinocytes. *J Biol Chem* 282, 22582–91. [PubMed: 17550901]
- Lehen'kyi V, Flourakis M, Skryma R and Prevarskaya N, 2007b. TRPV6 channel controls prostate cancer cell proliferation via Ca(2+)/NFAT-dependent pathways. *Oncogene* 26, 7380–5. [PubMed: 17533368]
- Lehen'kyi V and Prevarskaya N, 2011. Oncogenic TRP channels. *Adv Exp Med Biol* 704, 929–45. [PubMed: 21290334]
- Lehen'kyi V, Raphael M, Oulidi A, Flourakis M, Khalimonchuk S, Kondratskyi A, Gordienko DV, Mauroy B, Bonnal JL, Skryma R and Prevarskaya N, 2011a. TRPV6 determines the effect of vitamin D3 on prostate cancer cell growth. *PLoS One* 6, e16856. [PubMed: 21347289]
- Lehen'kyi V, Raphael M and Prevarskaya N, 2012. The role of the TRPV6 channel in cancer. *J Physiol* 590, 1369–76. [PubMed: 22331416]
- Lehen'kyi V, Vandenberghe M, Belaubre F, Julie S, Castex-Rizzi N, Skryma R and Prevarskaya N, 2011b. Acceleration of keratinocyte differentiation by transient receptor potential vanilloid (TRPV6) channel activation. *J Eur Acad Dermatol Venereol* 25 Suppl 1, 12–8.
- Levin VA, Jiang X and Kagan R, 2018. Estrogen therapy for osteoporosis in the modern era. *Osteoporos Int* 29, 1049–1055. [PubMed: 29520604]
- Li SH, Yin HB, Ren MR, Wu MJ, Huang XL, Li JJ, Luan YP and Wu YL, 2019. TRPV5 and TRPV6 are expressed in placenta and bone tissues during pregnancy in mice. *Biotech Histochem* 94, 244–251. [PubMed: 30916584]
- Lieben L, Benn BS, Ajibade D, Stockmans I, Moermans K, Hediger MA, Peng JB, Christakos S, Bouillon R and Carmeliet G, 2010. Trpv6 mediates intestinal calcium absorption during calcium restriction and contributes to bone homeostasis. *Bone* 47, 301–8. [PubMed: 20399919]
- Lieben L and Carmeliet G, 2012. The Involvement of TRP Channels in Bone Homeostasis. *Frontiers in endocrinology* 3, 99–99. [PubMed: 22934090]
- Lin Y, Liang A, He Y, Li Z, Li Z, Wang G and Sun F, 2019. Proteomic analysis of seminal extracellular vesicle proteins involved in asthenozoospermia by iTRAQ. *Mol Reprod Dev* 86, 1094–1105. [PubMed: 31215738]
- Little R, Muimo R, Robson L, Harris K and Grabowski PS, 2011. The transient receptor potential ion channel TRPV6 is expressed at low levels in osteoblasts and has little role in osteoblast calcium uptake. *PLoS One* 6, e28166. [PubMed: 22163264]
- Long SB, Tao X, Campbell EB and MacKinnon R, 2007. Atomic structure of a voltage-dependent K⁺ channel in a lipid membrane-like environment. *Nature* 450, 376–82. [PubMed: 18004376]
- Lu P, Boros S, Chang Q, Bindels RJ and Hoenderop JG, 2008. The beta-glucuronidase klotho exclusively activates the epithelial Ca²⁺ channels TRPV5 and TRPV6. *Nephrol Dial Transplant* 23, 3397–402. [PubMed: 18495742]
- Masamune A, Kotani H, Sorgel FL, Chen JM, Hamada S, Sakaguchi R, Masson E, Nakano E, Kakuta Y, Niihori T, Funayama R, Shirota M, Hirano T, Kawamoto T, Hosokoshi A, Kume K, Unger L, Ewers M, Laumen H, Bugert P, Mori MX, Tsvilovskyy V, Weissgerber P, Kriebs U, Fecher-Trost C, Freichel M, Diakopoulos KN, Berninger A, Lesina M, Ishii K, Itoi T, Ikeura T, Okazaki K, Kaune T, Rosendahl J, Nagasaki M, Uezono Y, Algul H, Nakayama K, Matsubara Y, Aoki Y, Ferec C, Mori Y, Witt H and Shimosegawa T, 2020. Variants That Affect Function of Calcium Channel TRPV6 Are Associated With Early-Onset Chronic Pancreatitis. *Gastroenterology* 158, 1626–1641 e8. [PubMed: 31930989]
- Mason AE, Grier D, Smithson SF, Burren CP and Gradhand E, 2020. Post-mortem histology in transient receptor potential cation channel subfamily V member 6 (TRPV6) under-mineralising skeletal dysplasia suggests postnatal skeletal recovery: a case report. *BMC Med Genet* 21, 64. [PubMed: 32228492]
- McGoldrick LL, Singh AK, Saotome K, Yelshanskaya MV, Twomey EC, Grassucci RA and Sobolevsky AI, 2018. Opening of the human epithelial calcium channel TRPV6. *Nature* 553, 233–237. [PubMed: 29258289]

- Mercado J, Gordon-Shaag A, Zagotta WN and Gordon SE, 2010. Ca²⁺-dependent desensitization of TRPV2 channels is mediated by hydrolysis of phosphatidylinositol 4,5-bisphosphate. *J Neurosci* 30, 13338–47. [PubMed: 20926660]
- Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, Witman GB, Terry A, Salamov A, Fritz-Laylin LK, Marechal-Drouard L, Marshall WF, Qu LH, Nelson DR, Sanderfoot AA, Spalding MH, Kapitonov VV, Ren Q, Ferris P, Lindquist E, Shapiro H, Lucas SM, Grimwood J, Schmutz J, Cardol P, Cerutti H, Chanfreau G, Chen CL, Cognat V, Croft MT, Dent R, Dutcher S, Fernandez E, Fukuzawa H, Gonzalez-Ballester D, Gonzalez-Halphen D, Hallmann A, Hanikenne M, Hippler M, Inwood W, Jabbari K, Kalanon M, Kuras R, Lefebvre PA, Lemaire SD, Lobanov AV, Lohr M, Manuell A, Meier I, Mets L, Mittag M, Mittelmeier T, Moroney JV, Moseley J, Napoli C, Nedelcu AM, Niyogi K, Novoselov SV, Paulsen IT, Pazour G, Purton S, Ral JP, Riano-Pachon DM, Riekhof W, Rymarquis L, Schroda M, Stern D, Umen J, Willows R, Wilson N, Zimmer SL, Allmer J, Balk J, Bisova K, Chen CJ, Elias M, Gendler K, Hauser C, Lamb MR, Ledford H, Long JC, Minagawa J, Page MD, Pan J, Pootakham W, Roje S, Rose A, Stahlberg E, Terauchi AM, Yang P, Ball S, Bowler C, Dieckmann CL, Gladyshev VN, Green P, Jorgensen R, Mayfield S, Mueller-Roeber B, Rajamani S, Sayre RT, Brokstein P, et al. , 2007. The Chlamydomonas genome reveals the evolution of key animal and plant functions. *Science* 318, 245–50. [PubMed: 17932292]
- Meyer MB, Watanuki M, Kim S, Shevde NK and Pike JW, 2006. The human transient receptor potential vanilloid type 6 distal promoter contains multiple vitamin D receptor binding sites that mediate activation by 1,25-dihydroxyvitamin D₃ in intestinal cells. *Mol Endocrinol* 20, 1447–61. [PubMed: 16574738]
- Meyer MB, Zella LA, Nerenz RD and Pike JW, 2007. Characterizing early events associated with the activation of target genes by 1,25-dihydroxyvitamin D₃ in mouse kidney and intestine in vivo. *J Biol Chem* 282, 22344–52. [PubMed: 17556365]
- Minke B, Wu C and Pak WL, 1975. Induction of photoreceptor voltage noise in the dark in *Drosophila* mutant. *Nature* 258, 84–7. [PubMed: 810728]
- Miura S, Sato K, Kato-Negishi M, Teshima T and Takeuchi S, 2015. Fluid shear triggers microvilli formation via mechanosensitive activation of TRPV6. *Nat Commun* 6, 8871. [PubMed: 26563429]
- Mobasher A, Matta C, Uzielienė I, Budd E, Martín-Vasallo P and Bernotiene E, 2019. The chondrocyte channelome: A narrative review. *Joint Bone Spine* 86, 29–35. [PubMed: 29452304]
- Montell C, 2005. The TRP superfamily of cation channels. *Sci STKE* 2005, re3. [PubMed: 15728426]
- Montell C, Birnbaumer L, Flockerzi V, Bindels RJ, Bruford EA, Caterina MJ, Clapham DE, Harteneck C, Heller S, Julius D, Kojima I, Mori Y, Penner R, Prawitt D, Scharenberg AM, Schultz G, Shimizu N and Zhu MX, 2002. A unified nomenclature for the superfamily of TRP cation channels. *Mol Cell* 9, 229–31. [PubMed: 11864597]
- Moor MB and Bonny O, 2016. Ways of calcium reabsorption in the kidney. *Am J Physiol Renal Physiol* 310, F1337–50. [PubMed: 27009338]
- Moreau R, Daoud G, Bernatchez R, Simoneau L, Masse A and Lafond J, 2002a. Calcium uptake and calcium transporter expression by trophoblast cells from human term placenta. *Biochim Biophys Acta* 1564, 325–32. [PubMed: 12175914]
- Moreau R, Hamel A, Daoud G, Simoneau L and Lafond J, 2002b. Expression of calcium channels along the differentiation of cultured trophoblast cells from human term placenta. *Biol Reprod* 67, 1473–9. [PubMed: 12390878]
- Morgan CP, Zhao H, LeMasurier M, Xiong W, Pan B, Kazmierczak P, Avenarius MR, Bateschell M, Larisch R, Ricci AJ, Muller U and Barr-Gillespie PG, 2018. TRPV6, TRPM6 and TRPM7 Do Not Contribute to Hair-Cell Mechanotransduction. *Front Cell Neurosci* 12, 41. [PubMed: 29515374]
- Muller D, Hoenderop JG, Merckx GF, van Os CH and Bindels RJ, 2000. Gene structure and chromosomal mapping of human epithelial calcium channel. *Biochem Biophys Res Commun* 275, 47–52. [PubMed: 10944439]
- Munson S, Wang Y, Chang W and Bikle DD, 2019. Myosin 1a Regulates Osteoblast Differentiation Independent of Intestinal Calcium Transport. *Journal of the Endocrine Society* 3, 1993–2011. [PubMed: 31620669]

