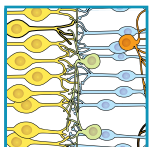


# THE PLASMA MEMBRANE CALCIUM ATP<sub>ASES</sub> AND THEIR ROLE AS MAJOR NEW PLAYERS IN HUMAN DISEASE

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**Stafford N, Wilson C, Oeandy D, Neyses L, Cartwright EJ.** The Plasma Membrane Calcium ATPases and Their Role as Major New Players in Human Disease. *Physiol Rev* 97: 1089–1125, 2017. Published May 31, 2017; doi:10.1152/physrev.00028.2016.—The  $\text{Ca}^{2+}$  extrusion function of the four mammalian isoforms of the plasma membrane calcium ATPases (PMCA) is well established. There is also some detail known of their roles in global and local  $\text{Ca}^{2+}$  homeostasis and intracellular signaling in a wide variety of cell types and tissues. It is becoming clear that the spatiotemporal patterns of expression of the PMCA and the fact that their abundances and relative levels vary from cell type to cell type both reflect and impact on their specific functions. Over recent years it has become increasingly apparent that these genes have significant roles in human health and disease, with PMCA1-4 being associated with muscular diseases, deafness, autism, ataxia, adenoma, and malarial resistance. This review brings together evidence of the variety of tissue-specific functions of PMCA and will highlight the roles these genes play in regulating normal physiological functions and the considerable impact they have on human disease.

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## I. INTRODUCTION

The presence of an ATP-dependent  $\text{Ca}^{2+}$  transporter located in the plasma membrane was first identified in erythrocytes half a century ago (327). The plasma membrane calcium ATPase (PMCA) has since been classified as a member of the P-type transport ATPase family, due to the covalent intermediate state that is formed upon phosphorylation of a highly conserved aspartate residue during its catalytic cycle (285). The pumps are high affinity ( $0.2\text{--}0.5\ \mu\text{M}$  under optimal conditions)  $\text{Ca}^{2+}$  extruders, binding a single intracellular  $\text{Ca}^{2+}$  ion which is then deposited to the extracellular space following conformational changes which first allow transport of the ion through the plasma membrane and finally dissociation from the pump through lowering its  $\text{Ca}^{2+}$  affinity (44, 225). This means that compared with the related ATPase of the sarco(endo)plasmic reticulum (SERCA), the PMCA has a lower capacity for  $\text{Ca}^{2+}$  clearance, removing only one  $\text{Ca}^{2+}$  ion per ATP molecule hydrolyzed as opposed to two (143). PMCA's export of  $\text{Ca}^{2+}$  occurs in conjunction with the import of one or more protons ( $\text{H}^+$ ), with reports ranging from an electrogenic  $<1$  to  $\sim 3\ \text{H}^+$  per  $\text{Ca}^{2+}$  in barnacle skeletal muscle to an electro-

neutral  $1\text{Ca}^{2+}:2\text{H}^{+}$  stoichiometry in human erythrocyte membrane preparations and snail neurons (93, 264, 371).

There are four known PMCA isoforms, encoded by separate genes *ATP2B1-4* located at human chromosomal loci 12q21-q23, 3p25-p26, Xq28, and 1q25-q32, respectively (358). In the mouse, a model which has been extensively used to understand the functional roles of the PMCA, these four genes are located on chromosomes 10, 6, X, and 1, respectively. In both humans and mouse, the PMCA 1–4 exhibit differential spatial patterns of expression, with isoforms 1 and 4 being more or less ubiquitous, while PMCA2 and 3 expression is restricted to specific cell types, most notably in the nervous system. In addition, a total of over 25 splice variants have been identified, including the more or less ubiquitous full-length “b” variants deemed to play housekeeping functions, and many others which show cell specific distributions (147, 353, 358). This has led to the consensus that each isoform and variant may perform unique functions.

## II. PMCAs: THE RELATIONSHIP BETWEEN STRUCTURE AND FUNCTION

This review article focuses its attention on the PMCAs in human health and disease and our current understanding of their physiological and pathophysiological roles. By way of introducing the PMCAs, we provide an overview of the structure of the PMCA isoforms and their regulation, but for more extensive and detailed information, we refer the

reader to a number of excellent published reviews (51, 94, 358).

## A. The Structure of PMCA

The general structure of the PMCA, like other members of the P-type ATPase family, consists of 10 hydrophobic trans-membrane (TM) domains flanked by cytosolic NH<sub>2</sub> and COOH terminals, and with two large intracellular loops (94, 358). More specifically, the PMCA belongs to the type II subset of P-type ATPases which include a number of proteins of physiological/pathophysiological importance in vertebrates due to their roles in the movement of a variety of ions across membranes. The NH<sub>2</sub> terminal of the PMCA contains the most variation among isoforms in its 80–90 amino acids. The two intracellular loops span TM domains 2–3 and 4–5, and each contains an autoinhibitory region that interacts with a calmodulin-binding site located on the long COOH terminal rendering the PMCA in a closed conformation in the absence of calmodulin and greatly reducing its Ca<sup>2+</sup> affinity (116, 117). The second intracellular loop contains the catalytic core of the pump, featuring conserved aspartate and lysine residues critical for catalytic phosphorylation and ATP binding, respectively.

The determination of the complete nucleotide sequence for the PMCA by two groups in the late 1980s began to clarify the structure of the enzyme (346, 387). The gene products among these isoforms share ~75–85% identity, while ~85–90% of the primary sequence is conserved (356). Importantly, rodent and human isoforms share ~99% sequence homology making rodents a suitable model in which to study PMCA function. The regions of the PMCA displaying least identity among isoforms are the NH<sub>2</sub> and COOH terminals (58, 357), which may be of note as the COOH terminal is especially rich in interaction partners (see sect. IID).

## B. Regulation of PMCA Activity

The major regulator of pump activity is calmodulin (CaM), which upon binding to its domain releases autoinhibition and raises pump Ca<sup>2+</sup> affinity to sub-micromolar levels rendering it active at cellular concentrations and increasing pump activity four- to sixfold (107, 112). CaM affinity is 5- to 10-fold higher in neuronal isoforms PMCA2 and 3 compared with the ubiquitous PMCA1 and 4 (44), while PMCA2 displays unusually high basal ATPase activity in the absence of CaM compared with other isoforms (107). Interestingly, there is evidence that the erythrocyte PMCA exists as both a CaM-activated monomer as well as in a Ca<sup>2+</sup>-dependent dimeric form which is fully activated in the absence of CaM (64, 190, 191). To date, there are no reports as to whether the self-activated oligomeric form is present in other cell types.

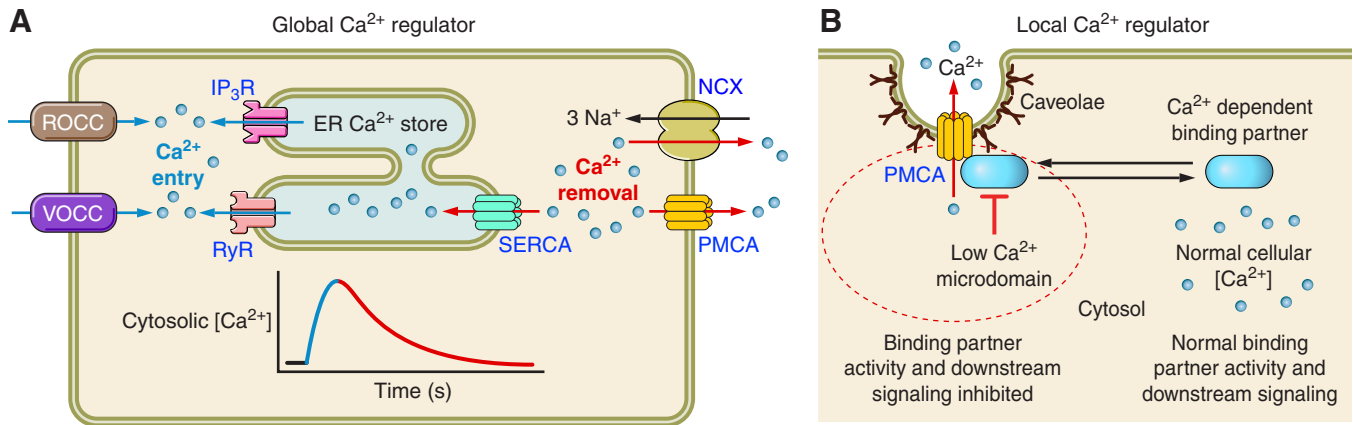
Further mediators of pump activation have also been identified including the action of acidic phospholipids, unsaturated fatty acids, and trypsin-mediated partial proteolysis of the enzyme (263). In addition, a rise in basal activity has been witnessed upon phosphorylation of the PMCA by protein kinase C (PKC) (113) and, in the case of PMCA1, protein kinase A (PKA) (142).

## C. PMCA and Regulation of Global and Local Intracellular Ca<sup>2+</sup>

Every cell type has unique requirements for optimal Ca<sup>2+</sup> homeostasis to perform their respective physiological functions. This can range from the maintenance of a low resting intracellular Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>) to prevent the activation of Ca<sup>2+</sup>-mediated cell death to dynamic control of beat-to-beat Ca<sup>2+</sup> levels in cardiomyocytes enabling muscle contraction and relaxation. In nonexcitable cells where the resting intracellular Ca<sup>2+</sup> levels remain low, the PMCA is generally the principal Ca<sup>2+</sup> removal system (423); however, excitable cells such as myocytes and neurons with their more complex needs for Ca<sup>2+</sup> extrusion demand higher capacity systems for cytosolic Ca<sup>2+</sup> clearance. In excitable cells, the three main pathways for removal of cytosolic Ca<sup>2+</sup> are via reuptake into the sarco(endo)plasmic reticulum via SERCA (221), and extrusion from the cell via the PMCA and the high capacity sodium-calcium exchanger (NCX) (45, 96), as illustrated in **FIGURE 1A**. The relative contributions of these three pathways to Ca<sup>2+</sup> clearance vary depending on cell type. In bladder smooth muscle for example, these are roughly 2:1:1 in favor of the NCX (138), whereas in cardiomyocytes, 70–92% of Ca<sup>2+</sup> (dependent on species) is returned to the internal store via SERCA with the remainder largely extruded via the NCX, leaving the PMCA along with a small amount of mitochondrial reuptake, together coined the “slow systems,” to contribute little more than 1% to global Ca<sup>2+</sup> clearance (26).

The PMCA is not only involved in global Ca<sup>2+</sup> homeostasis however, but also in the regulation of local intracellular Ca<sup>2+</sup> dynamics. Similarly to transient receptor potential canonical (TRPC) channels and inositol 1,4,5-trisphosphate receptors (InsP<sub>3</sub>Rs), which have been shown to activate Ca<sup>2+</sup>-dependent effectors through generating high Ca<sup>2+</sup> microdomains at the subplasmalemmal and perinuclear compartments, respectively (104, 406), the PMCA is able to regulate downstream signaling pathways. In the case of the PMCA, this is achieved through pump activity lowering the [Ca<sup>2+</sup>]<sub>i</sub> in its microdomain, thereby negatively regulating Ca<sup>2+</sup>-dependent interaction partners as illustrated in **FIGURE 1B**.

To gain an understanding of how the PMCA can influence local Ca<sup>2+</sup> dynamics and to directly monitor PMCA activity, we have recently developed a novel fusion protein by cloning the genetically encoded Ca<sup>2+</sup> indicator GCaMP2 to



**FIGURE 1.** A: cartoon illustrating global  $\text{Ca}^{2+}$  regulation in an excitable cell. The sources of  $\text{Ca}^{2+}$  entry to the cytosol and associated rise in global intracellular  $\text{Ca}^{2+}$  are highlighted in blue, while routes for cytosolic  $\text{Ca}^{2+}$  clearance and associated decay phase of the global transient are highlighted in red. B: diagram demonstrating regulation of local  $\text{Ca}^{2+}$  and  $\text{Ca}^{2+}$ -dependent signaling by PMCA. PMCA activity generates a low  $\text{Ca}^{2+}$  microdomain in its vicinity, negatively regulating  $\text{Ca}^{2+}$ -dependent binding partners by attracting them to its locale in caveolae, thereby influencing downstream signaling. VOCC, voltage-operated  $\text{Ca}^{2+}$  channel; ROCC, receptor-operated  $\text{Ca}^{2+}$  channel; IP<sub>3</sub>R, inositol trisphosphate receptor; RyR, ryanodine receptor; SERCA, sarco(endo)plasmic reticulum  $\text{Ca}^{2+}$ -ATPase; PMCA, plasma membrane  $\text{Ca}^{2+}$ -ATPase; NCX, Na<sup>+</sup>/Ca<sup>2+</sup> exchanger.

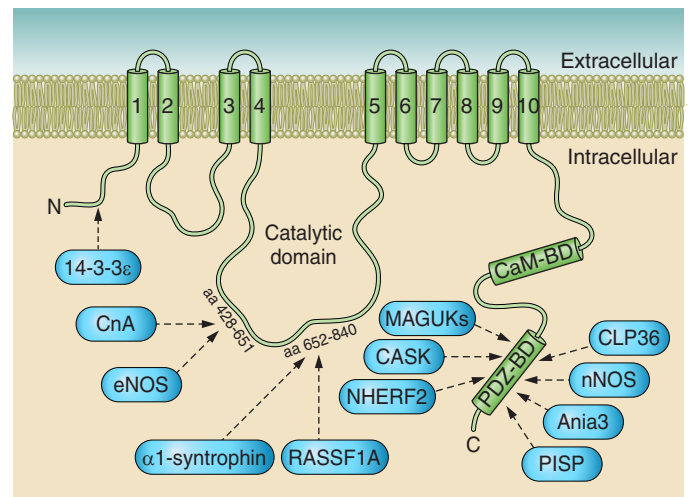
the NH<sub>2</sub> terminal of PMCA4. Upon transfection into cardiomyocytes, the PMCA4-GCaMP2 fusion protein was able to detect  $\text{Ca}^{2+}$  oscillations in the subsarcolemmal compartment which could be inhibited by specific pharmacological blockade of PMCA4, thus providing us with a tool to assess  $\text{Ca}^{2+}$  dynamics in the local vicinity of PMCA4 (241). It is clear therefore that the PMCA<sub>s</sub> can regulate both global and local intracellular  $\text{Ca}^{2+}$  levels depending on tissue type and PMCA isoform, while through the import of H<sup>+</sup> and hydrolysis of ATP they may also influence subplasmalemmal pH and ATP concentration (85, 93, 372). This regulation of local  $\text{Ca}^{2+}$  lends itself to the PMCA being a major mediator of  $\text{Ca}^{2+}$ -dependent signaling events (59, 267) as we will describe in the following section.

#### D. PMCA<sub>s</sub> as Signaling Molecules via Interactions With Protein Partners

Amidst large fluctuations in global  $\text{Ca}^{2+}$  during excitation-contraction coupling, excitable cells retain the ability to utilize  $\text{Ca}^{2+}$  as a second messenger in many signaling pathways involved in both normal physiological function and in disease progression (126). Over recent years the PMCA has emerged as a significant regulator of  $\text{Ca}^{2+}$ -dependent signaling, through protein-protein interactions and compartmentalization of the signal in the subplasmalemmal microdomain (267). A possible role in signal transduction was suggested based on evidence that PMCA4 is localized in protein-rich signaling hubs termed caveolae (128).

In subsequent years, our group and others have identified many PMCA interaction partners, some of which are common to all PMCA<sub>s</sub> and others unique to particular isoforms

(FIGURE 2). Many of these interactions occur at a PDZ ligand-binding domain located at the terminal end of the carboxyl tail. Interacting partners at this domain include members of the membrane-associated guanylate kinase (MAGUK) family, calcium/calmodulin-dependent serine protein kinase (CASK), LIM family protein CLP36, homer protein Ania-3, PMCA-interacting single-PDZ pro-



**FIGURE 2.** Schematic illustrating general PMCA structure, consisting of 10 transmembrane (TM) domains. The intracellular loop connecting TM 4 and 5 contains the catalytic core with phosphorylation and ATP binding sites. PMCA interaction partners are shown, with arrows detailing to which regions they bind. CnA, calcineurin A; eNOS, endothelial nitric oxide synthase; RASSF1A, Ras-associated factor 1A; NHERF2, Na/H exchanger regulatory factor 2; CASK, calcium/calmodulin-dependent serine protein kinase; MAGUK, membrane-associated guanylate kinase; PISP, PMCA-interacting single-PDZ protein; nNOS, neuronal nitric oxide synthase; PDZ-BD, PDZ protein-binding domain; CaM-BD, calmodulin-binding domain.



tein (PISP), Na/H exchanger regulatory factor 2 (NHERF2), and neuronal nitric oxide synthase (nNOS) (39, 91, 92, 137, 182, 334, 335, 339). We have found the PMCA4-nNOS interaction to occur in a macromolecular complex with  $\alpha$ -1 syntrophin and dystrophin, through binding a linker region on  $\alpha$ -1 syntrophin to the PMCA's second intracellular loop (395). Further interactions in this catalytic region of the PMCA occur with the  $\text{Ca}^{2+}$ -dependent phosphatase calcineurin, the tumor suppressor Ras-associated factor 1A (RASSF1A), and endothelial nitric oxide synthase (eNOS) (13, 46, 158). Meanwhile, isoform  $\epsilon$  of the trafficking protein 14-3-3 has been found to associate with PMCA4's NH<sub>2</sub>-terminal region (312). As we describe later in this review, some of these interactions have now been well characterized to be of functional significance, in particular those between PMCA and nNOS in the cardiovascular system, and PMCA and calcineurin in cardiovascular and breast cancer cells (61, 160).

It may in part be through the individualities of these interactions that isoforms can perform specific functions. For example, in identifying an inhibitory interaction between 14-3-3 $\epsilon$  and PMCA4, Carafoli and colleagues noted no association with PMCA2 (312). Similarly, while Strehler and colleagues found both isoforms 2 and 4 to interact with MAGUK family proteins, some family members bound selectively to PMCA4 (92), while NHERF2 interacts only with PMCA2 (91). These differences in the biochemical properties of the pumps mean that the various PMCA isoforms perform cell-specific roles, not only in health but also in human pathophysiology. The remainder of this review will focus on the tissue-specific physiological roles and disease associations of the PMCA pumps.

### III. PMCA AND ITS ASSOCIATIONS WITH HUMAN DISEASE

In recent years, the relevance of the PMCA in a number of human disease processes has come to light. Through examination of whole genomes among populations it is now possible to identify particular genetic variants associated with a certain disease. Following extensive data analysis, genome-wide association studies (GWAS) can detect particular disease-causing single nucleotide polymorphisms (SNPs) located within specific loci in the genome. Genetic mutations in the genes encoding each PMCA isoform have now been associated with human disease (Table 1 and FIGURE 3), highlighting the significance of this family of  $\text{Ca}^{2+}$  pumps in human health and disease.

#### A. PMCA1, Hypertension, and Cardiovascular Disease

Worldwide more people die from cardiovascular diseases (CVD) than any other cause. Currently, over 30% of all

deaths are attributed to CVD, and despite the size of the ongoing research focus, the World Health Organisation predicts that by 2030 the number of people that will die annually from this group of diseases will rise to ~22 million (1). Hypertension, which is a major risk factor for cardiovascular disease, affects ~30% of the adult population worldwide (201) and has long been thought to be influenced by genetic factors as well as environmental ones. In the past 5 years, GWAS of systolic and diastolic blood pressure, as well as hypertension in general, have reported the most significant SNPs associated with these diseases to occur at multiple loci in and around *ATP2B1*, the gene which encodes PMCA1 (76, 173, 178, 206, 366). This finding is consistent among multiple ethnicities including those of East Asian (Japanese, Korean and Chinese), South Asian, and European origin; however, the two studies carried out in African-American populations showed no association between *ATP2B1* and hypertension. Although the effects of *ATP2B1* variants on systolic and diastolic blood pressure are small at around 1 mmHg (105), Levy et al. (206) predicted the risk of developing hypertension to increase by 17 and 37% dependent on whether one or two respective alleles are affected. This finding of an association between *ATP2B1* and hypertension is not just limited to adults as one study has determined that the *ATP2B1* SNP rs2681472 is associated with an increased risk of hypertension in Chinese children (407), while the same SNP also predisposes Chinese women to early-onset preeclampsia during pregnancy (389). Using a gene-centric array in which ~50,000 SNPs in 2,100 genes previously implicated in cardiovascular, metabolic, and inflammatory processes, Fontana et al. (124) demonstrated a strong association between SNP rs12817819 in *ATP2B1* and resistant hypertension, a condition in which raised blood pressure is not controlled despite the use of at least three antihypertensive drugs.

It is beginning to emerge that it may be a reduction in the expression of *ATP2B1* which leads to raised blood pressure. Human umbilical artery smooth muscle cells carrying the risk allele of the *ATP2B1* SNP rs11105378 were found to have reduced *ATP2B1* mRNA expression (364). Studies in transgenic mice are also beginning to provide functional evidence of the role of *ATP2B1* in blood pressure control, with PMCA1 silencing leading to the development of hypertension associated with an increase in intracellular  $\text{Ca}^{2+}$  and vascular remodeling (187, 343).

Hypertension is not the only cardiovascular disease to be linked by genetic association and candidate gene studies with *ATP2B1*. A number of studies have now identified SNPs in the *ATP2B1* locus to carry increased risk of developing coronary artery disease in Asian and Caucasian populations (219, 367, 394) and myocardial infarction (119), while mutations also associate with several cardiovascular disease risk factors such as salt sensitivity

**Table 1.** Reported SNPs in ATP2B isoforms and their association with disease phenotypes

SNP	Disease Association	Ethnicity	Reference Nos.
ATP2B1-PMCA1			
rs1401982	Hypertension, SBP, DBP, arterial stiffness	Chinese	391
rs2070759	Hypertension	Japanese	364
rs2681472	Hypertension, SBP, DBP	Japanese	366
	SBP, DBP	European	206
	Salt sensitivity	Korean	309
	Preeclampsia	Han Chinese	389
	Coronary artery disease	East Asian	367
rs2681492	SBP, DBP	European	206
	Hypertension, SBP, DBP, arterial stiffness	Chinese	391
	MAP, PP	East Asian	180
rs7136259	Coronary artery disease	Han Chinese	219
rs10858911	Hypertension, SBP, DBP, arterial stiffness	Chinese	391
rs11105354	SBP, DBP	European	206
	Hypertension, SBP, DBP, MAP	European	173
	Coronary artery calcification in chronic kidney disease	European/African	119
	Myocardial infarction	European/African	119
rs11105378	Hypertension	Japanese	364
rs12817819	Resistant hypertension (in patient groups with CAD or ischemic HD)	European American and Hispanics	124
rs17249754	Hypertension, SBP, DBP, arterial stiffness	Chinese	391
	Hypertension, SBP, DBP	East Asian, South Asian, African	105
	Hypertension, SBP, DBP	Korean	162
	SBP, DBP	Asian	76
	Hypertension	Chinese children	407
	Hypertension, SBP, DBP	Chinese	220
	MAP, PP	East Asian	180
	Obesity and hypertension in children	Han Chinese	408
	Coronary artery disease	European	213
	Hyperlipidemia and diabetes	Korean	152
ATP2B2-PMCA2			
rs35678	Autism	European (Italian)	52
rs241509	Autism spectrum disorders	European (Italian)	296
rs3774179	Autism	Han Chinese	414
ATP2B4 - PMCA4			
rs4951074	Malaria in children	African	373
	Severe malaria/cerebral malaria/severe malarial anemia	African	314a
rs10900585	Malaria in pregnancy	African	21
	Malaria in children	African	373
	Severe malaria/cerebral malaria/severe malarial anemia	African	314a

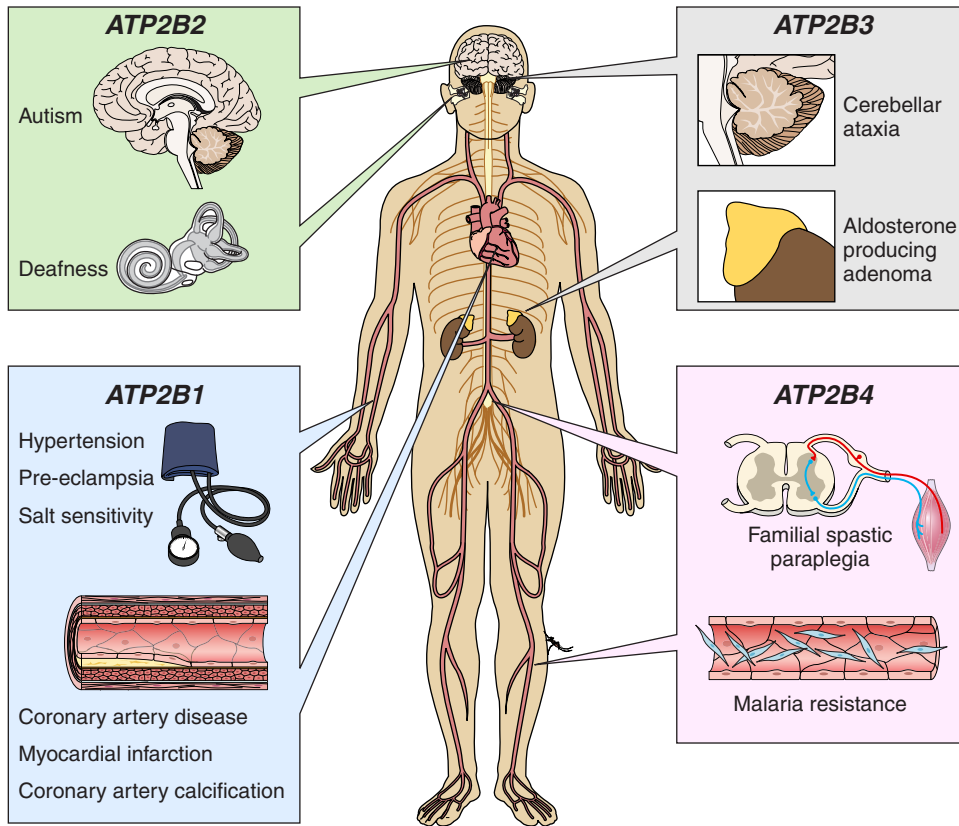
SNP, single nucleotide polymorphism; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; CAD, coronary artery disease; HD, heart disease.

and coronary artery calcification in chronic kidney disease (119, 309).

These data combined demonstrate the importance of PMCA1 in human health and its potential role in cardiovascular disease, and together suggest that PMCA1 may play a critical role in  $\text{Ca}^{2+}$  clearance and homeostasis in vascular smooth muscle and/or endothelial cells.

## B. PMCA2, Hereditary Deafness, and Autism

Hearing loss is known to be caused by both genetic and environmental factors and likely often a combination of the two. One of many genes that have been associated with deafness is *ATP2B2*, the gene that encodes PMCA2. Mutations in PMCA2 have been identified by two studies of



**FIGURE 3.** Cartoon depicting the diseases that have presently been found to be associated with genetic variants of each of the four PMCA isoforms in humans. Human disease associations of ATP2B1 are shown in blue, ATP2B2 in green, ATP2B3 in gray, and ATP2B4 in pink.

families with hereditary hearing loss (123, 336). While the mutations identified in each family were different, in both cases the mutation seems to potentiate the effects of a mutation in the *cadherin23* gene, resulting in severe hearing impairment. Studies in a number of mouse mutants appear to corroborate the fact that PMCA2 is essential for normal hearing function: the first of these was a mouse line known as “deafwaddler,” which as the name suggests has both deafness and motor imbalance. The mutation, which arose spontaneously, leads to a single point mutation in PMCA2 resulting in an amino acid change which affects sensory transduction in hair cells of the inner ear, in addition to neurotransmitter release from the basolateral membrane (355). A second spontaneous mutant known as “wriggle mouse sagami” also carries a PMCA2 point mutation, which differs from that of deafwaddler, and again results in hearing loss (365). Over the subsequent years other spontaneous mutations were identified, a gene knockout was generated, and a number of ethylnitrosourea (ENU) mutagenesis screens were performed which identified mutations in *Atp2b2* leading to deafness (38, 192, 236, 350, 410). The most recent of these ENU mutagenesis screens revealed two novel mutants of *Atp2b2*, one carrying a mutation in the 10th transmembrane domain and the other with a mutation in the catalytic core, both of which resulted in mild to moderate, progressive hearing loss in the heterozygous mutants and profound deafness in the homozygotes (56).

Recent links have also been made between PMCA2 and autism as a result of GWAS and candidate gene studies. Autism is a neurodevelopmental disorder with multiple genetic causes and an extremely high level of heritability. GWAS identified a number of chromosomal regions to have linkage to autism, including the region 3p25, where *ATP2B2* (as well as a number of other genes) is located (200, 235, 341). In a subsequent study comparing the gene expression profiles of lymphoblasts from autistic and nonaffected sibling pairs, *ATP2B2* was the only gene found to be differentially expressed of the 43 known genes in the identified region at 3p25 (164). Using Ingenuity Pathway Analysis, Hu et al. (164) identified that differential expression of *ATP2B2* may affect nervous system development and function via altering Purkinje cell morphology, cerebellar development, or synapse biogenesis. Intracellular  $\text{Ca}^{2+}$  levels and  $\text{Ca}^{2+}$ -signaling pathways are indeed important in the regulation of neuronal survival, differentiation, migration, and synaptogenesis, and it is likely that perturbations in these processes may play a role in the pathogenesis of autism spectrum disorders (193).

Based on this evidence, Carayol et al. (52) carried out a family-based association study that identified a number of SNPs in *ATP2B2* as having a significant association with autism in males only. Further studies have identified SNPs in *ATP2B2* as being associated with autism and autism

spectrum disorders in Italian families and in those of Han Chinese descent (296, 414).

### C. PMCA<sub>3</sub>, Cerebellar Ataxia, and Adenoma

*ATP2B3* mutations have been linked to two separate diseases affecting different organs. One was identified in a family with X-linked cerebellar ataxia, where three out of three descendants of a carrier female were found to be either affected (males) or a carrier (female) (420). The mutation, which was identified by exome sequencing, was found in the calmodulin binding domain of PMCA<sub>3</sub>, which was found in *in vitro* studies to impair Ca<sup>2+</sup> clearance in cell culture. Cali et al. (50) have identified another mutation of *ATP2B3*, which results in a missense mutation and impaired Ca<sup>2+</sup> extrusion, in a patient with cerebellar ataxia and developmental delay. The patient not only carried a PMCA<sub>3</sub> mutation but also two mutations in *LAMA1*, the gene encoding laminin 1 $\alpha$ , and it may be that the observed disease phenotype is dependent on simultaneous mutations in both of these genes.

Given the predominant expression of PMCA<sub>3</sub> in the nervous system, including the cerebellum where it has a role in neuronal Ca<sup>2+</sup> homeostasis, the association with cerebellar ataxia is not unexpected. However, what is more surprising is that two novel *ATP2B3* mutations have recently been found in tissue from a number of aldosterone-producing adenomas (APAs), a major factor in the development of primary aldosteronism which is the most common cause of secondary hypertension (27). Since Beuschlein et al.'s first report in 2013 (27), *ATP2B3* mutations have been identified in APAs present in western European, Japanese, Taiwanese, Chinese, and American populations occurring at frequencies ranging from 0.6 to 9% (6, 102, 121, 186, 272, 330, 331, 396, 404, 422). With the exception of one Taiwanese patient who exhibited a Tyr410Asp substitution (404), all mutations identified thus far involve the deletion of one or more amino acids between Thr-423 and Leu-433 lying in the M4 region of the plasma membrane, believed to be important in Ca<sup>2+</sup> binding during its transport to the extracellular space. Clinically APAs containing *ATP2B3* mutations are associated with higher levels of aldosterone secretion when compared with wild-type APAs (27, 186, 397), most likely as a result of an observed increase in aldosterone synthase CYP11B2 expression (250, 254, 396). Recently these associations have been confirmed through the transfection of *ATP2B3* carrying the common Leu425\_Val426 deletion into adrenocortical NCI-H295R cells, resulting in impaired Ca<sup>2+</sup> clearance accompanied by elevated basal Ca<sup>2+</sup>, CYP11B2 expression, and aldosterone secretion (370). This highlights a highly specialized function for this isoform in nonneuronal tissue.

### D. PMCA<sub>4</sub>, Malarial Resistance, and Familial Spastic Paraplegia

The Ca<sup>2+</sup>-ATPase of the membrane of erythrocytes was the first to be characterized, and has since been found to consist predominately of PMCA<sub>4</sub> (352). Recently a GWAS identified a novel locus within *ATP2B4* where several SNPs conferred resistance to a severe form of malaria among children in a West African population (373). One of the SNPs identified in this study was also shown to confer protection against malaria and associated maternal anemia in pregnant Ghanaian women (21), which may make PMCA<sub>4</sub> an interesting target for anti-malarial medication (246). This association between PMCA<sub>4</sub> and malaria was strengthened by the findings of a large multi-center study in which several *ATP2B4* SNPs were analyzed in ~12,000 cases of severe malaria from 12 different locations in Africa, Asia, and Oceania (224).

A single nucleotide variant of *ATP2B4* which results in a missense mutation has been found in a Chinese family with familial spastic paraplegia, a disease leading to muscle weakness and spasticity of the lower limbs (212). In a subsequent study, the authors have demonstrated that this mutation in *ATP2B4* results in altered Ca<sup>2+</sup> homeostasis, perhaps suggesting a link between disrupted Ca<sup>2+</sup> regulation and familial spastic paraplegia (156).

## IV. TISSUE-SPECIFIC FUNCTIONS OF THE PMCA<sub>s</sub>

It can be said that PMCA is ubiquitously expressed, with all cell types expressing at least one isoform of this gene family. The level, localization, and temporal nature of the expression of the PMCA<sub>s</sub> likely reflect their specific functional roles during embryonic development and in the adult in both health and disease.

*In situ* hybridization has provided evidence that PMCA<sub>1</sub> is expressed by day 9.5 post coitum (pc) in the developing mouse embryo and is abundant and ubiquitous thereafter, although most concentrated in the heart, nervous system, skeletal muscle, and intestine (415). All four isoforms are expressed by day 12.5 pc, with PMCA<sub>2</sub> confined to the brain and PMCA<sub>3</sub> to the nervous system, lung, and skeletal muscle. PMCA<sub>4</sub> expression is ubiquitous though less abundant than PMCA<sub>1</sub>, with the strongest expression found in the brain, bladder, heart, and spinal cord (415). The early and widespread nature of PMCA<sub>1</sub> expression has led to the opinion that this isoform is the major housekeeping isoform.

The ubiquitous pattern of PMCA<sub>1</sub> expression persists after birth and throughout adulthood in both rat and human, while PMCA<sub>4</sub> is also present in most cells. The ratio of PMCA<sub>1</sub> expression to that of isoform 4 is roughly 2:1 in



human adult lung, liver, kidney, stomach, and skeletal muscle (353). In the heart, however, the ratio between the two isoforms is roughly 1:1. PMCA2 demonstrates a more specific expression pattern, being prominent in Purkinje and inner ear cells, as well as in lactating mammary glands, while isoform 3 expression is restricted largely to neurons in the neonate and adult (358).

