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Structural Conservation of the Genes Encoding CaT1, CaT2, and Related Cation Channels

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We report here the genomic structures of the genes encoding human calcium transport proteins CaT1 and CaT2, which belong to a recently identified class of highly selective calcium entry channels. The mRNA for CaT1 was expressed more abundantly than that for CaT2 in three major tissues involved in transcellular calcium transport, namely intestine, kidney, and placenta, as determined by quantitative PCR. The genes encoding CaT1 and CaT2, *ECAC2* and *ECAC1*, respectively, are completely conserved in terms of exon size in the coding regions. They also share similar intron–exon structures with the genes encoding the closely related, nonselective cation channels VR1, VRL-1, OTRPC4 (also known as VR-OAC, Trp12, and VRL-2), and a hypothetical protein, VRL-3. We conclude that *ECAC2* and *ECAC1*, which encode calcium selective channels, share a common ancestral gene with the genes encoding the related nonselective cation channels.

Key words: gene, calcium, channel, CaT1, CaT2, ECaC, ECAC1, ECAC2

INTRODUCTION

Calcium (Ca^{2+}) is a major inorganic component of the skeleton and serves as a key extracellular [1] and intracellular [2] messenger. Ca^{2+}-permeable channels participate in biological processes such as neurotransmission, muscle contraction, and exocytosis by providing Ca^{2+} as an intracellular second messenger. Based on their gating properties, Ca^{2+} channels have been traditionally classified as voltage-operated, ligand-gated, mechanosensitive, and Ca^{2+}-store operated.

The Ca^{2+} channels CaT1 [3,4] and CaT2 (also known as ECaC) [5,6] mediate cellular Ca^{2+} uptake as part of the transcellular pathway of Ca^{2+} transport in intestine, kidney, and placenta. They are part of a family of cation channels which is distinct from the well-characterized "classical" channel families such as the CACN voltage-dependent calcium channel family (see http://www.gene.ucl.ac.uk/nomenclature/). CaT1 and CaT2 (encoded by the genes *ECAC2* and *ECAC1*, respectively) are elements within a multicomponent system that mediates and regulates Ca^{2+} influx into cells and/or transepithelial movement of Ca^{2+} between the outside environment and the body. In renal and intestinal epithelial cells, transcellular Ca^{2+} transport includes the following steps: (1) luminal Ca^{2+} entry across the apical membranes of the absorptive/reabsorptive cells; (2) transcellular Ca^{2+} diffusion of Ca^{2+}; and (3) exit of Ca^{2+} through the basolateral aspect of the cell. The CaT Ca^{2+} uptake channels function at a macroscopic level as facilitated transporters that enable Ca^{2+} to move down its electrochemical gradient from the outside to the inside of the intestinal and renal cells. Thus these channel proteins, in their capacity to mediate apical Ca^{2+} entry, are key "gatekeepers" that determine how much Ca^{2+} enters or leaves the body by intestinal and renal routes.

Recently, CaT1 was shown to share all the biophysical properties of the previously described calcium-release-activated calcium channel [7]. The latter is a channel through which Ca^{2+} enters cells in response to depletion of internal Ca^{2+} stores, which is known as capacitative Ca^{2+} entry or store-operated Ca^{2+} entry [reviewed in 8,9]. Thus, in addition to its postulated roles in transepithelial Ca^{2+} transport in intestine, kidney, and placenta, CaT1 (or, in some cases, CaT2 or both channels) may participate in store-operated Ca^{2+} entry, especially in exocrine organs such as pancreas, prostate, and salivary gland, where CaT1 is highly expressed [4,10,11].

The CaT proteins [3–6] constitute a novel subclass of Ca^{2+} transporter/channels. They share roughly 30% amino acid sequence identity with the capsaicin receptor, VR1 [12], the stretch-inhibitable nonselective cation channel (SIC) [13], the VRL-1 protein [14] (also known as growth factor-regulated channels [15]), and the osmolarity-sensitive channel OTRPC4 [16] (also known as VR-OAC [17], Trp12 [18], and VRL-2 [19]). All of these proteins are structurally related to the transient receptor potential (TRP) family of nonselective, Ca^{2+}-permeable, cation channels [20–22]. Most contain

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ankyrin repeats in their amino-terminal regions, show similar predicted transmembrane topologies, and share substantial sequence similarity in a stretch of amino acids encompassing their respective pore regions and transmembrane domain 6. They likewise show sequence similarities in their putative pore regions and transmembrane segment 6 to the polycystin-2 (PC-2) [23] and polycystin-like (PC-L) proteins [24–26].