- Na T and Peng JB, 2014. TRPV5: a Ca(2+) channel for the fine-tuning of Ca(2+) reabsorption. *Handb Exp Pharmacol* 222, 321–57. [PubMed: 24756712]
- Nakaya K, Harbidge DG, Wangemann P, Schultz BD, Green ED, Wall SM and Marcus DC, 2007. Lack of pendrin HCO₃⁻ transport elevates vestibular endolymphatic [Ca²⁺] by inhibition of acid-sensitive TRPV5 and TRPV6 channels. *Am J Physiol Renal Physiol* 292, F1314–21. [PubMed: 17200157]
- Neuberger A, Nadezhdin KD, Sobolevsky AI 2021. Structural mechanisms of TRPV6 inhibition by ruthenium red and Econazole. *Nat Commun* 12, 6284. [PubMed: 34725357]
- Nie X, Jin H, Wen G, Xu J, An J, Liu X, Xie R and Tuo B, 2020. Estrogen Regulates Duodenal Calcium Absorption Through Differential Role of Estrogen Receptor on Calcium Transport Proteins. *Dig Dis Sci*.
- Niemeyer BA, Bergs C, Wissenbach U, Flockerzi V and Trost C, 2001. Competitive regulation of Ca^T-like-mediated Ca²⁺ entry by protein kinase C and calmodulin. *Proc Natl Acad Sci U S A* 98, 3600–5. [PubMed: 11248124]
- Nijenhuis T, Hoenderop JG and Bindels RJ, 2005. TRPV5 and TRPV6 in Ca(2+) (re)absorption: regulating Ca(2+) entry at the gate. *Pflugers Arch* 451, 181–92. [PubMed: 16044309]
- Nijenhuis T, Hoenderop JG, Löffing J, van der Kemp AW, van Os CH and Bindels RJ, 2003a. Thiazide-induced hypocalciuria is accompanied by a decreased expression of Ca²⁺ transport proteins in kidney. *Kidney Int* 64, 555–64. [PubMed: 12846750]
- Nijenhuis T, Hoenderop JG, Nilius B and Bindels RJ, 2003b. (Patho)physiological implications of the novel epithelial Ca²⁺ channels TRPV5 and TRPV6. *Pflugers Arch* 446, 401–9. [PubMed: 12748856]
- Nijenhuis T, Hoenderop JG, van der Kemp AW and Bindels RJ, 2003c. Localization and regulation of the epithelial Ca²⁺ channel TRPV6 in the kidney. *J Am Soc Nephrol* 14, 2731–40. [PubMed: 14569082]
- Nilius B, 2007. TRP channels in disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1772, 805–812. [PubMed: 17368864]
- Nilius B and Owsianik G, 2011. The transient receptor potential family of ion channels. *Genome Biology* 12, 218. [PubMed: 21401968]
- Nilius B, Prenen J, Hoenderop JG, Vennekens R, Hoefs S, Weidema AF, Droogmans G and Bindels RJ, 2002. Fast and slow inactivation kinetics of the Ca²⁺ channels ECaC1 and ECaC2 (TRPV5 and TRPV6). Role of the intracellular loop located between transmembrane segments 2 and 3. *J Biol Chem* 277, 30852–8. [PubMed: 12077127]
- Nilius B, Vennekens R, Prenen J, Hoenderop JG, Droogmans G and Bindels RJ, 2001. The single pore residue Asp542 determines Ca²⁺ permeation and Mg²⁺ block of the epithelial Ca²⁺ channel. *J Biol Chem* 276, 1020–5. [PubMed: 11035011]
- Oddsson A, Sulem P, Helgason H, Edvardsson VO, Thorleifsson G, Sveinbjornsson G, Haraldsdottir E, Eyjolfsson GI, Sigurdardottir O, Olafsson I, Masson G, Holm H, Gudbjartsson DF, Thorsteinsdottir U, Indridason OS, Palsson R and Stefansson K, 2015. Common and rare variants associated with kidney stones and biochemical traits. *Nat Commun* 6, 7975. [PubMed: 26272126]
- Ohata Y, Ozono K and Michigami T, 2016. Current concepts in perinatal mineral metabolism. *Clinical pediatric endocrinology : case reports and clinical investigations : official journal of the Japanese Society for Pediatric Endocrinology* 25, 9–17.
- Owsianik G, Talavera K, Voets T and Nilius B, 2006. Permeation and selectivity of TRP channels. *Annu Rev Physiol* 68, 685–717. [PubMed: 16460288]
- Oz OK, Hajibeigi A, Howard K, Cummins CL, van Abel M, Bindels RJ, Word RA, Kuro-o M, Pak CY and Zerwekh JE, 2007. Aromatase deficiency causes altered expression of molecules critical for calcium reabsorption in the kidneys of female mice *. *J Bone Miner Res* 22, 1893–902. [PubMed: 17708714]
- Palmada M, Poppendieck S, Embark HM, van de Graaf SF, Boehmer C, Bindels RJ and Lang F, 2005. Requirement of PDZ domains for the stimulation of the epithelial Ca²⁺ channel TRPV5 by the NHE regulating factor NHERF2 and the serum and glucocorticoid inducible kinase SGK1. *Cell Physiol Biochem* 15, 175–82. [PubMed: 15665527]

- Pan Z, Yang H and Reinach PS, 2011. Transient receptor potential (TRP) gene superfamily encoding cation channels. *Hum Genomics* 5, 108–16. [PubMed: 21296744]
- Park SY, Yoo YM, Jung EM and Jeung EB, 2020. The effect of steroid hormone on the expression of the calcium-processing proteins in the immature female rat brain. *J Chem Neuroanat* 105, 101767. [PubMed: 32061997]
- Payandeh J, Scheuer T, Zheng N and Catterall WA, 2011. The crystal structure of a voltage-gated sodium channel. *Nature* 475, 353–8. [PubMed: 21743477]
- Peleg S, Sellin JH, Wang Y, Freeman MR and Umar S, 2010. Suppression of aberrant transient receptor potential cation channel, subfamily V, member 6 expression in hyperproliferative colonic crypts by dietary calcium. *Am J Physiol Gastrointest Liver Physiol* 299, G593–601. [PubMed: 20508153]
- Peng JB, 2011. TRPV5 and TRPV6 in transcellular Ca(2+) transport: regulation, gene duplication, and polymorphisms in African populations. *Adv Exp Med Biol* 704, 239–75. [PubMed: 21290300]
- Peng JB, Brown EM and Hediger MA, 2001a. Structural conservation of the genes encoding CaT1, CaT2, and related cation channels. *Genomics* 76, 99–109. [PubMed: 11549322]
- Peng JB, Brown EM and Hediger MA, 2003. Epithelial Ca²⁺ entry channels: transcellular Ca²⁺ transport and beyond. *J Physiol* 551, 729–40. [PubMed: 12869611]
- Peng JB, Chen XZ, Berger UV, Vassilev PM, Tsukaguchi H, Brown EM and Hediger MA, 1999. Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. *J Biol Chem* 274, 22739–46. [PubMed: 10428857]
- Peng JB, Chen XZ, Berger UV, Weremowicz S, Morton CC, Vassilev PM, Brown EM and Hediger MA, 2000. Human calcium transport protein CaT1. *Biochem Biophys Res Commun* 278, 326–32. [PubMed: 11097838]
- Peng JB, Suzuki Y, Gyimesi G and Hediger MA, 2018. TRPV5 and TRPV6 Calcium-Selective Channels, in: Kozak JA and Putney JW Jr. (Eds.), *Calcium Entry Channels in Non-Excitable Cells*. Boca Raton (FL), pp. 241–274.
- Peng JB, Zhuang L, Berger UV, Adam RM, Williams BJ, Brown EM, Hediger MA and Freeman MR, 2001b. CaT1 expression correlates with tumor grade in prostate cancer. *Biochem Biophys Res Commun* 282, 729–34. [PubMed: 11401523]
- Perrouin-Verbe MA, Schoentgen N, Talagas M, Garlantezec R, Uguen A, Doucet L, Rosec S, Marcocelles P, Potier-Cartereau M, Vandier C, Ferec C, Fromont G, Fournier G, Valeri A and Mignen O, 2019. Overexpression of certain transient receptor potential and Orai channels in prostate cancer is associated with decreased risk of systemic recurrence after radical prostatectomy. *Prostate* 79, 1793–1804. [PubMed: 31475744]
- Peters AA, Simpson PT, Bassett JJ, Lee JM, Da Silva L, Reid LE, Song S, Parat MO, Lakhani SR, Kenny PA, Roberts-Thomson SJ and Monteith GR, 2012. Calcium channel TRPV6 as a potential therapeutic target in estrogen receptor-negative breast cancer. *Mol Cancer Ther* 11, 2158–68. [PubMed: 22807578]
- Phelps CB, Huang RJ, Lishko PV, Wang RR and Gaudet R, 2008. Structural analyses of the ankyrin repeat domain of TRPV6 and related TRPV ion channels. *Biochemistry* 47, 2476–84. [PubMed: 18232717]
- Pigozzi D, Ducret T, Tajeddine N, Gala JL, Tombal B and Gailly P, 2006. Calcium store contents control the expression of TRPC1, TRPC3 and TRPV6 proteins in LNCaP prostate cancer cell line. *Cell Calcium* 39, 401–15. [PubMed: 16529812]
- Pike JW and Christakos S, 2017. Biology and Mechanisms of Action of the Vitamin D Hormone. *Endocrinol Metab Clin North Am* 46, 815–843. [PubMed: 29080638]
- Pike JW, Meyer MB, Watanuki M, Kim S, Zella LA, Fretz JA, Yamazaki M and Shevde NK, 2007a. Perspectives on mechanisms of gene regulation by 1,25-dihydroxyvitamin D₃ and its receptor. *J Steroid Biochem Mol Biol* 103, 389–95. [PubMed: 17223545]
- Pike JW, Zella LA, Meyer MB, Fretz JA and Kim S, 2007b. Molecular actions of 1,25-dihydroxyvitamin D₃ on genes involved in calcium homeostasis. *J Bone Miner Res* 22 Suppl 2, V16–9. [PubMed: 18290714]

- Qiu A and Hogstrand C, 2004. Functional characterisation and genomic analysis of an epithelial calcium channel (ECaC) from pufferfish, *Fugu rubripes*. *Gene* 342, 113–23. [PubMed: 15527971]
- Rahman MS, Kwon W-S and Pang M, 2014. Calcium Influx and Male Fertility in the Context of the Sperm Proteome: An Update. *Journal of Biomedicine and Biotechnology* 2014.
- Raphael M, Lehen'kyi V, Vandenberghe M, Beck B, Khalimonchik S, Vanden Abeele F, Farsetti L, Germain E, Bokhobza A, Mihalache A, Gosset P, Romanin C, Clezardin P, Skryma R and Prevarskaya N, 2014. TRPV6 calcium channel translocates to the plasma membrane via Orai1-mediated mechanism and controls cancer cell survival. *Proc Natl Acad Sci U S A* 111, E3870–9. [PubMed: 25172921]
- Replogle RA, Li Q, Wang L, Zhang M and Fleet JC, 2014. Gene-by-diet interactions influence calcium absorption and bone density in mice. *J Bone Miner Res* 29, 657–65. [PubMed: 23955923]
- Rourke KM, Coe S, Kohn CW, Rosol TJ, Mendoza FJ and Toribio RE, 2010. Cloning, comparative sequence analysis and mRNA expression of calcium-transporting genes in horses. *Gen Comp Endocrinol* 167, 6–10. [PubMed: 20226785]
- Rual JF, Venkatesan K, Hao T, Hirozane-Kishikawa T, Dricot A, Li N, Berriz GF, Gibbons FD, Dreze M, Ayivi-Guedehoussou N, Klitgord N, Simon C, Boxem M, Milstein S, Rosenberg J, Goldberg DS, Zhang LV, Wong SL, Franklin G, Li S, Albala JS, Lim J, Fraughton C, Llamas E, Cevik S, Bex C, Lamesch P, Sikorski RS, Vandenhaute J, Zoghbi HY, Smolyar A, Bosak S, Sequerra R, Doucette-Stamm L, Cusick ME, Hill DE, Roth FP and Vidal M, 2005. Towards a proteome-scale map of the human protein-protein interaction network. *Nature* 437, 1173–8. [PubMed: 16189514]
- Saito H and Harada S, 2014. Eldecalcitol replaces endogenous calcitriol but does not fully compensate for its action in vivo. *J Steroid Biochem Mol Biol* 144 Pt A, 189–96. [PubMed: 24291401]
- Sakipov S, Sobolevsky AI and Kurnikova MG, 2018. Ion Permeation Mechanism in Epithelial Calcium Channel TRPV6. *Sci Rep* 8, 5715. [PubMed: 29632318]
- Saotome K, Singh AK and Sobolevsky AI, 2018. Determining the Crystal Structure of TRPV6, in: Kozak JA and Putney JW Jr. (Eds.), *Calcium Entry Channels in Non-Excitable Cells*. Boca Raton (FL), pp. 275–292.
- Saotome K, Singh AK, Yelshanskaya MV and Sobolevsky AI, 2016. Crystal structure of the epithelial calcium channel TRPV6. *Nature* 534, 506–11. [PubMed: 27296226]
- Sato T, Yamamoto H, Sawada N, Nashiki K, Tsuji M, Nikawa T, Arai H, Morita K, Taketani Y and Takeda E, 2006. Immobilization decreases duodenal calcium absorption through a 1,25-dihydroxyvitamin D-dependent pathway. *J Bone Miner Metab* 24, 291–9. [PubMed: 16816923]
- Schindl R, Fritsch R, Jardin I, Frischauf I, Kahr H, Muik M, Riedl MC, Groschner K and Romanin C, 2012. Canonical transient receptor potential (TRPC) 1 acts as a negative regulator for vanilloid TRPV6-mediated Ca²⁺ influx. *J Biol Chem* 287, 35612–20. [PubMed: 22932896]
- Schoeber JP, Topala CN, Lee KP, Lambers TT, Ricard G, van der Kemp AW, Huynen MA, Hoenderop JG and Bindels RJ, 2008. Identification of Nipsnap1 as a novel auxiliary protein inhibiting TRPV6 activity. *Pflugers Arch* 457, 91–101. [PubMed: 18392847]
- Schoeber JP, Topala CN, Wang X, Diepens RJ, Lambers TT, Hoenderop JG and Bindels RJ, 2006. RGS2 inhibits the epithelial Ca²⁺ channel TRPV6. *J Biol Chem* 281, 29669–74. [PubMed: 16895908]
- Schroder B, Wilkens MR, Ricken GE, Leonhard-Marek S, Fraser DR and Breves G, 2015. Calcium transport in bovine rumen epithelium as affected by luminal Ca concentrations and Ca sources. *Physiol Rep* 3.
- Schwarz EC, Wissenbach U, Niemeyer BA, Strauss B, Philipp SE, Flockerzi V and Hoth M, 2006. TRPV6 potentiates calcium-dependent cell proliferation. *Cell Calcium* 39, 163–73. [PubMed: 16356545]
- Semenova SB, Vassilieva IO, Fomina AF, Runov AL and Negulyaev YA, 2009. Endogenous expression of TRPV5 and TRPV6 calcium channels in human leukemia K562 cells. *Am J Physiol Cell Physiol* 296, C1098–104. [PubMed: 19295174]
- Shaker JL and Deftos L, 2000. Calcium and Phosphate Homeostasis, in: Feingold KR, Anawalt B, Boyce A, Chrousos G, Dungan K, Grossman A, Hershman JM, Kaltsas G, Koch C, Kopp P, Korbonits M, McLachlan R, Morley JE, New M, Perreault L, Purnell J, Rebar R, Singer F,