## A. Role of PMCAs in the Development of the Embryo

Of the two largely ubiquitously expressed isoforms of PMCA (PMCA1 and 4), PMCA1 has been given the status of the housekeeping isoform based on the fact that it is the isoform to be expressed earliest in the development of the embryo; that it is widely expressed in both the embryo and the adult; and that its deletion leads to embryonic lethality. Other than the fact that there are no live births of PMCA1 knockout mice, we currently know little of the role of PMCA1 during development. The same is also true of the other PMCA isoforms; PMCA2 and PMCA4 knockout mice are viable, and while they are both expressed during development, their role has not been studied and we know even less of PMCA3 as a knockout mouse has never been reported.

### 1. PMCA and the placenta

The transport of  $\text{Ca}^{2+}$  from mother to baby via the placenta is tightly regulated during pregnancy to ensure normal fetal development and in particular skeletal mineralization. PMCAs are among many  $\text{Ca}^{2+}$  handling pumps, channels, and exchangers that move  $\text{Ca}^{2+}$  across the placenta. The syncytiotrophoblast, which is a polarized epithelium with a maternal microvillus membrane and a fetal-facing basal membrane, is a critical site involved in this transfer of  $\text{Ca}^{2+}$  from the maternal to the fetal circulation (290). During this transepithelial transfer,  $\text{Ca}^{2+}$  enters the syncytiotrophoblast on the brush-border membrane via transient receptor potential vanilloid (TRPV) channels, from where it is carried to the fetal side of the syncytiotrophoblast by  $\text{Ca}^{2+}$ -binding proteins, and then extruded by the PMCAs. The importance of the PMCAs in this process is evident, as ATP-dependent  $\text{Ca}^{2+}$  transport increases linearly across the syncytiotrophoblast basal membrane during the third trimester of pregnancy in line with the demand for fetal skeletal mineralization (360), and the level of PMCA3 gene expression correlates with the level of bone mineral content in newborn babies (232).

Given this vital role in materno-fetal  $\text{Ca}^{2+}$  transfer, it is no surprise that alterations in PMCA activity and expression have been witnessed in pregnancies complicated by a number of diseases. As already mentioned, the common SNP rs2681472 in *ATP2B1* has been associated with the risk of early-onset preeclampsia, a leading cause of maternal and

fetal mortality, preterm labor, and intrauterine growth restriction present in ~5% of pregnancies (389). Myometrial and subcutaneous resistance arteries from preeclamptic women display impaired relaxation and PMCA attributable  $\text{Ca}^{2+}$  clearance (398) while preeclamptic myometrium and syncytiotrophoblasts display a 50% reduction in PMCA activity and reduced PMCA1 and 4 expression (57, 145). In addition to changes during preeclampsia, altered ATP-dependent  $\text{Ca}^{2+}$  transport has also been witnessed in the placenta in pregnancies complicated by intrauterine growth restriction and insulin-dependent diabetes mellitus (359). These observations highlight the importance of the PMCA in tightly regulating  $\text{Ca}^{2+}$  transport between the mother and fetus.

### 2. PMCA and the spermatozoa

As in many cell types,  $\text{Ca}^{2+}$  is essential for the regulation of a number of functions of the spermatozoa, including motility, the acrosome reaction, and fusion of sperm and egg membranes (83). The PMCA, by acting as an extrusion pump, is known to provide tight control of intracellular  $\text{Ca}^{2+}$  levels which is required for normal sperm function and hence male fertility. While both PMCA1 and PMCA4 are expressed in spermatozoa, it has been shown that PMCA4 is the predominant isoform and is localized to the principal piece of the sperm tail (269). More recently it has been shown that in mouse sperm both major PMCA4 splice variants (PMCA4a and PMCA4b) are expressed throughout the sperm maturation process (283). Gene knockout studies have provided very clear evidence of the functional role PMCA4 has in sperm motility. Two separate mouse models in which PMCA4 has been deleted, our own model which carries a null deletion resulting in lack of expression of all PMCA4 splice variants (332), and that of Okunade et al. (269) which produces a functionally inactive mutant protein, both lead to male infertility as a result of impaired motility. Homozygous PMCA4 knockout mice are born at the expected Mendelian ratio when mice carrying a heterozygous PMCA4 deletion are bred; however, breeding of male and female PMCA4 KO mice did not result in any pups being born, even though mating behavior was normal. Investigations revealed sperm and testes morphology were normal, but assessment of parameters of motility showed a lack of progressive, directional, and hyperactivated motility (269, 332). Given that PMCA1 knockout mice are embryonic lethal, it cannot be ruled out that PMCA1 also has an important role in sperm motility, but mice carrying a heterozygous deletion of PMCA1 are not known to have impaired fertility (269).

## B. Role of PMCAs in the Sensory Systems

Due to the nervous system being one of the only areas of the human body where all four PMCA isoforms are expressed, numerous studies have looked at the role of PMCA in neuronal health and disease. As previously mentioned, PMCA2 and PMCA3 have been established as being clinically related to



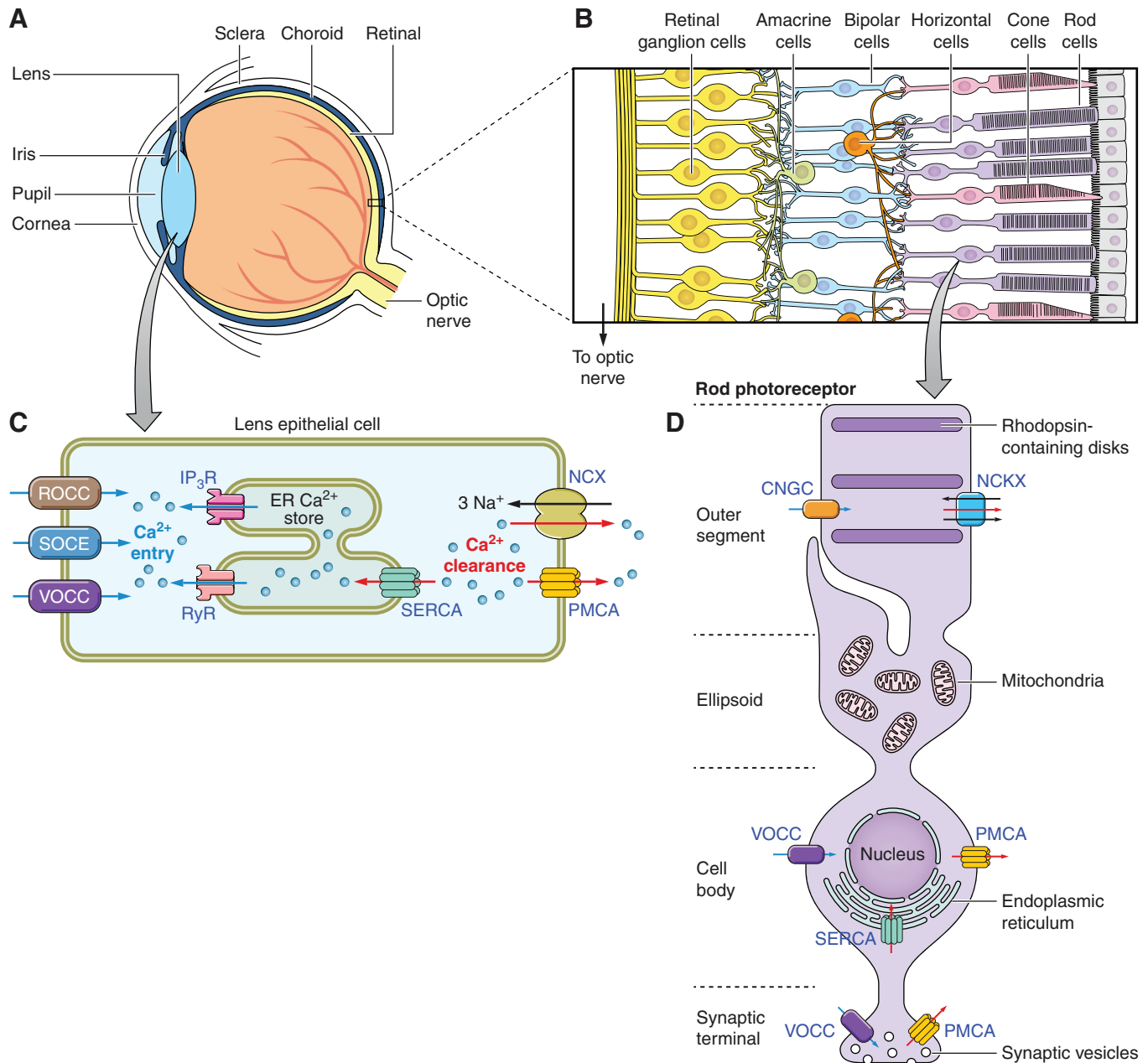
deafness and cerebellar ataxia, respectively (134, 420). Furthermore, PMCA has been reported to be involved in other sensory roles such as vision as well as numerous neuronal conditions associated with balance dysfunction and aging.

### 1. PMCA and the eye

PMCA isoform expression has been reported in various regions of the eye including lens epithelial cells (all PMCA isoforms), the corneal epithelium (all isoforms), lamina cribrosa

cells of the optic nerve head (PMCA1 and 4), various cell types of the inner retina (all PMCA isoforms), and the photoreceptors (PMCA1, 2, and 4) (195, 226, 237, 369). For ease of navigation, the macrostructure of the eye is depicted in **FIGURE 4A**.

The maintenance of low  $[Ca^{2+}]_i$  levels in the lens against the very high concentration in the surrounding aqueous humor requires tight regulation over  $Ca^{2+}$  homeostasis, and the PMCA<sub>s</sub> are among the mechanisms involved in ensuring this control, thus preventing lens opacity (310) (**FIGURE 4B**).



**FIGURE 4.** Cartoon illustrating the macrostructure of the eye (**A**) and the composition of the retina (**B**), in addition to the routes for cytosolic  $Ca^{2+}$  entry and clearance in a lens epithelial cell (**C**) and a rod photoreceptor (**D**). VOCC, voltage-operated  $Ca^{2+}$  channel; SOCE, store-operated  $Ca^{2+}$  entry; ROCC, receptor-operated  $Ca^{2+}$  channel; IP<sub>3</sub>R, inositol trisphosphate receptor; RyR, ryanodine receptor; SERCA, sarco(endo)plasmic reticulum  $Ca^{2+}$ -ATPase; PMCA, plasma membrane  $Ca^{2+}$ -ATPase; NCX, Na<sup>+</sup>/Ca<sup>2+</sup> exchanger; CNGC, cyclic nucleotide-gated  $Ca^{2+}$  channel; NCKX, Na<sup>+</sup>/K<sup>+</sup>/Ca<sup>2+</sup> exchanger.

The PMCA predominantly removes  $\text{Ca}^{2+}$  from lens epithelial cells, whereas expression is absent from fiber cells where the NCX takes on increased importance. It has been known for many years that intracellular  $\text{Ca}^{2+}$  is increased in cataractous lenses (100), and it is not surprising therefore that  $\text{Ca}^{2+}$ -ATPase activity is reduced by 50% in lenses from cataractous donors (284) despite a possibly compensatory upregulation in PMCA2 expression (227). Further evidence for a potentially protective role of the PMCA in cataract prevention has been found in cultured human lens epithelial cells exposed to two major factors involved in cataract development: an increase in PMCA1 expression was observed in response to raised  $[\text{Ca}^{2+}]_i$  (228), and both PMCA1 and 2 expression increased in response to oxidative stress (229).

Altered PMCA expression has also been witnessed in glial fibrillary acid-negative protein lamina cribrosa cells of the optic nerve head obtained from donors with glaucoma, which play an integral role in characteristic extracellular matrix remodelling during this condition. Both PMCA1 and 4 expression were found to be reduced, accompanied by an elevation in  $[\text{Ca}^{2+}]_i$ , most likely due to increased oxidative stress (237).

All four PMCA isoforms are expressed in the human corneal epithelium, and while PMCA4 is the predominant isoform, it appears that each isoform localizes to specific cell layers, which perhaps reflects the different  $\text{Ca}^{2+}$  requirements across the epithelium (369). Isoform-specific functions in the cornea are largely unknown, although experiments using siRNA to knockdown PMCA4 in cultured human corneal epithelial cells suggest an important role in corneal wound healing (368).

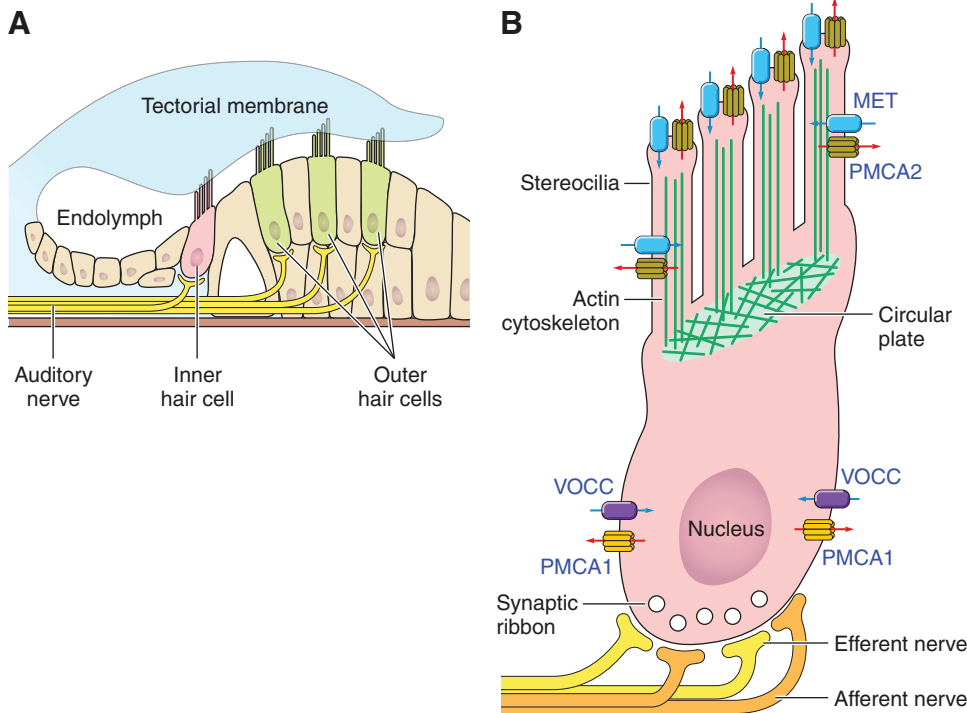
$\text{Ca}^{2+}$  plays myriad roles in the retina, the area of the eye that when hit by light begins the cascade of events which results in stimulation of the optic nerve, from light transduction in photoreceptors to subsequent neurotransmitter release from these and retinal neurons, in addition to its actions as a neuromodulatory intracellular second messenger (8). The retina is composed of rod and cone photoreceptors (FIGURE 4C), with rod cells being of more abundance and higher sensitivity and cone cells being responsible for color vision. Early studies, in the tree shrew and salamander, suggested PMCA was crucial in regulating intracellular  $\text{Ca}^{2+}$  concentration at these photoreceptor terminals to allow the transmission of visual signals in the retina by providing the main  $\text{Ca}^{2+}$  extrusion pathway from inner segments and synaptic terminals of photoreceptor cells (194, 252) as highlighted in FIGURE 4D. PMCA2 has since been identified to be heavily abundant in rod cells, but not cone cells, where it forms a complex with other proteins including membrane palmitoylated protein 4 (Mpp4) (413). Further animal studies suggested this restricted expression of PMCA2 in rod cells is related to visual signaling in this area of the eye. Mice lacking functional PMCA2 have

significantly impaired rod-mediated signaling, with the level of light-evoked synaptic transmission being halved compared with control animals, possibly related to the loss of high-affinity  $\text{Ca}^{2+}$  extrusion causing synaptic delays (101). In addition, PMCA has been shown to regulate  $[\text{Ca}^{2+}]_i$  in rod bipolar cell synaptic terminals both at rest and when stimulated, suggesting a role in adapting to background luminance (390). Finally, there has been a recent report that PMCA1 expression is reduced in the retina of diabetic retinopathy patients as well as fat-fed mice (69).

## 2. PMCA and the ear

The ear is one of the tissues in which we know most about the physiological role of the PMCA, with the cardiovascular system being the other area that has been widely studied, as will be described below. It is PMCA isoform 2 that is of great importance in hearing, with mutations in the protein or its complete loss resulting in impaired hearing or even deafness.

The inner and outer hair cells of the inner ear (FIGURE 5A) have an essential role in converting sound waves, which enter the cochlea, to electrical signals to be transmitted to the brain. The tight regulation of intracellular  $\text{Ca}^{2+}$  concentrations is governed by the mechanoelectrical transduction channels which bring  $\text{Ca}^{2+}$  into the cells and by PMCA2 which is involved in its extrusion (FIGURE 5B). PMCA2 is expressed in specialized regions of the hair cells known as stereocilia that are present in bundles at the apical end of the cell from where they protrude into the endolymph; it is the movement of the stereocilia caused by vibration of the endolymph that leads to opening of the mechanoelectrical transduction channels and subsequent  $\text{Ca}^{2+}$  entry into the cell (as reviewed in Ref. 135). By extruding  $\text{Ca}^{2+}$  from the stereocilia to the endolymph, PMCA2 has a dual role: 1) in maintaining the low  $[\text{Ca}^{2+}]_i$  concentration required within the stereocilia and 2) in supplying essential  $\text{Ca}^{2+}$  to the endolymph which influences the mechanical response of the stereocilia to vibrations (135). PMCA2 is localized to the stereocilia of both the inner and outer hair cells, where its expression progresses along the length of stereocilia in a temporal manner. At birth its expression is localized just to the base, then over a period of several days expression extends along the stereocilia all the way to the apex. This changing pattern of expression mirrors the maturation of the mechanoelectrical transduction channels (73). Using immunogold labeling, Chen et al. (73) were also able to determine that while PMCA2 was expressed on the inner hair cells it was present at a considerably higher density in all three layers of outer hair cells. PMCA2 is not the only isoform to be expressed in the sensory hair cells; the ubiquitously expressed PMCA1 is also expressed. It does however have a pattern of expression distinct from PMCA2 and has been found to be localized to the basolateral plasma membrane of the hair cells, suggesting specialized functions for the two isoforms within the hair cell (99, 139).



**FIGURE 5.** Cartoon illustrating the position of the hair cells of the cochlea within an organ of Corti (A) and sites of  $\text{Ca}^{2+}$  entry and PMCA-mediated extrusion in an inner hair cell (B), namely via PMCA2 in the stereocilia and PMCA1 at the basolateral membrane. VOCC, voltage-operated  $\text{Ca}^{2+}$  channel; MET, mechano-electrical transduction channel; 1, PMCA1; 2, PMCA2.

As has been described above, mutations in PMCA2 have been linked to deafness and hearing loss in humans and in genetically modified mice carrying a variety of PMCA2 mutations. Together this provides substantial support that PMCA2 is crucial in maintaining tight control of the  $\text{Ca}^{2+}$  concentrations required for the hair cells to perform their essential role in the hearing process (38, 123, 336, 355, 365, 392).

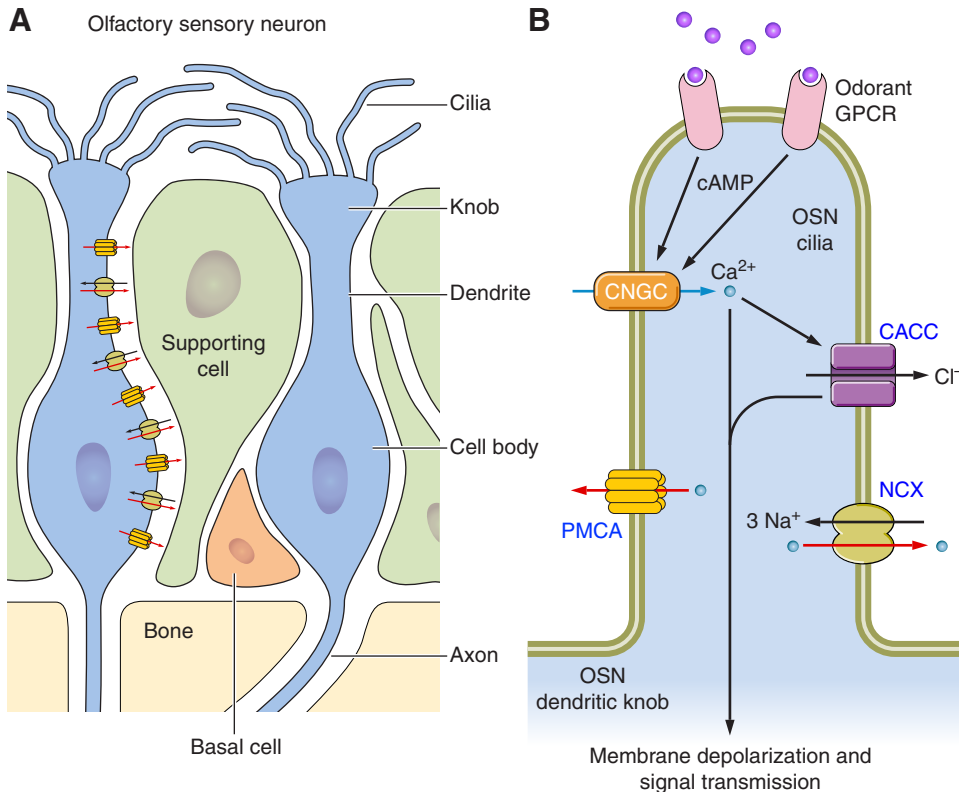
### 3. PMCA and the olfactory system

Initiation of excitation in olfactory sensory neurons (OSN; **FIGURE 6A**) occurs upon binding of odorants to G protein-coupled receptors in the cilia, allowing  $\text{Ca}^{2+}$  entry through cAMP-gated channels and subsequent activation of  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels (238) (**FIGURE 6B**). Signal transduction occurs as the rise in  $[\text{Ca}^{2+}]_i$  travels through the neuron from dendritic knob to dendrite to cell body, and subsequent removal of  $\text{Ca}^{2+}$  to basal levels terminates the signal (321). The PMCA<sub>s</sub>, in addition to NCX and the ER  $\text{Ca}^{2+}$  pump, are responsible for this  $\text{Ca}^{2+}$  clearance, and all four PMCA isoforms have been found to be expressed throughout mouse OSNs with the exception of PMCA3 which is absent from cilia and PMCA4 which has not been reported in the dendrite (393). Treatment of isolated toad OSNs with the PMCA inhibitor carboxyeosin significantly prolonged relaxation of whole cell current in the cilia and slowed  $\text{Ca}^{2+}$  clearance in mouse OSN knobs and cell bodies (62, 321). Saidu et al. (321) found inhibition of PMCA, SERCA, and NCX following OSN stimulation to each delay the rate constant of  $\text{Ca}^{2+}$  clearance by roughly 30%, suggesting a

similar contribution for each system. While the specific function of each isoform has yet to be elucidated, OSNs isolated from PMCA2 knockout mice exhibited significantly impaired  $\text{Ca}^{2+}$  clearance following stimulation to an extent similar to total PMCA inhibition, suggesting a major role for this isoform (321).

### C. Role of PMCA<sub>s</sub> in the Central Nervous System and Neurodegenerative Disease

Neuronal  $\text{Ca}^{2+}$  signaling has many unique functions, being critical to processes such as the regulation of synaptic transmission in the control of neurotransmitter release, and the formation and consolidation of memory (43). In addition,  $\text{Ca}^{2+}$  regulates processes common to other cell types such as differentiation and cell death. In vitro studies in pheochromocytoma cells have shown that knockdown of PMCA1 impairs neurite extension when stimulated with nerve growth factor (40), as does antisense oligonucleotide treatment of PMCA2 and 3 (362), suggesting a role for the PMCA in neuronal differentiation. There is also evidence for a role in the regulation of neuronal cell viability as pheochromocytoma cell survival is respectively improved or impaired by PMCA4 overexpression and knockdown under conditions of  $\text{Ca}^{2+}$  overload (131). Similarly, rat primary neurons and human SH-SY5Y neuroblastoma cells transfected with PMCA2 siRNA display increased levels of basal  $\text{Ca}^{2+}$  and impaired  $\text{Ca}^{2+}$  clearance following stimulation, and increased cell death upon exposure to excitotoxic concentrations of agents increasing  $[\text{Ca}^{2+}]_i$  as well as the glutamate receptor agonist *N*-methyl-D-aspartic acid



**FIGURE 6.** Cartoon illustrating the structure and routes for  $\text{Ca}^{2+}$  extrusion along an olfactory sensory neuron (A) and the mechanisms of excitation and  $\text{Ca}^{2+}$  extrusion in an OSN cilia (B). Following odorant binding to G protein-coupled receptors, generated cAMP activates  $\text{Ca}^{2+}$  entry via cyclic nucleotide-gated  $\text{Ca}^{2+}$  channels which in turn stimulates  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels leading to membrane depolarization and signal transmission along the OSN. Extrusion of  $\text{Ca}^{2+}$  via PMCA and NCX terminates the signal.

(NMDA) (120). Furthermore, there is evidence that a number of neurotoxic agents cause proteolytic degradation of the PMCA in rat cortical cultures, highlighting a potential role in neurodegeneration caused by multiple etiologies (146). Specifically, PMCA1 and 2 expressions have been shown to be reduced in rat hippocampal pyramidal cells following kainate-induced seizures (130), and PMCA4 and 2 each shows displacement from the plasma membrane and internalization in rat hippocampal cells upon exposure to toxic concentrations of glutamate in association with reduced  $\text{Ca}^{2+}$  efflux (295). The following sections will further explore the associations of the PMCA with specific neurodegenerative diseases.

### 1. PMCA and cerebellar disorders

The cerebellar cortex, composed principally of Purkinje neurons, is the region of the brain predominantly involved in motor control and sensory perception. The PMCA in conjunction with NCX is responsible for precise control of local  $\text{Ca}^{2+}$  at excitatory parallel fiber-Purkinje neuron synapse terminals, thus modulating neurotransmitter release (316). Immunolocalization analysis of rat cerebellum suggests that isoforms display specific patterns of expression, with “neuronal” isoforms PMCA2 and 3 being predominantly concentrated in post- and presynaptic terminals, respectively (48). In contrast, the “ubiquitous” isoforms are expressed at lower levels (47), where they may reside in lipid rafts of cerebellar granule neurons in association with NMDA receptors to regulate nitric oxide production (231).

As previously mentioned, two independent mutations in the PMCA3 gene affecting calmodulin binding and  $\text{Ca}^{2+}$  ejection, respectively, have been identified in patients presenting with cerebellar ataxia (50, 420); however, there is substantial evidence from PMCA2 mutant mice that this isoform is also critical to cerebellar function. PMCA2 is strongly expressed in Purkinje cells, and both pharmacological inhibition and genetic ablation of PMCA2 from Purkinje neurons impair dendritic growth (109, 342). Furthermore, in addition to the aforementioned hearing loss, PMCA2 null mutant mice display overt cerebellar ataxia apparent 12 days after birth accompanied by a severe inability to maintain balance and reduction in body weight (192). Similarly, the *wriggle mouse sagami* strain, which has a point mutation in the PMCA2 gene, displays involuntary dystonic movements of the extremities, writhing and wriggling of the trunk and neck, and difficulty maintaining an upright posture (166, 376). Mice with heterozygous inactivation of the gene, however, appear outwardly normal at rest, but experience abnormal coordination of the hindlimbs upon exercise (111).

Studies in these transgenic mice have identified a number of defects in cerebellar neuronal function and  $\text{Ca}^{2+}$  homeostasis. Cerebellar slices from *wriggle mouse sagami* for example display a smaller rise in  $[\text{Ca}^{2+}]_i$  and impaired clearance following stimulation (376) in addition to a reduction in synaptic connections between parallel fibers and Purkinje dendritic spines (166). Purkinje neurons from PMCA2 knockouts meanwhile exhibit lower presynaptic  $\text{Ca}^{2+}$  ex-



trusion at these junctions, together with increased basal  $\text{Ca}^{2+}$  and impaired clearance, and increased firing of post-synaptic inhibitory neurons which contributes to reduced and irregular firing of action potentials in the Purkinje cells (108–110). The phenotype of the PMCA2 knockout mouse may be further exacerbated by the identification of a novel association with the metabotropic glutamate receptor 1 (mGluR1), known to play essential roles in motor coordination, synaptic plasticity, and associative learning, which is downregulated in the knockout cerebellum along with subsequent downstream signaling (197). While the phenotype in heterozygous null mutants is less overt, there are reports of impaired neuronal function in the Purkinje cells. These include increased amplitude and slower recovery times of  $\text{Ca}^{2+}$  transients, reduced firing frequency of action potentials, and loss of Purkinje cells by 20 wk of age (111, 115).

## 2. PMCA<sub>s</sub> in diseases of the spinal cord

There is a growing body of evidence to suggest that the PMCA may play a protective role in preventing spinal cord pathology. This may be highlighted by the finding that PMCA1 and 3 are both downregulated in brain lesions obtained from multiple sclerosis (MS) patients at autopsy (217). Work in the rat using the experimental autoimmune encephalomyelitis (EAE) model of MS, in which demyelination and subsequent ascending weakness and paralysis is induced through immunization by myelin injection into the foot, indicates a dramatic reduction in PMCA2 expression in spinal cord neurons in a pattern mirroring disease course, which becomes restored during recovery (261, 262). A similar downregulation of PMCA2 expression has also been witnessed upon exposure of spinal cord neurons to the glutamate receptor agonist kainate (198, 262).

Further evidence of a vital role for the PMCA in maintaining  $\text{Ca}^{2+}$  homeostasis and neuronal health in the spinal cord has been detected upon pharmacological inhibition in cultured rat spinal cord neurons. Kurnellas et al. (199) found the pan-PMCA inhibitor carboxyeosin delayed  $\text{Ca}^{2+}$  clearance with subsequent promotion of caspase activity and neuronal death. The authors also found PMCA2 knockout and functionally inactive *deafwaddler* mice to exhibit a significant loss of spinal cord motor neurons (199), with further exploration revealing that PMCA2 silencing in these cells reduced the expression of collapsing response mediator protein 1 (CRMP1) before neuronal degeneration (198). A recent study also suggests that PMCA2 and 3, in complex with NCX1 in lipid rafts, are critical for the function of the neuronal glycine transporter 2 (GlyT2), and hence regulating inhibitory glycinergic signaling, in brain stem and spinal cord neurons, disturbances which are known to be a major cause of the rare condition hyperekplexia (87).

## 3. PMCA and age-related neurological disorders

Advancing age often coincides with a progressive decline in neuronal function, thought to be largely driven by the actions of reactive oxygen species (ROS). PMCA activity and abundance declines substantially with age in synaptic plasma membranes from rats, as does the extent of PMCA activation by aged calmodulin (CaM) due to the oxidation of CaM methionine residues (172, 240, 418). Likewise, a range of oxidative agents have been shown to induce proteolytic degradation and a reduction of PMCA activity in rat synaptic membranes and cortical neurons (417, 419), as well as causing PMCA internalization and loss of expression in primary hippocampal neurons (184). Given the critical importance of the PMCA in maintaining  $[\text{Ca}^{2+}]_i$ , neuronal function, and viability, it is therefore highly likely that ROS-induced inactivation of the PMCA may contribute to age-related neurodegeneration.

In addition to the aging brain, PMCA expression and activity have also been shown to be reduced in the cortex of brains excised post mortem from patients with Alzheimer's disease (24, 25). Alzheimer's disease is characterized by an accumulation of amyloid  $\beta$ -peptide ( $\text{A}\beta$ ) and tau protein, and studies have found each of these to have an inhibitory effect on PMCA activity in membrane vesicles from human hippocampus and cerebral cortex, with  $\text{A}\beta$  specifically affecting the PMCA4 isoform (24, 25, 230). Recent data suggest that PMCA activity may also be impaired in human brain tissue from Parkinson's disease patients (416), a disease characterized by loss of neurons in the substantia nigra region of the midbrain leading to insufficient dopamine secretion. This observation is supported by studies using the Parkinsonian mimetic 1-methyl-4-phenylpyridinium ( $\text{MPP}^+$ ), which causes an increase in  $[\text{Ca}^{2+}]_i$  and reduction in PMCA2 expression in SH-SY5Y cells and rat primary midbrain neurons (42). Interestingly Brendel et al. (42) also demonstrated that transfection of PMCA2 siRNA reduced neuronal viability while overexpression rendered cells resistant to  $\text{MPP}^+$ -induced toxicity.

Overall, there is a substantial body of evidence to suggest that multiple PMCA isoforms play a vital role in maintaining neuronal viability and synaptic function through precise regulation of  $\text{Ca}^{2+}$  homeostasis and local signaling. It may be that future therapies for excitotoxic and age-related neurodegeneration may look to stabilizing or restoring PMCA expression and function to prevent neuronal loss.

## D. Role of PMCA<sub>s</sub> in the Cardiovascular System

### 1. PMCA and the heart

It is now well established that  $\text{Ca}^{2+}$  has a dual role in the heart: 1) it is the driving force for myofilament contraction

and relaxation, and 2) it has the ability to regulate the intracellular signaling events, several of which dictate pathological cardiac remodeling associated with hypertrophy and heart failure.

With the bulk of intracellular  $\text{Ca}^{2+}$  being extruded from the cytosol into the sarcoplasmic reticulum (SR) of the cardiomyocyte by the SERCA and out of the cell via the NCX, the PMCA4s make only a minor contribution to  $\text{Ca}^{2+}$  extrusion during diastole and consequently have traditionally been considered relatively insignificant in terms of normal  $\text{Ca}^{2+}$  homeostasis in the heart (26, 77, 266, 405).

The presence of two isoforms (PMCA1 and PMCA4) suggests specialized functions, and in recent years, through the use of genetically modified mice, a role has emerged for PMCA4 in the regulation of signal transduction processes. In contrast, there is still little known of the role of PMCA1.

With its 10 transmembrane domains and large intracellular loops, PMCA acts as a structural protein providing a scaffold to anchor interacting proteins at the plasma membrane. Within the heart, these interacting proteins include nNOS and  $\alpha$ 1-syntrophin which interact with both PMCA4 and PMCA1, calcineurin A, and Ras-associated factor 1 (RASSF1) (13, 46, 335, 395). While the precise function is not yet known of some of these interactions, others have been well characterized to be of functional significance.

Of particular note in the cardiovascular system, the binding of PMCA4 and nNOS at its PDZ domain has been shown to influence basal contractility and  $\beta$ -adrenergic responsiveness as well as, as will be discussed in the next section, to have an influence on vascular tone (60, 61). This interaction with nNOS was first identified in cultured cells, where PMCA4 overexpression resulted in a reduction of nNOS activity, postulated to occur due to the decreased availability of local  $\text{Ca}^{2+}$ , while a mutant nNOS molecule lacking its PDZ domain was unaffected (335). A functional interaction was subsequently identified in the heart, where PMCA4 overexpression was found to attenuate the inotropic response to  $\beta$ -adrenergic stimulation to an extent comparable to that seen following nNOS-specific inhibition in controls, but not in mice overexpressing a mutant form of PMCA4 unable to interact with nNOS (266). This pathway has since been characterized to affect local cGMP and hence cAMP levels, thus modulating PKA activity at the SR (244, 245). Interestingly, the latter of these studies found PMCA4 knockout mice to display a similar attenuation of the  $\beta$ -adrenergic response, but have enhanced basal contractility correlating with an increase in systolic  $\text{Ca}^{2+}$  due to phosphorylation of the RyR at PKA-dependent serine residues. Furthermore, the PMCA4-nNOS interaction may also have disease implications following myocardial infarction, when nNOS in association with its adaptor protein CAPON

(COOH-terminal PDZ ligand of NOS1), have been shown to be recruited to the sarcolemma to bind to PMCA4 (22).