- Trence DL, Vinik A and Wilson DP (Eds.), Endotext. [MDText.com](https://www.mdtext.com/), Inc., South Dartmouth (MA).
- Sharma D, Shastri S and Sharma P, 2016. Intrauterine Growth Restriction: Antenatal and Postnatal Aspects. *Clinical medicine insights. Pediatrics* 10, 67–83. [PubMed: 27441006]
- Shin Y-C, Shin S-Y, So I, Kwon D and Jeon J-H, 2010. TRIP Database: a manually curated database of protein–protein interactions for mammalian TRP channels. *Nucleic Acids Research* 39, D356–D361. [PubMed: 20851834]
- Simonin C, Awale M, Brand M, van Deursen R, Schwartz J, Fine M, Kovacs G, Hafliger P, Gyimesi G, Sithampari A, Charles RP, Hediger MA and Reymond JL, 2015. Optimization of TRPV6 Calcium Channel Inhibitors Using a 3D Ligand-Based Virtual Screening Method. *Angew Chem Int Ed Engl* 54, 14748–52. [PubMed: 26457814]
- Singh AK, McGoldrick LL, Twomey EC and Sobolevsky AI, 2018a. Mechanism of calmodulin inactivation of the calcium-selective TRP channel TRPV6. *Sci Adv* 4, eaau6088. [PubMed: 30116787]
- Singh AK, Saotome K, McGoldrick LL and Sobolevsky AI, 2018b. Structural bases of TRP channel TRPV6 allosteric modulation by 2-APB. *Nat Commun* 9, 2465. [PubMed: 29941865]
- Singh AK, Saotome K and Sobolevsky AI, 2017. Swapping of transmembrane domains in the epithelial calcium channel TRPV6. *Sci Rep* 7, 10669. [PubMed: 28878326]
- Skrzypski M, Kolodziejewski PA, Mergler S, Khajavi N, Nowak KW and Strowski MZ, 2016. TRPV6 modulates proliferation of human pancreatic neuroendocrine BON-1 tumour cells. *Biosci Rep* 36.
- So CL, Milevskiy MJG and Monteith GR, 2020a. Transient receptor potential cation channel subfamily V and breast cancer. *Lab Invest* 100, 199–206. [PubMed: 31822791]
- Soejima M, Tachida H and Koda Y, 2009. Sequence analysis of human TRPV6 suggests positive selection outside Africa. *Biochem Genet* 47, 147–53. [PubMed: 19169858]
- Song H, Dong M, Zhou J, Sheng W, Li X and Gao W, 2018. Expression and prognostic significance of TRPV6 in the development and progression of pancreatic cancer. *Oncol Rep* 39, 1432–1440. [PubMed: 29344675]
- Song T, Ma J, Guo L, Yang P, Zhou X and Ye T, 2017. Regulation of chondrocyte functions by transient receptor potential cation channel V6 in osteoarthritis. *J Cell Physiol* 232, 3170–3181. [PubMed: 28063212]
- Song Y and Fleet JC, 2004. 1,25 dihydroxycholecalciferol-mediated calcium absorption and gene expression are higher in female than in male mice. *J Nutr* 134, 1857–61. [PubMed: 15284366]
- Song Y, Kato S and Fleet JC, 2003a. Vitamin D receptor (VDR) knockout mice reveal VDR-independent regulation of intestinal calcium absorption and ECaC2 and calbindin D9k mRNA. *J Nutr* 133, 374–80. [PubMed: 12566470]
- Song Y, Peng X, Porta A, Takanaga H, Peng JB, Hediger MA, Fleet JC and Christakos S, 2003b. Calcium transporter 1 and epithelial calcium channel messenger ribonucleic acid are differentially regulated by 1,25 dihydroxyvitamin D3 in the intestine and kidney of mice. *Endocrinology* 144, 3885–94. [PubMed: 12933662]
- Sopjani M, Kunert A, Czarkowski K, Klaus F, Laufer J, Foller M and Lang F, 2010. Regulation of the Ca(2+) channel TRPV6 by the kinases SGK1, PKB/Akt, and PIKfyve. *J Membr Biol* 233, 35–41. [PubMed: 20041238]
- Sprekeler N, Kowalewski MP and Boos A, 2012. TRPV6 and Calbindin-D9k-expression and localization in the bovine uterus and placenta during pregnancy. *Reprod Biol Endocrinol* 10, 66. [PubMed: 22931437]
- Stajich JE and Hahn MW, 2005. Disentangling the effects of demography and selection in human history. *Mol Biol Evol* 22, 63–73. [PubMed: 15356276]
- Steinberg X, Lespay-Rebolledo C and Brauchi S, 2014. A structural view of ligand-dependent activation in thermoTRP channels. *Frontiers in Physiology* 5.
- Sternfeld L, Anderie I, Schmid A, Al-Shaldi H, Krause E, Magg T, Schreiner D, Hofer HW and Schulz I, 2007. Identification of tyrosines in the putative regulatory site of the Ca²⁺ channel TRPV6. *Cell Calcium* 42, 91–102. [PubMed: 17197020]

- Sternfeld L, Krause E, Schmid A, Anderie I, Latas A, Al-Shaldi H, Kohl A, Evers K, Hofer HW and Schulz I, 2005. Tyrosine phosphatase PTP1B interacts with TRPV6 in vivo and plays a role in TRPV6-mediated calcium influx in HEK293 cells. *Cell Signal* 17, 951–60. [PubMed: 15894168]
- Stewart JM, 2020. TRPV6 as A Target for Cancer Therapy. *J Cancer* 11, 374–387. [PubMed: 31897233]
- Stulc J, 1997. Placental transfer of inorganic ions and water. *Physiol Rev* 77, 805–36. [PubMed: 9234966]
- Stumpf T, Zhang Q, Hirnet D, Lewandrowski U, Sickmann A, Wissenbach U, Dorr J, Lohr C, Deitmer JW and Fecher-Trost C, 2008. The human TRPV6 channel protein is associated with cyclophilin B in human placenta. *J Biol Chem* 283, 18086–98. [PubMed: 18445599]
- Sun F, Xiao L, Jang XX, Xiong Y, Li Q, Yue XJ, Wei YJ, Wei YX, Ma YL and Yu YH, 2016. TRPV6 is a prognostic marker in early-stage cervical squamous cell carcinoma. *Tumour Biol*.
- Sunycz JA, 2008. The use of calcium and vitamin D in the management of osteoporosis. *Therapeutics and clinical risk management* 4, 827–836. [PubMed: 19209265]
- Suzuki M, Ishibashi K, Ooki G, Tsuruoka S and Imai M, 2000. Electrophysiologic characteristics of the Ca-permeable channels, ECaC and CaT, in the kidney. *Biochem Biophys Res Commun* 274, 344–9. [PubMed: 10913341]
- Suzuki Y, C.D., Sawada H, et al. , 2018. TRPV6 variants interfere with maternal-fetal calcium transport through the placenta and cause transient neonatal hyperparathyroidism. *Am. J. Hum. Genet* 102, 1104–1114. [PubMed: 29861107]
- Suzuki Y, Kovacs CS, Takanaga H, Peng JB, Landowski CP and Hediger MA, 2008a. Calcium channel TRPV6 is involved in murine maternal-fetal calcium transport. *J Bone Miner Res* 23, 1249–56. [PubMed: 18348695]
- Suzuki Y, Landowski CP and Hediger MA, 2008b. Mechanisms and regulation of epithelial Ca²⁺ absorption in health and disease. *Annu Rev Physiol* 70, 257–71. [PubMed: 17850211]
- Suzuki Y, Pasch A, Bonny O, Mohaupt MG, Hediger MA and Frey FJ, 2008d. Gain-of-function haplotype in the epithelial calcium channel TRPV6 is a risk factor for renal calcium stone formation. *Hum Mol Genet* 17, 1613–8. [PubMed: 18276610]
- Suzuki Y, Sawada H, Tokumasu T, Suzuki S, Ninomiya S, Shirai M, Mukai T, Saito CT, Nishimura G and Tominaga M, 2020. Novel TRPV6 mutations in the spectrum of transient neonatal hyperparathyroidism. *J Physiol Sci* 70, 33. [PubMed: 32646367]
- Tai V, Leung W, Grey A, Reid IR and Bolland MJ, 2015. Calcium intake and bone mineral density: systematic review and meta-analysis. *BMJ (Clinical research ed.)* 351, h4183–h4183.
- Takumida M, Ishibashi T, Hamamoto T, Hirakawa K and Anniko M, 2009. Age-dependent changes in the expression of klotho protein, TRPV5 and TRPV6 in mouse inner ear. *Acta Otolaryngol* 129, 1340–50. [PubMed: 19922080]
- Taparia S, Fleet JC, Peng JB, Wang XD and Wood RJ, 2006. 1,25-Dihydroxyvitamin D and 25-hydroxyvitamin D--mediated regulation of TRPV6 (a putative epithelial calcium channel) mRNA expression in Caco-2 cells. *Eur J Nutr* 45, 196–204. [PubMed: 16362534]
- Tawfik D, Zaccagnino A, Bernt A, Szczepanowski M, Klapper W, Schwab A, Kalthoff H and Trauzold A, 2020. The A818-6 system as an in-vitro model for studying the role of the transportome in pancreatic cancer. *BMC Cancer* 20, 264. [PubMed: 32228510]
- Teerapornpuntakit J, Dorkkam N, Wongdee K, Krishnamra N and Charoenphandhu N, 2009. Endurance swimming stimulates transepithelial calcium transport and alters the expression of genes related to calcium absorption in the intestine of rats. *Am J Physiol Endocrinol Metab* 296, E775–86. [PubMed: 19176351]
- Thyagarajan B, Benn BS, Christakos S and Rohacs T, 2009. Phospholipase C-mediated regulation of transient receptor potential vanilloid 6 channels: implications in active intestinal Ca²⁺ transport. *Mol Pharmacol* 75, 608–16. [PubMed: 19073818]
- Timmermans S, Souffriau J and Libert C, 2019. A General Introduction to Glucocorticoid Biology. *Front Immunol* 10, 1545. [PubMed: 31333672]
- Tomilin VN, Cherezova AL, Negulyaev YA and Semenova SB, 2016. TRPV5/V6 Channels Mediate Ca(2+) Influx in Jurkat T Cells Under the Control of Extracellular pH. *J Cell Biochem* 117, 197–206. [PubMed: 26096460]