$\alpha$ 1-Syntrophin is a cytoskeletal protein which like nNOS has a PDZ binding domain; however, although  $\alpha$ 1-syntrophin interacts directly with PMCA1 and PMCA4, it does so by binding to a domain on the catalytic second intracellular loop of PMCA. In the heart PMCA4 can form a ternary complex with nNOS and  $\alpha$ 1-syntrophin which results in a synergistic inhibition of nNOS-mediated NO production (395).

RASSF1 also binds to the same domain on the second intracellular loop of PMCA. A function of this interaction became apparent when PMCA4 was overexpressed in cultured HEK293 cells which resulted in the reduced activity of RASSF1A, and inhibition of the activation of Ras-mediated signaling (13). At the time RASSF1A, although a potential upstream regulator of mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling, was not known to be associated with the hypertrophic process. However, RASSF1 knockout hearts have since been shown to have an exacerbated response to pressure overload, through mediating ERK1/2 signaling (268). More recently, it has been demonstrated that RASSF1 mediates the TNF- $\alpha$ -induced contractile response and signaling in the heart; however, it is not yet known whether these roles of RASSF1 in the heart are directly influenced by its interaction with PMCA4 (247, 268).

An association between the PMCA and calcineurin has been witnessed in multiple isoforms in a number of cell types. The interaction was first witnessed in PMCA4 overexpressing HEK cells and mapped to the catalytic subunit of calcineurin A, with consequential attenuation of calcineurin-nuclear factor of activated T cells (NFAT) signaling (46). Calcineurin has since been noted to also interact with PMCA2 and 4 in breast cancer cells and PMCA1, 2, and 4 in endothelial cells (158, 159), as well as with PMCA4 in the PC12 pheochromocytoma cell line (189). Calcineurin-NFAT signaling is one of the best characterized pathways in the development of pathological hypertrophy and remodeling (248), and thus this has prompted research into the function of this interaction during the hypertrophic process. PMCA4 overexpression was found to significantly reduce the extent and progression of pathological remodeling through the attenuation of NFAT signaling (405), and interestingly, we have recently found that deletion of PMCA4 also attenuates hypertrophic remodeling, through a calcineurin-independent mechanism (242).

Using a number of mouse models in which PMCA4 was deleted globally, or specifically from the cardiomyocytes or from the fibroblasts, we demonstrated that PMCA4 is involved in the development of pathological cardiac hypertrophy by regulating  $\text{Ca}^{2+}$  signaling in cardiac fibroblasts

which in turn is essential in the regulation of cardiac hypertrophy (242). Total PMCA4 knockout and deletion of PMCA4 specifically from the fibroblasts protected the animals against pressure overload-induced cardiac hypertrophy, but this protection was not evident when PMCA4 was deleted specifically from the cardiomyocytes. Ablation of PMCA4 from cardiac fibroblasts led to an increase in  $[Ca^{2+}]_i$  which subsequently increased secreted frizzled related protein 2 (sFRP2) transcription, an inhibitor of the Wnt signaling pathway known to be protective against injury of the myocardium (151, 242). Secretion of sFRP2 from PMCA4 ablated fibroblasts then acted in a paracrine fashion to reduce cardiomyocyte hypertrophy and confer protection against the development of heart failure.

Cardiac hypertrophy almost invariably precedes the development of heart failure, and as an important determinant of disease progression and clinical outcome in heart failure patients, cardiac hypertrophy presents a clear target for disease prevention and treatment. With this in mind we set out to identify a pharmacological inhibitor of PMCA4 to use as an anti-hypertrophic agent. There is precedent that as an ATPase the PMCA would make a suitable drug target in that two of our most clinically successful drugs over the past years have been the cardiovascular drug ouabain and the antacid omeprazole, inhibitors of the  $Na^+-K^+-ATPase$  and the  $H^+-K^+-ATPase$ , respectively. The ease with which the inhibition of the ATPase activity of PMCA4 (and other ATPases) can be measured lends itself to drug screening and led to the identification of aurintricarboxylic acid (ATA) as a potent inhibitor of PMCA4 (58, 241, 243, 246). ATA was found to inhibit PMCA4 at low concentrations (having an  $IC_{50}$  of 150 nM) and to have minimal effect on PMCA1, the other isoform of PMCA present in the heart (241). This novel and potent inhibitor of PMCA4 was successfully used to both prevent the development of pathological cardiac hypertrophy in an *in vivo* mouse model, and of greater potential clinical relevance it was shown to have the ability to reverse established cardiac hypertrophy in the mouse (242). To add further weight to the notion of assaying PMCA4 inhibition as an anti-hypertrophic therapy, it has recently been shown that crossing PMCA4 null mice with mice expressing a mutant form of  $\alpha$ -tropomyosin prevents the development of spontaneous hypertrophic cardiomyopathy which would otherwise occur (297).

## 2. PMCA and the vasculature

In addition to its regulatory roles in contractile and hypertrophic processes in the heart, several roles have been identified for the PMCA in noncardiac cell types in the cardiovascular system (59). In contrast to cardiac muscle, it has been demonstrated that the PMCA accounts for the bulk of  $Ca^{2+}$  extrusion from vascular smooth muscle, and its inhibition prolongs aortic relaxation to a similar extent to that of SERCA while NCX inhibition has little effect (298), and in myometrial resistance arteries PMCA inhibition mimics

the phenotype of preeclamptic vessels (398). As described in section IIIA, PMCA1 has been identified in numerous studies as a leading candidate gene for the development of hypertension. It is perhaps not surprising therefore that both PMCA1 and 4 have been shown to influence vascular tone (129, 141, 187, 333, 343) and that they have been identified as potential targets for the treatment of essential hypertension (214).

Both PMCA1 and PMCA4 have been identified in vascular smooth muscle cells from a variety of species including mouse, rat, and pig (4, 187, 269, 276, 326, 363). Both have also been identified in endothelial cells (276, 363). Adding extra weight to the potential significance of the expression of the PMCA<sub>s</sub> in the vasculature is the fact that functional interactions between PMCA1 and 4 and eNOS, the major producer of the vasodilator NO in the vasculature, have been identified in endothelial cells (158). However, it is again through the study of a number of mouse models carrying genetic mutations in either PMCA1 or PMCA4 that we have gained our understanding of the role of these genes in the regulation of vascular tone and blood pressure.

There is an accumulation of evidence that PMCA1 is important in arterial contractility and blood pressure regulation and that modified expression results in hypertension. Three different mouse models, all of which exhibit reduced expression of PMCA1, develop raised blood pressure. Hypertension in a model in which PMCA1 was specifically deleted from the vascular smooth muscle cells was found to correlate with increased intracellular  $Ca^{2+}$  (187), global heterozygous deletion of PMCA1 also resulted in raised blood pressure (129), and PMCA1 knockdown by siRNA led to an elevation in blood pressure (343) associated with structural changes to the walls of the resistance arteries.

Although to date there is no GWAS data linking PMCA4 with hypertension, there is evidence from mouse models that it is important in the regulation of vascular tone and blood pressure. Mice overexpressing PMCA4 in smooth muscle cells have been shown to have elevated blood pressure (141, 333). While this may appear counterintuitive as increased expression of a  $Ca^{2+}$  extrusion pump would potentially lead to lower intracellular  $Ca^{2+}$  levels and subsequent vascular relaxation, it is in fact through the negative regulation of nNOS that PMCA4 acts to regulate vascular contractility (141, 333).

## E. Role of PMCA<sub>s</sub> in Hematopoietic Cells

With PMCA first discovered and isolated in human erythrocytes (327), numerous studies have looked at the role of the pump in hematopoietic cells. Perhaps more than any other cell population, the PMCA is the predominant  $Ca^{2+}$  extrusion system in blood cells (erythrocytes, leukocytes, and platelets) where maintenance of intracellular  $Ca^{2+}$  lev-



els is essential for the regulation of cellular function, signaling, metabolism, and blood rheology (33, 211, 313). Variations in PMCA activity can impact  $\text{Ca}^{2+}$  homeostasis in blood cells and have been associated with numerous aspects of health and disease, as we will highlight below.

### 1. PMCA in erythrocytes

Aside from some internal buffering capacity, the PMCA is the sole mechanism responsible for the maintenance of free cytosolic  $[\text{Ca}^{2+}]$  in erythrocytes (122, 210). Coupled with the relative ease with which patient samples can be obtained, this has made red blood cells and red blood cell ghosts (erythrocytes which do not contain hemoglobin or other cytoplasmic components but retain their shape) a popular choice in which to study the biochemical properties of human PMCA. Using these models, researchers have been able to identify abnormalities in PMCA activity in a number of disease states (28, 291). In addition, purified preparations of human erythrocyte PMCA have been widely used to identify molecular and pharmacological modulators of the pumps' activity such as F- and G-actin, acidic phospholipids, diacylglycerol, the bidentate chromium (III) ATP complex, the phytoalexin resveratrol, and inhibitory peptide caloxin1c2 (251, 263, 277, 278, 289, 383).

In erythrocytes,  $\text{Ca}^{2+}$  regulates numerous parameters such as cell volume and rheological properties, metabolic activity, redox state, and cell clearance (33). Human erythrocyte PMCA is composed primarily of PMCA4, with PMCA1 expression also having been identified (188, 352). PMCA activity declines as red blood cells age in line with increasing glycated hemoglobin, which may make them prone to  $\text{Ca}^{2+}$  overload and earmark them for clearance (207).

Elevated  $[\text{Ca}^{2+}]_i$  is a feature of a number of diseases of the erythrocyte associated with hemolytic anemia such as sickle cell disease (SCD),  $\beta$ -thalassaemia, and familial phosphofructokinase (PFK) deficiency (103, 315, 340). In SCD- and PFK-deficient cells,  $\text{Ca}^{2+}$  accumulates via increased entry through the red cell membrane, in a stochastic fashion in the case of sickled cells (209), likely causing a reduction in deformability (the ability for cells to change shape in response to flow) and leaving them prone to hemolysis (114, 315). Interestingly, in SCD a reduction in PMCA activity is likely to contribute to this  $\text{Ca}^{2+}$  accumulation (114), whereas in PFK deficiency a compensatory increase in PMCA activity to clear the  $\text{Ca}^{2+}$  leads to ATP depletion (315). There is also a suggestion that in an effort to prevent  $\text{Ca}^{2+}$  overload, both SCD and  $\beta$ -thalassaemia erythrocytes are able to store  $\text{Ca}^{2+}$  in endocytic inside-out vesicles, pumping it in via the PMCA (35, 208).

Hypertension is also associated with a decrease in red blood cell deformability, where it may contribute to an increased risk of vascular occlusion (78, 249). PMCA activity is re-

duced in erythrocytes from hypertensive patients and appears directly correlated with erythrocyte deformability. Studies suggest deformability reduces by 55% upon PMCA inhibition and increases upon PMCA activation with neutral or acidic phospholipids (249). Similarly, a 50% reduction in erythrocyte PMCA activity has been reported in women suffering from preeclampsia (259), and this has been ascribed to changes in lipid composition, specifically an increase in the level of lipid peroxidation (273).

### 2. PMCA in leukocytes

$\text{Ca}^{2+}$  signaling in leukocytes is required for processes such as lymphocyte activation, granule secretion in granulocytes, and the regulation of cell motility and death (211, 328). The presence of a PMCA, thought to be predominately isoform 4, has been demonstrated in all classes of lymphocytes, as well as neutrophils, monocytes, macrophages, and mast cells (67, 132, 177, 211, 329, 338).

The PMCA represents the major route for  $\text{Ca}^{2+}$  clearance from human T cells, where it regulates  $[\text{Ca}^{2+}]_i$  in conjunction with the main  $\text{Ca}^{2+}$  entry channels, calcium release-activated channels (CRAC, encoded by ORAI) (211). To facilitate T-cell activation, there is a redistribution of the PMCA to maximize  $\text{Ca}^{2+}$  entry through CRAC (299), and decreased PMCA-mediated efflux through an interaction with stromal interacting molecule-1 (STIM1) (314) which together increase cytosolic  $\text{Ca}^{2+}$  thus activating NFAT. In contrast, defective PMCA signaling, for example through decreased expression following exposure to ROS, can promote T-cell apoptosis (286). There is also evidence that through an interaction with CD147, PMCA4 is able to modulate the immune response in T cells by inhibiting the production of interleukin-2 (361). A rise in  $\text{Ca}^{2+}$  in B-lymphocytes meanwhile is important for their proliferation, differentiation, and antibody production, and studies have shown that through positive and negative PMCA regulation, respectively, CD22 and myc transcription factors are able to either inhibit or stimulate these processes (71, 144).

PMCA activity has also been shown to be altered in disease states in neutrophils. Both hepatocytes and neutrophils from patients with alcoholic liver disease exhibit elevated basal  $\text{Ca}^{2+}$  and impaired PMCA-mediated efflux (16), while the ability of PMCA inhibition to attenuate neutrophil apoptosis is lost in patients with uremia (80).

### 3. PMCA in platelets

Tight regulation of platelet  $\text{Ca}^{2+}$  homeostasis is essential for maintaining low resting  $\text{Ca}^{2+}$  levels and also allowing steep rises to enable platelet activation, platelet aggregation, and thrombus formation at sites of injury; underactive platelets are associated with bleeding disorders, while hyperactivation can result in thromboembolism (88, 313). As



is the case in erythrocytes and leukocytes, the PMCA is the major  $\text{Ca}^{2+}$  extrusion system in platelets (318), with expression studies showing PMCA4 to be the predominant isoform (281). Studies have found that PMCA4 is recruited to the cytoskeleton during platelet activation through an interaction with the LIM family protein CLP36 to facilitate clot formation (39). The critical role of the PMCA during aggregation has been highlighted by studies using platelets from PMCA4 knockout mice, which display impaired platelet aggregation when stimulated with collagen (174). Similar results were obtained upon incubation of platelets with the PMCA inhibitor carboxyeosin, which led to elevated resting  $\text{Ca}^{2+}$ , but smaller increases in  $\text{Ca}^{2+}$  upon stimulation alongside reduced aggregation (174).

Altered platelet PMCA activity has been witnessed in a number of disease states associated with an increased risk of clot formation or bleeding. As we will discuss in the following section, platelet PMCA4 expression is elevated during diabetes, but this is accompanied by an overall reduction in PMCA activity in type 2 diabetic platelets as a result of increased tyrosine phosphorylation (66, 168, 317). A similar reduction in PMCA activity has been reported in platelets from hypertensive patients in a manner that correlates with increasing diastolic blood pressure, again despite increased expression of PMCA4 (82, 89). As in diabetes, this occurs as a result of tyrosine phosphorylation, leaving platelets with elevated  $[\text{Ca}^{2+}]_i$  and in a potentially hyperactivated state prone to spontaneous aggregation and thrombus formation (30). Increased PMCA4 expression has also been reported in a family with type 2B von Willebrand disease, where it is associated with the production of immature megakaryocytes and severe thrombocytopenia (265), while increased PMCA activity has been found in platelets from obese patients associated with increased anisotropy (302).

## F. Role of PMCA<sub>s</sub> in Pancreatic $\beta$ -Cell Function and Diabetes

An increase in cytosolic  $\text{Ca}^{2+}$  is required to stimulate insulin release from pancreatic  $\beta$ -cells in response to a rise in glucose (401), and as such, disturbances in the regulation of  $[\text{Ca}^{2+}]_i$  can have profound effects upon  $\beta$ -cell function and may be a potential mechanism leading to the development of type 2 diabetes. In addition,  $\text{Ca}^{2+}$  signaling is important in regulating  $\beta$ -cell mass, apoptosis, and proliferation, key events in the cytokine and nutrient-induced destruction of pancreatic islets which occur in type 1 and type 2 diabetes, respectively (79, 154).

$\text{Ca}^{2+}$  clearance from pancreatic  $\beta$ -cells is regulated by the SERCA, NCX and PMCA, with relative contributions estimated to occur at around a 2:1:1 ratio among these three systems in mice (72). Surprisingly for nonneuronal cells, all four PMCA isoforms are expressed at the mRNA and pro-

tein level in rat islets, including up to eight splice variants (175), suggesting highly specialized functions. Unusually, the “neuronal” isoforms PMCA2 and 3 may have particular importance in  $\beta$ -cells as their expression is downregulated upon exposure of insulin-producing RINm5F cells to the inflammatory cytokine interleukin-1 $\beta$ , thought to be a major cause of autoimmune death in type 1 diabetes (349). In addition, glucose has been shown to decrease PMCA activity, suggesting a possible link to altered  $\beta$ -cell function in type 2 diabetes (153).

Gain- and loss-of-function studies have shown that altered expression of either NCX1 or PMCA2 is sufficient to modulate insulin secretion,  $\beta$ -cell mass, proliferation, and survival (155). PMCA2 overexpression in insulin-secreting BRIN-BD11 cells leads to reduced increases in intracellular  $\text{Ca}^{2+}$  in response to depolarization and glucose accompanied by increased glucose metabolism and insulin secretion (176), while depleting cytosolic, mitochondrial, and ER  $[\text{Ca}^{2+}]$  and triggering apoptosis via the mitochondrial pathway (171). In contrast, pancreatic islet cells isolated from mice with heterozygous PMCA2 deletion were found to display increased  $\text{Ca}^{2+}$  stores and glucose-induced insulin release, along with having higher rates of  $\beta$ -cell proliferation with greater mass, viability, and islet size (275).

Diabetes is of course a major risk factor for the development of various diseases, and PMCA activity has been found to be altered in numerous nonpancreatic cell types both clinically and in experimental models of diabetes. A number of studies have found abnormal PMCA function in hematopoietic, vascular smooth muscle, or endothelial cells which may contribute to the increased risk of cardiovascular complications such as thrombus formation and atherosclerosis in diabetic patients.

An examination of  $\text{Ca}^{2+}$  clearance proteins in platelets from diabetic patients has shown PMCA4 expression to be increased in both insulin-dependent and -independent diabetes, and this can be corrected upon insulin treatment in type 1 diabetics (66). In line with the increased expression, platelets from insulin-dependent diabetic patients have previously been found to have higher PMCA activity (234), while incubation of platelets from healthy donors with low-density lipoprotein (LDL) isolated from type 1 diabetic patients also increased PMCA activity along with resting  $[\text{Ca}^{2+}]_i$  and platelet aggregation responses (300). On the contrary, PMCA activity appears to be reduced in platelets from type 2 diabetic patients likely due to increased tyrosine phosphorylation as a result of oxidative stress (168, 317), as well as in lymphocytes which may contribute to higher basal  $[\text{Ca}^{2+}]_i$  in these cells (19).

Similar to the situation seen in platelets, human aortic endothelial cells incubated with type 1 diabetic patient LDL were found to have increased basal  $[\text{Ca}^{2+}]_i$  and higher

PMCA activity, along with alterations in the plasma membrane structure which could lead to an atherogenic phenotype (301). Meanwhile, coronary smooth muscle cells isolated from swine following induction of diabetic dyslipidemia have been found to have increased basal  $[Ca^{2+}]_i$  due to reduced PMCA-mediated efflux, a phenomenon which can be prevented through exercise training (399, 400).

Altered PMCA activity may also lead to disturbances in  $Ca^{2+}$  homeostasis in other cell types during diabetes. For example, basolateral membrane vesicles isolated from syncytiotrophoblast of insulin-dependent diabetic patients display increased PMCA activity (359), while reduced PMCA activity or expression has been identified in parotid and submandibular salivary glands, kidney, and brain synaptosomes of streptozotocin-induced diabetic rats (260, 347, 421).

## G. Role of PMCA in Visceral Smooth Muscle

Smooth muscle contraction is triggered by the influx of extracellular  $Ca^{2+}$  following membrane depolarization through  $Ca^{2+}$  channels at the sarcolemma, or via  $IP_3$ -mediated SR  $Ca^{2+}$  release following agonist-induced activation of G protein-coupled receptors. Through its binding of calmodulin, the rise in cytosolic  $Ca^{2+}$  activates myosin light chain kinase (MLCK) leading to phosphorylation of the myosin light chain (MLC), thus increasing actin-myosin crossbridge formation and stimulating contraction. Relaxation is brought about by a decrease in cytosolic  $Ca^{2+}$  via SERCA-mediated SR reuptake and sarcolemmal extrusion through the PMCA and NCX, with inactivation of MLCK and activation of MLC phosphatase leading to the dephosphorylation of the MLC. The physiological role of the PMCA in this process in visceral smooth muscle will be discussed below.

### 1. PMCA in uterine smooth muscle

The contractile state and function of the myometrium influences reproductive processes such as implantation and parturition and can contribute to a number of disease states including spontaneous miscarriage, preterm labor, dysmenorrhea, and endometriosis (5).

Experiments in rat uterine smooth muscle cells have determined that  $Ca^{2+}$  extrusion at the plasma membrane is required for full recovery of transients induced by both membrane depolarization and  $Ca^{2+}$  release from internal stores (345). In the nonpregnant myometrium, it is believed that the PMCA is responsible for producing ~85% of relaxation and 70% of  $Ca^{2+}$  extrusion, while work in PMCA4 ablated mice has identified that ~80% of the PMCA contribution can be attributed to this isoform (233). In the endometrium however, PMCA1 appears to be the dominant isoform, and

its expression has been found to increase along with TRPV6 during the proliferative phase of the menstrual cycle (412).

In contrast to nonpregnant myometrium, it has been suggested that up to 70% of  $Ca^{2+}$  efflux occurs via the NCX in the pregnant rat uterus (344). This switch in mode of  $Ca^{2+}$  extrusion may be explained by an examination of the effects of physiological concentrations of oxytocin on human myometrium taken at term, which found the hormone to strongly inhibit  $Ca^{2+}$ -ATPase activity and thus induce contraction (294), a phenomenon which has also been reciprocated at late stages of gestation in the rat (222). Surprisingly, oxytocin's inhibitory actions on PMCA and SERCA activity at term occur despite an increase in expression of both pumps in the human myometrium during active labor (374).

### 2. PMCA in bladder smooth muscle

Tight regulation of  $[Ca^{2+}]_i$  in detrusor smooth muscle of the urinary bladder is critical to allow for relaxation while filling and storing urine, and contraction during micturition. Disturbances in these processes can lead to lower urinary tract symptoms such as urgency, frequency, and incontinence, which primarily manifest themselves with increasing age (10). Indeed, studies in guinea pig smooth muscle have found  $Ca^{2+}$  extrusion to be impaired in aged animals as a result of decreased PMCA activity (138).

With the use of inhibitors to SERCA and NCX in PMCA gene-ablated mice, the relative contribution of the PMCA to relaxation of bladder smooth muscle rings was estimated to be ~25–30%, slightly greater than that of SERCA and with the NCX responsible for up to 70% of relaxation (216). This is largely compatible with data from isolated guinea pig detrusor smooth muscle cells which, using inhibitors to each system, estimated the relative contributions of PMCA, NCX, and SERCA to  $Ca^{2+}$  clearance to be 27, 55, and 31%, respectively (138). Interestingly, Liu et al. (216) also found PMCA ablation to impair the rate of contraction, and in a further study in which contraction and  $[Ca^{2+}]_i$  were measured simultaneously, it was determined that the loss of PMCA4 significantly inhibited the force and rate of contraction, as well as the rates of increase and decrease of  $[Ca^{2+}]_i$  following cholinergic stimulation (215). In contrast, the authors found heterozygous PMCA1 deletion to elicit larger increases in  $[Ca^{2+}]_i$  and rate and force of contraction following KCl stimulation, suggesting a greater role for this isoform in overall  $Ca^{2+}$  extrusion.

## H. Role of PMCA in Epithelial $Ca^{2+}$ Absorption and Tissue Mineralization

Regulated  $Ca^{2+}$  absorption is critical in maintaining serum  $[Ca^{2+}]$  at desirable levels for a wide range of normal physiological functions, in addition to the mineralization of cal-

cified tissues such as bone and enamel. In mammals, the bulk of  $\text{Ca}^{2+}$  transport occurs in the small intestine and kidney, either paracellularly directly from the lumen to the extracellular space via tight junctions, or transcellularly through epithelial cells (157) (FIGURE 7). The PMCA, along with NCX1, performs the final step in transcellular  $\text{Ca}^{2+}$  absorption, extruding  $\text{Ca}^{2+}$  from the epithelia to the interstitial space at the basolateral membrane following its entry from the lumen via TRPV5/6 channels and subsequent diffusion through the cytosol bound to calbindin. The importance of PMCA1, the major isoform in epithelial absorption, in maintenance of serum ionic balance can be highlighted by the genome-wide association of the SNPs rs7965584 in the region of *ATP2B1* with serum  $\text{Mg}^{2+}$  concentration (239). This section discusses the role of the PMCA in intestinal and renal  $\text{Ca}^{2+}$  absorption, how it is affected by disease states, and the implications this can have on skeletal and dental mineralization.

### 1. PMCA and intestinal $\text{Ca}^{2+}$ absorption

In the intestine, the majority of transcellular  $\text{Ca}^{2+}$  absorption occurs in the duodenum (181), where it is primarily extruded from enterocytes via the PMCA (95). In addition, it has been demonstrated that the PMCA plays an active role in  $\text{Ca}^{2+}$  transport in the large bowel, with basolateral membrane vesicles isolated from human colon able to uptake  $\text{Ca}^{2+}$  in a  $\text{Mg}^{2+}$ /ATP-dependent calmodulin-regulated manner (323).

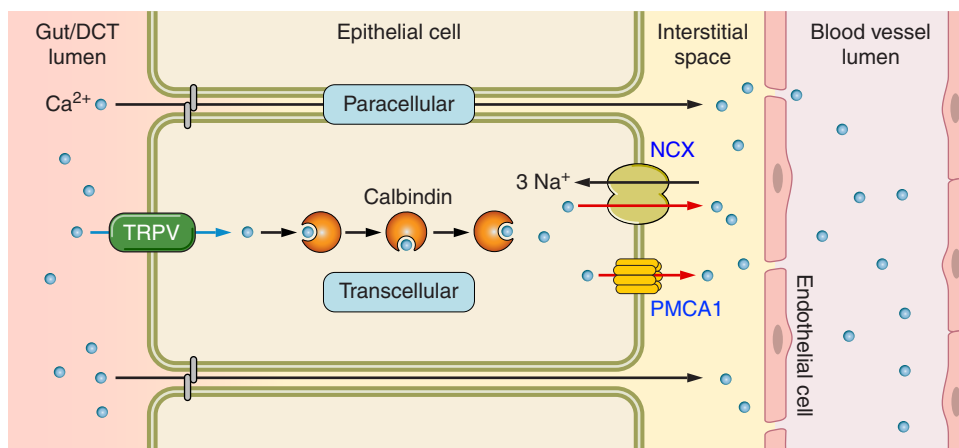
Expression studies have shown that PMCA1 is the predominant isoform in both human and rat duodenal mucosa, and therefore, this isoform is likely to be largely responsible for  $\text{Ca}^{2+}$  absorption in the intestine (163). Indeed, PMCA1 expression levels positively correlate with both intestinal  $\text{Ca}^{2+}$  absorption and bone mineral density in mice (11, 308). In rabbit and mouse small intestine it has been shown that PMCA1 expression is higher proximally in the duodenum than in the jejunum or ileum, and in the large bowel higher in the cecum and ascending colon than in the descending colon (9, 125). Alexander et al. (9) also found that

PMCA1 was the only isoform present in enterocytes throughout human and mouse intestine, whereas PMCA4 was the predominant isoform in smooth muscle layers, increasing in expression distally from small to large bowel.

As with most components of the transcellular pathway for  $\text{Ca}^{2+}$  absorption, duodenal PMCA1 expression is primarily regulated by the vitamin D metabolite  $1,25\text{-(OH)}_2\text{D}_3$ . Data from rat intestine indicate that PMCA1 expression decreases substantially in aged animals in line with serum  $1,25\text{-(OH)}_2\text{D}_3$  concentration (12). Treatment of human duodenal mucosal explants with  $1,25\text{-(OH)}_2\text{D}_3$ , as well as its in vivo administration in mice and chickens, has been shown to significantly increase PMCA1 expression (20, 49, 202, 388). Along with the upregulation of TRPV6 and calbindin- $\text{D}_{9\text{K}}$ , this enables the gut to increase  $\text{Ca}^{2+}$  absorption in an attempt to maintain  $\text{Ca}^{2+}$  balance under experimental conditions of dietary  $\text{Ca}^{2+}$  deficiency, as well as a dietary depletion or excess of phosphorus and chronic metabolic acidosis (49, 65, 70, 179).

There is also evidence for a role in the regulation of duodenal PMCA1 expression by estrogens, with ovariectomized rats and mice displaying reduced PMCA1 mRNA (97, 379). This experimental model results in negative  $\text{Ca}^{2+}$  balance and displays many characteristic features of postmenopausal osteoporosis. In support of this role, intestinal PMCA1 expression is increased in pregnant and lactating mice, and in response to  $17\beta$ -estradiol treatment in ovariectomized rats (378, 379).

The potential link between aberrant duodenal PMCA1 expression and inadequate bone mineralization can also be highlighted by recent data showing enterocyte-specific PMCA1 deletion in mice to lead to growth restriction and reduced bone mineral density (320). Furthermore, intestinal PMCA1 expression was found to be downregulated in ulcerative colitis patients and in mice fed on a high-fat diet, the latter finding also correlating with reduced bone mineral density (402, 409), and both inflammatory bowel disease and obese patients have a higher risk for the development of



**FIGURE 7.** Cartoon illustrating transepithelial  $\text{Ca}^{2+}$  absorption into the blood.  $\text{Ca}^{2+}$  moves passively from gut/kidney lumen through tight junctions between epithelial cells to the interstitial space (paracellular) or is actively transported across the epithelial cell (transcellular).  $\text{Ca}^{2+}$  enters the cell via transient receptor potential vanilloid channels, is transported across the cytoplasm bound to calbindins, and exits via the NCX and PMCA1 to the interstitial space.



bone-related disorders including osteoporosis (140, 150). In addition, duodenal PMCA1 levels are lower in pregnant hypoxic rats than normoxic controls which may contribute towards growth restriction in preeclamptic offspring (411).

## 2. PMCA and renal $\text{Ca}^{2+}$ reabsorption

In excess of 98% of the  $\text{Ca}^{2+}$  filtered through the glomeruli is reabsorbed into the plasma along the nephron. Approximately two-thirds of this transport occurs passively in the proximal convoluted tubule, 20% in the thick ascending loop of Henle, and 10 and 5% in the distal convoluted tubule (DCT) and connecting tubule, respectively, the main sites for active  $\text{Ca}^{2+}$  absorption (127, 157). Unlike in the intestine, it is NCX1 that is believed to reabsorb the majority of transcellular  $\text{Ca}^{2+}$  at the basolateral membrane, with the PMCA responsible for the remaining one-third (29).

The presence of a  $\text{Mg}^{2+}$ -dependent  $\text{Ca}^{2+}$ -ATPase was demonstrated throughout the rabbit nephron in the early 1980s, with activity highest in the DCT and cortical collecting tubule (98). Since then, mRNA for all four PMCA isoforms has been found with site-specific distribution along the nephron. Interestingly, PMCA3 expression appears to be restricted mainly to the thin descending loop of Henle in the rat nephron, whereas isoforms 1, 2, and 4 show more general distribution along the nephron (53, 55). PMCA1 and 4 predominate in mouse DCT cells where they appear targeted to the basolateral membrane (223), and immunohistochemical staining of the mouse nephron shows PMCA expression to peak in the late DCT and connecting tubule where transcellular  $\text{Ca}^{2+}$  transport occurs, before expression tapers off towards the collecting duct (218).

In contrast to intestinal absorption, recent data suggest that PMCA4 may be the major isoform involved in renal active  $\text{Ca}^{2+}$  transport, where it shows strong localization to distal tubular regions in human and mouse kidney (9). In a cultured murine model of the distal convolution, PMCA4, but not PMCA1, was found to be enriched when compared with total cortical expression along with the other components of renal transcellular transport TRPV5, calbindin- $\text{D}_{28\text{k}}$ , and NCX1 (381). Given this evidence regarding the expression of PMCA4 it was then interesting to note that in vivo deletion of PMCA4 from the mouse does not affect renal  $\text{Ca}^{2+}$  handling, with the mice displaying normal serum  $\text{Ca}^{2+}$  levels and urinary  $\text{Ca}^{2+}$  excretion (242, 382). As is the case in the digestive tract, active  $\text{Ca}^{2+}$  transport is primarily regulated via  $1,25\text{-(OH)}_2\text{D}_3$  in the distal tubule, and it has been shown that vitamin  $\text{D}_3$  treatment of Madin-Darby canine kidney cells leads to significant upregulation of basolateral PMCA4 expression in line with an increase in apical-to-basolateral membrane  $\text{Ca}^{2+}$  flux (185).

Interestingly, the pathways regulating PMCA expression in the renal cortex appear to show isoform specificity; whereas PMCA4 expression is induced by  $1,25\text{-(OH)}_2\text{D}_3$ , and

PMCA1 expression is dramatically reduced in the kidney of ovariectomized rats and estrogen-deficient aromatase knockout mice (97, 274). In contrast, a low phosphate diet induces PMCA2 and 3 downregulation and a corresponding increase in urinary  $\text{Ca}^{2+}$ , while PMCA1 expression is unaltered by dietary  $\text{Ca}^{2+}$  levels (54, 97).

Associations have been identified between abnormal PMCA activity and a number of disease states involving the kidney. Patients with idiopathic hypercalciuria, an inherited condition associated with an increased risk of kidney stones, display significantly higher erythrocyte PMCA activity than healthy controls (28). In contrast, red blood cell PMCA activity is reduced in line with an increase in cytosolic  $\text{Ca}^{2+}$  in both children and adults with chronic kidney disease (CKD) (253, 293). In children, erythrocyte PMCA activity and  $\text{Ca}^{2+}$  balance deteriorates as CKD progresses and can be only partially and transiently rescued by hemodialysis (291, 292). There is also evidence from the rat that gestational diabetes induced by streptozotocin (STZ) can impact on PMCA expression and urinary  $\text{Ca}^{2+}$  output in neonatal male offspring which persists into adulthood (34), while STZ-induced diabetes in the adult rat reduces renal PMCA1 expression and increases urinary  $\text{Ca}^{2+}$  excretion (421). In both these cases, the disturbance in  $\text{Ca}^{2+}$  balance ultimately leads to reduced trabecular bone formation, a likely mechanism contributing towards the development of diabetes-induced osteoporosis.

## 3. PMCA and bone mineralization

As detailed in the previous sections, it is clear that perturbations in duodenal and renal PMCA expression may impact on bone mineral density (12, 34, 320, 409, 421). Furthermore, altered maternal PMCA expression also appears to influence bone formation in offspring. This is evident both in the placenta, where PMCA3 levels correlate strongly with neonatal bone area and mineral content (232), as well as during lactation where a decrease in PMCA2 expression in mammary epithelial cells from  $1,25\text{-(OH)}_2\text{D}_3$ -deficient dams is associated with reduced cortical bone volume in pups (170). In fact, PMCA1, 2, and 4 mRNA and total PMCA protein each increases dramatically during lactation in rat mammary tissue, most notably that of PMCA2 which is upregulated some 1,500- and 100-fold at the RNA and protein levels, respectively (303, 304). The importance of this increase in PMCA2 expression in transport of  $\text{Ca}^{2+}$  from mammary epithelial cells into milk has since been verified through studies in transgenic mice, which show a 60–70% reduction in milk  $[\text{Ca}^{2+}]$  produced by PMCA2 null or functionally inactive *Deafwaddler* mice (306, 385).

In addition to serum and milk  $[\text{Ca}^{2+}]$ , the degree of bone mineralization depends on optimal  $\text{Ca}^{2+}$  homeostasis within the bone forming and resorbing cell types of the tissue itself, namely, the osteoblasts and osteoclasts. Bone is



a highly dynamic tissue that undergoes constant turnover. Osteoblasts lay down new osteoid through matrix,  $\text{Ca}^{2+}$ , and phosphate secretion, forming a specialized connective tissue hardened by hydroxyapatite. Meanwhile, osteoclasts simultaneously digest this osteoid, and the balance of these two processes impacts on bone growth and strength (84).

PMCA<sub>s</sub> are expressed in both cell types, although their precise functions are incompletely understood. PMCA<sub>1</sub>, 2, and 4 have all been found to be present in human osteoblasts (41, 196, 380). In human and rat osteoblasts, the PMCA appears targeted to the osteoidal domain, suggesting a role in the mineralization process (258, 380), although chick osteoblasts show localization to the apical and lateral membranes facing away from bone so there may be some interspecies variation in this respect (7, 351). In support of a role in basolateral  $\text{Ca}^{2+}$  transport, PMCA inhibition using ortho-vanadate has been shown to decrease mineralization of murine osteoblast precursor cultures (257), while patients suffering from adult idiopathic scoliosis appear to have a reduction in PMCA<sub>4</sub> expression (41).

It has been reported that PMCA<sub>1</sub> and 4 are expressed during osteoclast differentiation from human peripheral blood mononuclear cells, and each acts to inhibit osteoclastogenesis through regulation of  $\text{Ca}^{2+}$  oscillations, NFATc1, and in the case of PMCA<sub>4</sub>, NO-dependent osteoclast fusion (183). In mature human, mouse, and chick osteoclasts, the PMCA appears localized to the basolateral membrane facing away from bone (7, 183, 380). In addition to PMCA's role during osteoclastogenesis, they also appear to prevent apoptosis in mature cells, while both PMCA<sub>1</sub> heterozygous mice and PMCA<sub>4</sub> ablated mice exhibit reduced bone mineral density and trabecular bone volume associated with increased osteoclast number (183). Interestingly, this study also found a correlation between PMCA<sub>4</sub> expression and higher peak bone mass in Chinese women.

#### 4. PMCA and dental mineralization

Enamel is the hardest substance in the human body, being composed primarily of calcium phosphate in the form of hydroxyapatite. It is laid down during tooth development by ameloblasts, and constantly remineralized thereafter via  $\text{Ca}^{2+}$  and phosphate secreted in saliva (161).

PMCA has been shown to be localized to the enamel facing Tomes' processes and plasma membrane of human ameloblasts, indicating a likely role in enamel mineralization, and indeed PMCA<sub>1</sub> and 4 expressions have been shown to increase in parallel with the onset and progression of enamel development (37). The importance of PMCA<sub>1</sub> in the mineralization process may be highlighted by work using morpholino knockdown of the zebrafish *Atp2b1a* gene, which leads to inadequate calcification of developing pharyngeal teeth (136), while PMCA<sub>4</sub> expression is reduced in matrix

metalloproteinase-20 (MMP20) null mice in which enamel thickness is reduced by 50% (375).

Regulation of salivary  $\text{Ca}^{2+}$  levels is critical in preventing the incidence of dental caries and formation of calculus, and the PMCA is the principal route via which  $\text{Ca}^{2+}$  is extruded from acini into the salivary ducts. PMCA<sub>1</sub>, 2, and 4 are each expressed in the human parotid and submandibular glands, where they appear localized to the apical membrane of acinar cells (161). PMCA<sub>2</sub> was also found to be present in the secretory canaliculi between the cells, while labeling of all three isoforms was found in the cytoplasm of the interlobular and intralobular ducts of rabbit submandibular glands (36).

### I. Role of PMCA<sub>s</sub> in Cell Proliferation, Differentiation, and Death

$\text{Ca}^{2+}$  is known to be a widespread intracellular messenger, controlling numerous cellular processes from cell growth to cell death. When the level of cytosolic  $\text{Ca}^{2+}$  changes, a multitude of downstream signaling pathways driving these processes become activated and therefore the regulation of intracellular  $\text{Ca}^{2+}$  is a key element of cellular physiology (23). With the PMCA being the major regulator of  $[\text{Ca}^{2+}]_i$  in many cell types, it therefore plays a critical role in directing  $\text{Ca}^{2+}$ -mediated signaling, and as discussed elsewhere in this review can influence such processes as megakaryocyte, osteoclast, and neuronal differentiation (40, 41, 66, 82, 183, 362); the proliferation of B-lymphocytes and pancreatic  $\beta$ -cells (71, 144, 275); and apoptosis of  $\beta$ -cells, osteoclasts, and neurons (120, 131, 171, 183). Indeed, microarray analysis of PC12 cells following antisense-mediated suppression of PMCA<sub>2</sub> or PMCA<sub>3</sub> revealed altered expression patterns of many genes involved in the regulation of cell cycle, proliferation, migration, differentiation, and apoptosis (31). Furthermore, cellular proliferation, differentiation, and survival are key determinants in tumor progression which we will discuss in further detail in section IVJ.

#### 1. PMCA during differentiation

Direct evidence that the PMCA may play a role in regulating the differentiation process has been demonstrated upon overexpression of human PMCA<sub>4</sub> in rat L6 myoblasts, which led to reduced  $[\text{Ca}^{2+}]_i$ , increased myotube formation, and hence accelerated differentiation (148). A spike in intracellular  $\text{Ca}^{2+}$  is required for the maturation of a number of precursor cell types (23), while low resting cytosolic  $\text{Ca}^{2+}$  is a feature in most mature cells. Studies of *Xenopus* oocytes indicate that PMCA internalization, preventing PMCA-mediated efflux, contributes towards the sustained increase in cytosolic  $[\text{Ca}^{2+}]$  necessary for egg activation during maturation (106). A similar spike in  $\text{Ca}^{2+}$  is thought to be required for neuronal cell differentiation, and it has been shown that PMCA activity increases substantially in

line with PMCA2, 3, and 4 expressions as IMR-32 neuroblastoma cells become differentiated, postulated to allow for optimal  $\text{Ca}^{2+}$  signaling in mature cells (377). In contrast, PMCA expression is highly abundant in megakaryoblastoid precursor cells (primarily PMCA4) compared with mature platelets (281), while expression and activity also decrease during the differentiation process in keratinocytes (75).

Hence, the PMCA may play a complex role in cellular maturation, and there is a suggestion that cell-specific patterns of PMCA expression may provide a signature for terminal differentiation among different cell types. This has been put forth following studies examining the expression of alternate splice variants during differentiation of myogenic and neuronal cells alongside fibroblasts, smooth muscle, and endothelial cells, which have shown a switch from the “b” splice variants of PMCA1 and 4 during differentiation to also express isoforms 1c, 1d, and 4a and that this is unique to the excitable cell types (86, 147).

## 2. PMCA during proliferation

A role for the PMCA in proliferation has now been identified in a number of cell types. Of these, perhaps the best defined is in the regulation of the vascular smooth muscle cell (VSMC) cycle. Husain and colleagues (3, 165) have shown that repression of PMCA1 expression in rat VSMCs through the binding of transcription factor c-Myb to its promoter region leads to a subsequent reduction in the rate of PMCA-mediated  $\text{Ca}^{2+}$  efflux, which results in the increase in intracellular  $\text{Ca}^{2+}$  required for  $\text{G}_1/\text{S}$  stage transition in the cell cycle. Moreover, the authors (165) showed that PMCA1 overexpression inhibited S phase entry and reduced the rate of proliferation. The authors (4) have since found an opposing role for PMCA4 in cell cycle progression, with VSMCs from PMCA4 knockout mice displaying  $\text{G}_1$  phase arrest which could be rescued upon electroporation of either PMCA4a or 4b splice variants. Each of these splice variants appeared to regulate independent pathways with PMCA4a suppressing the anti-proliferative AP-2 $\beta$  and 4b downregulating the cyclin-dependent kinase inhibitor p15, while an isoform switch occurred from a 50:50 ratio in normal vessels to PMCA4b being the predominant variant following arterial injury (4). Interestingly studies of canine VSMCs have found PMCA4a to be present only in proliferating cell populations (2). Meanwhile, in contrast to VSMCs, PMCA4 has been shown to be downregulated in proliferating airway SMCs and inhibition to enhance proliferation, whereas nonselective inhibition (and thus additionally of PMCA1) had the opposite effect (74).

In addition to its roles in arterial smooth muscle cells, the actions of PMCA4 have recently been shown to mediate VEGF-induced angiogenic signaling in vascular endothelial cells (17). Baggott et al. (17) found PMCA4 to inhibit endothelial cell migration and blood vessel formation through

suppression of calcineurin/NFAT signaling while also demonstrating that PMCA4 knockout mice had improved hindlimb perfusion following femoral artery ligation.

## 3. PMCA in cell death

$\text{Ca}^{2+}$  signaling plays a critical role in cell viability; a rise in basal  $\text{Ca}^{2+}$  can lead to mitochondrial dysfunction and activate pro-apoptotic factors, while  $\text{Ca}^{2+}$  overload may cause cells to undergo necrosis (23, 271). As regulators of global  $[\text{Ca}^{2+}]_i$  and  $\text{Ca}^{2+}$ -mediated signaling, the PMCA have been implicated in both necrotic and programmed cell death in numerous cell types.

In some instances PMCA-mediated apoptosis is required for normal physiological functions. For example, mice exhibit a 95% reduction in PMCA2 expression in mammary epithelial cells (which as we discuss elsewhere is greatly elevated during lactation) as early as 24 h after weaning, thus leaving the cells in a state of high  $\text{Ca}^{2+}$ , triggering apoptosis and ultimately mammary gland involution (305, 384). Improper control of this process however, as evidenced by PMCA2 mutant *deafwaddler* mice, results in apoptosis of mammary epithelial cells during pregnancy (384). PMCA-dependent promotion of cell survival has also been demonstrated in follicular granulosa cells, which through a basic fibroblast growth factor-mediated increase in PKC $\delta$  activity are able to upregulate PMCA1 expression and  $\text{Ca}^{2+}$  efflux, thus preventing apoptosis in response to elevated  $[\text{Ca}^{2+}]_i$  (287, 288). In line with this protective role, antisense-mediated inhibition of PMCA1 and PMCA2, respectively, in rat VSMCs and PC12 cells induces apoptosis (32, 326).

PMCA's role in regulating cell death is somewhat complex however. A mutant form of PMCA4 which demonstrated greatly reduced expression in L929 fibrosarcoma cells conferred resistance to tumor necrosis factor-induced cell death despite elevated cytosolic  $\text{Ca}^{2+}$ , thought to result from the promotion of exocytosis of acidic lysosomes accumulating in the cytoplasm (270). In addition, the PMCA is particularly vulnerable to proteolysis, and it has consistently been shown that in the early stages of apoptosis the autoinhibitory COOH-terminal is cleaved from PMCA4 by caspase-3 leaving a properly targeted 120-kDa fragment active even in the absence of calmodulin (279, 280, 282). In theory, this would then increase  $\text{Ca}^{2+}$  extrusion and protect cells from overload; however, it appears that cleavage can eventually impair PMCA activity. This has been witnessed in hepatocytes transfected with hepatitis B virus X protein, which undergo apoptosis associated with increased PMCA4 cleavage by caspase-3, higher  $[\text{Ca}^{2+}]_i$ , and mitochondrial abnormalities (68). The same is true during ischemic brain injury in rats and in glutamate-treated cerebellar granule neurons, which demonstrate caspase-1-induced cleavage of PMCA2 leading to impaired activity and apoptosis (337). Schwab et al. (337) also determined that PMCA4 cleavage in nonex-

citable CHO cells resulted in impaired  $\text{Ca}^{2+}$  handling,  $\text{Ca}^{2+}$  overload, and secondary necrosis.

The mechanisms through which the PMCA pump becomes inactivated under these circumstances are unclear. One simple hypothesis would be that further cleavage would result in proteolytic degradation and reduced expression. There is also the suggestion that ATP depletion via mitochondrial dysfunction impairs PMCA activity resulting in  $\text{Ca}^{2+}$  overload and necrosis, as evidenced in pancreatic acinar cells exposed to palmitoleic acid in a model of acute pancreatitis (324). Further evidence that  $\text{Ca}^{2+}$  overload and necrosis occur as a result of reduced PMCA activity following ATP depletion has been identified in epithelial cells exposed to oxidative stress, where increased  $\text{Na}^+/\text{K}^+$ -ATPase activity induced by elevated  $[\text{Na}^+]_i$  effectively “steals” ATP from the PMCA rendering the pump inactive (63). Similarly, UVB irradiation-induced oxidative stress in human lens epithelial cells has been shown to reduce PMCA1 expression and activity resulting in increased  $\text{Ca}^{2+}$  and necrosis (403). Thus it appears the PMCA plays a complex role in directing cell death via apoptotic or necrotic pathways. This can be highlighted by its regulation by the anti-apoptotic protein Bcl-2; pancreatic acinar cells isolated from Bcl-2 knockout mice demonstrate enhanced PMCA-mediated  $\text{Ca}^{2+}$  clearance and a resistance to oxidation or  $\text{Ca}^{2+}$ -induced necrosis, but increased apoptosis. Bcl-2 overexpression meanwhile was shown to impair  $\text{Ca}^{2+}$  extrusion while PMCA inhibition led to increases in necrosis in pancreatic cells (118).

## J. Role of PMCA<sub>s</sub> in Cancer

Regulation of the  $\text{Ca}^{2+}$  signal can impact on many of the typical hallmarks of cancer such as sustained proliferation, resistance to cell death, and induction of angiogenesis (149, 354). This had led to the study of  $\text{Ca}^{2+}$  handling components during tumor development becoming an active field of research in recent years, with the TRP channel family leading the way, and may offer a number of novel pharmaceutical targets for the treatment of cancer (325). As we highlighted in the previous section, there is substantial evidence that PMCA expression and activity, like that of TRP channels, also impacts on many of the cellular processes defining tumorigenesis and progression. Indeed, first evidence for a potential role for the PMCA came from analysis of the expression of PMCA1 and 4 in human skin and lung fibroblastic cell lines following exposure to the oncogenic simian virus 40 (SV40), both of which were found to be downregulated (307). In this section we describe the expression changes and roles of the PMCA to have currently been identified in various cancers.

### 1. PMCA and breast cancer

As we discussed elsewhere in this review, changes in PMCA expression and particularly that of isoform 2 are inextricably

linked to mammary epithelial cell physiology, showing a dramatic upregulation during lactation and its immediate downregulation being essential for mammary gland involution upon weaning (303–305, 384). This highlights a potentially critical role for the pumps in regulating cell survival. PMCA isoforms 1, 2, and 4 are all expressed in a variety of human breast cancer cell lines (203), with PMCA2 levels being up to 100-fold higher in tumorigenic versus nontumorigenic human breast epithelial cell lines (204). Similarly, PMCA1 demonstrates an approximately threefold increase in expression in serum-starved MCF-7 breast cancer cells compared with control MCF-10A mammary epithelial cells, while PMCA4 levels appear lower in some cancerous cell lines compared with controls (203, 204).

Clinically, high levels of PMCA2 expression in breast tumors correlate with increased tumor grade, resistance to the chemotherapeutic drug docetaxel, and reduced 5-yr survival rates in breast cancer patients (169, 384). Each of these studies examined the effects of PMCA2 overexpression in breast cancer cells, reporting decreases in  $[\text{Ca}^{2+}]_i$  and apoptosis as well as higher incidence, reduced latency, and accelerated tumor growth when grown as xenografts in immunocompromised mice (169, 384). In agreement with this, crossing PMCA2-inactive mutant *deafwaddler* mice with mouse mammary tumor virus (MMTV)-Neu mice inhibited tumor formation (169). Mechanistically, these effects may well be multifaceted. Jeong et al. (169) found PMCA2 expression to correlate with human epidermal growth factor receptor 2 (HER2) in breast tumors, and through a physical interaction to regulate HER2 signaling as well as its internalization and degradation upon PMCA2 knockdown. In addition, PMCA2 has been found to interact with calcineurin in breast cancer cells, with overexpression leading to a reduction in NFAT transcriptional activity (159) and disruption of the complex causing NFAT activation with a consequential upregulation in proapoptotic Fas ligand expression, apoptosis, and increased cytotoxicity to the chemotherapeutic agent paclitaxel (18).

There is evidence that PMCA1 and 4 may also play important roles in signal regulation in breast cancer cells and that these are isoform specific. Upregulation of PMCA4 has been witnessed during induced differentiation of MCF-7 breast cancer cells alongside an increased rate of  $\text{Ca}^{2+}$  clearance (386). PMCA4 expression also increases during proliferation in these cells, and antisense PMCA inhibition was shown to result in reduced rates of proliferation while increasing  $[\text{Ca}^{2+}]_i$  and impairing PMCA-mediated  $\text{Ca}^{2+}$  clearance (205). Fewer cells were found to be in the S phase and more in the  $\text{G}_2/\text{M}$  phase of the cell cycle upon PMCA inhibition, with the authors concluding slower transition through this stage. Further experiments by the group, this time in the MDA-MB-231 breast cancer cell line, have identified that PMCA1 primarily regulates bulk  $\text{Ca}^{2+}$  extrusion



in these cells and that knockdown of this isoform leaves cells vulnerable to  $\text{Ca}^{2+}$ -induced necrotic cell death (81). PMCA4 silencing, on the other hand, was found to enhance apoptosis induced by Bcl-2 inhibition, likely through regulation of NF $\kappa$ B signaling.

Hence, there is evidence to suggest that inhibition of any of the three PMCA isoforms expressed in breast cancer cells may have the potential to be used as an anti-cancer therapy. This may be highlighted by research conducted using a new platinum-based compound [Pt(O,O0-acac)(g-acac)(DMS)], which is highly cytotoxic to cisplatin-resistant MCF-7 cells through inhibition of PMCA activity, thus raising  $[\text{Ca}^{2+}]_i$  and activating apoptotic pathways (255, 256). This may be a promising avenue to explore, particularly in tumors resistant to currently available chemotherapeutic agents.

## 2. PMCA and colorectal cancer

The finding that PMCA expression was altered during breast cancer tumorigenesis prompted the question as to whether this was also the case in other neoplastic cell types. Both PMCA1 and 4 have been shown to be expressed in a variety of human colon cancer cell lines, with isoform 1 predominating in undifferentiated cells (14, 311). During induced or postconfluent differentiation however, there is a marked upregulation of PMCA4 expression as well as enhanced PMCA-mediated  $\text{Ca}^{2+}$  efflux (14, 15, 311). In contrast, differentiated colon tumors exhibit significantly lower PMCA4 expression compared with healthy surrounding tissue (15). Aung et al. (15) then went on to determine that siRNA inhibition of PMCA4 did not alter HT-29 colon cancer cell viability following apoptotic stimulation, while overexpression led to a decrease in cell proliferation suggesting that the postdifferentiation downregulation of PMCA4 may facilitate tumor growth. In agreement with these findings, an examination of PMCA4 protein in normal mucosa compared with varying grades of colon tumor has found reduced levels only in high grade adenoma, adenocarcinoma, and lymph node metastasis indicating a potential role in tumor progression (319). Meanwhile, rectal tumors display elevated PMCA4 expression compared with normal mucosa (133).

## 3. PMCA and other cancers

In addition to the more extensively defined roles in breast and colon cancer, an association has also been found between the PMCA isoforms and tumors in a number of other tissues. PMCA1 has been found to be downregulated in oral squamous cell carcinoma and premalignant lesions, a possible result of increased methylation (322). Conversely, PMCA expression appears higher in mouse AS-30D hepatoma cells compared with normal liver, a pattern similar to that seen in regenerating hepatic cells (90). In addition, evidence that the PMCA isoforms may be a potential therapeutic target for che-

motherapy-resistant cancers has been found in two further cell types. First, PMCA1 expression is increased in cisplatin-resistant human ovarian adenocarcinoma cells compared with cisplatin-sensitive cells (348), and second, the resistance to cell death conferred upon pancreatic cancer cells by a switch in metabolism from mitochondrial to glycolytic pathways can be reversed by inhibiting glycolysis, an effect which leads to PMCA inhibition,  $\text{Ca}^{2+}$  overload, and cell death (167). Hence, although the field is still in its infancy, the identified roles for different PMCA isoforms in regulating tumorigenesis, progression, and survival in various tissues may lend themselves towards targeting for the treatment of cancers in specific cell types.

## V. CONCLUSIONS

The ubiquitous nature of PMCA expression combined with the tissue-specific distribution of the four isoforms provide an adaptable toolkit to control intracellular  $\text{Ca}^{2+}$  dynamics throughout the body. As regulators of bulk  $\text{Ca}^{2+}$  transport they are critical in the maintenance of desirable serum ionic balance for tissue mineralization from embryonic development through to adulthood, as well as influencing processes such as fertilization, smooth muscle tone, and cell survival. Meanwhile, as binding partners they are able to direct  $\text{Ca}^{2+}$ -dependent signaling to regulate a plethora of physiological processes from sensory transmission to cellular growth. Through genome-wide screening and the use of animal models, specific roles for each isoform have now been defined in the development and progression of a large number of human diseases affecting the cardiovascular, nervous, and musculoskeletal systems as well as in the fields of cancer, endocrinology, and infectious disease. Given the widespread spatiotemporal expression pattern of the PMCA isoforms, it is perhaps surprising that mutating or knocking out a particular isoform reveals a role localized to a particular tissue. However, it is likely that the specificity of action is dictated through a combination of isoform/splice variant, interaction partner, and local  $\text{Ca}^{2+}$  requirements. It is the cell-specific functions of these pumps combined with their nature as membrane-bound ATPases that may therefore make them suitable and druggable targets for the development of novel therapeutic strategies in wide-ranging fields including contraception, hypertension, cardiac hypertrophy, neurodegeneration, malaria, and cancer.

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## DISCLOSURES

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## REFERENCES

1. WHO. Cardiovascular diseases (CVDs). <http://www.who.int/mediacentre/factsheets/fs317/en/>.
2. Abramowitz J, Aydemir-Koksoy A, Helgason T, Jemelka S, Odeunmi T, Seidel CL, Allen JC. Expression of plasma membrane calcium ATPases in phenotypically distinct canine vascular smooth muscle cells. *J Mol Cell Cardiol* 32: 777–789, 2000. doi:10.1006/jmcc.2000.1120.
3. Afroze T, Husain M. c-Myb-binding sites mediate G<sub>i</sub>/S-associated repression of the plasma membrane Ca<sup>2+</sup>-ATPase-1 promoter. *J Biol Chem* 275: 9062–9069, 2000. doi:10.1074/jbc.275.12.9062.
4. Afroze T, Yang G, Khoshbin A, Tanwir M, Tabish T, Momen A, Husain M. Calcium efflux activity of plasma membrane Ca<sup>2+</sup> ATPase-4 (PMCA4) mediates cell cycle progression in vascular smooth muscle cells. *J Biol Chem* 289: 7221–7231, 2014. doi:10.1074/jbc.M113.533638.
5. Aguilar HN, Mitchell BF. Physiological pathways and molecular mechanisms regulating uterine contractility. *Hum Reprod Update* 16: 725–744, 2010. doi:10.1093/humupd/dmq016.
6. Åkerström T, Willenberg HS, Cupisti K, Ip J, Backman S, Moser A, Maharjan R, Robinson B, Iwen KA, Dralle H, D Volpe C, Bäckdahl M, Botling J, Ståhlberg P, Westin G, Walz MK, Lehnert H, Sidhu S, Zedenius J, Björklund P, Hellman P. Novel somatic mutations and distinct molecular signature in aldosterone-producing adenomas. *Endocr Relat Cancer* 22: 735–744, 2015. doi:10.1530/ERC-15-0321.
7. Akisaka T, Yamamoto T, Gay CV. Ultracytochemical investigation of calcium-activated adenosine triphosphatase (Ca<sup>2+</sup>-ATPase) in chick tibia. *J Bone Miner Res* 3: 19–25, 1988. doi:10.1002/jbmr.5650030105.
8. Akopian A, Witkovsky P. Calcium and retinal function. *Mol Neurobiol* 25: 113–132, 2002. doi:10.1385/MN:25:2:113.
9. Alexander RT, Beggs MR, Zamani R, Marcussen N, Frische S, Dimke H. Ultrastructural and immunohistochemical localization of plasma membrane Ca<sup>2+</sup>-ATPase 4 in Ca<sup>2+</sup>-transporting epithelia. *Am J Physiol Renal Physiol* 309: F604–F616, 2015. doi:10.1152/ajprenal.00651.2014.
10. Andersson KE, Arner A. Urinary bladder contraction and relaxation: physiology and pathophysiology. *Physiol Rev* 84: 935–986, 2004. doi:10.1152/physrev.00038.2003.
11. Armbricht HJ, Boltz MA, Hodam TL. Differences in intestinal calcium and phosphate transport between low and high bone density mice. *Am J Physiol Gastrointest Liver Physiol* 282: G130–G136, 2002. doi:10.1152/ajpgi.00175.2001.
12. Armbricht HJ, Boltz MA, Wongsurawat N. Expression of plasma membrane calcium pump mRNA in rat intestine: effect of age and 1,25-dihydroxyvitamin D. *Biochim Biophys Acta* 1195: 110–114, 1994. doi:10.1016/0005-2736(94)90016-7.
13. Armesilla AL, Williams JC, Buch MH, Pickard A, Emerson M, Cartwright EJ, Oceandy D, Vos MD, Gillies S, Clark GJ, Neynes L. Novel functional interaction between the plasma membrane Ca<sup>2+</sup> pump 4b and the proapoptotic tumor suppressor Ras-associated factor 1 (RASSF1). *J Biol Chem* 279: 31318–31328, 2004. doi:10.1074/jbc.M307557200.
14. Aung CS, Kruger WA, Poronnik P, Roberts-Thomson SJ, Monteith GR. Plasma membrane Ca<sup>2+</sup>-ATPase expression during colon cancer cell line differentiation. *Biochem Biophys Res Commun* 355: 932–936, 2007. doi:10.1016/j.bbrc.2007.02.050.
15. Aung CS, Ye W, Plowman G, Peters AA, Monteith GR, Roberts-Thomson SJ. Plasma membrane calcium ATPase 4 and the remodeling of calcium homeostasis in human colon cancer cells. *Carcinogenesis* 30: 1962–1969, 2009. doi:10.1093/carcin/bgp223.
16. Baffy G, Varga Z, Fóris G, Leövey A. Disturbed intracellular calcium-related processes of hepatocytes and neutrophils in human alcoholic liver disease. *Clin Biochem* 23: 241–245, 1990. doi:10.1016/0009-9120(90)90692-N.
17. Baggott RR, Alfranca A, López-Maderuelo D, Mohamed TM, Escolano A, Oller J, Ornes BC, Kurusamy S, Rowther FB, Brown JE, Oceandy D, Cartwright EJ, Wang W, Gómez-del Arco P, Martínez-Martínez S, Neynes L, Redondo JM, Armesilla AL. Plasma membrane calcium ATPase isoform 4 inhibits vascular endothelial growth factor-mediated angiogenesis through interaction with calcineurin. *Arterioscler Thromb Vasc Biol* 34: 2310–2320, 2014. doi:10.1161/ATVBAHA.114.304363.
18. Baggott RR, Mohamed TM, Oceandy D, Holton M, Blanc MC, Roux-Soro SC, Brown S, Brown JE, Cartwright EJ, Wang W, Neynes L, Armesilla AL. Disruption of the interaction between PMCA2 and calcineurin triggers apoptosis and enhances paclitaxel-induced cytotoxicity in breast cancer cells. *Carcinogenesis* 33: 2362–2368, 2012. doi:10.1093/carcin/bgs282.
19. Balasubramanyam M, Balaji RA, Subashini B, Mohan V. Evidence for mechanistic alterations of Ca<sup>2+</sup> homeostasis in type 2 diabetes mellitus. *Int J Exp Diabetes Res* 1: 275–287, 2001. doi:10.1155/EDR.2000.275.
20. Balesaria S, Sangha S, Walters JR. Human duodenum responses to vitamin D metabolites of TRPV6 and other genes involved in calcium absorption. *Am J Physiol Gastrointest Liver Physiol* 297: G1193–G1197, 2009. doi:10.1152/ajpgi.00237.2009.
21. Bedu-Addo G, Meese S, Mockenhaupt FP. An ATP2B4 polymorphism protects against malaria in pregnancy. *J Infect Dis* 207: 1600–1603, 2013. doi:10.1093/infdis/jit070.
22. Beigi F, Oskouei BN, Zheng M, Cooke CA, Lamirault G, Hare JM. Cardiac nitric oxide synthase-1 localization within the cardiomyocyte is accompanied by the adaptor protein, CAPON. *Nitric Oxide* 21: 226–233, 2009. doi:10.1016/j.niox.2009.09.005.
23. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signaling. *Nat Rev Mol Cell Biol* 1: 11–21, 2000. doi:10.1038/35036035.
24. Berrocal M, Corbacho I, Vázquez-Hernández M, Ávila J, Sepúlveda MR, Mata AM. Inhibition of PMCA activity by tau as a function of aging and Alzheimer's neuropathology. *Biochim Biophys Acta* 1852: 1465–1476, 2015. doi:10.1016/j.bbadis.2015.04.007.
25. Berrocal M, Marcos D, Sepúlveda MR, Pérez M, Ávila J, Mata AM. Altered Ca<sup>2+</sup> dependence of synaptosomal plasma membrane Ca<sup>2+</sup>-ATPase in human brain affected by Alzheimer's disease. *FASEB J* 23: 1826–1834, 2009. doi:10.1096/fj.08-121459.
26. Bers DM. Calcium fluxes involved in control of cardiac myocyte contraction. *Circ Res* 87: 275–281, 2000. doi:10.1161/01.RES.87.4.275.
27. Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD, Penton D, Schack VR, Amar L, Fischer E, Walther A, Tauber P, Schwarzmayr T, Diener S, Graf E, Allolio B, Samson-Couterie B, Benecke A, Quinkler M, Fallo F, Plouin PF, Mantero F, Meitinger T, Mulatero P, Jeunemaitre X, Warth R, Vilsen B, Zennaro MC, Strom TM, Reincke M. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat Genet* 45: 440–444, 2013. doi:10.1038/ng.2550.
28. Bianchi G, Vezzoli G, Cusi D, Cova T, Elli A, Soldati L, Tripodi G, Surian M, Ottaviano E, Rigatti P, Ortolani S. Abnormal red-cell calcium pump in patients with idiopathic hypercalciuria. *N Engl J Med* 319: 897–901, 1988. doi:10.1056/NEJM198810063191402.
29. Bindels RJ, Ramakers PL, Dempster JA, Hartog A, van Os CH. Role of Na<sup>+</sup>/Ca<sup>2+</sup> exchange in transcellular Ca<sup>2+</sup> transport across primary cultures of rabbit kidney collecting system. *Pflugers Arch* 420: 566–572, 1992. doi:10.1007/BF00374634.
30. Blankenship KA, Dawson CB, Aronoff GR, Dean WL. Tyrosine phosphorylation of human platelet plasma membrane Ca<sup>2+</sup>-ATPase in hypertension. *Hypertension* 35: 103–107, 2000. doi:10.1161/01.HYP.35.1.103.
31. Boczek T, Kozaczuk A, Ferenc B, Kosiorek M, Pikula S, Zylinska L. Gene expression pattern in PC12 cells with reduced PMCA2 or PMCA3 isoform: selective up-regulation of calmodulin and neuromodulin. *Mol Cell Biochem* 360: 89–102, 2012. doi:10.1007/s10101010-1047-3.