- Tran DN, Jung EM, Ahn C, Lee JH, Yoo YM and Jeung EB, 2018. Effects of Bisphenol A and 4-tert-Octylphenol on Embryo Implantation Failure in Mouse. *Int J Environ Res Public Health* 15.
- Turner TT, 2002. Necessity's Potion: Inorganic Ions and Small Organic Molecules in the Epididymal Lumen., in: Robaire B, H.B.T. (Ed.), *The Epididymis: From Molecules to Clinical Practice*. Springer, Boston, MA, pp. 131–150.
- Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigartyo CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J and Pontén F, 2015. Proteomics. Tissue-based map of the human proteome. *Science* 347, 1260419. [PubMed: 25613900]
- Vachel L, Norez C, Jayle C, Becq F and Vandebrouck C, 2015. The low PLC-delta 1 expression in cystic fibrosis bronchial epithelial cells induces upregulation of TRPV6 channel activity. *Cell Calcium* 57, 38–48. [PubMed: 25477137]
- van Abel M, Huybers S, Hoenderop JG, van der Kemp AW, van Leeuwen JP and Bindels RJ, 2006. Age-dependent alterations in Ca²⁺ homeostasis: role of TRPV5 and TRPV6. *Am J Physiol Renal Physiol* 291, F1177–83. [PubMed: 16705151]
- Van Cromphaut SJ, Dewerchin M, Hoenderop JG, Stockmans I, Van Herck E, Kato S, Bindels RJ, Collen D, Carmeliet P, Bouillon R and Carmeliet G, 2001. Duodenal calcium absorption in vitamin D receptor-knockout mice: functional and molecular aspects. *Proc Natl Acad Sci U S A* 98, 13324–9. [PubMed: 11687634]
- Van Cromphaut SJ, Rummens K, Stockmans I, Van Herck E, Dijcks FA, Ederveen AG, Carmeliet P, Verhaeghe J, Bouillon R and Carmeliet G, 2003. Intestinal calcium transporter genes are upregulated by estrogens and the reproductive cycle through vitamin D receptor-independent mechanisms. *J Bone Miner Res* 18, 1725–36. [PubMed: 14584880]
- Van Cromphaut SJ, Stockmans I, Torrekens S, Van Herck E, Carmeliet G and Bouillon R, 2007. Duodenal calcium absorption in dexamethasone-treated mice: functional and molecular aspects. *Arch Biochem Biophys* 460, 300–5. [PubMed: 17224125]
- van de Graaf SF, Boullart I, Hoenderop JG and Bindels RJ, 2004. Regulation of the epithelial Ca²⁺ channels TRPV5 and TRPV6 by 1alpha,25-dihydroxy Vitamin D3 and dietary Ca²⁺. *J Steroid Biochem Mol Biol* 89-90, 303–8. [PubMed: 15225790]
- van de Graaf SF, Chang Q, Mensenkamp AR, Hoenderop JG and Bindels RJ, 2006a. Direct interaction with Rab11a targets the epithelial Ca²⁺ channels TRPV5 and TRPV6 to the plasma membrane. *Mol Cell Biol* 26, 303–12. [PubMed: 16354700]
- van de Graaf SF, Hoenderop JG and Bindels RJ, 2006b. Regulation of TRPV5 and TRPV6 by associated proteins. *Am J Physiol Renal Physiol* 290, F1295–302. [PubMed: 16682485]
- van de Graaf SF, Hoenderop JG, Gkika D, Lamers D, Prenen J, Rescher U, Gerke V, Staub O, Nilius B and Bindels RJ, 2003. Functional expression of the epithelial Ca(2+) channels (TRPV5 and TRPV6) requires association of the S100A10-annexin 2 complex. *EMBO J* 22, 1478–87. [PubMed: 12660155]
- van de Graaf SF, Hoenderop JG, van der Kemp AW, Gisler SM and Bindels RJ, 2006c. Interaction of the epithelial Ca²⁺ channels TRPV5 and TRPV6 with the intestine- and kidney-enriched PDZ protein NHERF4. *Pflugers Arch* 452, 407–17. [PubMed: 16565876]
- van de Graaf SF, van der Kemp AW, van den Berg D, van Oorschot M, Hoenderop JG and Bindels RJ, 2006d. Identification of BSPRY as a novel auxiliary protein inhibiting TRPV5 activity. *J Am Soc Nephrol* 17, 26–30. [PubMed: 16380433]
- van der Eerden BC, Weissgerber P, Fratzl-Zelman N, Olausson J, Hoenderop JG, Schreuders-Koedam M, Eijken M, Roschger P, de Vries TJ, Chiba H, Klaushofer K, Flockerzi V, Bindels RJ, Freichel M and van Leeuwen JP, 2012. The transient receptor potential channel TRPV6 is dynamically expressed in bone cells but is not crucial for bone mineralization in mice. *J Cell Physiol* 227, 1951–9. [PubMed: 21732366]
- van der Eerden BCJ, Hoenderop JGJ, de Vries TJ, Schoenmaker T, Buurman CJ, Uitterlinden AG, Pols HAP, Bindels RJM and van Leeuwen JPTM, 2005. The epithelial Ca²⁺ channel TRPV5

- is essential for proper osteoclastic bone resorption. *Proceedings of the National Academy of Sciences of the United States of America* 102, 17507–17512. [PubMed: 16291808]
- van Goor MKC, Hoenderop JGJ and van der Wijst J, 2017. TRP channels in calcium homeostasis: from hormonal control to structure-function relationship of TRPV5 and TRPV6. *Biochim Biophys Acta Mol Cell Res* 1864, 883–893. [PubMed: 27913205]
- Vanden Abeele F, Roudbaraki M, Shuba Y, Skryma R and Prevarskaya N, 2003. Store-operated Ca²⁺ current in prostate cancer epithelial cells. Role of endogenous Ca²⁺ transporter type 1. *J Biol Chem* 278, 15381–9. [PubMed: 12584203]
- Vanoevelen J, Janssens A, Huitema LF, Hammond CL, Metz JR, Flik G, Voets T and Schulte-Merker S, 2011. Trpv5/6 is vital for epithelial calcium uptake and bone formation. *Faseb j* 25, 3197–207. [PubMed: 21670068]
- Vasil'eva IO, Neguliaeva Iu A, Marakhova II and Semenova SB, 2008. [TRPV5 and TRPV6 calcium channels in human T cells]. *Tsitologiya* 50, 953–7. [PubMed: 19140341]
- Vassilieva IO, Tomilin VN, Marakhova II, Shatrova AN, Negulyaev YA and Semenova SB, 2013. Expression of transient receptor potential vanilloid channels TRPV5 and TRPV6 in human blood lymphocytes and Jurkat leukemia T cells. *J Membr Biol* 246, 131–40. [PubMed: 23111462]
- Veldurthy V, Wei R, Oz L, Dhawan P, Jeon YH and Christakos S, 2016. Vitamin D, calcium homeostasis and aging. *Bone research* 4, 16041–16041. [PubMed: 27790378]
- Velisetty P, Borbiro I, Kasimova MA, Liu L, Badheka D, Carnevale V and Rohacs T, 2016. A molecular determinant of phosphoinositide affinity in mammalian TRPV channels. *Sci Rep* 6, 27652. [PubMed: 27291418]
- Venkatachalam K and Montell C, 2007. TRP channels. *Annu Rev Biochem* 76, 387–417. [PubMed: 17579562]
- Voets T, Janssens A, Droogmans G and Nilius B, 2004. Outer pore architecture of a Ca²⁺-selective TRP channel. *J Biol Chem* 279, 15223–30. [PubMed: 14736889]
- Voets T, Janssens A, Prenen J, Droogmans G and Nilius B, 2003. Mg²⁺-dependent gating and strong inward rectification of the cation channel TRPV6. *J Gen Physiol* 121, 245–60. [PubMed: 12601087]
- Walters JR, Balesaria S, Chavele KM, Taylor V, Berry JL, Khair U, Barley NF, van Heel DA, Field J, Hayat JO, Bhattacharjee A, Jeffery R and Poulosom R, 2006. Calcium channel TRPV6 expression in human duodenum: different relationships to the vitamin D system and aging in men and women. *J Bone Miner Res* 21, 1770–7. [PubMed: 17002582]
- Walters JR, Balesaria S, Khair U, Sangha S, Banks L and Berry JL, 2007. The effects of Vitamin D metabolites on expression of genes for calcium transporters in human duodenum. *J Steroid Biochem Mol Biol* 103, 509–12. [PubMed: 17204416]
- Wang L, Holmes RP and Peng JB, 2017. The L530R variation associated with recurrent kidney stones impairs the structure and function of TRPV5. *Biochem Biophys Res Commun* 492, 362–367. [PubMed: 28847730]
- Wang L, Klopot A, Freund JN, Dowling LN, Krasinski SD and Fleet JC, 2004. Control of differentiation-induced calbindin-D9k gene expression in Caco-2 cells by cdx-2 and HNF-1alpha. *Am J Physiol Gastrointest Liver Physiol* 287, G943–53. [PubMed: 15217781]
- Wangemann P, Nakaya K, Wu T, Maganti RJ, Itza EM, Sanneman JD, Harbidge DG, Billings S and Marcus DC, 2007. Loss of cochlear HCO₃⁻ secretion causes deafness via endolymphatic acidification and inhibition of Ca²⁺ reabsorption in a Pendred syndrome mouse model. *Am J Physiol Renal Physiol* 292, F1345–53. [PubMed: 17299139]
- Weber K, Erben RG, Rump A and Adamski J, 2001. Gene structure and regulation of the murine epithelial calcium channels ECaC1 and 2. *Biochem Biophys Res Commun* 289, 1287–94. [PubMed: 11741335]
- Weissgerber P, Kriebs U, Tsvilovskyy V, Olausson J, Kretz O, Stoerger C, Mannebach S, Wissenbach U, Vennekens R, Middendorff R, Flockerzi V and Freichel M, 2012. Excision of Trpv6 gene leads to severe defects in epididymal Ca²⁺ absorption and male fertility much like single D541A pore mutation. *J Biol Chem* 287, 17930–41. [PubMed: 22427671]

- Weissgerber P, Kriebs U, Tsvilovskyy V, Olausson J, Kretz O, Stoerger C, Vennekens R, Wissenbach U, Middendorff R, Flockerzi V and Freichel M, 2011. Male fertility depends on Ca²⁺ absorption by TRPV6 in epididymal epithelia. *Sci Signal* 4, ra27. [PubMed: 21540454]
- Weldenogodguad M, Pokharel K, Ming Y, Honkatukia M, Peippo J, Reilas T, Roed KH and Kantanen J, 2020. Genome sequence and comparative analysis of reindeer (*Rangifer tarandus*) in northern Eurasia. *Sci Rep* 10, 8980. [PubMed: 32488117]
- Wilkens MR, Kunert-Keil C, Brinkmeier H and Schroder B, 2009. Expression of calcium channel TRPV6 in ovine epithelial tissue. *Vet J* 182, 294–300. [PubMed: 18701326]
- Wilson S, Qi J and Filipp FV, 2016. Refinement of the androgen response element based on ChIP-Seq in androgen-insensitive and androgen-responsive prostate cancer cell lines. *Sci Rep* 6, 32611. [PubMed: 27623747]
- Wissenbach U, Niemeyer B, Himmerkus N, Fixemer T, Bonkhoff H and Flockerzi V, 2004. TRPV6 and prostate cancer: cancer growth beyond the prostate correlates with increased TRPV6 Ca²⁺ channel expression. *Biochem Biophys Res Commun* 322, 1359–63. [PubMed: 15336984]
- Wissenbach U and Niemeyer BA, 2007. Trpv6. *Handb Exp Pharmacol*, 221–34. [PubMed: 17217060]
- Wissenbach U, Niemeyer BA, Fixemer T, Schneidewind A, Trost C, Cavalie A, Reus K, Meese E, Bonkhoff H and Flockerzi V, 2001. Expression of CaT-like, a novel calcium-selective channel, correlates with the malignancy of prostate cancer. *J Biol Chem* 276, 19461–8. [PubMed: 11278579]
- Woo JS, Kim DH, Allen PD and Lee EH, 2008. TRPC3-interacting triadic proteins in skeletal muscle. *Biochem J* 411, 399–405. [PubMed: 18215135]
- Wood RJ, Tchack L and Taparia S, 2001. 1,25-Dihydroxyvitamin D₃ increases the expression of the CaT1 epithelial calcium channel in the Caco-2 human intestinal cell line. *BMC Physiol* 1, 11. [PubMed: 11545681]
- Worcester EM and Coe FL, 2008. New insights into the pathogenesis of idiopathic hypercalciuria. *Seminars in nephrology* 28, 120–132. [PubMed: 18359393]
- Woudenberg-Vrenken TE, Lameris AL, Weissgerber P, Olausson J, Flockerzi V, Bindels RJ, Freichel M and Hoenderop JG, 2012. Functional TRPV6 channels are crucial for transepithelial Ca²⁺ absorption. *Am J Physiol Gastrointest Liver Physiol* 303, G879–85. [PubMed: 22878123]
- Wu G, Zhang W, Na T, Jing H, Wu H and Peng JB, 2012. Suppression of intestinal calcium entry channel TRPV6 by OCRL, a lipid phosphatase associated with Lowe syndrome and Dent disease. *Am J Physiol Cell Physiol* 302, C1479–91. [PubMed: 22378746]
- Wu Y, Miyamoto T, Li K, Nakagomi H, Sawada N, Kira S, Kobayashi H, Zakohji H, Tsuchida T, Fukazawa M, Araki I and Takeda M, 2011. Decreased expression of the epithelial Ca²⁺ channel TRPV5 and TRPV6 in human renal cell carcinoma associated with vitamin D receptor. *J Urol* 186, 2419–25. [PubMed: 22019165]
- Xin Y, Malick A, Hu M, Liu C, Batah H, Xu H and Duan C, 2019. Cell-autonomous regulation of epithelial cell quiescence by calcium channel Trpv6. *Elife* 8.
- Xu S, Zhang L, Cheng X, Yu H, Bao J and Lu R, 2018. Capsaicin inhibits the metastasis of human papillary thyroid carcinoma BCPAP cells through the modulation of the TRPV1 channel. *Food Funct* 9, 344–354. [PubMed: 29185571]
- Xue H, Wang Y, MacCormack TJ, Lutes T, Rice C, Davey M, Dugourd D, Ilenchuk TT and Stewart JM, 2018. Inhibition of Transient Receptor Potential Vanilloid 6 channel, elevated in human ovarian cancers, reduces tumour growth in a xenograft model. *J Cancer* 9, 3196–3207. [PubMed: 30210643]
- Yamashita S, Mizumoto H, Sawada H, Suzuki Y and Hata D, 2019. TRPV6 Gene Mutation in a Dizygous Twin With Transient Neonatal Hyperparathyroidism. *J Endocr Soc* 3, 602–606. [PubMed: 30820485]
- Yamauchi D, Nakaya K, Raveendran NN, Harbidge DG, Singh R, Wangemann P and Marcus DC, 2010. Expression of epithelial calcium transport system in rat cochlea and vestibular labyrinth. *BMC Physiol* 10, 1. [PubMed: 20113508]
- Yamauchi D, Raveendran NN, Pondugula SR, Kampalli SB, Sanneman JD, Harbidge DG and Marcus DC, 2005. Vitamin D upregulates expression of ECaC1 mRNA in semicircular canal. *Biochem Biophys Res Commun* 331, 1353–7. [PubMed: 15883024]