32. Boczek T, Lisek M, Kowalski A, Pikula S, Niewiarowska J, Wiktorska M, Zylinska L. Downregulation of PMCA2 or PMCA3 reorganizes  $\text{Ca}^{2+}$  handling systems in differentiating PC12 cells. *Cell Calcium* 52: 433–444, 2012. doi:[10.1016/j.ceca.2012.08.002](https://doi.org/10.1016/j.ceca.2012.08.002).
33. Bogdanova A, Makhro A, Wang J, Lipp P, Kaestner L. Calcium in red blood cells: a perilous balance. *Int J Mol Sci* 14: 9848–9872, 2013. doi:[10.3390/ijms14059848](https://doi.org/10.3390/ijms14059848).
34. Bond H, Hamilton K, Balment RJ, Denton J, Freemont AJ, Garland HO, Glazier JD, Sibley CP. Diabetes in rat pregnancy alters renal calcium and magnesium reabsorption and bone formation in adult offspring. *Diabetologia* 48: 1393–1400, 2005. doi:[10.1007/s00125-005-1804-5](https://doi.org/10.1007/s00125-005-1804-5).
35. Bookchin RM, Ortiz OE, Shalev O, Tsurel S, Rachmilewitz EA, Hockaday A, Lew VL. Calcium transport and ultrastructure of red cells in beta-thalassemia intermedia. *Blood* 72: 1602–1607, 1988.
36. Borke JL, Zaki AE, Eisenmann DR, Ashrafi SH, Sharawy MM, Rahman SS. In situ hybridization and monoclonal antibody analysis of plasma membrane  $\text{Ca}$ -pump mRNA and protein in submandibular glands of rabbit, rat and man. *Scanning Microsc* 9: 817–823, 1995.
37. Borke JL, Zaki A-M, Eisenmann DR, Mednieks MI. Localization of plasma membrane  $\text{Ca}^{2+}$  pump mRNA and protein in human ameloblasts by in situ hybridization and immunohistochemistry. *Connect Tissue Res* 33: 139–144, 1995. doi:[10.3109/03008209509016993](https://doi.org/10.3109/03008209509016993).
38. Bortolozzi M, Brini M, Parkinson N, Crispino G, Scimemi P, De Sisti RD, Di Leva F, Parker A, Ortolano S, Arslan E, Brown SD, Carafoli E, Mammato F. The novel PMCA2 pump mutation Tommy impairs cytosolic calcium clearance in hair cells and links to deafness in mice. *J Biol Chem* 285: 37693–37703, 2010. doi:[10.1074/jbc.M110.170092](https://doi.org/10.1074/jbc.M110.170092).
39. Bozulic LD, Malik MT, Powell DW, Nanez A, Link AJ, Ramos KS, Dean WL. Plasma membrane  $\text{Ca}^{2+}$ -ATPase associates with CLP36,  $\alpha$ -actinin and actin in human platelets. *Thromb Haemost* 97: 587–597, 2007. doi:[10.1160/TH06-08-0438](https://doi.org/10.1160/TH06-08-0438).
40. Brandt PC, Siskin JE, Neve RL, Vanaman TC. Blockade of plasma membrane calcium pumping ATPase isoform I impairs nerve growth factor-induced neurite extension in pheochromocytoma cells. *Proc Natl Acad Sci USA* 93: 13843–13848, 1996. doi:[10.1073/pnas.93.24.13843](https://doi.org/10.1073/pnas.93.24.13843).
41. Bredoux R, Corvazier E, Dally S, Chaabane C, Bobe R, Raies A, Moreau A, Enouf J. Human platelet  $\text{Ca}^{2+}$ -ATPases: new markers of cell differentiation as illustrated in idiopathic scoliosis. *Platelets* 17: 421–433, 2006. doi:[10.1080/09537100600758719](https://doi.org/10.1080/09537100600758719).
42. Brendel A, Renziehausen J, Behl C, Hajieva P. Downregulation of PMCA2 increases the vulnerability of midbrain neurons to mitochondrial complex I inhibition. *Neurotoxicology* 40: 43–51, 2014. doi:[10.1016/j.neuro.2013.11.003](https://doi.org/10.1016/j.neuro.2013.11.003).
43. Brini M, Cali T, Ottolini D, Carafoli E. Neuronal calcium signaling: function and dysfunction. *Cell Mol Life Sci* 71: 2787–2814, 2014. doi:[10.1007/s00018-013-1550-7](https://doi.org/10.1007/s00018-013-1550-7).
44. Brini M, Carafoli E. Calcium pumps in health and disease. *Physiol Rev* 89: 1341–1378, 2009. doi:[10.1152/physrev.00032.2008](https://doi.org/10.1152/physrev.00032.2008).
45. Brini M, Carafoli E. The plasma membrane  $\text{Ca}^{2+}$  ATPase and the plasma membrane sodium calcium exchanger cooperate in the regulation of cell calcium. *Cold Spring Harb Perspect Biol* 3: a004168, 2011. doi:[10.1101/cshperspect.a004168](https://doi.org/10.1101/cshperspect.a004168).
46. Buch MH, Pickard A, Rodriguez A, Gillies S, Maass AH, Emerson M, Cartwright EJ, Williams JC, Oceandy D, Redondo JM, Neyses L, Armesilla AL. The sarcolemmal calcium pump inhibits the calcineurin/nuclear factor of activated T-cell pathway via interaction with the calcineurin A catalytic subunit. *J Biol Chem* 280: 29479–29487, 2005. doi:[10.1074/jbc.M501326200](https://doi.org/10.1074/jbc.M501326200).
47. Burette A, Rockwood JM, Strehler EE, Weinberg RJ. Isoform-specific distribution of the plasma membrane  $\text{Ca}^{2+}$  ATPase in the rat brain. *J Comp Neurol* 467: 464–476, 2003. doi:[10.1002/cne.10933](https://doi.org/10.1002/cne.10933).
48. Burette A, Weinberg RJ. Perisynaptic organization of plasma membrane calcium pumps in cerebellar cortex. *J Comp Neurol* 500: 1127–1135, 2007. doi:[10.1002/cne.21237](https://doi.org/10.1002/cne.21237).
49. Cai Q, Chandler JS, Wasserman RH, Kumar R, Penniston JT. Vitamin D and adaptation to dietary calcium and phosphate deficiencies increase intestinal plasma membrane calcium pump gene expression. *Proc Natl Acad Sci USA* 90: 1345–1349, 1993. doi:[10.1073/pnas.90.4.1345](https://doi.org/10.1073/pnas.90.4.1345).
50. Cali T, Lopreaiato R, Shimony J, Vineyard M, Frizzarin M, Zanni G, Zanotti G, Brini M, Shinawi M, Carafoli E. A novel mutation in isoform 3 of the plasma membrane  $\text{Ca}^{2+}$  pump impairs cellular  $\text{Ca}^{2+}$  homeostasis in a patient with cerebellar ataxia and laminin subunit 1 $\alpha$  mutations. *J Biol Chem* 290: 16132–16141, 2015. doi:[10.1074/jbc.M115.656496](https://doi.org/10.1074/jbc.M115.656496).
51. Carafoli E. Calcium pump of the plasma membrane. *Physiol Rev* 71: 129–153, 1991.
52. Carayol J, Sacco R, Tores F, Rousseau F, Lewin P, Hager J, Persico AM. Converging evidence for an association of ATP2B2 allelic variants with autism in male subjects. *Biol Psychiatry* 70: 880–887, 2011. doi:[10.1016/j.biopsych.2011.05.020](https://doi.org/10.1016/j.biopsych.2011.05.020).
53. Caride AJ, Chini EN, Homma S, Penniston JT, Dousa TP. mRNA encoding four isoforms of the plasma membrane calcium pump and their variants in rat kidney and nephron segments. *J Lab Clin Med* 132: 149–156, 1998. doi:[10.1016/S0022-2143\(98\)90010-5](https://doi.org/10.1016/S0022-2143(98)90010-5).
54. Caride AJ, Chini EN, Penniston JT, Dousa TP. Selective decrease of mRNAs encoding plasma membrane calcium pump isoforms 2 and 3 in rat kidney. *Kidney Int* 56: 1818–1825, 1999. doi:[10.1046/j.1523-1755.1999.00736.x](https://doi.org/10.1046/j.1523-1755.1999.00736.x).
55. Caride AJ, Chini EN, Yamaki M, Dousa TP, Penniston JT. Unique localization of mRNA encoding plasma membrane  $\text{Ca}^{2+}$  pump isoform 3 in rat thin descending loop of Henle. *Am J Physiol Renal Physiol* 269: F681–F685, 1995.
56. Carpinelli MR, Manning MG, Kile BT, Burt RA. Two ENU-induced alleles of Atp2b2 cause deafness in mice. *PLoS One* 8: e67479, 2013. doi:[10.1371/journal.pone.0067479](https://doi.org/10.1371/journal.pone.0067479).
57. Carrera F, Casart YC, Proverbio T, Proverbio F, Marín R. Preeclampsia and calcium-ATPase activity of plasma membranes from human myometrium and placental trophoblast. *Hypertens Pregnancy* 22: 295–304, 2003. doi:[10.1081/PRG-120024033](https://doi.org/10.1081/PRG-120024033).
58. Cartwright EJ, Neyses L. Evaluation of plasma membrane calcium/calmodulin-dependent ATPase isoform 4 as a potential target for fertility control. *Handb Exp Pharmacol* 198: 79–95, 2010. doi:[10.1007/978-3-642-02062-9\\_6](https://doi.org/10.1007/978-3-642-02062-9_6).
59. Cartwright EJ, Oceandy D, Austin C, Neyses L.  $\text{Ca}^{2+}$  signalling in cardiovascular disease: the role of the plasma membrane calcium pumps. *Sci China Life Sci* 54: 691–698, 2011. doi:[10.1007/s11427-011-4199-1](https://doi.org/10.1007/s11427-011-4199-1).
60. Cartwright EJ, Oceandy D, Neyses L. Physiological implications of the interaction between the plasma membrane calcium pump and nNOS. *Pflugers Arch* 457: 665–671, 2009. doi:[10.1007/s00424-008-0455-z](https://doi.org/10.1007/s00424-008-0455-z).
61. Cartwright EJ, Oceandy D, Neyses L. Plasma membrane calcium ATPase and its relationship to nitric oxide signaling in the heart. *Ann NY Acad Sci* 1099: 247–253, 2007. doi:[10.1196/annals.1387.007](https://doi.org/10.1196/annals.1387.007).
62. Castillo K, Delgado R, Bacigalupo J. Plasma membrane  $\text{Ca}^{2+}$ -ATPase in the cilia of olfactory receptor neurons: possible role in  $\text{Ca}^{2+}$  clearance. *Eur J Neurosci* 26: 2524–2531, 2007. doi:[10.1111/j.1460-9568.2007.05863.x](https://doi.org/10.1111/j.1460-9568.2007.05863.x).
63. Castro J, Ruminot I, Porras OH, Flores CM, Hermosilla T, Verdugo E, Venegas F, Härtel S, Michea L, Barros LF. ATP steal between cation pumps: a mechanism linking  $\text{Na}^{+}$  influx to the onset of necrotic  $\text{Ca}^{2+}$  overload. *Cell Death Differ* 13: 1675–1685, 2006. doi:[10.1038/sj.cdd.4401852](https://doi.org/10.1038/sj.cdd.4401852).
64. Cavieses JD. Calmodulin and the target size of the  $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -ATPase of human red-cell ghosts. *Biochim Biophys Acta* 771: 241–244, 1984. doi:[10.1016/0005-2736\(84\)90539-X](https://doi.org/10.1016/0005-2736(84)90539-X).
65. Centeno VA, Díaz de Barboza GE, Marchionatti AM, Alisio AE, Dallorso ME, Nasif R, Tolosa de Talamoni NG. Dietary calcium deficiency increases  $\text{Ca}^{2+}$  uptake and  $\text{Ca}^{2+}$  extrusion mechanisms in chick enterocytes. *Comp Biochem Physiol A Mol Integr Physiol* 139: 133–141, 2004. doi:[10.1016/j.cbpa.2004.08.002](https://doi.org/10.1016/j.cbpa.2004.08.002).
66. Chaabane C, Dally S, Corvazier E, Bredoux R, Bobe R, Ftouhi B, Raies A, Enouf J. Platelet PMCA- and SERCA-type  $\text{Ca}^{2+}$ -ATPase expression in diabetes: a novel signature of abnormal megakaryocytopoiesis. *J Thromb Haemost* 5: 2127–2135, 2007. doi:[10.1111/j.1538-7836.2007.02709.x](https://doi.org/10.1111/j.1538-7836.2007.02709.x).
67. Chakravarty N, Nielsen EH.  $\text{Ca}^{2+}$ - $\text{Mg}^{2+}$ -activated adenosine triphosphatase in plasma and granule membranes in non-secreting and secreting mast cells. *Exp Cell Res* 130: 175–184, 1980. doi:[10.1016/0014-4827\(80\)90054-3](https://doi.org/10.1016/0014-4827(80)90054-3).
68. Chami M, Ferrari D, Nicotera P, Paterlini-Bréchet P, Rizzuto R. Caspase-dependent alterations of  $\text{Ca}^{2+}$  signaling in the induction of apoptosis by hepatitis B virus X protein. *J Biol Chem* 278: 31745–31755, 2003. doi:[10.1074/jbc.M304202200](https://doi.org/10.1074/jbc.M304202200).

69. Chang RC, Shi L, Huang CC, Kim AJ, Ko ML, Zhou B, Ko GY. High-fat diet-induced retinal dysfunction. *Invest Ophthalmol Vis Sci* 56: 2367–2380, 2015. doi:10.1167/iov.14-16143.
70. Charoenphandhu N, Tudpor K, Pulsook N, Krishnamra N. Chronic metabolic acidosis stimulated transcellular and solvent drag-induced calcium transport in the duodenum of female rats. *Am J Physiol Gastrointest Liver Physiol* 291: G446–G455, 2006. doi:10.1152/ajpgi.00108.2006.
71. Chen J, McLean PA, Neel BG, Okunade G, Shull GE, Wortis HH. CD22 attenuates calcium signaling by potentiating plasma membrane calcium-ATPase activity. *Nat Immunol* 5: 651–657, 2004. doi:10.1038/ni1072.
72. Chen L, Koh DS, Hille B. Dynamics of calcium clearance in mouse pancreatic beta-cells. *Diabetes* 52: 1723–1731, 2003. doi:10.2337/diabetes.52.7.1723.
73. Chen Q, Mahendrasingam S, Tickle JA, Hackney CM, Furness DN, Fettiplace R. The development, distribution and density of the plasma membrane calcium ATPase 2 calcium pump in rat cochlear hair cells. *Eur J Neurosci* 36: 2302–2310, 2012. doi:10.1111/j.1460-9568.2012.08159.x.
74. Chen YF, Cao J, Zhong JN, Chen X, Cheng M, Yang J, Gao YD. Plasma membrane  $\text{Ca}^{2+}$ -ATPase regulates  $\text{Ca}^{2+}$  signaling and the proliferation of airway smooth muscle cells. *Eur J Pharmacol* 740: 733–741, 2014. doi:10.1016/j.ejphar.2014.05.055.
75. Cho JK, Bikle DD. Decrease of  $\text{Ca}^{2+}$ -ATPase activity in human keratinocytes during calcium-induced differentiation. *J Cell Physiol* 172: 146–154, 1997. doi:10.1002/(SICI)1097-4652(199708)172:2<146::AID-JCP2>3.0.CO;2-O.
76. Cho YS, Go MJ, Kim YJ, Heo JY, Oh JH, Ban HJ, Yoon D, Lee MH, Kim DJ, Park M, Cha SH, Kim JW, Han BG, Min H, Ahn Y, Park MS, Han HR, Jang HY, Cho EY, Lee JE, Cho NH, Shin C, Park T, Park JW, Lee JK, Cardon L, Clarke G, McCarthy MI, Lee JY, Lee JK, Oh B, Kim HL. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat Genet* 41: 527–534, 2009. doi:10.1038/ng.357.
77. Choi HS, Eisner DA. The role of sarcolemmal  $\text{Ca}^{2+}$ -ATPase in the regulation of resting calcium concentration in rat ventricular myocytes. *J Physiol* 515: 109–118, 1999. doi:10.1111/j.1469-7793.1999.109ad.x.
78. Cicco G, Pirrelli A. Red blood cell (RBC) deformability, RBC aggregability and tissue oxygenation in hypertension. *Clin Hemorheol Microcirc* 21: 169–177, 1999.
79. Cnop M, Welsh N, Jonas JC, Jörns A, Lenzen S, Eizirik DL. Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. *Diabetes* 54, Suppl 2: S97–S107, 2005. doi:10.2337/diabetes.54.supp\_2.S97.
80. Cohen G, Raupachova J, Wimmer T, Deicher R, Hörl WH. The uraemic retention solute para-hydroxy-hippuric acid attenuates apoptosis of polymorphonuclear leukocytes from healthy subjects but not from haemodialysis patients. *Nephrol Dial Transplant* 23: 2512–2519, 2008. doi:10.1093/ndt/gfn098.
81. Curry MC, Luk NA, Kenny PA, Roberts-Thomson SJ, Monteith GR. Distinct regulation of cytoplasmic calcium signals and cell death pathways by different plasma membrane calcium ATPase isoforms in MDA-MB-231 breast cancer cells. *J Biol Chem* 287: 28598–28608, 2012. doi:10.1074/jbc.M112.364737.
82. Dally S, Chaabane C, Corvazier E, Bredoux R, Bobe R, Ftouhi B, Slimane H, Raies A, Enouf J. Increased expression of plasma membrane  $\text{Ca}^{2+}$ -ATPase 4b in platelets from hypertensives: a new sign of abnormal thrombopoiesis? *Platelets* 18: 543–549, 2007. doi:10.1080/09537100701501646.
83. Darszon A, Nishigaki T, Beltran C, Treviño CL. Calcium channels in the development, maturation, and function of spermatozoa. *Physiol Rev* 91: 1305–1355, 2011. doi:10.1152/physrev.00028.2010.
84. Datta HK, Ng WF, Walker JA, Tuck SP, Varanasi SS. The cell biology of bone metabolism. *J Clin Pathol* 61: 577–587, 2008. doi:10.1136/jcp.2007.048868.
85. Daugirdas JT, Arrieta J, Ye M, Flores G, Battle DC. Intracellular acidification associated with changes in free cytosolic calcium. Evidence for  $\text{Ca}^{2+}/\text{H}^{+}$  exchange via a plasma membrane  $\text{Ca}^{2+}$ -ATPase in vascular smooth muscle cells. *J Clin Invest* 95: 1480–1489, 1995. doi:10.1172/JCI117819.
86. De Jaegere S, Wuytack F, De Smedt H, Van den Bosch L, Casteels R. Alternative processing of the gene transcripts encoding a plasma-membrane and a sarco/endoplasmic reticulum  $\text{Ca}^{2+}$  pump during differentiation of BC3H1 muscle cells. *Biochim Biophys Acta* 1173: 188–194, 1993. doi:10.1016/0167-4781(93)90180-L.
87. De Juan-Sanz J, Núñez E, Zafra F, Berrocal M, Corbacho I, Ibáñez I, Arribas-González E, Marcos D, López-Corcuera B, Mata AM, Aragón C. Presynaptic control of glycine transporter 2 (GlyT2) by physical and functional association with plasma membrane  $\text{Ca}^{2+}$ -ATPase (PMCA) and  $\text{Na}^{+}$ - $\text{Ca}^{2+}$  exchanger (NCX). *J Biol Chem* 289: 34308–34324, 2014. doi:10.1074/jbc.M114.586966.
88. Dean WL. Role of platelet plasma membrane  $\text{Ca}$ -ATPase in health and disease. *World J Biol Chem* 1: 265–270, 2010. doi:10.4331/wjbc.v1.i9.265.
89. Dean WL, Pope JE, Brier ME, Aronoff GR. Platelet calcium transport in hypertension. *Hypertension* 23: 31–37, 1994. doi:10.1161/01.HYP.23.1.31.
90. Delgado-Coello B, Santiago-García J, Zarain-Herzberg A, Mas-Oliva J. Plasma membrane  $\text{Ca}^{2+}$ -ATPase mRNA expression in murine hepatocarcinoma and regenerating liver cells. *Mol Cell Biochem* 247: 177–184, 2003. doi:10.1023/A:1024119831983.
91. DeMarco SJ, Chicka MC, Strehler EE. Plasma membrane  $\text{Ca}^{2+}$  ATPase isoform 2b interacts preferentially with  $\text{Na}^{+}/\text{H}^{+}$  exchanger regulatory factor 2 in apical plasma membranes. *J Biol Chem* 277: 10506–10511, 2002. doi:10.1074/jbc.M111616200.
92. DeMarco SJ, Strehler EE. Plasma membrane  $\text{Ca}^{2+}$ -ATPase isoforms 2b and 4b interact promiscuously and selectively with members of the membrane-associated guanylate kinase family of PDZ (PSD95/Dlg/ZO-1) domain-containing proteins. *J Biol Chem* 276: 21594–21600, 2001. doi:10.1074/jbc.M101448200.
93. DeSantiago J, Batlle D, Khilnani M, Dedhia S, Kulczyk J, Duque R, Ruiz J, Pena-Rasgado C, Rasgado-Flores H.  $\text{Ca}^{2+}/\text{H}^{+}$  exchange via the plasma membrane  $\text{Ca}^{2+}$  ATPase in skeletal muscle. *Front Biosci* 12: 4641–4660, 2007. doi:10.2741/2414.
94. Di Leva F, Domi T, Fedrizzi L, Lim D, Carafoli E. The plasma membrane  $\text{Ca}^{2+}$  ATPase of animal cells: structure, function and regulation. *Arch Biochem Biophys* 476: 65–74, 2008. doi:10.1016/j.abb.2008.02.026.
95. Diaz de Barboza G, Guizzardi S, Tolosa de Talamoni N. Molecular aspects of intestinal calcium absorption. *World J Gastroenterol* 21: 7142–7154, 2015.
96. Di Polo R, Beaugé L. The calcium pump and sodium-calcium exchange in squid axons. *Annu Rev Physiol* 45: 313–324, 1983. doi:10.1146/annurev.ph.45.030183.001525.
97. Dong XL, Zhang Y, Wong MS. Estrogen deficiency-induced Ca balance impairment is associated with decrease in expression of epithelial Ca transport proteins in aged female rats. *Life Sci* 96: 26–32, 2014. doi:10.1016/j.lfs.2013.12.025.
98. Doucet A, Katz AI. High-affinity  $\text{Ca}$ - $\text{Mg}$ -ATPase along the rabbit nephron. *Am J Physiol Renal Physiol* 242: F346–F352, 1982.
99. Dumont RA, Lins U, Filoteo AG, Penniston JT, Kachar B, Gillespie PG. Plasma membrane  $\text{Ca}^{2+}$ -ATPase isoform 2a is the PMCA of hair bundles. *J Neurosci* 21: 5066–5078, 2001.
100. Duncan G, Bushell AR. Ion analyses of human cataractous lenses. *Exp Eye Res* 20: 223–230, 1975. doi:10.1016/0014-4835(75)90136-0.
101. Duncan JL, Yang H, Doan T, Silverstein RS, Murphy GJ, Nune G, Liu X, Copenhagen D, Tempel BL, Rieke F, Krizaj D. Scotopic visual signaling in the mouse retina is modulated by high-affinity plasma membrane calcium extrusion. *J Neurosci* 26: 7201–7211, 2006. doi:10.1523/JNEUROSCI.5230-05.2006.
102. Dutta RK, Welander J, Brauckhoff M, Walz M, Alesina P, Arnesen T, Söderkvist P, Gimm O. Complementary somatic mutations of KCNJ5, ATP1A1, and ATP2B3 in sporadic aldosterone producing adrenal adenomas. *Endocr Relat Cancer* 21: L1–L4, 2014. doi:10.1530/ERC-13-0466.
103. Eaton JW, Skelton TD, Swofford HS, Kolpin CE, Jacob HS. Elevated erythrocyte calcium in sickle cell disease. *Nature* 246: 105–106, 1973. doi:10.1038/246105a0.
104. Eder P, Molkentin JD. TRPC channels as effectors of cardiac hypertrophy. *Circ Res* 108: 265–272, 2011. doi:10.1161/CIRCRESAHA.110.225888.
105. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, Smith AV, Tobin MD, Verwoert GC, Hwang SJ, Pihur V, Vollenweider P, O'Reilly PF, Amin N, Bragg-Gresham JL, Teumer A, Glazer NL, Launer L, Zhao JH, Aulchenko Y, Heath S, Söber S, Parsa A, Luan J, Arora P, Dehghan A, Zhang F, Lucas G, Hicks AA, Jackson AU, Peden JF, Tanaka T, Wild SH, Rudan I, Igl W, Milaneschi Y, Parker AN, Fava C, Chambers JC, Fox ER, Kumari M, Go MJ, van der Harst P, Kao WH, Sjögren M, Vinay DG, Alexander M, Tabara Y, Shaw-Hawkins S, Whincup PH, Liu Y, Shi G, Kuusisto J, Tayo B, Seielstad M, Sim X, Nguyen KD, Lehtimäki T, Matullo G, Wu Y, Gaunt TR, Onland-Moret NC, Cooper MN, Platou CG, Org E, Hardy R, Dahgam S, Palmen J, Vitart V, Braund PS, Kuznetsova T, Uitterwaal CS, Adeyemo A, Palmas W, Campbell



- H, Ludwig B, Tomaszewski M, Tzoulaki I, Palmer ND, Aspelund T, Garcia M, Chang YP, O'Connell JR, Steinle NI, Grobbee DE, Arking DE, Kardina SL, Morrison AC, Hernandez D, Najjar S, McArdle WL, Hadley D, Brown MJ, Connell JM, Hingorani AD, Day IN, Lawlor DA, Beilby JP, Lawrence RW, Clarke R, Hopewell JC, Ongen H, Dreisbach AW, Li Y, Young JH, Bis JC, Kähönen M, Viikari J, Adair LS, Lee NR, Chen MH, Olden M, Pattaro C, Bolton JA, Kottgen A, Bergmann S, Mooser V, Chaturvedi N, Frayling TM, Islam M, Jafar TH, Erdmann J, Kulkarni SR, Bornstein SR, Grässler J, Groop L, Voight BF, Kettunen J, Howard P, Taylor A, Guarrera S, Ricceri F, Emilsson V, Plump A, Barroso I, Khaw KT, Weder AB, Hunt SC, Sun YV, Bergman RN, Collins FS, Bonnycastle LL, Scott LJ, Stringham HM, Peltonen L, Perola M, Vartiainen E, Brand SM, Staessen JA, Wang TJ, Burton PR, Soler Artigas M, Dong Y, Snieder H, Wang X, Zhu H, Lohman KK, Rudock ME, Heckbert SR, Smith NL, Wiggins KL, Doumatey A, Shriner D, Veldre G, Viigimaa M, Kinra S, Prabhakaran D, Tripathy V, Langefeld CD, Rosengren A, Thelle DS, Corsi AM, Singleton A, Forrester T, Hilton G, McKenzie CA, Salako T, Iwai N, Kita Y, Ogihara T, Ohkubo T, Okamura T, Ueshima H, Umemura S, Eyheramendy S, Meitinger T, Wichmann HE, Cho YS, Kim HL, Lee JY, Scott J, Sehmi JS, Zhang W, Hedblad B, Nilsson P, Smith GD, Wong A, Narisu N, Stančáková A, Raffel LJ, Yao J, Kathiresan S, O'Donnell CJ, Schwartz SM, Ikram MA, Longstreth WT Jr, Mosley TH, Seshadri S, Shrine PR, Wain LV, Morken MA, Swift AJ, Laitinen J, Prokopenko I, Zitting P, Cooper JA, Humphries SE, Danesh J, Rasheed A, Goel A, Hamsten A, Watkins H, Bakker SJ, van Gilst WH, Janipalli CS, Mani KR, Yajnik CS, Hofman A, Mattace-Raso FU, Oostra BA, Demirkan A, Isaacs A, Rivadeneira F, Lakatta EG, Orru M, Scuteri A, Ala-Korpela M, Kangas AJ, Lyytikäinen LP, Soininen P, Tukiainen T, Würtz P, Ong RT, Dörr M, Kroemer HK, Völker U, Völzke H, Galan P, Hercberg S, Lathrop M, Zelenika D, Deloukas P, Mangino M, Spector TD, Zhai G, Meschia JF, Nalls MA, Sharma P, Terzic J, Kumar MV, Denniff M, Zukowska-Szczekowska E, Wagenknecht LE, Fowkes FG, Charchar FJ, Schwarz PE, Hayward C, Guo X, Rotimi C, Bots ML, Brand E, Samani NJ, Polasek O, Talmud PJ, Nyberg F, Kuh D, Laan M, Hveem K, Palmer LJ, van der Schouw YT, Casas JP, Mohlke KL, Vineis P, Raitakari O, Ganesh SK, Wong TY, Tai ES, Cooper RS, Laakso M, Rao DC, Harris TB, Morris RW, Dominiczak AF, Kivimäki M, Marmot MG, Miki T, Saleheen D, Chandak GR, Coresh J, Navis G, Salomaa V, Han BG, Zhu X, Koener JS, Melander O, Ridker PM, Bandinelli S, Gyllenstein UB, Wright AF, Wilson JF, Ferrucci L, Farrall M, Tuomilehto J, Pramstaller PP, Elosua R, Soranzo N, Sijbrands EJ, Altschuler D, Loos RJ, Shuldiner AR, Gieger C, Meneton P, Uitterlinden AG, Wareham NJ, Gudnason V, Rotter JJ, Rettig R, Uda M, Strachan DP, Witteman JC, Hartikainen AL, Beckmann JS, Boerwinkle E, Vasan RS, Boehnke M, Larson MG, Jarvelin MR, Psaty BM, Abecasis GR, Chakravarti A, Elliott P, van Duijn CM, Newton-Cheh C, Levy D, Caulfield MJ, Johnson T International Consortium for Blood Pressure Genome-Wide Association StudiesCARDIoGRAM consortiumCKDGen ConsortiumKidneyGen ConsortiumECHOGen consortium; CHARGE-HF consortium. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* 478: 103–109, 2011. doi:10.1038/nature10405.
106. El-Jouni W, Jang B, Haun S, Machaca K. Calcium signaling differentiation during *Xenopus* oocyte maturation. *Dev Biol* 288: 514–525, 2005. doi:10.1016/j.ydbio.2005.10.034.
107. Elwess NL, Filoteo AG, Enyedi A, Penniston JT. Plasma membrane  $\text{Ca}^{2+}$  pump isoforms 2a and 2b are unusually responsive to calmodulin and  $\text{Ca}^{2+}$ . *J Biol Chem* 272: 17981–17986, 1997. doi:10.1074/jbc.272.29.17981.
108. Empson RM, Akemann W, Knöpfel T. The role of the calcium transporter protein plasma membrane calcium ATPase PMCA2 in cerebellar Purkinje neuron function. *Funct Neurol* 25: 153–158, 2010.
109. Empson RM, Garside ML, Knöpfel T. Plasma membrane  $\text{Ca}^{2+}$  ATPase 2 contributes to short-term synapse plasticity at the parallel fiber to Purkinje neuron synapse. *J Neurosci* 27: 3753–3758, 2007. doi:10.1523/JNEUROSCI.0069-07.2007.
110. Empson RM, Huang H, Nagaraja RY, Roome CJ, Knöpfel T. Enhanced synaptic inhibition in the cerebellar cortex of the ataxic PMCA2(–/–) knockout mouse. *Cerebellum* 12: 667–675, 2013. doi:10.1007/s12111-013-0472-0.
111. Empson RM, Turner PR, Nagaraja RY, Beesley PW, Knöpfel T. Reduced expression of the  $\text{Ca}^{2+}$  transporter protein PMCA2 slows  $\text{Ca}^{2+}$  dynamics in mouse cerebellar Purkinje neurones and alters the precision of motor coordination. *J Physiol* 588: 907–922, 2010. doi:10.1113/jphysiol.2009.182196.
112. Enyedi A, Flura M, Sarkadi B, Gardos G, Carafoli E. The maximal velocity and the calcium affinity of the red cell calcium pump may be regulated independently. *J Biol Chem* 262: 6425–6430, 1987.
113. Enyedi A, Verma AK, Filoteo AG, Penniston JT. Protein kinase C activates the plasma membrane  $\text{Ca}^{2+}$  pump isoform 4b by phosphorylation of an inhibitory region downstream of the calmodulin-binding domain. *J Biol Chem* 271: 32461–32467, 1996. doi:10.1074/jbc.271.50.32461.
114. Etzion Z, Tiffert T, Bookchin RM, Lew VL. Effects of deoxygenation on active and passive  $\text{Ca}^{2+}$  transport and on the cytoplasmic  $\text{Ca}^{2+}$  levels of sickle cell anemia red cells. *J Clin Invest* 92: 2489–2498, 1993. doi:10.1172/JCI116857.
115. Fakira AK, Gaspers LD, Thomas AP, Li H, Jain MR, Elkabes S. Purkinje cell dysfunction and delayed death in plasma membrane calcium ATPase 2-heterozygous mice. *Mol Cell Neurosci* 51: 22–31, 2012. doi:10.1016/j.mcn.2012.07.001.
116. Falchetto R, Vorherr T, Brunner J, Carafoli E. The plasma membrane  $\text{Ca}^{2+}$  pump contains a site that interacts with its calmodulin-binding domain. *J Biol Chem* 266: 2930–2936, 1991.
117. Falchetto R, Vorherr T, Carafoli E. The calmodulin-binding site of the plasma membrane  $\text{Ca}^{2+}$  pump interacts with the transduction domain of the enzyme. *Protein Sci* 1: 1613–1621, 1992. doi:10.1002/pro.5560011209.
118. Ferdek PE, Gerasimenko JV, Peng S, Tepikin AV, Petersen OH, Gerasimenko OV. A novel role for Bcl-2 in regulation of cellular calcium extrusion. *Curr Biol* 22: 1241–1246, 2012. doi:10.1016/j.cub.2012.05.002.
119. Ferguson JF, Matthews GJ, Townsend RR, Raj DS, Kanetsky PA, Budoff M, Fischer MJ, Rosas SE, Kanthety R, Rahman M, Master SR, Qasim A, Li M, Mehta NN, Shen H, Mitchell BD, O'Connell JR, Shuldiner AR, Ho WK, Young R, Rasheed A, Danesh J, He J, Kusek JW, Ojo AO, Flack J, Go AS, Gadegbeku CA, Wright JT Jr, Saleheen D, Feldman HI, Rader DJ, Foulkes AS, Reilly MP, CRIC Study Principal Investigators. Candidate gene association study of coronary artery calcification in chronic kidney disease: findings from the CRIC study (Chronic Renal Insufficiency Cohort). *J Am Coll Cardiol* 62: 789–798, 2013. doi:10.1016/j.jacc.2013.01.103.
120. Fernandes D, Zaidi A, Bean J, Hui D, Michaelis ML. RNA-induced silencing of the plasma membrane  $\text{Ca}^{2+}$ -ATPase 2 in neuronal cells: effects on  $\text{Ca}^{2+}$  homeostasis and cell viability. *J Neurochem* 102: 454–465, 2007. doi:10.1111/j.1471-4159.2007.04592.x.
121. Fernandes-Rosa FL, Williams TA, Riester A, Steichen O, Beuschlein F, Boulkroun S, Strom TM, Monticone S, Amar L, Meatchi T, Mantero F, Cicala MV, Quinkler M, Fallo F, Allolio B, Bernini G, Maccario M, Giacchetti G, Jeunemaitre X, Mulatero P, Reincke M, Zennaro MC. Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. *Hypertension* 64: 354–361, 2014. doi:10.1161/HYPERTENSIONAHA.114.03419.
122. Ferreira HG, Lew VL. Use of ionophore A23187 to measure cytoplasmic Ca buffering and activation of the Ca pump by internal Ca. *Nature* 259: 47–49, 1976. doi:10.1038/259047a0.
123. Ficarella R, Di Leva F, Bortolozzi M, Ortolano S, Donaudy F, Petrillo M, Melchionda S, Lelli A, Domi T, Fedrizzi L, Lim D, Shull GE, Gasparini P, Brini M, Mammano F, Carafoli E. A functional study of plasma-membrane calcium-pump isoform 2 mutants causing digenic deafness. *Proc Natl Acad Sci USA* 104: 1516–1521, 2007. doi:10.1073/pnas.0609775104.
124. Fontana V, McDonough CW, Gong Y, El Rouby NM, Sá AC, Taylor KD, Chen YD, Gums JG, Chapman AB, Turner ST, Pepine CJ, Johnson JA, Cooper-DeHoff RM. Large-scale gene-centric analysis identifies polymorphisms for resistant hypertension. *J Am Heart Assoc* 3: e001398, 2014. doi:10.1161/JAHA.114.001398.
125. Freeman TC, Howard A, Bentsen BS, Legon S, Walters JR. Cellular and regional expression of transcripts of the plasma membrane calcium pump PMCA1 in rabbit intestine. *Am J Physiol Gastrointest Liver Physiol* 269: G126–G131, 1995.
126. Frey N, McKinsey TA, Olson EN. Decoding calcium signals involved in cardiac growth and function. *Nat Med* 6: 1221–1227, 2000. doi:10.1038/81321.
127. Friedman PA, Gesek FA. Cellular calcium transport in renal epithelia: measurement, mechanisms, and regulation. *Physiol Rev* 75: 429–471, 1995.
128. Fujimoto T. Calcium pump of the plasma membrane is localized in caveolae. *J Cell Biol* 120: 1147–1157, 1993. doi:10.1083/jcb.120.5.1147.
129. Fujiwara A, Hirawa N, Fujita M, Kobayashi Y, Okuyama Y, Yatsu K, Katsumata M, Yamamoto Y, Ichihara N, Saka S, Toba Y, Yasuda G, Goshima Y, Tabara Y, Miki T, Ueshima H, Ishikawa Y, Umemura S. Impaired nitric oxide production and increased blood pressure in systemic heterozygous ATP2B1 null mice. *J Hypertens* 32: 1415–1423, 2014. doi:10.1097/HJH.0000000000000206.
130. Garcia ML, Murray KD, Garcia VB, Strehler EE, Isackson PJ. Seizure-induced alterations of plasma membrane calcium ATPase isoforms 1, 2 and 3 mRNA and protein in rat hippocampus. *Brain Res Mol Brain Res* 45: 230–238, 1997. doi:10.1016/S0169-328X(96)00253-7.



131. Garcia ML, Usachev YM, Thayer SA, Strehler EE, Windebank AJ. Plasma membrane calcium ATPase plays a role in reducing Ca<sup>2+</sup>-mediated cytotoxicity in PC12 cells. *J Neurosci Res* 64: 661–669, 2001. doi:10.1002/jnr.1120.
132. Gennaro R, Mottola C, Schneider C, Romeo D. Ca<sup>2+</sup>-dependent ATPase activity of alveolar macrophage plasma membrane. *Biochim Biophys Acta* 567: 238–246, 1979. doi:10.1016/0005-2744(79)90190-6.
133. Geyik E, Igci YZ, Pala E, Suner A, Borazan E, Bozgeyik I, Bayraktar E, Bayraktar R, Ergun S, Cakmak EA, Gokalp A, Arslan A. Investigation of the association between ATP2B4 and ATP5B genes with colorectal cancer. *Gene* 540: 178–182, 2014. doi:10.1016/j.gene.2014.02.050.
134. Giacomello M, De Mario A, Lopreiato R, Primerano S, Campeol M, Brini M, Carafoli E. Mutations in PMCA2 and hereditary deafness: a molecular analysis of the pump defect. *Cell Calcium* 50: 569–576, 2011. doi:10.1016/j.ceca.2011.09.004.
135. Giacomello M, De Mario A, Primerano S, Brini M, Carafoli E. Hair cells, plasma membrane Ca<sup>2+</sup> ATPase and deafness. *Int J Biochem Cell Biol* 44: 679–683, 2012. doi:10.1016/j.biocel.2012.02.006.
136. Go W, Korzh V. Plasma membrane Ca(2+) ATPase Atp2b1a regulates bone mineralization in zebrafish. *Bone* 54: 48–57, 2013. doi:10.1016/j.bone.2013.01.026.
137. Goellner GM, DeMarco SJ, Strehler EE. Characterization of PISP, a novel single-PDZ protein that binds to all plasma membrane Ca<sup>2+</sup>-ATPase b-splice variants. *Ann NY Acad Sci* 986: 461–471, 2003. doi:10.1111/j.1749-6632.2003.tb07230.x.
138. Gomez-Pinilla PJ, Pozo MJ, Baba A, Matsuda T, Camello PJ. Ca<sup>2+</sup> extrusion in aged smooth muscle cells. *Biochem Pharmacol* 74: 860–869, 2007. doi:10.1016/j.bcp.2007.06.037.
139. Grati M, Aggarwal N, Strehler EE, Wenthold RJ. Molecular determinants for differential membrane trafficking of PMCA1 and PMCA2 in mammalian hair cells. *J Cell Sci* 119: 2995–3007, 2006. doi:10.1242/jcs.03030.
140. Greco EA, Lenzi A, Migliaccio S. The obesity of bone. *Ther Adv Endocrinol Metab* 6: 273–286, 2015. doi:10.1177/2042018815611004.
141. Gros R, Afroze T, You XM, Kabir G, Van Wert R, Kalair W, Hoque AE, Mungrue IN, Husain M. Plasma membrane calcium ATPase overexpression in arterial smooth muscle increases vasomotor responsiveness and blood pressure. *Circ Res* 93: 614–621, 2003. doi:10.1161/01.RES.0000092142.19896.D9.
142. Guerini D, Pan B, Carafoli E. Expression, purification, and characterization of isoform I of the plasma membrane Ca<sup>2+</sup> pump: focus on calpain sensitivity. *J Biol Chem* 278: 38141–38148, 2003. doi:10.1074/jbc.M302400200.
143. Guerini D, Zecca-Mazza A, Carafoli E. Single amino acid mutations in transmembrane domain 5 confer to the plasma membrane Ca<sup>2+</sup> pump properties typical of the Ca<sup>2+</sup> pump of endo(sarco)plasmic reticulum. *J Biol Chem* 275: 31361–31368, 2000. doi:10.1074/jbc.M003474200.
144. Habib T, Park H, Tsang M, de Alborán IM, Nicks A, Wilson L, Knoepfler PS, Andrews S, Rawlings DJ, Eisenman RN, Iritani BM. Myc stimulates B lymphocyte differentiation and amplifies calcium signaling. *J Cell Biol* 179: 717–731, 2007. doi:10.1083/jcb.200704173.
145. Haché S, Takser L, LeBellego F, Weiler H, Leduc L, Forest JC, Giguère Y, Masse A, Barbeau B, Lafond J. Alteration of calcium homeostasis in primary preeclamptic syncytiotrophoblasts: effect on calcium exchange in placenta. *J Cell Mol Med* 15: 654–667, 2011. doi:10.1111/j.1582-4934.2010.01039.x.
146. Hajimohammadreza I, Raser KJ, Nath R, Nadimpalli R, Scott M, Wang KK. Neuronal nitric oxide synthase and calmodulin-dependent protein kinase IIα undergo neurotoxin-induced proteolysis. *J Neurochem* 69: 1006–1013, 1997. doi:10.1046/j.1471-4159.1997.69031006.x.
147. Hammes A, Oberdorf S, Strehler EE, Stauffer T, Carafoli E, Vetter H, Neyes L. Differentiation-specific isoform mRNA expression of the calmodulin-dependent plasma membrane Ca(2+)-ATPase. *FASEB J* 8: 428–435, 1994.
148. Hammes A, Oberdorf-Maass S, Jenatschke S, Pelzer T, Maass A, Gollnick F, Meyer R, Afflerbach J, Neyes L. Expression of the plasma membrane Ca<sup>2+</sup>-ATPase in myogenic cells. *J Biol Chem* 271: 30816–30822, 1996. doi:10.1074/jbc.271.48.30816.
149. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 144: 646–674, 2011. doi:10.1016/j.cell.2011.02.013.
150. Harpavat M, Keljo DJ, Regueiro MD. Metabolic bone disease in inflammatory bowel disease. *J Clin Gastroenterol* 38: 218–224, 2004. doi:10.1097/00004836-200403000-00005.
151. He W, Zhang L, Ni A, Zhang Z, Mirotsoy M, Mao L, Pratt RE, Dzau VJ. Exogenously administered secreted frizzled related protein 2 (Sfrp2) reduces fibrosis and improves cardiac function in a rat model of myocardial infarction. *Proc Natl Acad Sci USA* 107: 21110–21115, 2010. doi:10.1073/pnas.1004708107.
152. Heo SG, Hwang JY, Uhm S, Go MJ, Oh B, Lee JY, Park JW. Male-specific genetic effect on hypertension and metabolic disorders. *Hum Genet* 133: 311–319, 2014. doi:10.1007/s00439-013-1382-4.
153. Herchuelz A, Kamagate A, Ximenes H, Van Eylen F. Role of Na/Ca exchange and the plasma membrane Ca<sup>2+</sup>-ATPase in beta cell function and death. *Ann NY Acad Sci* 1099: 456–467, 2007. doi:10.1196/annals.1387.048.
154. Herchuelz A, Nguidjoe E, Jiang L, Pachera N. β-Cell preservation and regeneration in diabetes by modulation of β-cell Ca<sup>2+</sup> homeostasis. *Diabetes Obes Metab* 14, Suppl 3: 136–142, 2012. doi:10.1111/j.1463-1326.2012.01649.x.
155. Herchuelz A, Nguidjoe E, Jiang L, Pachera N. Na(+)/Ca(2+) exchange and the plasma membrane Ca(2+)-ATPase in β-cell function and diabetes. *Adv Exp Med Biol* 961: 385–394, 2013. doi:10.1007/978-1-4614-4756-6\_33.
156. Ho PW, Pang SY, Li M, Tse ZH, Kung MH, Sham PC, Ho SL. PMCA4 (ATP2B4) mutation in familial spastic paraplegia causes delay in intracellular calcium extrusion. *Brain Behav* 5: e00321, 2015. doi:10.1002/brb3.321.
157. Hoenderop JG, Nilius B, Bindels RJ. Calcium absorption across epithelia. *Physiol Rev* 85: 373–422, 2005. doi:10.1152/physrev.00003.2004.
158. Holton M, Mohamed TM, Oceandy D, Wang W, Lamas S, Emerson M, Neyes L, Armesilla AL. Endothelial nitric oxide synthase activity is inhibited by the plasma membrane calcium ATPase in human endothelial cells. *Cardiovasc Res* 87: 440–448, 2010. doi:10.1093/cvr/cvq077.
159. Holton M, Yang D, Wang W, Mohamed TM, Neyes L, Armesilla AL. The interaction between endogenous calcineurin and the plasma membrane calcium-dependent ATPase is isoform specific in breast cancer cells. *FEBS Lett* 581: 4115–4119, 2007. doi:10.1016/j.febslet.2007.07.054.
160. Holton ML, Wang W, Emerson M, Neyes L, Armesilla AL. Plasma membrane calcium ATPase proteins as novel regulators of signal transduction pathways. *World J Biol Chem* 1: 201–208, 2010. doi:10.4331/wjbc.v1.i6.201.
161. Homann V, Kinne-Saffran E, Arnold WH, Gaengler P, Kinne RK. Calcium transport in human salivary glands: a proposed model of calcium secretion into saliva. *Histochem Cell Biol* 125: 583–591, 2006. doi:10.1007/s00418-005-0100-2.
162. Hong KW, Go MJ, Jin HS, Lim JE, Lee JY, Han BG, Hwang SY, Lee SH, Park HK, Cho YS, Oh B. Genetic variations in ATP2B1, CSK, ARSG and CSMD1 loci are related to blood pressure and/or hypertension in two Korean cohorts. *J Hum Hypertens* 24: 367–372, 2010. doi:10.1038/jhh.2009.86.
163. Howard A, Legon S, Walters JR. Human and rat intestinal plasma membrane calcium pump isoforms. *Am J Physiol Gastrointest Liver Physiol* 265: G917–G925, 1993.
164. Hu VW, Nguyen A, Kim KS, Steinberg ME, Sarachana T, Scully MA, Soldin SJ, Liu T, Lee NH. Gene expression profiling of lymphoblasts from autistic and nonaffected sib pairs: altered pathways in neuronal development and steroid biosynthesis. *PLoS One* 4: e5775, 2009. doi:10.1371/journal.pone.0005775.
165. Husain M, Jiang L, See V, Bein K, Simons M, Alper SL, Rosenberg RD. Regulation of vascular smooth muscle cell proliferation by plasma membrane Ca(2+)-ATPase. *Am J Physiol Cell Physiol* 272: C1947–C1959, 1997.
166. Inoue Y, Matsumura Y, Inoue K, Ichikawa R, Takayama C. Abnormal synaptic architecture in the cerebellar cortex of a new dystonic mutant mouse, Wrinkle Mouse Sagami. *Neurosci Res* 16: 39–48, 1993. doi:10.1016/0168-0102(93)90007-D.
167. James AD, Chan A, Erice O, Siriwardena AK, Bruce JI. Glycolytic ATP fuels the plasma membrane calcium pump critical for pancreatic cancer cell survival. *J Biol Chem* 288: 36007–36019, 2013. doi:10.1074/jbc.M113.502948.
168. Jardin I, Redondo PC, Salido GM, Pariente JA, Rosado JA. Endogenously generated reactive oxygen species reduce PMCA activity in platelets from patients with non-insulin-dependent diabetes mellitus. *Platelets* 17: 283–288, 2006. doi:10.1080/09537100600745187.

169. Jeong J, VanHouten JN, Dann P, Kim W, Sullivan C, Yu H, Liotta L, Espina V, Stern DF, Friedman PA, Wysolmerski JJ. PMCA2 regulates HER2 protein kinase localization and signaling and promotes HER2-mediated breast cancer. *Proc Natl Acad Sci USA* 113: E282–E290, 2016. doi:[10.1073/pnas.1516138113](https://doi.org/10.1073/pnas.1516138113).
170. Ji J, Lu R, Zhou X, Xue Y, Shi C, Goltzman D, Miao D. 1,25-Dihydroxyvitamin D<sub>3</sub> contributes to regulating mammary calcium transport and modulates neonatal skeletal growth and turnover cooperatively with calcium. *Am J Physiol Endocrinol Metab* 301: E889–E900, 2011. doi:[10.1152/ajpendo.00173.2011](https://doi.org/10.1152/ajpendo.00173.2011).
171. Jiang L, Allagnat F, Nguidjoe E, Kamagate A, Pachera N, Vanderwinden JM, Brini M, Carafoli E, Eizirik DL, Cardozo AK, Herchuelz A. Plasma membrane Ca<sup>2+</sup>-ATPase overexpression depletes both mitochondrial and endoplasmic reticulum Ca<sup>2+</sup> stores and triggers apoptosis in insulin-secreting BRIN-BD11 cells. *J Biol Chem* 285: 30634–30643, 2010. doi:[10.1074/jbc.M110.116681](https://doi.org/10.1074/jbc.M110.116681).
172. Jiang L, Bechtel MD, Galeva NA, Williams TD, Michaelis EK, Michaelis ML. Decreases in plasma membrane Ca<sup>2+</sup>-ATPase in brain synaptic membrane rafts from aged rats. *J Neurochem* 123: 689–699, 2012. doi:[10.1111/j.1471-4159.2012.07918.x](https://doi.org/10.1111/j.1471-4159.2012.07918.x).
173. Johnson T, Gaunt TR, Newhouse SJ, Padmanabhan S, Tomaszewski M, Kumari M, Morris RW, Tzoulaki I, O'Brien ET, Poulter NR, Sever P, Shields DC, Thom S, Wamnamethee SG, Whincup PH, Brown MJ, Connell JM, Dobson RJ, Howard PJ, Mein CA, Onipinla A, Shaw-Hawkins S, Zhang Y, Davey Smith G, Day IN, Lawlor DA, Goodall AH, Fowkes FG, Abecasis GR, Elliott P, Gateva V, Braund PS, Burton PR, Nelson CP, Tobin MD, van der Harst P, Glorioso N, Neuvirth H, Salvi E, Staessen JA, Stucchi A, Devos N, Jeunemaitre X, Plouin PF, Tichet J, Juhanson P, Org E, Putku M, Söber S, Veldre G, Viigimaa M, Levinsson A, Rosengren A, Thelle DS, Hastie CE, Hedner T, Lee WK, Melander O, Wahlstrand B, Hardy R, Wong A, Cooper JA, Palmer J, Chen L, Stewart AF, Wells GA, Westra HJ, Wolfs MG, Clarke R, Franzosi MG, Farrall M, Hamsten A, Lathrop M, Peden JF, Seedorf U, Watkins H, Ouwehand WH, Sambrook J, Stephens J, Casas JP, Drenos F, Holmes MV, Kivimaki M, Shah S, Shah T, Talmud PJ, Whittaker J, Wallace C, Delles C, Laan M, Kuh D, Humphries SE, Nyberg F, Cusi D, Roberts R, Newton-Cheh C, Franke L, Stanton AV, Dominiczak AF, Farrall M, Hingorani AD, Samani NJ, Caulfield MJ, Munroe PB, Cardiogenics Consortium, Global BGen Consortium. Blood pressure loci identified with a gene-centric array. *Am J Hum Genet* 89: 688–700, 2011. doi:[10.1016/j.ajhg.2011.10.013](https://doi.org/10.1016/j.ajhg.2011.10.013).
174. Jones S, Solomon A, Sanz-Rosa D, Moore C, Holbrook L, Cartwright EJ, Neyeses L, Emerson M. The plasma membrane calcium ATPase modulates calcium homeostasis, intracellular signaling events and function in platelets. *J Thromb Haemost* 8: 2766–2774, 2010. doi:[10.1111/j.1538-7836.2010.04076.x](https://doi.org/10.1111/j.1538-7836.2010.04076.x).
175. Kamagate A, Herchuelz A, Bollen A, Van Eylen F. Expression of multiple plasma membrane Ca(2+)-ATPases in rat pancreatic islet cells. *Cell Calcium* 27: 231–246, 2000. doi:[10.1054/ceca.2000.0116](https://doi.org/10.1054/ceca.2000.0116).
176. Kamagate A, Herchuelz A, Van Eylen F. Plasma membrane Ca(2+)-ATPase overexpression reduces Ca(2+) oscillations and increases insulin release induced by glucose in insulin-secreting BRIN-BD11 cells. *Diabetes* 51: 2773–2788, 2002. doi:[10.2337/diabetes.51.9.2773](https://doi.org/10.2337/diabetes.51.9.2773).
177. Kang YH. Effects of bacterial lipopolysaccharide and calmodulin on Ca(2+)-ATPase and calcium in human natural killer cells, studied by a combined technique of immunoelectron microscopy and ultracytochemistry. *J Histochem Cytochem* 38: 359–370, 1990. doi:[10.1177/38.3.2137483](https://doi.org/10.1177/38.3.2137483).
178. Kato N, Takeuchi F, Tabara Y, Kelly TN, Go MJ, Sim X, Tay WT, Chen CH, Zhang Y, Yamamoto K, Katsuya T, Yokota M, Kim YJ, Ong RT, Nabika T, Gu D, Chang LC, Kokubo Y, Huang W, Ohnaka K, Yamori Y, Nakashima E, Jaquish CE, Lee JY, Seielstad M, Isono M, Hixson JE, Chen YT, Miki T, Zhou X, Zhou X, Sugiyama T, Jeon JP, Liu JJ, Takayanagi R, Kim SS, Aung T, Sung YJ, Zhang X, Wong TY, Han BG, Kobayashi S, Ogiwara T, Zhu D, Iwai N, Wu JY, Teo YY, Tai ES, Cho YS, He J. Meta-analysis of genome-wide association studies identifies common variants associated with blood pressure variation in east Asians. *Nat Genet* 43: 531–538, 2011. doi:[10.1038/ng.834](https://doi.org/10.1038/ng.834).
179. Katsumata S, Matsuzaki H, Katsumata-Tsuboi R, Uehara M, Suzuki K. Effects of high phosphorus diet on bone metabolism-related gene expression in young and aged mice. *J Nutr Metab* 2014: 575932, 2014. doi:[10.1155/2014/575932](https://doi.org/10.1155/2014/575932).
180. Kelly TN, Takeuchi F, Tabara Y, Edwards TL, Kim YJ, Chen P, Li H, Wu Y, Yang CF, Zhang Y, Gu D, Katsuya T, Ohkubo T, Gao YT, Go MJ, Teo YY, Lu L, Lee NR, Chang LC, Peng H, Zhao Q, Nakashima E, Kita Y, Shu XO, Kim NH, Tai ES, Wang Y, Adair LS, Chen CH, Zhang S, Li C, Nabika T, Umemura S, Cai Q, Cho YS, Wong TY, Zhu J, Wu JY, Gao X, Hixson JE, Cai H, Lee J, Cheng CY, Rao DC, Xiang YB, Cho MC, Han BG, Wang A, Tsai FJ, Mohlke K, Lin X, Ikram MK, Lee JY, Zheng W, Tetsuro M, Kato N, He J. Genome-wide association study meta-analysis reveals transethnic replication of mean arterial and pulse pressure loci. *Hypertension* 62: 853–859, 2013. doi:[10.1161/HYPERTENSIONAHA.113.01148](https://doi.org/10.1161/HYPERTENSIONAHA.113.01148).
181. Khanal RC, Nemere I. Regulation of intestinal calcium transport. *Annu Rev Nutr* 28: 179–196, 2008. doi:[10.1146/annurev.nutr.010308.161202](https://doi.org/10.1146/annurev.nutr.010308.161202).
182. Kim E, DeMarco SJ, Marfatia SM, Chishti AH, Sheng M, Strehler EE. Plasma membrane Ca<sup>2+</sup> ATPase isoform 4b binds to membrane-associated guanylate kinase (MAGUK) proteins via their PDZ (PSD-95/Dlg/ZO-1) domains. *J Biol Chem* 273: 1591–1595, 1998. doi:[10.1074/jbc.273.3.1591](https://doi.org/10.1074/jbc.273.3.1591).
183. Kim HJ, Prasad V, Hyung SW, Lee ZH, Lee SW, Bhargava A, Pearce D, Lee Y, Kim HH. Plasma membrane calcium ATPase regulates bone mass by fine-tuning osteoclast differentiation and survival. *J Cell Biol* 199: 1145–1158, 2012. doi:[10.1083/jcb.201204067](https://doi.org/10.1083/jcb.201204067).
184. Kip SN, Strehler EE. Rapid downregulation of NCX and PMCA in hippocampal neurons following H<sub>2</sub>O<sub>2</sub> oxidative stress. *Ann NY Acad Sci* 1099: 436–439, 2007. doi:[10.1196/annals.1387.005](https://doi.org/10.1196/annals.1387.005).
185. Kip SN, Strehler EE. Vitamin D<sub>3</sub> upregulates plasma membrane Ca<sup>2+</sup>-ATPase expression and potentiates apico-basal Ca<sup>2+</sup> flux in MDCK cells. *Am J Physiol Renal Physiol* 286: F363–F369, 2004. doi:[10.1152/ajprenal.00076.2003](https://doi.org/10.1152/ajprenal.00076.2003).
186. Kitamoto T, Suematsu S, Yamazaki Y, Nakamura Y, Sasano H, Matsuzawa Y, Saito J, Omura M, Nishikawa T. Clinical and steroidogenic characteristics of aldosterone-producing adenomas with ATPase or CACNA1D gene mutations. *J Clin Endocrinol Metab* 101: 494–503, 2016. doi:[10.1210/jc.2015-3284](https://doi.org/10.1210/jc.2015-3284).
187. Kobayashi Y, Hirawa N, Tabara Y, Muraoka H, Fujita M, Miyazaki N, Fujiwara A, Ichikawa Y, Yamamoto Y, Ichihara N, Saka S, Wakui H, Yoshida S, Yatsu K, Toya Y, Yasuda G, Kohara K, Kita Y, Takei K, Goshima Y, Ishikawa Y, Ueshima H, Miki T, Umemura S. Mice lacking hypertension candidate gene ATP2B1 in vascular smooth muscle cells show significant blood pressure elevation. *Hypertension* 59: 854–860, 2012. doi:[10.1161/HYPERTENSIONAHA.110.165068](https://doi.org/10.1161/HYPERTENSIONAHA.110.165068).
188. Kocsis I, Vászárhelyi B, Héninger E, Vér A, Tulassay T. Expression and activity of the Ca(2+)-ATPase enzyme in human neonatal erythrocytes. *Biol Neonate* 80: 215–218, 2001. doi:[10.1159/000047145](https://doi.org/10.1159/000047145).
189. Kosiorek M, Podrzywalow-Bartnicka P, Zylinska L, Zablocki K, Pikula S. Interaction of plasma membrane Ca(2+)-ATPase isoform 4 with calcineurin A: implications for catecholamine secretion by PC12 cells. *Biochem Biophys Res Commun* 411: 235–240, 2011. doi:[10.1016/j.bbrc.2011.06.098](https://doi.org/10.1016/j.bbrc.2011.06.098).
190. Kosk-Kosicka D, Bzdega T. Activation of the erythrocyte Ca<sup>2+</sup>-ATPase by either self-association or interaction with calmodulin. *J Biol Chem* 263: 18184–18189, 1988.
191. Kosk-Kosicka D, Bzdega T, Wawrzynow A. Fluorescence energy transfer studies of purified erythrocyte Ca<sup>2+</sup>-ATPase. Ca<sup>2+</sup>-regulated activation by oligomerization. *J Biol Chem* 264: 19495–19499, 1989.
192. Kozel PJ, Friedman RA, Erway LC, Yamoah EN, Liu LH, Riddle T, Duffy JJ, Doetschman T, Miller ML, Cardell EL, Shull GE. Balance and hearing deficits in mice with a null mutation in the gene encoding plasma membrane Ca<sup>2+</sup>-ATPase isoform 2. *J Biol Chem* 273: 18693–18696, 1998. doi:[10.1074/jbc.273.30.18693](https://doi.org/10.1074/jbc.273.30.18693).
193. Krey JF, Dolmetsch RE. Molecular mechanisms of autism: a possible role for Ca<sup>2+</sup> signaling. *Curr Opin Neurobiol* 17: 112–119, 2007. doi:[10.1016/j.conb.2007.01.010](https://doi.org/10.1016/j.conb.2007.01.010).
194. Krizaj D, Copenhagen DR. Compartmentalization of calcium extrusion mechanisms in the outer and inner segments of photoreceptors. *Neuron* 21: 249–256, 1998. doi:[10.1016/S0896-6273\(00\)80531-0](https://doi.org/10.1016/S0896-6273(00)80531-0).
195. Krizaj D, Liu X, Copenhagen DR. Expression of calcium transporters in the retina of the tiger salamander (*Ambystoma tigrinum*). *J Comp Neurol* 475: 463–480, 2004. doi:[10.1002/cne.20170](https://doi.org/10.1002/cne.20170).
196. Kumar R, Haugen JD, Penniston JT. Molecular cloning of a plasma membrane calcium pump from human osteoblasts. *J Bone Miner Res* 8: 505–513, 1993. doi:[10.1002/jbmr.5650080415](https://doi.org/10.1002/jbmr.5650080415).
197. Kurnellas MP, Lee AK, Li H, Deng L, Ehrlich DJ, Elkabes S. Molecular alterations in the cerebellum of the plasma membrane calcium ATPase 2 (PMCA2)-null mouse indicate abnormalities in Purkinje neurons. *Mol Cell Neurosci* 34: 178–188, 2007. doi:[10.1016/j.mcn.2006.10.010](https://doi.org/10.1016/j.mcn.2006.10.010).
198. Kurnellas MP, Li H, Jain MR, Giraud SN, Nicot AB, Ratnayake A, Heary RF, Elkabes S. Reduced expression of plasma membrane calcium ATPase 2 and collapsin response

- mediator protein 1 promotes death of spinal cord neurons. *Cell Death Differ* 17: 1501–1510, 2010. doi:[10.1038/cdd.2010.54](https://doi.org/10.1038/cdd.2010.54).
199. Kurnellas MP, Nicot A, Shull GE, Elkabes S. Plasma membrane calcium ATPase deficiency causes neuronal pathology in the spinal cord: a potential mechanism for neurodegeneration in multiple sclerosis and spinal cord injury. *FASEB J* 19: 298–300, 2005. doi:[10.1096/fj.04-2549fje](https://doi.org/10.1096/fj.04-2549fje).
200. Lauritsen MB, Als TD, Dahl HA, Flint TJ, Wang AG, Vang M, Kruse TA, Ewald H, Mors O. A genome-wide search for alleles and haplotypes associated with autism and related pervasive developmental disorders on the Faroe Islands. *Mol Psychiatry* 11: 37–46, 2006. doi:[10.1038/sj.mp.4001754](https://doi.org/10.1038/sj.mp.4001754).
201. Lawes CM, Vander Hoorn S, Rodgers A, International Society of Hypertension. Global burden of blood-pressure-related disease, 2001. *Lancet* 371: 1513–1518, 2008. doi:[10.1016/S0140-6736\(08\)60655-8](https://doi.org/10.1016/S0140-6736(08)60655-8).
202. Lee SM, Riley EM, Meyer MB, Benkusky NA, Plum LA, DeLuca HF, Pike JW. 1,25-Dihydroxyvitamin D<sub>3</sub> controls a cohort of vitamin D receptor target genes in the proximal intestine that is enriched for calcium-regulating components. *J Biol Chem* 290: 18199–18215, 2015. doi:[10.1074/jbc.M115.665794](https://doi.org/10.1074/jbc.M115.665794).
203. Lee WJ, Roberts-Thomson SJ, Holman NA, May FJ, Lehrbach GM, Monteith GR. Expression of plasma membrane calcium pump isoform mRNAs in breast cancer cell lines. *Cell Signal* 14: 1015–1022, 2002. doi:[10.1016/S0898-6568\(02\)00049-9](https://doi.org/10.1016/S0898-6568(02)00049-9).
204. Lee WJ, Roberts-Thomson SJ, Monteith GR. Plasma membrane calcium-ATPase 2 and 4 in human breast cancer cell lines. *Biochem Biophys Res Commun* 337: 779–783, 2005. doi:[10.1016/j.bbrc.2005.09.119](https://doi.org/10.1016/j.bbrc.2005.09.119).
205. Lee WJ, Robinson JA, Holman NA, McCall MN, Roberts-Thomson SJ, Monteith GR. Antisense-mediated inhibition of the plasma membrane calcium-ATPase suppresses proliferation of MCF-7 cells. *J Biol Chem* 280: 27076–27084, 2005. doi:[10.1074/jbc.M414142200](https://doi.org/10.1074/jbc.M414142200).
206. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Aspelund T, Aulchenko Y, Lumley T, Köttgen A, Vasan RS, Rivadeneira F, Eiriksdottir G, Guo X, Arking DE, Mitchell GF, Mattace-Raso FU, Smith AV, Taylor K, Scharpf RB, Hwang SJ, Sijbrands EJ, Bis J, Harris TB, Ganesh SK, O'Donnell CJ, Hofman A, Rotter JI, Coresh J, Benjamin EJ, Uitterlinden AG, Heiss G, Fox CS, Witteman JC, Boerwinkle E, Wang TJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, van Duijn CM. Genome-wide association study of blood pressure and hypertension. *Nat Genet* 41: 677–687, 2009. doi:[10.1038/ng.384](https://doi.org/10.1038/ng.384).
207. Lew VL, Daw N, Etzion Z, Tiffert T, Muoma A, Vanagas L, Bookchin RM. Effects of age-dependent membrane transport changes on the homeostasis of senescent human red blood cells. *Blood* 110: 1334–1342, 2007. doi:[10.1182/blood-2006-11-057232](https://doi.org/10.1182/blood-2006-11-057232).
208. Lew VL, Hockaday A, Sepulveda MI, Somlyo AP, Somlyo AV, Ortiz OE, Bookchin RM. Compartmentalization of sickle-cell calcium in endocytic inside-out vesicles. *Nature* 315: 586–589, 1985. doi:[10.1038/315586a0](https://doi.org/10.1038/315586a0).
209. Lew VL, Ortiz OE, Bookchin RM. Stochastic nature and red cell population distribution of the sickling-induced Ca<sup>2+</sup> permeability. *J Clin Invest* 99: 2727–2735, 1997. doi:[10.1172/JCI119462](https://doi.org/10.1172/JCI119462).
210. Lew VL, Tsien RY, Miner C, Bookchin RM. Physiological [Ca<sup>2+</sup>]<sub>i</sub> level and pump-leak turnover in intact red cells measured using an incorporated Ca chelator. *Nature* 298: 478–481, 1982. doi:[10.1038/298478a0](https://doi.org/10.1038/298478a0).
211. Lewis RS. Calcium signaling mechanisms in T lymphocytes. *Annu Rev Immunol* 19: 497–521, 2001. doi:[10.1146/annurev.immunol.19.1.497](https://doi.org/10.1146/annurev.immunol.19.1.497).
212. Li M, Ho PW, Pang SY, Tse ZH, Kung MH, Sham PC, Ho SL. PMCA4 (ATP2B4) mutation in familial spastic paraplegia. *PLoS One* 9: e104790, 2014. doi:[10.1371/journal.pone.0104790](https://doi.org/10.1371/journal.pone.0104790).
213. Lieb W, Jansen H, Loley C, Pencina MJ, Nelson CP, Newton-Cheh C, Kathiresan S, Reilly MP, Assimes TL, Boerwinkle E, Hall AS, Hengstenberg C, Laaksonen R, McPherson R, Thorsteinsdottir U, Ziegler A, Peters A, Thompson JR, König IR, Erdmann J, Samani NJ, Vasan RS, Schunkert H, CARDIoGRAM. Genetic predisposition to higher blood pressure increases coronary artery disease risk. *Hypertension* 61: 995–1001, 2013. doi:[10.1161/HYPERTENSIONAHA.111.00275](https://doi.org/10.1161/HYPERTENSIONAHA.111.00275).
214. Little R, Cartwright EJ, Neyes L, Austin C. Plasma membrane calcium ATPases (PMCAs) as potential targets for the treatment of essential hypertension. *Pharmacol Ther* 159: 23–34, 2016. doi:[10.1016/j.pharmthera.2016.01.013](https://doi.org/10.1016/j.pharmthera.2016.01.013).
215. Liu L, Ishida Y, Okunade G, Pyne-Geithman GJ, Shull GE, Paul RJ. Distinct roles of PMCA isoforms in Ca<sup>2+</sup> homeostasis of bladder smooth muscle: evidence from PMCA gene-ablated mice. *Am J Physiol Cell Physiol* 292: C423–C431, 2007. doi:[10.1152/ajpcell.00313.2006](https://doi.org/10.1152/ajpcell.00313.2006).
216. Liu L, Ishida Y, Okunade G, Shull GE, Paul RJ. Role of plasma membrane Ca<sup>2+</sup>-ATPase in contraction-relaxation processes of the bladder: evidence from PMCA gene-ablated mice. *Am J Physiol Cell Physiol* 290: C1239–C1247, 2006. doi:[10.1152/ajpcell.00440.2005](https://doi.org/10.1152/ajpcell.00440.2005).
217. Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H, Langer-Gould A, Strober S, Cannella B, Allard J, Klonowski P, Austin A, Lad N, Kaminski N, Galli SJ, Oksenberg JR, Raine CS, Heller R, Steinman L. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nat Med* 8: 500–508, 2002. doi:[10.1038/nm0502-500](https://doi.org/10.1038/nm0502-500).
218. Loffing J, Loffing-Cueni D, Valderrabano V, Kläusli L, Hebert SC, Rossier BC, Hoenderop JG, Bindels RJ, Kaissling B. Distribution of transcellular calcium and sodium transport pathways along mouse distal nephron. *Am J Physiol Renal Physiol* 281: F1021–F1027, 2001. doi:[10.1152/ajprenal.0085.2001](https://doi.org/10.1152/ajprenal.0085.2001).
219. Lu X, Wang L, Chen S, He L, Yang X, Shi Y, Cheng J, Zhang L, Gu CC, Huang J, Wu T, Ma Y, Li J, Cao J, Chen J, Ge D, Fan Z, Li Y, Zhao L, Li H, Zhou X, Chen L, Liu D, Chen J, Duan X, Hao Y, Wang L, Lu F, Liu Z, Yao C, Shen C, Pu X, Yu L, Fang X, Xu L, Mu J, Wu X, Zheng R, Wu N, Zhao Q, Li Y, Liu X, Wang M, Yu D, Hu D, Ji X, Guo D, Sun D, Wang Q, Yang Y, Liu F, Mao Q, Liang X, Ji J, Chen P, Mo X, Li D, Chai G, Tang Y, Li X, Du Z, Liu X, Dou C, Yang Z, Meng Q, Wang D, Wang R, Yang J, Schunkert H, Samani NJ, Kathiresan S, Reilly MP, Erdmann J, Peng X, Wu X, Liu D, Yang Y, Chen R, Qiang B, Gu D. Coronary Artery Disease Genome-Wide Replication And Meta-Analysis (CARDIoGRAM) Consortium. Genome-wide association study in Han Chinese identifies four new susceptibility loci for coronary artery disease. *Nat Genet* 44: 890–894, 2012. doi:[10.1038/ng.2337](https://doi.org/10.1038/ng.2337).
220. Lu X, Wang L, Lin X, Huang J, Charles Gu C, He M, Shen H, He J, Zhu J, Li H, Hixson JE, Wu T, Dai J, Lu L, Shen C, Chen S, He L, Mo Z, Hao Y, Mo X, Yang X, Li J, Cao J, Chen J, Fan Z, Li Y, Zhao L, Li H, Lu F, Yao C, Yu L, Xu L, Mu J, Wu X, Deng Y, Hu D, Zhang W, Ji X, Guo D, Guo Z, Zhou Z, Yang Z, Wang R, Yang J, Zhou X, Yan W, Sun N, Gao P, Gu D. Genome-wide association study in Chinese identifies novel loci for blood pressure and hypertension. *Hum Mol Genet* 24: 865–874, 2015. doi:[10.1093/hmg/ddu478](https://doi.org/10.1093/hmg/ddu478).
221. Lyttton J, Zarain-Herzberg A, Periasamy M, MacLennan DH. Molecular cloning of the mammalian smooth muscle sarco(endo)plasmic reticulum Ca<sup>2+</sup>-ATPase. *J Biol Chem* 264: 7059–7065, 1989.
222. Magocsi M, Penniston JT. Oxytocin pretreatment of pregnant rat uterus inhibits Ca<sup>2+</sup> uptake in plasma membrane and sarcoplasmic reticulum. *Biochim Biophys Acta* 1063: 7–14, 1991. doi:[10.1016/0005-2736\(91\)90346-A](https://doi.org/10.1016/0005-2736(91)90346-A).
223. Magyar CE, White KE, Rojas R, Apodaca G, Friedman PA. Plasma membrane Ca<sup>2+</sup>-ATPase and NCX1 Na<sup>+</sup>/Ca<sup>2+</sup> exchanger expression in distal convoluted tubule cells. *Am J Physiol Renal Physiol* 283: F29–F40, 2002. doi:[10.1152/ajprenal.00252.2000](https://doi.org/10.1152/ajprenal.00252.2000).
225. Mangialavori I, Ferreira-Gomes M, Pignataro MF, Strehler EE, Rossi JP. Determination of the dissociation constants for Ca<sup>2+</sup> and calmodulin from the plasma membrane Ca<sup>2+</sup> pump by a lipid probe that senses membrane domain changes. *J Biol Chem* 285: 123–130, 2010. doi:[10.1074/jbc.M109.076679](https://doi.org/10.1074/jbc.M109.076679).
226. Marian MJ, Li H, Borchman D, Paterson CA. Plasma membrane Ca<sup>2+</sup>-ATPase expression in the human lens. *Exp Eye Res* 81: 57–64, 2005. doi:[10.1016/j.exer.2005.01.011](https://doi.org/10.1016/j.exer.2005.01.011).
227. Marian MJ, Mukhopadhyay P, Borchman D, Paterson CA. Plasma membrane Ca-ATPase isoform expression in human cataractous lenses compared to age-matched clear lenses. *Ophthalmic Res* 40: 86–93, 2008. doi:[10.1159/000113886](https://doi.org/10.1159/000113886).
228. Marian MJ, Mukhopadhyay P, Borchman D, Tang D, Paterson CA. Regulation of sarco/endoplasmic and plasma membrane calcium ATPase gene expression by calcium in cultured human lens epithelial cells. *Cell Calcium* 41: 87–95, 2007. doi:[10.1016/j.ceca.2006.05.003](https://doi.org/10.1016/j.ceca.2006.05.003).
229. Marian MJ, Mukhopadhyay P, Borchman D, Tang D, Paterson CA. The effect of hydrogen peroxide on sarco/endoplasmic and plasma membrane calcium ATPase gene expression in cultured human lens epithelial cells. *Open Ophthalmol J* 2: 123–129, 2008. doi:[10.2174/1874364100802010123](https://doi.org/10.2174/1874364100802010123).