- Yang D and Kim J, 2020. Emerging role of transient receptor potential (TRP) channels in cancer progression. *BMB Rep* 53, 125–132. [PubMed: 32172727]
- Yang H, Ahn C and Jeung EB, 2015. Differential expression of calcium transport genes caused by COMT inhibition in the duodenum, kidney and placenta of pregnant mice. *Mol Cell Endocrinol* 401, 45–55. [PubMed: 25486511]
- Yang H, An BS, Choi KC and Jeung EB, 2013a. Change of genes in calcium transport channels caused by hypoxic stress in the placenta, duodenum, and kidney of pregnant rats. *Biol Reprod* 88, 30. [PubMed: 23255337]
- Yang H, Choi KC, Hyun SH and Jeung EB, 2011. Coexpression and estrogen-mediated regulation of TRPV6 and PMCA1 in the human endometrium during the menstrual cycle. *Mol Reprod Dev* 78, 274–82. [PubMed: 21400627]
- Yang H, Kim TH, An BS, Choi KC, Lee HH, Kim JM and Jeung EB, 2013b. Differential expression of calcium transport channels in placenta primary cells and tissues derived from preeclamptic placenta. *Mol Cell Endocrinol* 367, 21–30. [PubMed: 23267838]
- Yang JH, Zhao ZH, Hou JF, Zhou ZL, Deng YF and Dai JJ, 2013c. Expression of TRPV6 and CaBP-D28k in the egg shell gland (uterus) during the oviposition cycle of the laying hen. *Br Poult Sci* 54, 398–406. [PubMed: 23796121]
- Yang W, Lee HW, Hellinga H and Yang JJ, 2002. Structural analysis, identification, and design of calcium-binding sites in proteins. *Proteins* 47, 344–56. [PubMed: 11948788]
- Yelshanskaya MV, Nadezhdin KD, Kurnikova MG and Sobolevsky AI, 2020. Structure and function of the calcium-selective TRP channel TRPV6. *J Physiol*.
- Yue L, Peng JB, Hediger MA and Clapham DE, 2001. CaT1 manifests the pore properties of the calcium-release-activated calcium channel. *Nature* 410, 705–9. [PubMed: 11287959]
- Zakharian E, Cao C and Rohacs T, 2011. Intracellular ATP supports TRPV6 activity via lipid kinases and the generation of PtdIns(4,5) P(2). *FASEB J* 25, 3915–28. [PubMed: 21810903]
- Zhang SS, Xie X, Wen J, Luo KJ, Liu QW, Yang H, Hu Y and Fu JH, 2016a. TRPV6 plays a new role in predicting survival of patients with esophageal squamous cell carcinoma. *Diagn Pathol* 11, 14. [PubMed: 26818094]
- Zhang W, Na T and Peng JB, 2008. WNK3 positively regulates epithelial calcium channels TRPV5 and TRPV6 via a kinase-dependent pathway. *Am J Physiol Renal Physiol* 295, F1472–84. [PubMed: 18768590]
- Zhang W, Na T, Wu G, Jing H and Peng JB, 2010. Down-regulation of intestinal apical calcium entry channel TRPV6 by ubiquitin E3 ligase Nedd4-2. *J Biol Chem* 285, 36586–96. [PubMed: 20843805]
- Zhang X and Giovannucci E, 2011. Calcium, vitamin D and colorectal cancer chemoprevention. *Best Pract Res Clin Gastroenterol* 25, 485–94. [PubMed: 22122765]
- Zhang Y, Shao J, Wang Z, Yang T, Liu S, Liu Y, Fan X and Ye W, 2016b. Aqueous extract of pomegranate seed attenuates glucocorticoid-induced bone loss and hypercalciuria in mice: A comparative study with alendronate. *Int J Mol Med* 38, 491–8. [PubMed: 27278225]
- Zhao D, Han X, Huang L, Wang J, Zhang X, Jeon JH, Zhao Q and Dong JT, 2019. Transcription factor ZFH3 regulates calcium influx in mammary epithelial cells in part via the TRPV6 calcium channel. *Biochem Biophys Res Commun* 519, 366–371. [PubMed: 31519324]
- Zheng XE, Wang Z, Liao MZ, Lin YS, Shuhart MC, Schuetz EG and Thummel KE, 2012. Human PXR-mediated induction of intestinal CYP3A4 attenuates 1 α ,25-dihydroxyvitamin D(3) function in human colon adenocarcinoma LS180 cells. *Biochem Pharmacol* 84, 391–401. [PubMed: 22562045]
- Zhuang L, Peng JB, Tou L, Takanaga H, Adam RM, Hediger MA and Freeman MR, 2002. Calcium-selective ion channel, CaT1, is apically localized in gastrointestinal tract epithelia and is aberrantly expressed in human malignancies. *Lab Invest* 82, 1755–64. [PubMed: 12480925]
- Zou WB, Wang YC, Ren XL, Wang L, Deng SJ, Mao XT, Li ZS and Liao Z, 2020. TRPV6 variants confer susceptibility to chronic pancreatitis in the Chinese population. *Hum Mutat*.

Highlights

- TRPV6 is a vitamin D-regulated Ca^{2+} -selective channel required for Ca^{2+} homeostasis
- TRPV6 mediates the first step in the transcellular Ca^{2+} transport pathway
- TRPV6 is required for mammalian male fertility and maternal-fetal transport of Ca^{2+}
- Mutations compromising TRPV6 function cause transient neonatal hyperparathyroidism
- TRPV6 dysfunction is linked to chronic pancreatitis and cancer aggressiveness.

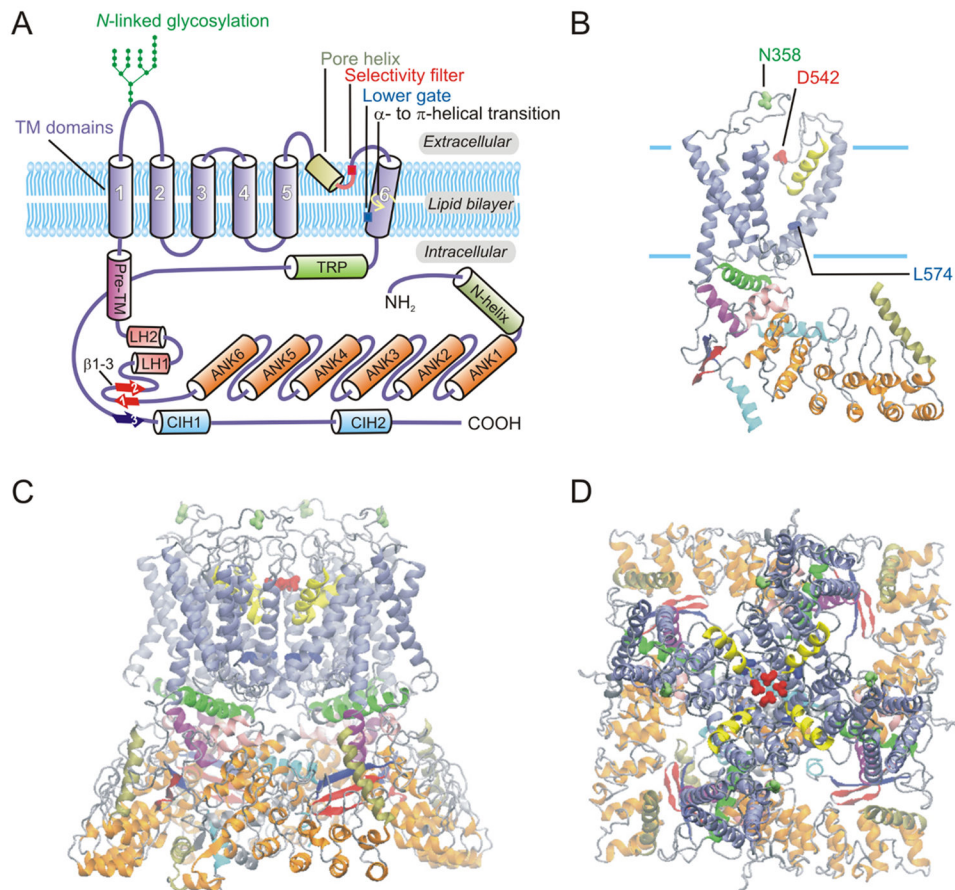
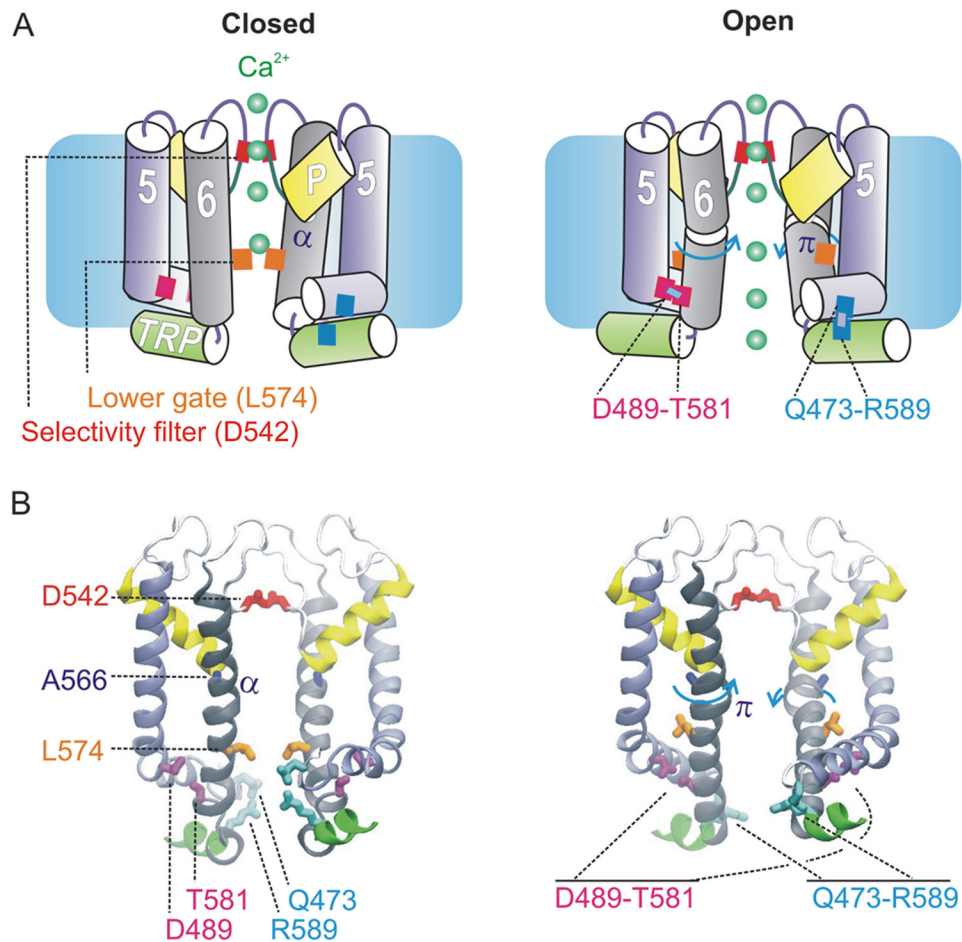


Figure 1.

Structure and domain organization of TRPV6. A. Domain organization of human TRPV6. TRPV6 monomer subunit contains the following secondary structure elements: an N-terminal helix (in light green), an ankyrin repeat domain with six ankyrin repeats (ANK1-6 in orange), a linker domain comprised of a β -hairpin (β 1 and β 2 in pink) and two linker helices (LH1 and LH2 in red), a pre-S1 helix (in magenta) connects the linker domain and the transmembrane (TM) domain that comprises of six TM helices (S1-S6 in silver-blue) and a pore helix (in yellow) connecting S5 and S6, an amphipathic TRP helix, a β -strand (β 3 in blue) that forms a β -sheet with β 1 and β 2, and two C-terminal interacting helices (CIH1 and CIH2 in cyan). The positions of the glycosylation site (N358), the key selective residue (D542) in the selective filter, the representative residue (L574) in the lower gate, and the site for α - to π -helical transition (A566) during the opening of the lower gate are also labeled. B. Structure of TRPV6 subunit. The two cyan lines represent the surfaces of the membrane. C and D. Side and top views of TRPV6 tetramer. Four subunits of TRPV6 arrange to form a four-fold symmetric channel. The structures shown in panels B, C, and D are based on the cryo-electron microscopy structure of human TRPV6 (PDB: 6E2F) with the colors of the structural domains matching those in panel A.