230. Mark RJ, Hensley K, Butterfield DA, Mattson MP. Amyloid beta-peptide impairs ion-motive ATPase activities: evidence for a role in loss of neuronal  $\text{Ca}^{2+}$  homeostasis and cell death. *J Neurosci* 15: 6239–6249, 1995.
231. Marques-da-Silva D, Gutierrez-Merino C. Caveolin-rich lipid rafts of the plasma membrane of mature cerebellar granule neurons are microcompartments for calcium/reactive oxygen and nitrogen species cross-talk signaling. *Cell Calcium* 56: 108–123, 2014. doi:10.1016/j.ceca.2014.06.002.
232. Martin R, Harvey NC, Crozier SR, Poole JR, Javaid MK, Dennison EM, Inskip HM, Hanson M, Godfrey KM, Cooper C, Lewis R, SWS Study Group. Placental calcium transporter (PMCA3) gene expression predicts intrauterine bone mineral accrual. *Bone* 40: 1203–1208, 2007. doi:10.1016/j.bone.2006.12.060.
233. Matthew A, Shmygol A, Wray S.  $\text{Ca}^{2+}$  entry, efflux and release in smooth muscle. *Biol Res* 37: 617–624, 2004. doi:10.4067/S0716-97602004000400017.
234. Mazzanti L, Rabini RA, Fumelli P, Martarelli D, Staffolani R, Salvolini E, Curatola G. Altered platelet membrane dynamic properties in type I diabetes. *Diabetes* 46: 2069–2074, 1997. doi:10.2337/diab.46.12.2069.
235. McCauley JL, Li C, Jiang L, Olson LM, Crockett G, Gainer K, Folstein SE, Haines JL, Sutcliffe JS. Genome-wide and ordered-subset linkage analyses provide support for autism loci on 17q and 19p with evidence of phenotypic and interlocus genetic correlates. *BMC Med Genet* 6: 1, 2005. doi:10.1186/1471-2350-6-1.
236. McCullough BJ, Tempel BL. Haplo-insufficiency revealed in deafwaddler mice when tested for hearing loss and ataxia. *Hear Res* 195: 90–102, 2004. doi:10.1016/j.heares.2004.05.003.
237. McElnea EM, Quill B, Docherty NG, Irnaten M, Siah WF, Clark AF, O'Brien CJ, Wallace DM. Oxidative stress, mitochondrial dysfunction and calcium overload in human lamina cribrosa cells from glaucoma donors. *Mol Vis* 17: 1182–1191, 2011.
238. Menini A. Calcium signalling and regulation in olfactory neurons. *Curr Opin Neurobiol* 9: 419–426, 1999. doi:10.1016/S0959-4388(99)80063-4.
239. Meyer TE, Verwoert GC, Hwang SJ, Glazer NL, Smith AV, van Rooij FJ, Ehret GB, Boerwinkle E, Felix JF, Leak TS, Harris TB, Yang Q, Dehghan A, Aspelund T, Katz R, Homuth G, Kocher T, Rettig R, Ried JS, Gieger C, Prucha H, Pfeufer A, Meitinger T, Coresh J, Hofman A, Sarnak MJ, Chen YD, Uitterlinden AG, Chakravarti A, Psaty BM, van Duijn CM, Kao WH, Witteman JC, Gudnason V, Siscovick DS, Fox CS, Köttgen A, Genetic Factors for Osteoporosis Consortium, Meta Analysis of Glucose and Insulin Related Traits Consortium. Genome-wide association studies of serum magnesium, potassium, and sodium concentrations identify six Loci influencing serum magnesium levels. *PLoS Genet* 6: e1001045, 2010. doi:10.1371/journal.pgen.1001045.
240. Michaelis ML, Bigelow DJ, Schöneich C, Williams TD, Ramonda L, Yin D, Hühmer AF, Yao Y, Gao J, Squier TC. Decreased plasma membrane calcium transport activity in aging brain. *Life Sci* 59: 405–412, 1996. doi:10.1016/0024-3205(96)00319-0.
241. Mohamed TM, Abou-Leisa R, Baudoin F, Stafford N, Neyses L, Cartwright EJ, Oceandy D. Development and characterization of a novel fluorescent indicator protein PMCA4-GCaMP2 in cardiomyocytes. *J Mol Cell Cardiol* 63: 57–68, 2013. doi:10.1016/j.jmcc.2013.07.007.
242. Mohamed TM, Abou-Leisa R, Stafford N, Maqsood A, Zi M, Prehar S, Baudoin-Stanley F, Wang X, Neyses L, Cartwright EJ, Oceandy D. The plasma membrane calcium ATPase 4 signalling in cardiac fibroblasts mediates cardiomyocyte hypertrophy. *Nat Commun* 7: 11074, 2016. doi:10.1038/ncomms11074.
243. Mohamed TM, Baudoin-Stanley FM, Abou-Leisa R, Cartwright E, Neyses L, Oceandy D. Measurement of plasma membrane calcium-calmodulin-dependent ATPase (PMCA) activity. *Methods Mol Biol* 637: 333–342, 2010. doi:10.1007/978-1-60761-700-6\_18.
244. Mohamed TM, Oceandy D, Prehar S, Alatiwi N, Hegab Z, Baudoin FM, Pickard A, Zaki AO, Nadif R, Cartwright EJ, Neyses L. Specific role of neuronal nitric-oxide synthase when tethered to the plasma membrane calcium pump in regulating the beta-adrenergic signal in the myocardium. *J Biol Chem* 284: 12091–12098, 2009. doi:10.1074/jbc.M809112200.
245. Mohamed TM, Oceandy D, Zi M, Prehar S, Alatiwi N, Wang Y, Shaheen MA, Abou-Leisa R, Schelcher C, Hegab Z, Baudoin F, Emerson M, Mamas M, Di Benedetto G, Zaccolo M, Lei M, Cartwright EJ, Neyses L. Plasma membrane calcium pump (PMCA4)-neuronal nitric-oxide synthase complex regulates cardiac contractility through modulation of a compartmentalized cyclic nucleotide microdomain. *J Biol Chem* 286: 41520–41529, 2011. doi:10.1074/jbc.M111.290411.
246. Mohamed TM, Zakeri SA, Baudoin F, Wolf M, Oceandy D, Cartwright EJ, Gul S, Neyses L. Optimisation and validation of a high throughput screening compatible assay to identify inhibitors of the plasma membrane calcium ATPase pump—a novel therapeutic target for contraception and malaria. *J Pharm Pharm Sci* 16: 217–230, 2013. doi:10.18433/J3PG68.
247. Mohamed TM, Zi M, Prehar S, Maqsood A, Abou-Leisa R, Nguyen L, Pfeifer GP, Cartwright EJ, Neyses L, Oceandy D. The tumour suppressor Ras-association domain family protein 1A (RASSF1A) regulates TNF- $\alpha$  signalling in cardiomyocytes. *Cardiovasc Res* 103: 47–59, 2014. doi:10.1093/cvr/cvu111.
248. Molkenkin JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, Grant SR, Olson EN. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* 93: 215–228, 1998. doi:10.1016/S0092-8674(00)81573-1.
249. Monesterolo NE, Nigra AD, Campetelli AN, Santander VS, Rivelli JF, Arce CA, Casale CH. PMCA activity and membrane tubulin affect deformability of erythrocytes from normal and hypertensive human subjects. *Biochim Biophys Acta* 1848, Pt A: 2813–2820, 2015. doi:10.1016/j.bbame.2015.08.011.
250. Monticone S, Castellano I, Versace K, Lucatello B, Veglio F, Gomez-Sanchez CE, Williams TA, Mulatero P. Immunohistochemical, genetic and clinical characterization of sporadic aldosterone-producing adenomas. *Mol Cell Endocrinol* 411: 146–154, 2015. doi:10.1016/j.mce.2015.04.022.
251. Moreira OC, Rios PF, Barrabin H. Inhibition of plasma membrane  $\text{Ca}^{2+}$ -ATPase by CrATP. LaATP but not CrATP stabilizes the  $\text{Ca}^{2+}$ -occluded state. *Biochim Biophys Acta* 1708: 411–419, 2005. doi:10.1016/j.bbabi.2005.05.010.
252. Morgans CW, El Far O, Berntson A, Wässle H, Taylor WR. Calcium extrusion from mammalian photoreceptor terminals. *J Neurosci* 18: 2467–2474, 1998.
253. Morvová M Jr, Lajdová I, Spustová V, Zvarik M, Šikurová L. The effect of vitamin D<sub>3</sub> supplementation on intracellular calcium and plasma membrane calcium ATPase activity in early stages of chronic kidney disease. *Physiol Res* 63, Suppl 4: S593–S599, 2014.
254. Murakami M, Yoshimoto T, Minami I, Bouchi R, Tsuchiya K, Hashimoto K, Izumiyama H, Fujii Y, Endo T, Akashi T, Nishimoto K, Mukai K, Kihara K, Ogawa Y. A novel somatic deletion mutation of ATP2B3 in aldosterone-producing adenoma. *Endocr Pathol* 26: 328–333, 2015. doi:10.1007/s12022-015-9400-9.
255. Muscella A, Calabriso N, Fanizzi FP, De Pascali SA, Urso L, Ciccarese A, Migoni D, Marsigliante S. [Pt(O,O'-acac)(gamma-acac)(DMS)], a new Pt compound exerting fast cytotoxicity in MCF-7 breast cancer cells via the mitochondrial apoptotic pathway. *Br J Pharmacol* 153: 34–49, 2008. doi:10.1038/sj.bjp.0707576.
256. Muscella A, Calabriso N, Vetrugno C, Fanizzi FP, De Pascali SA, Storelli C, Marsigliante S. The platinum (II) complex [Pt(O,O'-acac)(gamma-acac)(DMS)] alters the intracellular calcium homeostasis in MCF-7 breast cancer cells. *Biochem Pharmacol* 81: 91–103, 2011. doi:10.1016/j.bcp.2010.09.012.
257. Nakano Y, Addison WN, Kaartinen MT. ATP-mediated mineralization of MC3T3-E1 osteoblast cultures. *Bone* 41: 549–561, 2007. doi:10.1016/j.bone.2007.06.011.
258. Nakano Y, Beertsen W, van den Bos T, Kawamoto T, Oda K, Takano Y. Site-specific localization of two distinct phosphatases along the osteoblast plasma membrane: tissue non-specific alkaline phosphatase and plasma membrane calcium ATPase. *Bone* 35: 1077–1085, 2004. doi:10.1016/j.bone.2004.07.009.
259. Nardulli G, Proverbio F, Limongi FG, Marín R, Proverbio T. Preeclampsia and calcium adenosine triphosphatase activity of red blood cell ghosts. *Am J Obstet Gynecol* 171: 1361–1365, 1994. doi:10.1016/0002-9378(94)90161-9.
260. Nicolau J, De Souza DN, Simões A. Alteration of  $\text{Ca}^{2+}$ -ATPase activity in the homogenate, plasma membrane and microsomes of the salivary glands of streptozotocin-induced diabetic rats. *Cell Biochem Funct* 27: 128–134, 2009. doi:10.1002/cbf.1544.
261. Nicot A, Kurnellas M, Elkabes S. Temporal pattern of plasma membrane calcium ATPase 2 expression in the spinal cord correlates with the course of clinical symptoms in two rodent models of autoimmune encephalomyelitis. *Eur J Neurosci* 21: 2660–2670, 2005. doi:10.1111/j.1460-9568.2005.04086.x.
262. Nicot A, Ratnakar PV, Ron Y, Chen CC, Elkabes S. Regulation of gene expression in experimental autoimmune encephalomyelitis indicates early neuronal dysfunction. *Brain* 126: 398–412, 2003. doi:10.1093/brain/awg041.



263. Niggli V, Adunyah ES, Carafoli E. Acidic phospholipids, unsaturated fatty acids, and limited proteolysis mimic the effect of calmodulin on the purified erythrocyte  $\text{Ca}^{2+}$ -ATPase. *J Biol Chem* 256: 8588–8592, 1981.
264. Niggli V, Sigel E, Carafoli E. The purified  $\text{Ca}^{2+}$  pump of human erythrocyte membranes catalyzes an electroneutral  $\text{Ca}^{2+}$ - $\text{H}^{+}$  exchange in reconstituted liposomal systems. *J Biol Chem* 257: 2350–2356, 1982.
265. Nurden P, Debili N, Vainchenker W, Bobe R, Bredoux R, Corvazier E, Combrie R, Fressinaud E, Meyer D, Nurden AT, Enouf J. Impaired megakaryocytopoiesis in type 2B von Willebrand disease with severe thrombocytopenia. *Blood* 108: 2587–2595, 2006. doi:10.1182/blood-2006-03-009449.
266. Oceandy D, Cartwright EJ, Emerson M, Prehar S, Baudoin FM, Zi M, Alatiwi N, Venetucci L, Schuh K, Williams JC, Armesilla AL, Neyses L. Neuronal nitric oxide synthase signaling in the heart is regulated by the sarcolemmal calcium pump 4b. *Circulation* 115: 483–492, 2007. doi:10.1161/CIRCULATIONAHA.106.643791.
267. Oceandy D, Mohamed TM, Cartwright EJ, Neyses L. Local signals with global impacts and clinical implications: lessons from the plasma membrane calcium pump (PMCA4). *Biochim Biophys Acta* 1813: 974–978, 2011. doi:10.1016/j.bbamcr.2010.12.007.
268. Oceandy D, Pickard A, Prehar S, Zi M, Mohamed TM, Stanley PJ, Baudoin-Stanley F, Nadif R, Tommasi S, Pfeifer GP, Armesilla AL, Cartwright EJ, Neyses L. Tumor suppressor Ras-association domain family 1 isoform A is a novel regulator of cardiac hypertrophy. *Circulation* 120: 607–616, 2009. doi:10.1161/CIRCULATIONAHA.109.868554.
269. Okunade GW, Miller ML, Pyne GJ, Sutliff RL, O'Connor KT, Neumann JC, Andringa A, Miller DA, Prasad V, Doetschman T, Paul RJ, Shull GE. Targeted ablation of plasma membrane  $\text{Ca}^{2+}$ -ATPase (PMCA) 1 and 4 indicates a major housekeeping function for PMCA1 and a critical role in hyperactivated sperm motility and male fertility for PMCA4. *J Biol Chem* 279: 33742–33750, 2004. doi:10.1074/jbc.M404628200.
270. Ono K, Wang X, Han J. Resistance to tumor necrosis factor-induced cell death mediated by PMCA4 deficiency. *Mol Cell Biol* 21: 8276–8288, 2001. doi:10.1128/MCB.21.24.8276-8288.2001.
271. Orrenius S, Gogvadze V, Zhivotovsky B. Calcium and mitochondria in the regulation of cell death. *Biochem Biophys Res Commun* 460: 72–81, 2015. doi:10.1016/j.bbrc.2015.01.137.
272. Oßwald A, Fischer E, Degenhart C, Quinkler M, Bidlingmaier M, Pallauf A, Lang K, Mussack T, Hallfeldt K, Beuschlein F, Reincke M. Lack of influence of somatic mutations on steroid gradients during adrenal vein sampling in aldosterone-producing adenoma patients. *Eur J Endocrinol* 169: 657–663, 2013. doi:10.1530/EJF-13-0551.
273. Oviedo NJ, Benaim G, Cervino V, Proverbio T, Proverbio F, Marin R. The plasma membrane  $\text{Ca}^{2+}$ -ATPase protein from red blood cells is not modified in preeclampsia. *Biochim Biophys Acta* 1762: 381–385, 2006. doi:10.1016/j.bbadis.2005.12.001.
274. Oz OK, Hajibeigi A, Howard K, Cummins CL, van Abel M, Bindels RJ, Word RA, Kuro-o M, Pak CY, Zerwekh JE. Aromatase deficiency causes altered expression of molecules critical for calcium reabsorption in the kidneys of female mice. *J Bone Miner Res* 22: 1893–1902, 2007. doi:10.1359/jbmr.070808.
275. Pachera N, Papin J, Zummo FP, Rahier J, Mast J, Meyerovich K, Cardozo AK, Herculez A. Heterozygous inactivation of plasma membrane  $\text{Ca}(2+)$ -ATPase in mice increases glucose-induced insulin release and beta cell proliferation, mass and viability. *Diabetologia* 58: 2843–2850, 2015. doi:10.1007/s00125-015-3745-y.
276. Pande J, Mallhi KK, Sawh A, Szwedczyk MM, Simpson F, Grover AK. Aortic smooth muscle and endothelial plasma membrane  $\text{Ca}^{2+}$  pump isoforms are inhibited differently by the extracellular inhibitor caloxin 1b1. *Am J Physiol Cell Physiol* 290: C1341–C1349, 2006. doi:10.1152/ajpcell.00573.2005.
277. Pande J, Szwedczyk MM, Kuszcak I, Grover S, Escher E, Grover AK. Functional effects of caloxin 1c2, a novel engineered selective inhibitor of plasma membrane  $\text{Ca}(2+)$ -pump isoform 4, on coronary artery. *J Cell Mol Med* 12: 1049–1060, 2008. doi:10.1111/j.1582-4934.2008.00140.x.
278. Pandey KB, Rizvi SI. Role of resveratrol in regulation of membrane transporters and integrity of human erythrocytes. *Biochem Biophys Res Commun* 453: 521–526, 2014. doi:10.1016/j.bbrc.2014.09.117.
279. Pászty K, Antalffy G, Hegedüs L, Padányi R, Penheiter AR, Filoteo AG, Penniston JT, Enyedi A. Cleavage of the plasma membrane  $\text{Ca}^{2+}$ -ATPase during apoptosis. *Ann NY Acad Sci* 1099: 440–450, 2007. doi:10.1196/annals.1387.003.
280. Pászty K, Antalffy G, Penheiter AR, Homolya L, Padányi R, Illás A, Filoteo AG, Penniston JT, Enyedi A. The caspase-3 cleavage product of the plasma membrane  $\text{Ca}^{2+}$ -ATPase 4b is activated and appropriately targeted. *Biochem J* 391: 687–692, 2005. doi:10.1042/BJ20051012.
281. Pászty K, Kovács T, Lacabartz-Porret C, Papp B, Enouf J, Filoteo AG, Penniston JT, Enyedi A. Expression of hPMCA4b, the major form of the plasma membrane calcium pump in megakaryoblastoid cells is greatly reduced in mature human platelets. *Cell Calcium* 24: 129–135, 1998. doi:10.1016/S0143-4160(98)90080-X.
282. Pászty K, Verma AK, Padányi R, Filoteo AG, Penniston JT, Enyedi A. Plasma membrane  $\text{Ca}^{2+}$ -ATPase isoform 4b is cleaved and activated by caspase-3 during the early phase of apoptosis. *J Biol Chem* 277: 6822–6829, 2002. doi:10.1074/jbc.M109548200.
283. Patel R, Al-Dossary AA, Stabley DL, Barone C, Galileo DS, Strehler EE, Martin-DeLeon PA. Plasma membrane  $\text{Ca}^{2+}$ -ATPase 4 in murine epididymis: secretion of splice variants in the luminal fluid and a role in sperm maturation. *Biol Reprod* 89: 6, 2013. doi:10.1095/biolreprod.113.108712.
284. Paterson CA, Zeng J, Hussein Z, Borchman D, Delamere NA, Garland D, Jimenez-Asensio J. Calcium ATPase activity and membrane structure in clear and cataractous human lenses. *Curr Eye Res* 16: 333–338, 1997. doi:10.1076/ceyr.16.4.333.10689.
285. Pedersen PL, Carafoli E. Ion motive ATPases. I. Ubiquity, properties, and significance to cell function. *Trends Biochem Sci* 12: 146–150, 1987. doi:10.1016/0968-0004(87)90071-5.
286. Pellegrini M, Finetti F, Petronilli V, Olivieri C, Giusti F, Lupetti P, Giorgio M, Pellicci PG, Bernardi P, Baldari CT. p66SHC promotes T cell apoptosis by inducing mitochondrial dysfunction and impaired  $\text{Ca}^{2+}$  homeostasis. *Cell Death Differ* 14: 338–347, 2007. doi:10.1038/sj.cdd.4401997.
287. Peluso JJ. Basic fibroblast growth factor (bFGF) regulation of the plasma membrane calcium ATPase (PMCA) as part of an anti-apoptotic mechanism of action. *Biochem Pharmacol* 66: 1363–1369, 2003. doi:10.1016/S0006-2952(03)00486-6.
288. Peluso JJ, Pappalardo A, Fernandez G. Basic fibroblast growth factor maintains calcium homeostasis and granulosa cell viability by stimulating calcium efflux via a PKC delta-dependent pathway. *Endocrinology* 142: 4203–4211, 2001. doi:10.1210/endo.142.10.8460.
289. Pérez-Gordones MC, Lugo MR, Winkler M, Cervino V, Benaim G. Diacylglycerol regulates the plasma membrane calcium pump from human erythrocytes by direct interaction. *Arch Biochem Biophys* 489: 55–61, 2009. doi:10.1016/j.abb.2009.07.010.
290. Pitkin RM. Calcium metabolism in pregnancy and the perinatal period: a review. *Am J Obstet Gynecol* 151: 99–109, 1985. doi:10.1016/0002-9378(85)90434-X.
291. Polak-Jonkisz D, Purzyc L, Laszki-Szczachor K, Musiał K, Zvolinska D. The endogenous modulators of  $\text{Ca}^{2+}$ - $\text{Mg}^{2+}$ -dependent ATPase in children with chronic kidney disease (CKD). *Nephrol Dial Transplant* 25: 438–444, 2010. doi:10.1093/ndt/gfp436.
292. Polak-Jonkisz D, Purzyc L, Zvolinska D.  $\text{Ca}(2+)$ - $\text{Mg}(2+)$ -dependent ATPase activity in hemodialyzed children. Effect of a hemodialysis session. *Pediatr Nephrol* 25: 2501–2507, 2010. doi:10.1007/s00467-010-1634-7.
293. Polak-Jonkisz D, Zvolinska D, Purzyc L, Musiał K.  $\text{Ca}^{2+}$ - $\text{Mg}^{2+}$ -dependent ATPase activity and calcium homeostasis in children with chronic kidney disease. *Pediatr Nephrol* 22: 414–419, 2007. doi:10.1007/s00467-006-0329-6.
294. Popescu LM, Nutu O, Panoiu C. Oxytocin contracts the human uterus at term by inhibiting the myometrial  $\text{Ca}^{2+}$ -extrusion pump. *Biosci Rep* 5: 21–28, 1985. doi:10.1007/BF01117437.
295. Pottorf WJ II, Johans TM, Derrington SM, Strehler EE, Enyedi A, Thayer SA. Glutamate-induced protease-mediated loss of plasma membrane  $\text{Ca}^{2+}$  pump activity in rat hippocampal neurons. *J Neurochem* 98: 1646–1656, 2006. doi:10.1111/j.1471-4159.2006.04063.x.
296. Prandini P, Pasquali A, Malerba G, Marostica A, Zusi C, Xumerle L, Muglia P, Da Ros L, Ratti E, Trabetti E, Pignatti PF, Italian Autism Network (ITAN). The association of rs4307059 and rs35678 markers with autism spectrum disorders is replicated in Italian families. *Psychiatr Genet* 22: 177–181, 2012. doi:10.1097/YPG.0b013e32835185c9.
297. Prasad V, Lorenz JN, Lasko VM, Nieman ML, Jiang M, Gao X, Rubinstein J, Wiecekorek DF, Shull GE. Ablation of plasma membrane  $\text{Ca}(2+)$ -ATPase isoform 4 prevents development of hypertrophy in a model of hypertrophic cardiomyopathy. *J Mol Cell Cardiol* 77: 53–63, 2014. doi:10.1016/j.jmcc.2014.09.025.

298. Pritchard TJ, Bowman PS, Jefferson A, Tosun M, Lynch RM, Paul RJ.  $\text{Na}^+/\text{K}^+$ -ATPase and  $\text{Ca}^{2+}$  clearance proteins in smooth muscle: a functional unit. *Am J Physiol Heart Circ Physiol* 299: H548–H556, 2010. doi:10.1152/ajpheart.00527.2009.
299. Quintana A, Pasche M, Junker C, Al-Ansary D, Rieger H, Kummerow C, Nuñez L, Villalobos C, Meraner P, Becherer U, Rettig J, Niemeyer BA, Hoth M. Calcium microdomains at the immunological synapse: how ORAI channels, mitochondria and calcium pumps generate local calcium signals for efficient T-cell activation. *EMBO J* 30: 3895–3912, 2011. doi:10.1038/emboj.2011.289.
300. Rabini RA, Staffolani R, Martarelli D, Fumelli P, Ravaglia F, Dousset N, Curatola G, Mazzanti L. Influence of low density lipoprotein from insulin-dependent diabetic patients on platelet functions. *J Clin Endocrinol Metab* 84: 3770–3774, 1999. doi:10.1210/jcem.84.10.6044.
301. Rabini RA, Vignini A, Salvolini E, Staffolani R, Martarelli D, Moretti N, Mazzanti L. Activation of human aortic endothelial cells by LDL from Type 1 diabetic patients: an in vitro study. *Atherosclerosis* 165: 69–77, 2002. doi:10.1016/S0021-9150(02)00197-1.
302. Raffaelli F, Nanetti L, D'Angelo M, Montecchiani G, Alidori A, Montesi L, Faloia E, Vignini A, Mazzanti L. Interactions between lipoproteins and platelet membranes in obesity. *Obesity (Silver Spring)* 17: 1375–1380, 2009. doi:10.1038/oby.2008.654.
303. Reinhardt TA, Filoteo AG, Penniston JT, Horst RL.  $\text{Ca}^{2+}$ -ATPase protein expression in mammary tissue. *Am J Physiol Cell Physiol* 279: C1595–C1602, 2000.
304. Reinhardt TA, Horst RL.  $\text{Ca}^{2+}$ -ATPases and their expression in the mammary gland of pregnant and lactating rats. *Am J Physiol Cell Physiol* 276: C796–C802, 1999.
305. Reinhardt TA, Lippolis JD. Mammary gland involution is associated with rapid down regulation of major mammary  $\text{Ca}^{2+}$ -ATPases. *Biochem Biophys Res Commun* 378: 99–102, 2009. doi:10.1016/j.bbrc.2008.11.004.
306. Reinhardt TA, Lippolis JD, Shull GE, Horst RL. Null mutation in the gene encoding plasma membrane  $\text{Ca}^{2+}$ -ATPase isoform 2 impairs calcium transport into milk. *J Biol Chem* 279: 42369–42373, 2004. doi:10.1074/jbc.M407788200.
307. Reisner PD, Brandt PC, Vanaman TC. Analysis of plasma membrane  $\text{Ca}^{2+}$ -ATPase expression in control and SV40-transformed human fibroblasts. *Cell Calcium* 21: 53–62, 1997. doi:10.1016/S0143-4160(97)90096-8.
308. Replogle RA, Li Q, Wang L, Zhang M, Fleet JC. Gene-by-diet interactions influence calcium absorption and bone density in mice. *J Bone Miner Res* 29: 657–665, 2014. doi:10.1002/jbmr.2065.
309. Rhee MY, Yang SJ, Oh SW, Park Y, Kim CI, Park HK, Park SW, Park CY. Novel genetic variations associated with salt sensitivity in the Korean population. *Hypertens Res* 34: 606–611, 2011. doi:10.1038/hr.2010.278.
310. Rhodes JD, Sanderson J. The mechanisms of calcium homeostasis and signalling in the lens. *Exp Eye Res* 88: 226–234, 2009. doi:10.1016/j.exer.2008.10.025.
311. Ribiczey P, Tordai A, Andrikovics H, Filoteo AG, Penniston JT, Enouf J, Enyedi A, Papp B, Kovács T. Isoform-specific up-regulation of plasma membrane  $\text{Ca}^{2+}$ -ATPase expression during colon and gastric cancer cell differentiation. *Cell Calcium* 42: 590–605, 2007. doi:10.1016/j.ceca.2007.02.003.
312. Rimessi A, Coletto L, Pinton P, Rizzuto R, Brini M, Carafoli E. Inhibitory interaction of the 14-3-3epsilon protein with isoform 4 of the plasma membrane  $\text{Ca}^{2+}$ -ATPase pump. *J Biol Chem* 280: 37195–37203, 2005. doi:10.1074/jbc.M504921200.
313. Rink TJ, Sage SO. Calcium signaling in human platelets. *Annu Rev Physiol* 52: 431–449, 1990. doi:10.1146/annurev.ph.52.030190.002243.
314. Ritchie MF, Samakai E, Soboloff J. STIM1 is required for attenuation of PMCA-mediated  $\text{Ca}^{2+}$  clearance during T-cell activation. *EMBO J* 31: 1123–1133, 2012. doi:10.1038/emboj.2011.495.
- 314a. Rockett KA, Clarke GM, Fitzpatrick K, Hubbard C, Jeffreys AE, Rowlands K, Craik R, Jallow M, Conway DJ, Bojang KA, Pinder M, Usen S, Sisay-Joof F, Sirugo G, Toure O, Thera MA, Konate S, Sissoko S, Niangaly A, Poudiougou B, Mangano VD, Bougouma EC, Sirima SB, Modiano D, Amenga-Etego LN, Ghansah A, Koram KA, Wilson MD, Enimil A, Evans J, Amodu O, Olaniyan S, Apinijoh T, Mugri R, Ndi A, Ndila CM, Uyoga S, Macharia A, Peshu N, Williams TN, Manjurano A, Riley E, Drakeley C, Reyburn H, Nyirongo V, Kachala D, Molyneux M, Dunstan SJ, Phu NH, Quyen NTN, Thai CQ, Hien TT, Manning L, Laman M, Siba P, Karunajeewa H, Allen S, Allen A, Davis TME, Michon P, Mueller I, Green A, Molloy S, Johnson KJ, Kerasidou A, Cornelius V, Hart L, Vanderwal A, Sanjoaquin M, Band G, Le SQ, Pirinen M, Sepúlveda N, Spencer CCA, Clark TG, Agbenyega T, Achidi E, Doumbo O, Farrar J, Marsh K, Taylor T, Kwiatkowski DP, Malaria Genomic Epidemiology Network, Malaria Genomic Epidemiology Network. Reappraisal of known malaria resistance loci in a large multicenter study. *Nat Genet* 46: 1197–1204, 2014. doi:10.1038/ng.3107.
315. Ronquist G, Rudolph O, Engström I, Waldenström A. Familial phosphofructokinase deficiency is associated with a disturbed calcium homeostasis in erythrocytes. *J Intern Med* 249: 85–95, 2001. doi:10.1046/j.1365-2796.2001.00780.x.
316. Roome CJ, Empson RM. The contribution of the sodium-calcium exchanger (NCX) and plasma membrane  $\text{Ca}^{2+}$ -ATPase (PMCA) to cerebellar synapse function. *Adv Exp Med Biol* 961: 251–263, 2013. doi:10.1007/978-1-4614-4756-6\_21.
317. Rosado JA, Saavedra FR, Redondo PC, Hernández-Cruz JM, Salido GM, Pariente JA. Reduced plasma membrane  $\text{Ca}^{2+}$ -ATPase function in platelets from patients with non-insulin-dependent diabetes mellitus. *Haematologica* 89: 1142–1144, 2004.
318. Rosado JA, Sage SO. Regulation of plasma membrane  $\text{Ca}^{2+}$ -ATPase by small GTPases and phosphoinositides in human platelets. *J Biol Chem* 275: 19529–19535, 2000. doi:10.1074/jbc.M001319200.
319. Rüschoff JH, Brandenburger T, Strehler EE, Filoteo AG, Heinmöller E, Aumüller G, Wilhelm B. Plasma membrane calcium ATPase expression in human colon multistep carcinogenesis. *Cancer Invest* 30: 251–257, 2012. doi:10.3109/07357907.2012.657817.
320. Ryan ZC, Craig TA, Filoteo AG, Westendorf JJ, Cartwright EJ, Neyes L, Strehler EE, Kumar R. Deletion of the intestinal plasma membrane calcium pump, isoform 1, Atp2b1, in mice is associated with decreased bone mineral density and impaired responsiveness to 1, 25-dihydroxyvitamin D3. *Biochem Biophys Res Commun* 467: 152–156, 2015. doi:10.1016/j.bbrc.2015.09.087.
321. Saidu SP, Weeraratne SD, Valentine M, Delay R, Van Houten JL. Role of plasma membrane calcium ATPases in calcium clearance from olfactory sensory neurons. *Chem Senses* 34: 349–358, 2009. doi:10.1093/chemse/bjp008.
322. Saito K, Uzawa K, Endo Y, Kato Y, Nakashima D, Ogawara K, Shiba M, Bukawa H, Yokoe H, Tanzawa H. Plasma membrane  $\text{Ca}^{2+}$ -ATPase isoform 1 down-regulated in human oral cancer. *Oncol Rep* 15: 49–55, 2006.
323. Saksena S, Ammar MS, Tyagi S, Elsharydah A, Gill RK, Ramaswamy K, Dudeja PK. Mechanisms of calcium transport in human colonic basolateral membrane vesicles. *Dig Dis Sci* 47: 2306–2315, 2002. doi:10.1023/A:1020151730940.
324. Samad A, James A, Wong J, Mankad P, Whitehouse J, Patel W, Alves-Simoes M, Siriwardena AK, Bruce JL. Insulin protects pancreatic acinar cells from palmitoleic acid-induced cellular injury. *J Biol Chem* 289: 23582–23595, 2014. doi:10.1074/jbc.M114.589440.
325. Santoni G, Farfariello V. TRP channels and cancer: new targets for diagnosis and chemotherapy. *Endocr Metab Immune Disord Drug Targets* 11: 54–67, 2011. doi:10.2174/187153011794982068.
326. Sasamura S, Furukawa K, Shiratori M, Motomura S, Ohizumi Y. Antisense-inhibition of plasma membrane  $\text{Ca}^{2+}$  pump induces apoptosis in vascular smooth muscle cells. *Jpn J Pharmacol* 90: 164–172, 2002. doi:10.1254/jjp.90.164.
327. Schatzmann HJ. ATP-dependent  $\text{Ca}^{2+}$ -extrusion from human red cells. *Experientia* 22: 364–365, 1966. doi:10.1007/BF01901136.
328. Schneider C, Mottola C, Dolzani L, Romeo D, Babior BM. ATP-driven  $\text{Ca}^{2+}$  pump activity of macrophage and neutrophil plasma membrane. *Adv Exp Med Biol* 141: 463–472, 1982. doi:10.1007/978-1-4684-8088-7\_44.
329. Schneider C, Mottola C, Romeo D. Calcium ion-dependent adenosine triphosphatase activity and plasma-membrane phosphorylation in the human neutrophil. *Biochem J* 182: 655–660, 1979. doi:10.1042/bj1820655.
330. Scholl UI, Goh G, Stölting G, de Oliveira RC, Choi M, Overton JD, Fonseca AL, Korah R, Starker LF, Kunstman JW, Prasad ML, Hartung EA, Mauras N, Benson MR, Brady T, Shapiro JR, Loring E, Nelson-Williams C, Libutti SK, Mane S, Hellman P, Westin G, Åkerström G, Björklund P, Carling T, Fahlke C, Hidalgo P, Lifton RP. Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. *Nat Genet* 45: 1050–1054, 2013. doi:10.1038/ng.2695.
331. Scholl UI, Healy JM, Thiel A, Fonseca AL, Brown TC, Kunstman JW, Horne MJ, Dietrich D, Riemer J, Kücüköylü S, Reimer EN, Reis AC, Goh G, Kristiansen G, Mahajan A, Korah R, Lifton RP, Prasad ML, Carling T. Novel somatic mutations in

- primary hyperaldosteronism are related to the clinical, radiological and pathological phenotype. *Clin Endocrinol (Oxf)* 83: 779–789, 2015. doi:[10.1111/cen.12873](https://doi.org/10.1111/cen.12873).
332. Schuh K, Cartwright EJ, Jankevics E, Bundschu K, Liebermann J, Williams JC, Armesilla AL, Emerson M, Oceandy D, Knobloch KP, Neyses L. Plasma membrane  $\text{Ca}^{2+}$  ATPase 4 is required for sperm motility and male fertility. *J Biol Chem* 279: 28220–28226, 2004. doi:[10.1074/jbc.M312599200](https://doi.org/10.1074/jbc.M312599200).
333. Schuh K, Quaschnig T, Knauer S, Hu K, Kocak S, Roethlein N, Neyses L. Regulation of vascular tone in animals overexpressing the sarcolemmal calcium pump. *J Biol Chem* 278: 41246–41252, 2003. doi:[10.1074/jbc.M307606200](https://doi.org/10.1074/jbc.M307606200).
334. Schuh K, Uldrijan S, Gambaryan S, Roethlein N, Neyses L. Interaction of the plasma membrane  $\text{Ca}^{2+}$  pump 4b/Cl with the  $\text{Ca}^{2+}$ /calmodulin-dependent membrane-associated kinase CASK. *J Biol Chem* 278: 9778–9783, 2003. doi:[10.1074/jbc.M212507200](https://doi.org/10.1074/jbc.M212507200).
335. Schuh K, Uldrijan S, Telkamp M, Roethlein N, Neyses L. The plasmamembrane calmodulin-dependent calcium pump: a major regulator of nitric oxide synthase I. *J Cell Biol* 155: 201–205, 2001. doi:[10.1083/jcb.200104131](https://doi.org/10.1083/jcb.200104131).
336. Schultz JM, Yang Y, Caride AJ, Filoteo AG, Penheiter AR, Lagziel A, Morell RJ, Mohiddin SA, Fananapazir L, Madeo AC, Penniston JT, Griffith AJ. Modification of human hearing loss by plasma-membrane calcium pump PMCA2. *N Engl J Med* 352: 1557–1564, 2005. doi:[10.1056/NEJMoa043899](https://doi.org/10.1056/NEJMoa043899).
337. Schwab BL, Guerini D, Didszun C, Bano D, Ferrando-May E, Fava E, Tam J, Xu D, Xanthoudakis S, Nicholson DW, Carafoli E, Nicotera P. Cleavage of plasma membrane calcium pumps by caspases: a link between apoptosis and necrosis. *Cell Death Differ* 9: 818–831, 2002. doi:[10.1038/sj.cdd.4401042](https://doi.org/10.1038/sj.cdd.4401042).
338. Scully SP, Segel GB, Lichtman MA. Plasma membrane vesicles prepared from unadhered monocytes: characterization of calcium transport and the calcium ATPase. *Cell Calcium* 3: 515–530, 1982. doi:[10.1016/0143-4160\(82\)90042-2](https://doi.org/10.1016/0143-4160(82)90042-2).
339. Sgambato-Faure V, Xiong Y, Berke JD, Hyman SE, Strehler EE. The Homer-1 protein Ania-3 interacts with the plasma membrane calcium pump. *Biochem Biophys Res Commun* 343: 630–637, 2006. doi:[10.1016/j.bbrc.2006.03.020](https://doi.org/10.1016/j.bbrc.2006.03.020).
340. Shalev O, Mogilner S, Shinar E, Rachmilewitz EA, Schrier SL. Impaired erythrocyte calcium homeostasis in beta-thalassemia. *Blood* 64: 564–566, 1984.
341. Shao Y, Wolpert CM, Raiford KL, Menold MM, Donnelly SL, Ravan SA, Bass MP, McClain C, von Wendt L, Vance JM, Abramson RH, Wright HH, Ashley-Koch A, Gilbert JR, DeLong RG, Cuccaro ML, Pericak-Vance MA. Genomic screen and follow-up analysis for autistic disorder. *Am J Med Genet* 114: 99–105, 2002. doi:[10.1002/ajmg.10153](https://doi.org/10.1002/ajmg.10153).
342. Sherkhane P, Kapfhammer JP. The plasma membrane  $\text{Ca}^{2+}$ -ATPase2 (PMCA2) is involved in the regulation of Purkinje cell dendritic growth in cerebellar organotypic slice cultures. *Neural Plast* 2013: 321685, 2013. doi:[10.1155/2013/321685](https://doi.org/10.1155/2013/321685).
343. Shin YB, Lim JE, Ji SM, Lee HJ, Park SY, Hong KW, Lim M, McCarthy MI, Lee YH, Oh B. Silencing of Atp2b1 increases blood pressure through vasoconstriction. *J Hypertens* 31: 1575–1583, 2013. doi:[10.1097/HJH.0b013e32836189e9](https://doi.org/10.1097/HJH.0b013e32836189e9).
344. Shmigol A, Eisner DA, Wray S. Carboxy eosin decreases the rate of decay of the  $[\text{Ca}^{2+}]_i$  transient in uterine smooth muscle cells isolated from pregnant rats. *Pflügers Arch* 437: 158–160, 1998. doi:[10.1007/s004240050761](https://doi.org/10.1007/s004240050761).
345. Shmigol AV, Eisner DA, Wray S. The role of the sarcoplasmic reticulum as a  $\text{Ca}^{2+}$  sink in rat uterine smooth muscle cells. *J Physiol* 520: 153–163, 1999. doi:[10.1111/j.1469-7793.1999.00153.x](https://doi.org/10.1111/j.1469-7793.1999.00153.x).
346. Shull GE, Greb J. Molecular cloning of two isoforms of the plasma membrane  $\text{Ca}^{2+}$ -transporting ATPase from rat brain. Structural and functional domains exhibit similarity to  $\text{Na}^+$ ,  $\text{K}^+$ - and other cation transport ATPases. *J Biol Chem* 263: 8646–8657, 1988.
347. Singhal K, Sandhir R. L-type calcium channel blocker ameliorates diabetic encephalopathy by modulating dysregulated calcium homeostasis. *J Neurosci Res* 93: 296–308, 2015. doi:[10.1002/jnr.23478](https://doi.org/10.1002/jnr.23478).
348. Solár P, Sytkowski AJ. Differentially expressed genes associated with cisplatin resistance in human ovarian adenocarcinoma cell line A2780. *Cancer Lett* 309: 11–18, 2011. doi:[10.1016/j.canlet.2011.05.008](https://doi.org/10.1016/j.canlet.2011.05.008).
349. Souza KL, Elsner M, Mathias PC, Lenzen S, Tiedge M. Cytokines activate genes of the endocytotic pathway in insulin-producing RINm5F cells. *Diabetologia* 47: 1292–1302, 2004. doi:[10.1007/s00125-004-1435-2](https://doi.org/10.1007/s00125-004-1435-2).
350. Spiden SL, Bortolozzi M, Di Leva F, de Angelis MH, Fuchs H, Lim D, Ortolano S, Ingham NJ, Brini M, Carafoli E, Mammano F, Steel KP. The novel mouse mutation Oblivion inactivates the PMCA2 pump and causes progressive hearing loss. *PLoS Genet* 4: e1000238, 2008. doi:[10.1371/journal.pgen.1000238](https://doi.org/10.1371/journal.pgen.1000238).
351. Stains JP, Weber JA, Gay CV. Expression of  $\text{Na}^+/\text{Ca}^{2+}$  exchanger isoforms (NCX1 and NCX3) and plasma membrane  $\text{Ca}^{2+}$  ATPase during osteoblast differentiation. *J Cell Biochem* 84: 625–635, 2002. doi:[10.1002/jcb.10050](https://doi.org/10.1002/jcb.10050).
352. Stauffer TP, Guerini D, Carafoli E. Tissue distribution of the four gene products of the plasma membrane  $\text{Ca}^{2+}$  pump. A study using specific antibodies. *J Biol Chem* 270: 12184–12190, 1995. doi:[10.1074/jbc.270.20.12184](https://doi.org/10.1074/jbc.270.20.12184).
353. Stauffer TP, Hilfiker H, Carafoli E, Strehler EE. Quantitative analysis of alternative splicing options of human plasma membrane calcium pump genes. *J Biol Chem* 268: 25993–26003, 1993.
354. Stewart TA, Yapa KT, Monteith GR. Altered calcium signaling in cancer cells. *Biochim Biophys Acta* 1848, Pt B: 2502–2511, 2015. doi:[10.1016/j.bbame.2014.08.016](https://doi.org/10.1016/j.bbame.2014.08.016).
355. Street VA, McKee-Johnson JW, Fonseca RC, Tempel BL, Noben-Trauth K. Mutations in a plasma membrane  $\text{Ca}^{2+}$ -ATPase gene cause deafness in deafwaddler mice. *Nat Genet* 19: 390–394, 1998. doi:[10.1038/1284](https://doi.org/10.1038/1284).
356. Strehler EE. Recent advances in the molecular characterization of plasma membrane  $\text{Ca}^{2+}$  pumps. *J Membr Biol* 120: 1–15, 1991. doi:[10.1007/BF01868586](https://doi.org/10.1007/BF01868586).
357. Strehler EE, Filoteo AG, Penniston JT, Caride AJ. Plasma-membrane  $\text{Ca}^{2+}$  pumps: structural diversity as the basis for functional versatility. *Biochem Soc Trans* 35: 919–922, 2007. doi:[10.1042/BST0350919](https://doi.org/10.1042/BST0350919).
358. Strehler EE, Zacharias DA. Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. *Physiol Rev* 81: 21–50, 2001.
359. Strid H, Bucht E, Jansson T, Wennergren M, Powell TL. ATP dependent  $\text{Ca}^{2+}$  transport across basal membrane of human syncytiotrophoblast in pregnancies complicated by intrauterine growth restriction or diabetes. *Placenta* 24: 445–452, 2003. doi:[10.1053/plac.2002.0941](https://doi.org/10.1053/plac.2002.0941).
360. Strid H, Powell TL. ATP-dependent  $\text{Ca}^{2+}$  transport is up-regulated during third trimester in human syncytiotrophoblast basal membranes. *Pediatr Res* 48: 58–63, 2000. doi:[10.1203/00006450-200007000-00012](https://doi.org/10.1203/00006450-200007000-00012).
361. Supper V, Schiller HB, Paster W, Forster F, Boulégue C, Mitulovic G, Leksa V, Ohradanova-Repic A, Machacek C, Schatzlmaier P, Zlabinger GJ, Stockinger H. Association of CD147 and calcium exporter PMCA4 uncouples IL-2 expression from early TCR signaling. *J Immunol* 196: 1387–1399, 2016. doi:[10.4049/jimmunol.1501889](https://doi.org/10.4049/jimmunol.1501889).
362. Szemraj J, Kawecka I, Bartkowiak J, Zylinska L. The effect of antisense oligonucleotide treatment of plasma membrane  $\text{Ca}^{2+}$ -ATPase in PC12 cells. *Cell Mol Biol Lett* 9: 451–464, 2004.
363. Szewczyk MM, Pande J, Akolkar G, Grover AK. Caloxin 1b3: a novel plasma membrane  $\text{Ca}^{2+}$ -pump isoform 1 selective inhibitor that increases cytosolic  $\text{Ca}^{2+}$  in endothelial cells. *Cell Calcium* 48: 352–357, 2010. doi:[10.1016/j.ccca.2010.10.008](https://doi.org/10.1016/j.ccca.2010.10.008).
364. Tabara Y, Kohara K, Kita Y, Hirawa N, Katsuya T, Ohkubo T, Hiura Y, Tajima A, Morisaki T, Miyata T, Nakayama T, Takashima N, Nakura J, Kawamoto R, Takahashi N, Hata A, Soma M, Imai Y, Kokubo Y, Okamura T, Tomoike H, Iwai N, Ogihara T, Inoue I, Tokunaga K, Johnson T, Caulfield M, Munroe P, Umemura S, Ueshima H, Miki T, Global Blood Pressure Genetics Consortium. Common variants in the ATP2B1 gene are associated with susceptibility to hypertension: the Japanese Millennium Genome Project. *Hypertension* 56: 973–980, 2010. doi:[10.1161/HYPERTENSIONAHA.110.153429](https://doi.org/10.1161/HYPERTENSIONAHA.110.153429).
365. Takahashi K, Kitamura K. A point mutation in a plasma membrane  $\text{Ca}^{2+}$ -ATPase gene causes deafness in Wriggle Mouse Sagami. *Biochem Biophys Res Commun* 261: 773–778, 1999. doi:[10.1006/bbrc.1999.1102](https://doi.org/10.1006/bbrc.1999.1102).
366. Takeuchi F, Isono M, Katsuya T, Yamamoto K, Yokota M, Sugiyama T, Nabika T, Fujioka A, Ohnaka K, Asano H, Yamori Y, Yamaguchi S, Kobayashi S, Takayanagi R, Ogihara T, Kato N. Blood pressure and hypertension are associated with 7 loci in the Japanese population. *Circulation* 121: 2302–2309, 2010. doi:[10.1161/CIRCULATIONAHA.109.904664](https://doi.org/10.1161/CIRCULATIONAHA.109.904664).



367. Takeuchi F, Isono M, Yamamoto K, Yokota M, Akiyama K, Katsuya T, Kim HS, Park JE, Jang Y, Lee JY, Lee JY, Kato N, AGEN Consortium. Heterogeneous effects of association between blood pressure loci and coronary artery disease in east Asian individuals. *Circ J* 79: 830–838, 2015. doi:10.1253/circj.CJ-14-0841.
368. Talarico EF Jr. Plasma membrane calcium-ATPase isoform four distribution changes during corneal epithelial wound healing. *Mol Vis* 16: 2259–2272, 2010.
369. Talarico EF Jr, Kennedy BG, Marfurt CF, Loeffler KU, Mangini NJ. Expression and immunolocalization of plasma membrane calcium ATPase isoforms in human corneal epithelium. *Mol Vis* 11: 169–178, 2005.
370. Tauber P, Aichinger B, Christ C, Stindl J, Rhayem Y, Beuschlein F, Warth R, Bandulik S. Cellular pathophysiology of an adrenal adenoma-associated mutant of the plasma membrane  $\text{Ca}(2+)$ -ATPase ATP2B3. *Endocrinology* 157: 2489–2499, 2016. doi:10.1210/en.2015-2029.
371. Thomas RC. The plasma membrane calcium ATPase (PMCA) of neurones is electro-neutral and exchanges 2  $\text{H}^+$  for each  $\text{Ca}^{2+}$  or  $\text{Ba}^{2+}$  ion extruded. *J Physiol* 587: 315–327, 2009. doi:10.1113/jphysiol.2008.162453.
372. Tiffert T, Lew VL. Elevated intracellular  $\text{Ca}^{2+}$  reveals a functional membrane nucleotide pool in intact human red blood cells. *J Gen Physiol* 138: 381–391, 2011. doi:10.1085/jgp.201110660.
373. Timmann C, Thye T, Vens M, Evans J, May J, Ehmen C, Sievertsen J, Muntau B, Ruge G, Loag W, Ansong D, Antwi S, Asafa-Adjei E, Nguah SB, Kwakye KO, Akoto AO, Sylverken J, Brendel M, Schuldt K, Loley C, Franke A, Meyer CG, Agbenyega T, Ziegler A, Horstmann RD. Genome-wide association study indicates two novel resistance loci for severe malaria. *Nature* 489: 443–446, 2012. doi:10.1038/nature11334.
374. Tribe RM, Moriarty P, Poston L. Calcium homeostatic pathways change with gestation in human myometrium. *Biol Reprod* 63: 748–755, 2000. doi:10.1095/biolreprod63.3.748.
375. Tye CE, Sharma R, Smith CE, Bartlett JD. Altered ion-responsive gene expression in Mmp20 null mice. *J Dent Res* 89: 1421–1426, 2010. doi:10.1177/0022034510384625.
376. Ueno T, Kameyama K, Hirata M, Ogawa M, Hatsuse H, Takagaki Y, Ohmura M, Osawa N, Kudo Y. A mouse with a point mutation in plasma membrane  $\text{Ca}^{2+}$ -ATPase isoform 2 gene showed the reduced  $\text{Ca}^{2+}$  influx in cerebellar neurons. *Neurosci Res* 42: 287–297, 2002. doi:10.1016/S0168-0102(02)00008-1.
377. Usachev YM, Toutenhoofd SL, Goellner GM, Strehler EE, Thayer SA. Differentiation induces up-regulation of plasma membrane  $\text{Ca}(2+)$ -ATPase and concomitant increase in  $\text{Ca}(2+)$  efflux in human neuroblastoma cell line IMR-32. *J Neurochem* 76: 1756–1765, 2001. doi:10.1046/j.1471-4159.2001.00169.x.
378. Van Abel M, Hoenderop JG, van der Kemp AW, van Leeuwen JP, Bindels RJ. Regulation of the epithelial  $\text{Ca}^{2+}$  channels in small intestine as studied by quantitative mRNA detection. *Am J Physiol Gastrointest Liver Physiol* 285: G78–G85, 2003. doi:10.1152/ajpgi.00036.2003.
379. Van Cromphaut SJ, Rummens K, Stockmans I, Van Herck E, Dijcks FA, Ederveen AG, Carmeliet P, Verhaeghe J, Bouillon R, Carmeliet G. Intestinal calcium transporter genes are upregulated by estrogens and the reproductive cycle through vitamin D receptor-independent mechanisms. *J Bone Miner Res* 18: 1725–1736, 2003. doi:10.1359/jbmr.2003.18.10.1725.
380. 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, van Leeuwen JP. 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: 1951–1959, 2012. doi:10.1002/jcp.22923.
381. Van der Hagen EA, Lavrijsen M, van Zeeland F, Praetorius J, Bonny O, Bindels RJ, Hoenderop JG. Coordinated regulation of TRPV5-mediated  $\text{Ca}^{2+}$  transport in primary distal convolution cultures. *Pflugers Arch* 466: 2077–2087, 2014. doi:10.1007/s00424-014-1470-x.
382. Van Loon EP, Little R, Prehar S, Bindels RJ, Cartwright EJ, Hoenderop JG. Calcium extrusion pump PMCA4: a new player in renal calcium handling? *PLoS One* 11: e0153483, 2016. doi:10.1371/journal.pone.0153483.
383. Vanagas L, de la Fuente MC, Dalghi M, Ferreira-Gomes M, Rossi RC, Strehler EE, Mangialavori IC, Rossi JP. Differential effects of G- and F-actin on the plasma membrane calcium pump activity. *Cell Biochem Biophys* 66: 187–198, 2013. doi:10.1007/s12013-012-9467-6.
384. VanHouten J, Sullivan C, Bazinet C, Ryoo T, Camp R, Rimm DL, Chung G, Wysolmerski J. PMCA2 regulates apoptosis during mammary gland involution and predicts outcome in breast cancer. *Proc Natl Acad Sci USA* 107: 11405–11410, 2010. doi:10.1073/pnas.0911186107.
385. VanHouten JN, Neville MC, Wysolmerski JJ. The calcium-sensing receptor regulates plasma membrane calcium adenosine triphosphatase isoform 2 activity in mammary epithelial cells: a mechanism for calcium-regulated calcium transport into milk. *Endocrinology* 148: 5943–5954, 2007. doi:10.1210/en.2007-0850.
386. Varga K, Pászty K, Padányi R, Hegedűs L, Brouland JP, Papp B, Enyedi A. Histone deacetylase inhibitor- and PMA-induced upregulation of PMCA4b enhances  $\text{Ca}^{2+}$  clearance from MCF-7 breast cancer cells. *Cell Calcium* 55: 78–92, 2014. doi:10.1016/j.ceca.2013.12.003.
387. Verma AK, Filoteo AG, Stanford DR, Wieben ED, Penniston JT, Strehler EE, Fischer R, Heim R, Vogel G, Mathews S, et al. Complete primary structure of a human plasma membrane  $\text{Ca}^{2+}$  pump. *J Biol Chem* 263: 14152–14159, 1988.
388. Walters JR, Balesaria S, Khair U, Sangha S, Banks L, Berry JL. The effects of vitamin D metabolites on expression of genes for calcium transporters in human duodenum. *J Steroid Biochem Mol Biol* 103: 509–512, 2007. doi:10.1016/j.jsbmb.2006.11.013.
389. Wan JP, Wang H, Li CZ, Zhao H, You L, Shi DH, Sun XH, Lv H, Wang F, Wen ZQ, Wang XT, Chen ZJ. The common single-nucleotide polymorphism rs2681472 is associated with early-onset preeclampsia in Northern Han Chinese women. *Reprod Sci* 21: 1423–1427, 2014. doi:10.1177/1933719114527354.
390. Wan QF, Nixon E, Heidelberger R. Regulation of presynaptic calcium in a mammalian synaptic terminal. *J Neurophysiol* 108: 3059–3067, 2012. doi:10.1152/jn.00213.2012.
391. Wang Y, Zhang Y, Li Y, Zhou X, Wang X, Gao P, Jin L, Zhang X, Zhu D. Common variants in the ATP2B1 gene are associated with hypertension and arterial stiffness in Chinese population. *Mol Biol Rep* 40: 1867–1873, 2013. doi:10.1007/s11033-012-2242-3.
392. Watson CJ, Tempel BL. A new Atp2b2 deafwaddler allele, dfw(i5), interacts strongly with Cdh23 and other auditory modifiers. *Hear Res* 304: 41–48, 2013. doi:10.1016/j.heares.2013.06.003.
393. Weeraratne SD, Valentine M, Cusick M, Delay R, Van Houten JL. Plasma membrane calcium pumps in mouse olfactory sensory neurons. *Chem Senses* 31: 725–730, 2006. doi:10.1093/chemse/bjl014.
394. Weng L, Taylor KD, Chen YD, Sopko G, Kelsey SF, Bairey Merz CN, Pepine CJ, Miller VM, Rotter JI, Gulati M, Goodarzi MO, Cooper-DeHoff RM. Genetic loci associated with nonobstructive coronary artery disease in Caucasian women. *Physiol Genomics* 48: 12–20, 2016. doi:10.1152/physiolgenomics.00067.2015.
395. Williams JC, Armesilla AL, Mohamed TM, Hagarty CL, McIntyre FH, Schomburg S, Zaki AO, Oceandy D, Cartwright EJ, Buch MH, Emerson M, Neynes L. The sarcolemmal calcium pump, alpha-1 syntrophin, and neuronal nitric-oxide synthase are parts of a macromolecular protein complex. *J Biol Chem* 281: 23341–23348, 2006. doi:10.1074/jbc.M513341200.
396. Williams TA, Monticone S, Schack VR, Stindl J, Burrello J, Buffolo F, Annaratone L, Castellano I, Beuschlein F, Reincke M, Lucatello B, Ronconi V, Fallo F, Bernini G, Maccario M, Giacchetti G, Veglio F, Warth R, Vilsen B, Mulatero P. Somatic ATP1A1, ATP2B3, and KCNJ5 mutations in aldosterone-producing adenomas. *Hypertension* 63: 188–195, 2014. doi:10.1161/HYPERTENSIONAHA.113.01733.
397. Williams TA, Peitzsch M, Dietz AS, Dekkers T, Bidlingmaier M, Riest A, Treitl M, Rhayem Y, Beuschlein F, Lenders JW, Deinum J, Eisenhofer G, Reincke M. Genotype-specific steroid profiles associated with aldosterone-producing adenomas. *Hypertension* 67: 139–145, 2016. doi:10.1161/HYPERTENSIONAHA.115.06186.
398. Wimalasundera RC, Wijetunge S, Thom SM, Regan L, Hughes AD. Impaired recovery of intracellular calcium and force after activation in isolated myometrial and subcutaneous resistance arteries from women with preeclampsia. *J Hypertens* 28: 568–574, 2010. doi:10.1097/HJH.0b013e328334f20b.
399. Witczak CA, Sturek M. Exercise prevents diabetes-induced impairment in superficial buffer barrier in porcine coronary smooth muscle. *J Appl Physiol (1985)* 96: 1069–1079, 2004. doi:10.1152/japplphysiol.00460.2003.
400. Witczak CA, Wamhoff BR, Sturek M. Exercise training prevents  $\text{Ca}^{2+}$  dysregulation in coronary smooth muscle from diabetic dyslipidemic yucatan swine. *J Appl Physiol (1985)* 101: 752–762, 2006. doi:10.1152/japplphysiol.00235.2006.



401. Wollheim CB, Sharp GW. Regulation of insulin release by calcium. *Physiol Rev* 61: 914–973, 1981.
402. Wu F, Dassopoulos T, Cope L, Maitra A, Brant SR, Harris ML, Bayless TM, Parmigiani G, Chakravarti S. Genome-wide gene expression differences in Crohn's disease and ulcerative colitis from endoscopic pinch biopsies: insights into distinctive pathogenesis. *Inflamm Bowel Dis* 13: 807–821, 2007. doi:10.1002/ibd.20110.
403. Wu Q, Guo D, Bi H, Wang D, Du Y. UVB irradiation-induced dysregulation of plasma membrane calcium ATPase1 and intracellular calcium homeostasis in human lens epithelial cells. *Mol Cell Biochem* 382: 263–272, 2013. doi:10.1007/s11010-013-1743-2.
404. Wu VC, Huang KH, Peng KY, Tsai YC, Wu CH, Wang SM, Yang SY, Lin LY, Chang CC, Lin YH, Lin SL, Chu TS, Wu KD. Prevalence and clinical correlates of somatic mutation in aldosterone producing adenoma-Taiwanese population. *Sci Rep* 5: 11396, 2015. doi:10.1038/srep11396.
405. Wu X, Chang B, Blair NS, Sargent M, York AJ, Robbins J, Shull GE, Molkenin JD. Plasma membrane Ca<sup>2+</sup>-ATPase isoform 4 antagonizes cardiac hypertrophy in association with calcineurin inhibition in rodents. *J Clin Invest* 119: 976–985, 2009.
406. Wu X, Zhang T, Bossuyt J, Li X, McKinsey TA, Dedman JR, Olson EN, Chen J, Brown JH, Bers DM. Local InsP3-dependent perinuclear Ca<sup>2+</sup> signaling in cardiac myocyte excitation-transcription coupling. *J Clin Invest* 116: 675–682, 2006. doi:10.1172/JCI27374.
407. Xi B, Shen Y, Zhao X, Chandak GR, Cheng H, Hou D, Li Y, Ott J, Zhang Y, Wang X, Mi J. Association of common variants in/near six genes (ATP2B1, CSK, MTHFR, CYP17A1, STK39 and FGF5) with blood pressure/hypertension risk in Chinese children. *J Hum Hypertens* 28: 32–36, 2014. doi:10.1038/jhh.2013.50.
408. Xi B, Zhao X, Chandak GR, Shen Y, Cheng H, Hou D, Wang X, Mi J. Influence of obesity on association between genetic variants identified by genome-wide association studies and hypertension risk in Chinese children. *Am J Hypertens* 26: 990–996, 2013. doi:10.1093/ajh/hpt046.
409. Xiao Y, Cui J, Shi YH, Sun J, Wang ZP, Le GW. Effects of duodenal redox status on calcium absorption and related genes expression in high-fat diet-fed mice. *Nutrition* 26: 1188–1194, 2010. doi:10.1016/j.nut.2009.11.021.
410. Xu L, Wang Z, Xiong X, Gu X, Gao X, Gao X. Identification of a novel point mutation of mouse Atp2b2 induced by N-ethyl-N-nitrosourea mutagenesis. *Exp Anim* 60: 71–78, 2011. doi:10.1538/expanim.60.71.
411. Yang H, An BS, Choi KC, Jeung EB. Change of genes in calcium transport channels caused by hypoxic stress in the placenta, duodenum, and kidney of pregnant rats. *Biol Reprod* 88: 30, 2013. doi:10.1095/biolreprod.112.103705.
412. Yang H, Choi KC, Hyun SH, Jeung EB. Coexpression and estrogen-mediated regulation of TRPV6 and PMCA1 in the human endometrium during the menstrual cycle. *Mol Reprod Dev* 78: 274–282, 2011. doi:10.1002/mrd.21303.
413. Yang J, Pawlyk B, Wen XH, Adamian M, Soloviev M, Michaud N, Zhao Y, Sandberg MA, Makino CL, Li T. Mpp4 is required for proper localization of plasma membrane calcium ATPases and maintenance of calcium homeostasis at the rod photoreceptor synaptic terminals. *Hum Mol Genet* 16: 1017–1029, 2007. doi:10.1093/hmg/ddm047.
414. Yang W, Liu J, Zheng F, Jia M, Zhao L, Lu T, Ruan Y, Zhang J, Yue W, Zhang D, Wang L. The evidence for association of ATP2B2 polymorphisms with autism in Chinese Han population. *PLoS One* 8: e61021, 2013. doi:10.1371/journal.pone.0061021.
415. Zacharias DA, Kappen C. Developmental expression of the four plasma membrane calcium ATPase (Pmca) genes in the mouse. *Biochim Biophys Acta* 1428: 397–405, 1999. doi:10.1016/S0304-4165(99)00058-6.
416. Zaidi A. Plasma membrane Ca-ATPases: targets of oxidative stress in brain aging and neurodegeneration. *World J Biol Chem* 1: 271–280, 2010. doi:10.4331/wjbc.v1.i9.271.
417. Zaidi A, Fernandes D, Bean JL, Michaelis ML. Effects of paraquat-induced oxidative stress on the neuronal plasma membrane Ca(2+)-ATPase. *Free Radic Biol Med* 47: 1507–1514, 2009. doi:10.1016/j.freeradbiomed.2009.08.018.
418. Zaidi A, Gao J, Squier TC, Michaelis ML. Age-related decrease in brain synaptic membrane Ca<sup>2+</sup>-ATPase in F344/BNF1 rats. *Neurobiol Aging* 19: 487–495, 1998. doi:10.1016/S0197-4580(98)00078-5.
419. Zaidi A, Michaelis ML. Effects of reactive oxygen species on brain synaptic plasma membrane Ca(2+)-ATPase. *Free Radic Biol Med* 27: 810–821, 1999. doi:10.1016/S0891-5849(99)00128-8.
420. Zanni G, Cali T, Kalscheuer VM, Ottolini D, Barresi S, Lebrun N, Montecchi-Palazzi L, Hu H, Chelly J, Bertini E, Brini M, Carafoli E. Mutation of plasma membrane Ca<sup>2+</sup> ATPase isoform 3 in a family with X-linked congenital cerebellar ataxia impairs Ca<sup>2+</sup> homeostasis. *Proc Natl Acad Sci USA* 109: 14514–14519, 2012. doi:10.1073/pnas.1207488109.
421. Zhang Y, Papasian CJ, Deng HW. Alteration of vitamin D metabolic enzyme expression and calcium transporter abundance in kidney involved in type I diabetes-induced bone loss. *Osteoporos Int* 22: 1781–1788, 2011. doi:10.1007/s00198-010-1404-1.
422. Zheng FF, Zhu LM, Nie AF, Li XY, Lin JR, Zhang K, Chen J, Zhou WL, Shen ZJ, Zhu YC, Wang JG, Zhu DL, Gao PJ. Clinical characteristics of somatic mutations in Chinese patients with aldosterone-producing adenoma. *Hypertension* 65: 622–628, 2015. doi:10.1161/HYPERTENSIONAHA.114.03346.
423. Zylińska L, Soszyński M. Plasma membrane Ca<sup>2+</sup>-ATPase in excitable and nonexcitable cells. *Acta Biochim Pol* 47: 529–539, 2000.