**Figure 2.**

Gating mechanism of TRPV6. The closed and open conformations of TRPV6 are represented by cartoon (A) and cryo-electron microscopy structural view (B). The gating of TRPV6 is achieved by the conformational change and electrostatic bond formation affecting the lower gate without affecting the conformation of the selectivity filter. In the closed conformation, residues L574 (a representative residue in the lower gate) form a constriction and hydrophobically seals the pore. In the open conformation, residues L574 rotate away from the ion permeation pathway and widen the pore size, allowing Ca²⁺ ions to pass through the lower gate. The opening of the lower gate is caused by an α - to the π -helical transition of the transmembrane helix S6 at residue A566, which induces the intracellular part of S6 bends by about 11° and rotates by about 100°. At the open state, a hydrogen bond between D489 (in S5) and T581 (in S6) and a salt bridge between Q473 (in S4-S5 elbow) and R589 (in TRP helix) are formed. These electrostatic interactions may offset the high energetic cost of unfavorable α -to- π helical transition.

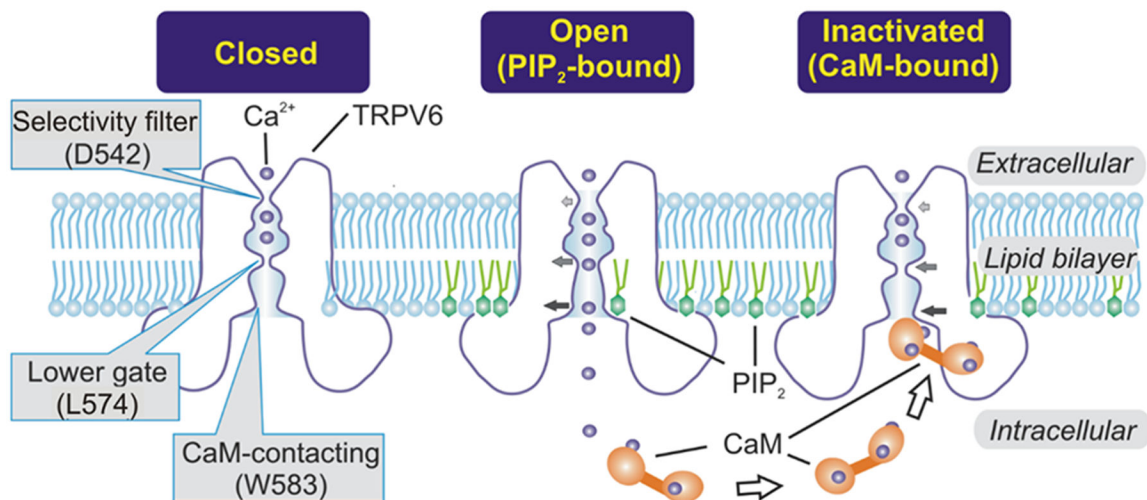
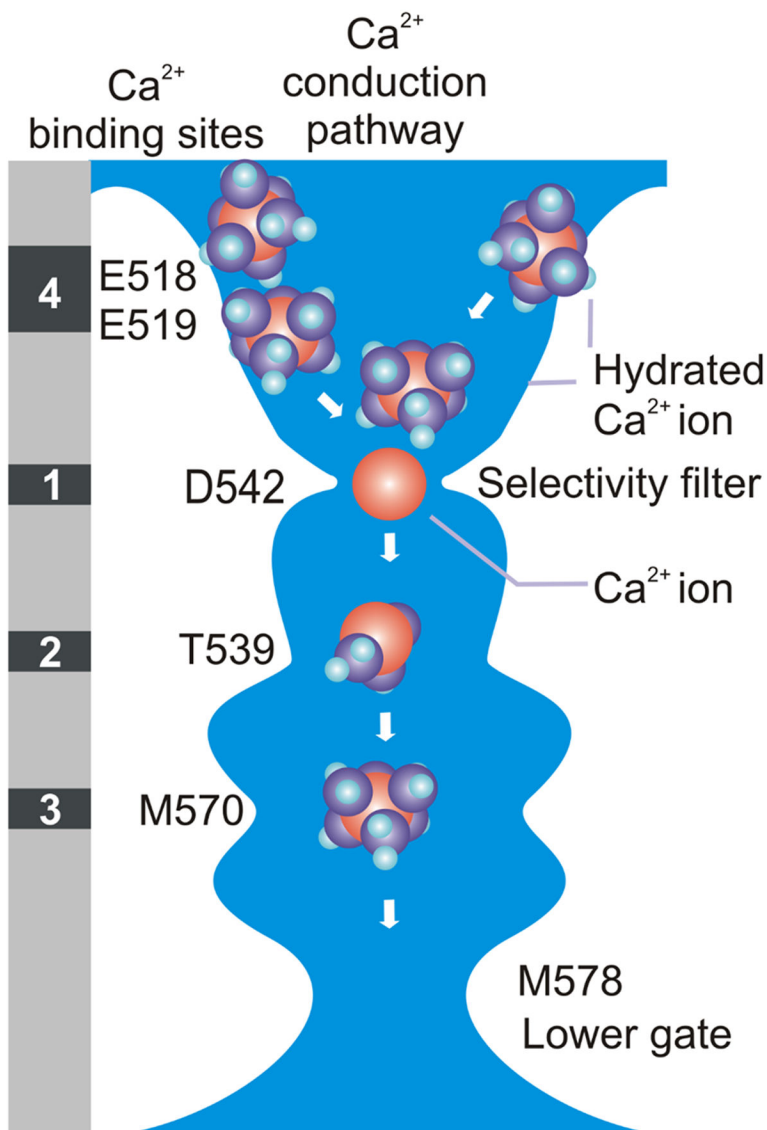


Figure 3.

Regulation of TRPV6 by endogenous modulators phosphatidylinositol 4,5-bisphosphate (PIP₂) and Calmodulin (CaM). The binding of PIP₂ induces the activation of TRPV6, whereas the binding of CaM causes the inactivation of the channel. In the closed conformation, the residues in the lower gate (represented by L574) form a constriction point and seal the pore. When PIP₂ binds to TRPV6, conformational changes occur. The changes include the upward motion of selective residue D542 and opening of the lower gate, which keeps TRPV6 active. When the intracellular [Ca²⁺] increases, extra Ca²⁺ ions bind to CaM. The Ca²⁺-CaM complex in turn seals the pore through the interaction between residues K155 in CaM and residues (such as W583) that form the intracellular orifice of the lower gate of TRPV6. During the activation and inactivation processes, the change of diameter in the ion conduction path is more pronounced at the lower gate and the intracellular opening than at the selective filter (indicated by the length of the arrows). This ensures the alteration in ion selectivity is minimum during activation/inactivation processes.

**Figure 4:**

Ca^{2+} ion conduction pathway of human TRPV6. There are four cation-binding sites proposed in the pore regions of TRPV6. The binding sites 1, 2, and 3 are formed by residues D542, T539, and M570, respectively. These three residues are corresponding to D541, T538, and M569 in rat TRPV6. The site 4 is formed by E518 and E519, which are corresponding to D517 and E518 in rat TRPV6. In the structure of rat TRPV6, D547 is also involved in forming the site 4. However, it is an asparagine residue in this position of human TRPV6, and thus the residue is not shown in this figure. Hydrated Ca^{2+} ions are recruited by site 4. Ca^{2+} ions bind to the selectivity filter (site 1) in dehydrated form, and they are knocked off by incoming Ca^{2+} ions. Partially rehydrated Ca^{2+} ions bind to site 2, and subsequently move down in fully hydrated form and interact with site 3. The lower gate represented by M578 (M577 in rat TRPV6) allows the permeation of hydrated Ca^{2+} ions in open state. Based on the results described in Saotome et al., 2016, Sakipov et al., 2018.

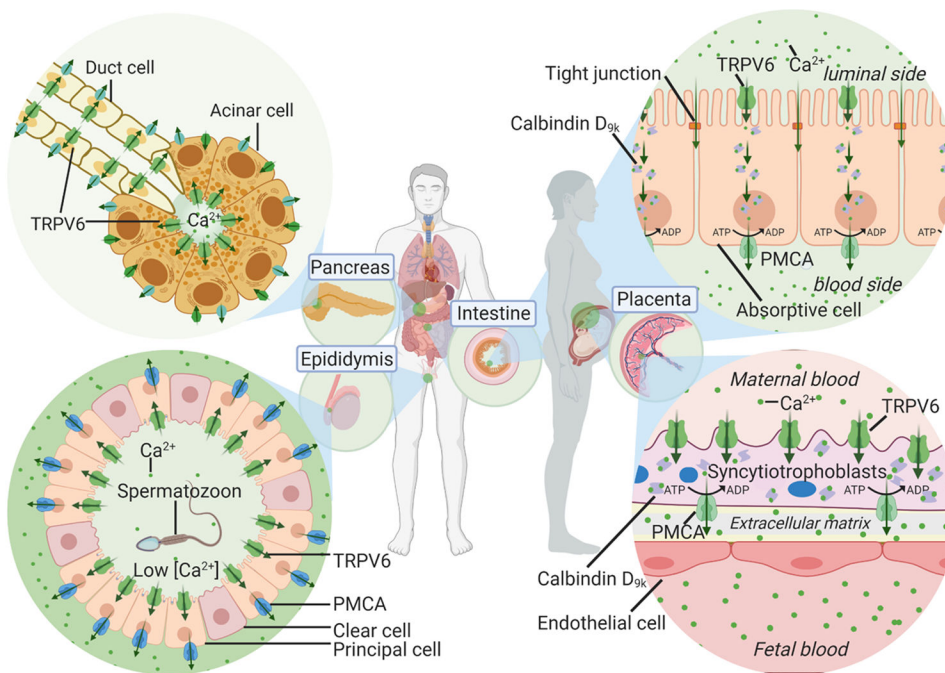


Figure 5.

Representative physiological roles of TRPV6. Firstly, TRPV6 mediates Ca^{2+} entry across the plasma membrane as the first step in the transcellular pathway of Ca^{2+} transport. This is considered the rate-limiting step in Ca^{2+} absorption by the duodenal enterocytes (upper inset) and maternal-fetal Ca^{2+} transport by placental syncytiotrophoblasts (lower inset). The transcellular pathway enables the transport of Ca^{2+} against a $[\text{Ca}^{2+}]$ gradient to ensure Ca^{2+} absorption when the luminal $[\text{Ca}^{2+}]$ is lower than that in the blood side; it also establishes a higher $[\text{Ca}^{2+}]$ in fetal blood relative to maternal blood to meet the demand of Ca^{2+} for fetal skeleton growth. The Ca^{2+} binding protein calbindin- D_{9k} and plasma membrane Ca^{2+} ATPase (PMCA) are known components in these transcellular pathways. Secondly, TRPV6 plays a role in the exocrine function. In the pancreas (upper left inset), TRPV6 is likely involved in reuptake of Ca^{2+} to replenish the Ca^{2+} store and secretory granules in acinar cells and in removing excessive Ca^{2+} by the duct cells to prevent premature activation of enzymes following the release of secretory cargo. Thirdly, TRPV6 plays a critical role in maintaining a low local $[\text{Ca}^{2+}]$. In Cauda epididymis (lower left inset), TRPV6-mediated uptake of Ca^{2+} by principal cells is required for lowering $[\text{Ca}^{2+}]$ in the intraluminal fluid to maintain the fertilization capacity of sperm. For simplicity, not all the components in these processes are labeled. Created with [BioRender.com](https://www.bio-render.com/).

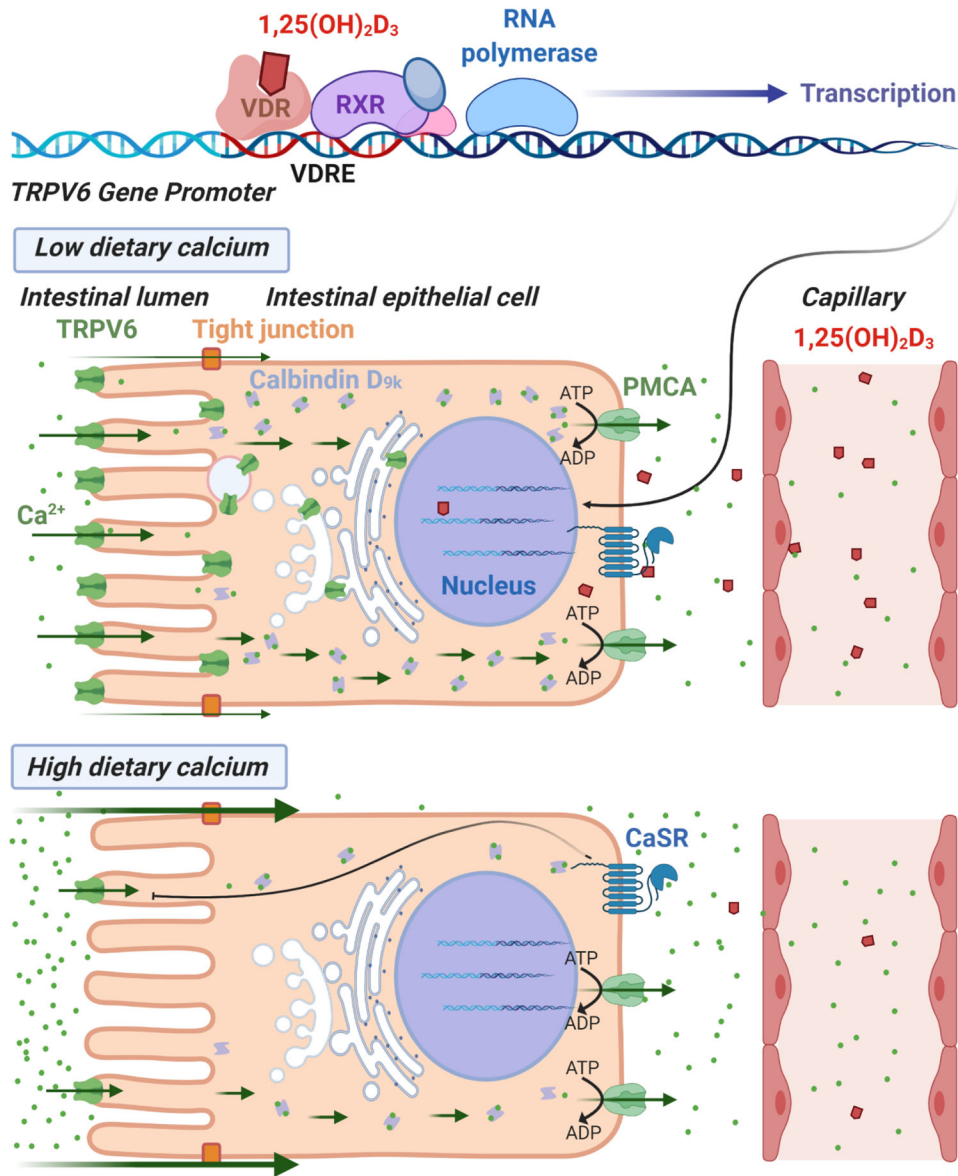


Figure 6. Regulation of TRPV6-mediated Ca²⁺ absorption by 1,25(OH)₂D₃ and dietary Ca²⁺. The transcellular pathway and paracellular pathway are two main routes of Ca²⁺ absorption operate in the intestine. The transcellular absorption of Ca²⁺ by enterocytes can be described in three main steps: 1) TRPV6-mediated entry of Ca²⁺ ions across the brush border membrane; 2) buffering of Ca²⁺ ions by calbindin-D_{9k} and diffusion of Ca²⁺ to the basolateral side as facilitated by calbindin-D_{9k}, and 3) basolateral exit mainly through the plasma membrane Ca²⁺ ATPase (PMCA). When luminal [Ca²⁺] is low (i.e., low dietary calcium, upper panel), the level of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) is elevated. The transcription of the *TRPV6* gene is then upregulated because of the presence of multiple vitamin D responsive elements (VDRE) in its promoter (only one VDRE is shown for simplicity). While PMCA transcription is relatively stable, calbindin-D_{9k} gene transcription is also upregulated following an increase in TRPV6-mediated Ca²⁺ influx.

When luminal $[Ca^{2+}]$ is high (i.e., high dietary calcium, lower panel), the $[Ca^{2+}]$ gradient favors paracellular transport, which becomes the predominant route of Ca^{2+} absorption. The level of $1,25(OH)_2D_3$ is reduced when the plasma $[Ca^{2+}]$ is slightly increased under high calcium dietary. TRPV6 expression is subsequently reduced, and so is the transcellular route of Ca^{2+} absorption. Also shown in the figure is the Ca^{2+} -sensing receptor (CaSR) on the basolateral membrane can sense the increase in $[Ca^{2+}]$ and attenuates Ca^{2+} absorption via TRPV6 to prevent hypercalcemia. Created with [BioRender.com](https://www.biorender.com).

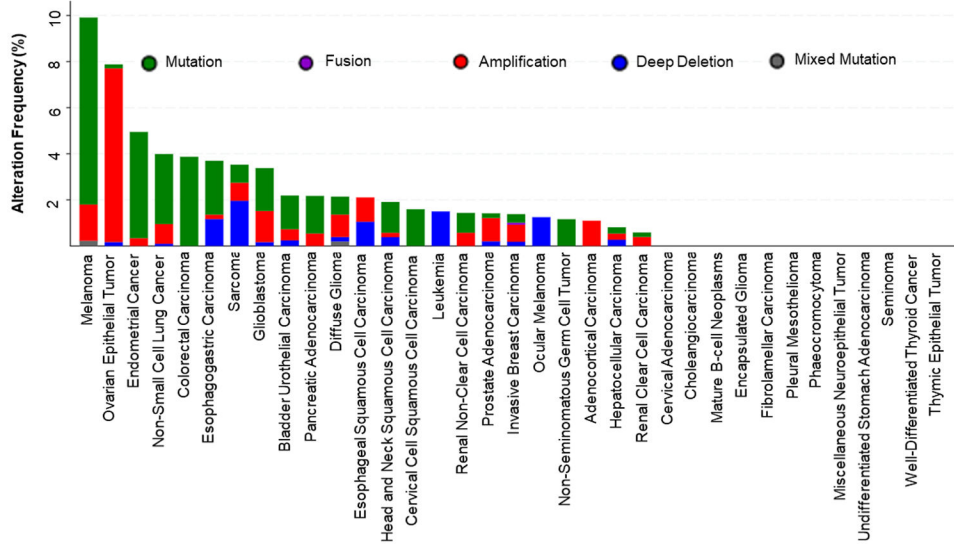


Figure 7. Frequency and expression of TRPV6 genomic aberrations across different cancer subtypes: To gather insights regarding the expression of TRPV6 transcripts and genomic aberrations in different cancer subtypes, TRPV6 was queried in the cBioPortal Cancer Genomic pipeline (thresholds set at “Min. # Total Cases” – 0 and “Min. % Altered Cases” – 0). The portal analyzed the TRPV6 mutation profile across 32 TCGA cancer datasets comprising data from 10953 patients. Outputs reporting frequency of copy number alterations of different TRPV6 mutations across TCGA datasets are reported. The graphs report TRPV6 copy number alteration (CNA) frequency (as a percentage) across 32 TCGA datasets. The distribution of different types of CNAs (percentage) across each cancer subtype is shown. The key on the top denotes the type of TRPV6 genomic aberration: green – “Mutation”; purple – “Fusion”; Red – “Amplification”; Blue – “Deep Deletion”; Grey – “Mixed Mutations”.

Table 1:

TRPV6: Important Genome and Proteome identifiers

Species	Human	Rat	Mous
Chromosomal Location	7q33-q34	4q22	6 B2
Annotated AA Length	725	727	727
Putative <i>in vivo</i> AA Length	765	767	767
Molecular weight (kDa)*	87.3	87.4	87.4
Gene Symbol	<i>TRPV6</i>	<i>Trpv6</i>	<i>Trpv6</i>
Ensembl Gene	ENSG00000165125	ENSRNOG00000014714	ENSM
Entrez Gene	55503	114246	6417
RefSeq Nucleotide	NM_018646	NM_053686	NM_
RefSeq Protein	NP_061116	NP_446138	NP_0
UniProtKB	Q9H1D0	Q9R186	Q91W
OMIM	606680	N/A	N/A
KEGG Gene	hsa:55503	rno:114246 (Rn)	mmu:
Human Protein Atlas ID	ENSG00000165125	N/A	N/A
Mouse Genome Informatics (MGI) ID	N/A	N/A	MGI:
PDB IDs	6BO8,6D7S,6BOA,6BO9,6D7T,6E2F	6BOB,6D7Q,6D7V,6D7X,6D7P,6D7O,5WOA,5WO8,5WO7,5WO9,5IWT,5IWK,5IWP,5IWR,5WO6,6E2G	N/A

Abbreviations

AA: Amino Acids; ENSG: Ensembl Gene; ENSMUSG: Ensembl Gene (Mus Musculus); ENSRNOG: Ensembl Gene (Rattus Norvegicus); KEGG: Kyoto Encyclopedia of Genes and Genomes; NM: Natural mRNA (RefSeq Identifier); NP: Natural Protein (RefSeq Identifier) OMIM: Online Mendelian Inheritance in Man; PDB: Protein Data Bank; UniProt KB: UniProt Knowledgebase.

* Calculated based on the molecular weight of the long form of TRPV6. The observed molecular weight of TRPV6 protein differs based on glycosylation status, oligomerization state, denaturation conditions (e.g. reducing vs. non-reducing etc.), antibody employed, and the version of TRPV6 construct being used for expression and characterization. The annotated version of human TRPV6 used in many studies lacks a 40-aa-long extension that has been observed *in vivo*.

Table 2:

TRPV6 Tissue Distribution

Species	Human	Rat	Mouse
Detection	<i>In situ</i> hybridization, Western Blot, Northern Blot, RT-PCR.	<i>In situ</i> hybridization, Northern Blot, RT-PCR.	Immunocytochemistry, RT-PCR, Northern Blot, Western Blot.
Tissues	Intestines, stomach, placenta, salivary glands, liver, prostate, pancreas, kidney, testes, mammary glands	Intestines, kidney, brain.	Intestines, stomach, kidney, lung, spleen, liver, brain, placenta, prostate, pancreas, adrenal glands, skin, bone, epididymis
Major References	(Peng et al., 2000; Hoenderop et al., 2001; Peng et al., 2001a; Peng et al., 2001b; Wissenbach et al., 2001; Zhuang et al., 2002; Hirnet et al., 2003)	(Peng et al., 1999)	(Weber et al., 2001; Zhuang et al., 2002; Hirnet et al., 2003; Weissgerber et al., 2011; Weissgerber et al., 2012; Beggs et al., 2019)

TRPV6 Protein Interactions (Shin et al., 2010).

Table 3:

Protein Interactor	Screening methods	Validation Methods		Characterization		Functional Consequence(s)			Reference(s)
		In vitro	In vivo	Method(s)	TRPV6 Region	Method(s)	Effect		
BSPRY	Y2H	FP-PD	N/A	N/A	N/A	N/A	N/A	N/A	(van de Graaf et al., 2006d)
Calbindin-D_{28k}	Inference	FP-PD	N/A	N/A	N/A	N/A	N/A	N/A	(Lambers et al., 2006)
Calmodulin	Inference	FP-PD	Co-IP, FRET	Fluorescence probe, FP-PD	327-577 (Rab) 88-97 (Rab) 643-656 (Rab) 691-711 (Hu) ARD Domain (Hu)	Ca ²⁺ measurement Patch-clamp	Inhibition		(Niemeier et al., 2001; Hirnet et al., 2003; Lambers et al., 2004; Derler et al., 2006; Mercado et al., 2010)
Cyclophilin B	Affinity purification-MS	N/A	Co-IF, FRET	N/A	N/A	Ca ²⁺ measurement	Activation		(Stumpf et al., 2008)
FYN	Inference	N/A	N/A	N/A	N/A	In vitro PO ₄ lyation	PO ₄ lyation		(Sternfeld et al., 2007)
I-MEA	Y2H	N/A	N/A	N/A	N/A	N/A	N/A		(Rual et al., 2005)
Klotho	Inference	N/A	N/A	N/A	N/A	Ca ²⁺ measurement Patch-clamp In vitro PTM Assay	Activation, Glycosylation (Asn-357)		(Chang et al., 2005; Lu et al., 2008)
NHERF4	Y2H	FP-PD	Co-IP	FP-PD	724-727 (Mo)	Patch clamp	Activation		(van de Graaf et al., 2006c; Kim et al., 2007)
Nipsnap1	Inference	FP-PD	Co-IP	FP-PD	83-118 (Mo)	Patch clamp	Inhibition		(Schoeber et al., 2008)
Numb	Inference	N/A	Co-IP	Co-IP	716 (Hu)	Ca²⁺ measurement	Inhibition		(Kim et al., 2013; Kim et al., 2014)
PTEN	Inference	N/A	Co-IP	N/A	N/A	N/A	N/A		(Kim et al., 2014)
PTP1B	Inference	Yeast two-hybrid	BFC	BFC	1-191 (Rat)	Ca ²⁺ measurement In vitro PO ₄ lyation In vivo PO ₄ lyation	DePO ₄ lyation (Tyr-161 and Tyr-162)		(Sternfeld et al., 2005; Sternfeld et al., 2007)
Rab11a	Y2H	FP-PD	Co-IF	N/A	N/A	Co-IF	Activation, Increase in Plasma membrane level		(van de Graaf et al., 2006a)
RGS2	Y2H	FP-PD	N/A	N/A	N/A	Patch-clamp	N/A		(Schoeber et al., 2006)
RyR1	Affinity purification-MS	N/A	N/A	N/A	N/A	N/A	N/A		(Woo et al., 2008)

Protein Interactor	Screening methods	Validation Methods		Characterization		Functional Consequence(s)		Reference(s)
		In vitro	In vivo	Method(s)	TRPV6 Region	Method(s)	Effect	
SI00A10	Inference, Y2H	FP-PD	Co-IF, Co-IP	FP-PD	597-601 (Mo)	Patch clamp, Co-IF	Activation, Increase in Plasma membrane level	(van de Graaf et al., 2003; Rual et al., 2005; Borthwick et al., 2008)
Src	Inference	N/A	Co-IP	N/A	N/A	In vitro PO ₄ lytation	PO ₄ lytation (Tyr-161, 162)	(Sternfeld et al., 2007)
TRPC1	Inference	N/A	Co-IP, FRET	FRET	1-198 (Hu)	Cell Surface Biotinylation	Retains in ER, Inhibition	(Schindl et al., 2012; Raphael et al., 2014)
TRPML3	Inference	N/A	FRET	N/A	N/A	N/A	N/A	(Guo et al., 2013)
TRPV5	Inference	N/A	Co-IP, Co-IF, FRET	N/A	N/A	Patch-clamp	Tetramer formation, New Channel creation	(Hoenderop et al., 2003b; Hellwig et al., 2005; Semenova et al., 2009; Guo et al., 2013)

Abbreviations

Methods

BFC: Biomolecular fluorescence complementation, Co-IF: Co-immunofluorescence, Co-IP: Co-immunoprecipitation, FRET: Fluorescence Resonance Energy transfer, FP-PD: Fusion Protein Pull-Down, PO₄lytation: Phosphorylation, Y2H: Yeast Two-Hybrid,

Protein Interactor

BSPRY: B-Box and Spry Domain Containing Protein; FYN: Fyn Kinase Belonging Src Family of Kinases; I-MFA: Myo D Family Inhibitor; NHERF: Na Exchanger Regulatory Factor; NIPSNAP1 4-Nitrophenylphosphatase Domain and Non-Neuronal SNAP25-Like Protein Homolog 1; Numb: Drosophila mutation that removes most of the sensory neurons in the developing peripheral nervous system; PTP: Protein Tyrosine Phosphatase; Rab11a: Member RAS Oncogene Family; RGS2: Regulator Of G-Protein Signaling 2; RyR1: Ryanodine Receptor 1; TRPC1: Transient receptor potential canonical 1; TRPML3: Transient receptor potential mucolipin-3.

Rab. – Rabbit; Hu. – Human; Mo. – Mouse; ER: Endoplasmic Reticulum

Table 4:

TRPV6 Mutations Linked to Human Genetic Diseases

Disease		Position (Structural Location)	Suggested Mechanism for dysfunctions	References
Transient Neonatal Hyperparathyroidism (TNHP)		C212Y, I223T (AR4)	Impaired protein stability. Loss of Ca ²⁺ ion import.	(Suzuki et al., 2018) (Suzuki et al., 2020)
		R425Q, G428R (S2) R483W (S3)	Decreased localization/trafficking at the plasma membrane.	(Suzuki et al., 2018) (Suzuki et al., 2020)
		G451E (S2-S3 Loop)	Increased Ca ²⁺ ion import (intracellular Ca ²⁺ overload) induces cell death.	(Suzuki et al., 2018) (Suzuki et al., 2020)
		T198R	Not analyzed	(Almidani et al., 2020)
		G291S (AR6)	Not analyzed	(Suzuki et al., 2020)
		R390H (S1)	Not analyzed	(Suzuki et al., 2020)
		G660R (C-terminal hook)	Not analyzed	(Suzuki et al., 2020)
Skeletal Dysplasia and Under-mineralization		G660R (C-terminal hook)	Impaired tetramer stability	(Burren et al., 2018; Mason et al., 2020)
		R510Ter	Nonsense-mediated decay	
Chronic Pancreatitis	Japanese	R174X, A210V, Y262X, R345C, L392F, R425Q, G428R, R483Q, V504F, I580F, A606T, L608R, L609F	Reduced TRPV6 activity and/or protein *	(Masamune et al., 2020)
		A18S, R73Q, A135T, R174Q, H205Y, I223T, D319N, D324N, R363Q, I434T, G451A, M537T, Y549C, E558K, R661W, R672W, R672Q, G695S	Not analyzed	
	French	K82Rfs, N102Tfs, R174X, G311V, R345C, R483W, G489R, E575K	Reduced TRPV6 activity and/or protein **	
		P10L, A18S, G42D, R179C, R193G, H205R, R220Q, D263N, L299Q, T309M, R363Q, I381V, D404N, T432S, M494V, V505I, R661W, R730C	Not analyzed	
	German	R342Q, R345H, G365E, R483W, R483Q, H622Gfs, A626P, A626P, C659X	Reduced TRPV6 activity and/or protein ***	
		A18S, P23T, S72N, R73G, R73Q, R194H, I223T, L299Q, T309M, I323V, V492L	Not analyzed	

* In the Japanese cohort L392F, R425Q, G428R, R483Q reduced TRPV6 activity but not protein level

** In the French cohort R483W resulted in a decrease in TRPV6 activity but not in protein level

*** In the German cohort G365E resulted in a decrease in TRPV6 activity but not in protein level

Table 5:

TRPV6 expression in Human Cancer Tissues and Cancer Cell lines

		Methods	References
Cancer Tissues			
Prostate Cancer		RT-PCR, Northern, ISH	(Peng et al., 2001b; Wissenbach et al., 2001; Fixemer et al., 2003; Wissenbach et al., 2004)
Breast Cancer		RT-PCR, Immunodetection	(Zhuang et al., 2002; Bolanz et al., 2008)
Ovarian		Immunodetection	(Zhuang et al., 2002)
Thyroid		Immunodetection	(Zhuang et al., 2002)
Endometrial		ISH	(Wissenbach and Niemeyer, 2007)
Colon/Colorectal		Immunodetection	(Zhuang et al., 2002)
Pancreatic		RT-PCR	(Song et al., 2018)
Esophageal		Immunodetection	(Zhang et al., 2016a)
Lung (NSCLC)		Immunodetection, RT-PCR	(Fan et al., 2014; Lau et al., 2014)
Cancer Cell Lines			
Prostate	LNCaP	Northern, Western	(Peng et al., 2001b; Bodding et al., 2003)
	DU-145	RT-PCR	(Peng et al., 2001b; Lehen'kyi et al., 2011a)
	PC-3	RT-PCR	(Peng et al., 2001b)
Breast	MCF-7	RT-PCR, Western	(Bolanz et al., 2008)
	T47D	MS/MS, RT-PCR	(Bolanz et al., 2008), (Peters et al., 2012; Fecher-Trost et al., 2013)
	MDA-MB-231	RT-PCR	(Peters et al., 2012)
	MDA-MB-468	RT-PCR	(Peters et al., 2012)
	ZR-75	RT-PCR	(Peters et al., 2012)
	SKBR3	RT-PCR	(Peters et al., 2012)
	SW480	Northern	(Peng et al., 2000)
	LS-180	RT-PCR, Western	(Zheng et al., 2012)
	Caco-2	RT-PCR, Western	(Fleet et al., 2002; Taparia et al., 2006)
Ovarian	SKOV3	Peptide-based staining	(Bowen et al., 2013)
Hematologic malignancies	K562	Northern	(Heise et al., 2010)
	Jurkat	Northern, RT-PCR, Western	(Cui et al., 2002; Peng et al., 2003; Tomilin et al., 2016; Kever et al., 2019)
Gastric	AGS	Western	(Chow et al., 2007)
Pancreatic	BxPC-3	RT-PCR, Western	(Song et al., 2018)
	AsPC-1	RT-PCR, Western	(Song et al., 2018)
	SW1990	RT-PCR, Western	(Song et al., 2018)
	PANC-1	RT-PCR, Western	(Song et al., 2018)
	Capan	RT-PCR, Western	(Song et al., 2018)
Esophageal	KYSE140	RT-PCR	(Zhang et al., 2016a)
	KYSE180	RT-PCR	(Zhang et al., 2016a)
	KYSE410	RT-PCR	(Zhang et al., 2016a)

		Methods	References
	KYSE510	RT-PCR	(Zhang et al., 2016a)
	KYSE520	RT-PCR	(Zhang et al., 2016a)
	HKESC1	RT-PCR	(Zhang et al., 2016a)
	CE81T	RT-PCR	(Zhang et al., 2016a)
	EC109	RT-PCR	(Zhang et al., 2016a)
	EC9706	RT-PCR	(Zhang et al., 2016a)
Lung	NCI-H82	RT-PCR, Western	(Lau et al., 2014)
	NCI-H69	RT-PCR, Western	(Lau et al., 2014)
	DMS 53	RT-PCR, Western	(Lau et al., 2014)
	DMS 114	RT-PCR, Western	(Lau et al., 2014)

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Table 6:Strategies employed for Pharmacological targeting of TRPV6 Ca²⁺-selective channel

Compound	TRPV6 IC ₅₀ (μM)	TRPV5 IC ₅₀ (μM)	Remarks	References
Ruthenium Red	9	0.12	<ul style="list-style-type: none"> • First known blocker of TRPV5/6 activity • Inhibits other CRAC channels • Nonselective Pharmacological tool to study Ca²⁺ channels 	(Hoenderop et al., 2001)
TH-1177 Compounds	0.44-0.50	N/A	<ul style="list-style-type: none"> • 9 related compounds • Blocks Ca²⁺ entry in LNCaP and PC-3 • Ester-bonded phenolic group required for inhibition • Extend the average life span of mice carrying prostate tumors by up to 38% without toxicity 	(Landowski et al., 2011)
2-APB	20.7	N/A	<ul style="list-style-type: none"> • Binds to TRPV6 in a pocket formed by the cytoplasmic half of the S1–S4 transmembrane helix bundle. • Induces TRPV6 channel closure by modulating protein-lipid interactions. • Non-selective: Competitive antagonist of IP₃R, and agonist for TRPV2 	(Kovacs et al., 2012; Singh et al., 2018b)
2-APB derivative 22b	5	N/A	<ul style="list-style-type: none"> • Store-operated Ca²⁺ channel blocker 	(Hofer et al., 2013)
Econazole (Miconazole)	190 (201)	296 (442)	<ul style="list-style-type: none"> • Broad-spectrum antifungal agent approved in the US and Canada for treatment of fungal infections 	(Schwarz et al., 2006; Landowski et al., 2011)
Piperazine derivative Cis-22a	0.32	2.4	<ul style="list-style-type: none"> • Non-selective. Acts on TRPV1, TRPV3, TRPV5, TRPM8, SOCE 	(Simonin et al., 2015)
Capsaicin	25-50	Not tested	<ul style="list-style-type: none"> • Induces apoptosis in SCLC and gastric carcinoma cells (in vitro). Reduces SCLC xenograft growth 	(Chow et al., 2007; Lau et al., 2014; Xu et al., 2018)
⁹-tetrahydrocannabivarin (THCV)	9.4	4.8	<ul style="list-style-type: none"> • Extracted from the cannabis plant • Ligands of the G protein-coupled cannabinoid receptors CB1 and CB2 • Nonselective: acts on other TRPV6 channels 	(Janssens et al., 2018)
SOR-C13	0.014	No effect	<ul style="list-style-type: none"> • 13-amino-acid-peptide derived from Soricidin: a 54-amino acid peptide found in the paralytic venom of the northern short-tailed shrew (<i>Blarina brevicauda</i>) • Blunts ovarian and prostate cancer growth <i>in vitro</i> and <i>in vivo</i> • Phase 1 trial completed • Demonstrated potential for <i>in vivo</i> diagnostic cancer imaging 	(Bowen et al., 2013; Fu et al., 2017; Xue et al., 2018)
Lidocaine	N/A	N/A	<ul style="list-style-type: none"> • Approved anesthetic • Reduces migration and invasion in MDA-MB-231 • Mechanism of action on TRPV6 unknown 	(Jiang et al., 2016)

Abbreviations

2-APB: 2-Aminoethoxydiphenyl Borate; CB: Cannabinoid Receptor; CRAC: Ca²⁺-Release-Activated Channel; SOR-C13: 13-amino-acid-peptide derived from the biological compound Soricidin: a 54-amino acid peptide found in the paralytic venom of the northern short-tailed shrew.

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