Despite their overall structural similarities, however, the functions of these various channel proteins differ substantially. VR1 is a ligand-gated and heat-activated nonselective cation channel [12]. SIC and OTRP4 are mechanosensitive and osmoreceptive cation channels [13,16,17]. Some of the TRP channels are putative Ca^{2+} store-operated channels [22], and PCL is a Ca^{2+}-regulated, nonselective cation channel [27]. CaT1 and CaT2 also exhibit distinctive functional properties, including saturation kinetics and constitutive activity that confer upon them some of the attributes of facilitated transporters [3,4,6]. Single channel activities can be detected, however, when Na^+ or K^+ is used as a charge carrier [7,28,29]. An additional feature of the CaT1/CaT2 subfamily of channels is activation of both CaT1 and CaT2 by membrane hyperpolarization [7,28,29]. CaT1 is also activated by depletion of intracellular Ca^{2+} stores [7]. CaT1 is inactivated by intracellular Ca^{2+} [7], and Ca^{2+}-dependent calmodulin binding to CaT1 is one mechanism that mediates inactivation of CaT1 [30]. So far, no endogenous ligands have been identified that regulate the activities of CaT1 and CaT2.

Functional domains of proteins are often encoded by different exons. Thus analysis of gene structure can provide clues to underlying structure-function relationships and might represent an additional approach to understanding the evolutionary relationships of CaT1, CaT2, and the other channels noted above to one another. Here we have compared the intronic and exonic structures of human *ECAC2* and *ECAC1* and their relationships to the corresponding cDNAs. Furthermore, we have compared the structures of *ECAC2* and *ECAC1* with those of the genes encoding VR1 and related proteins, some members of the TRPC family, and the polycystins.

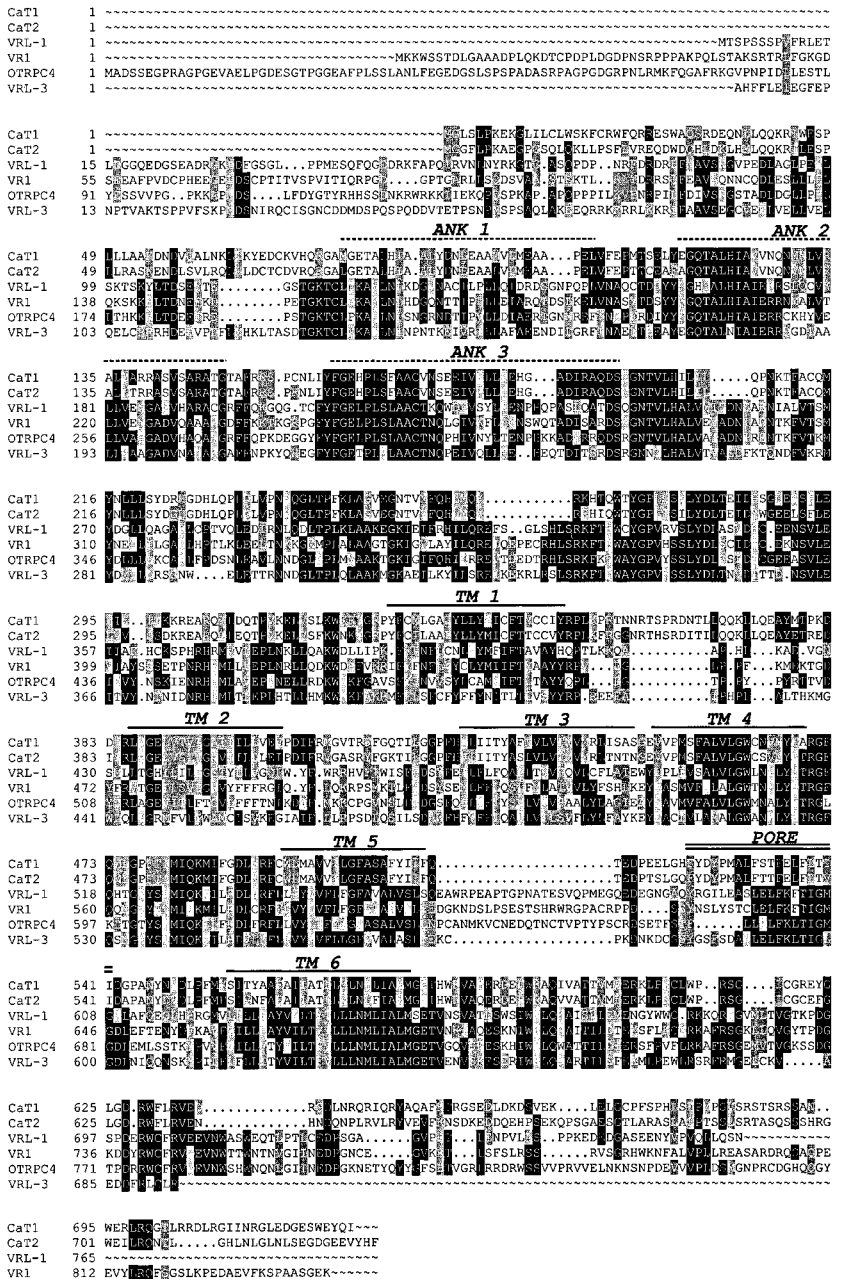

FIG. 1. Alignment of the amino acid sequences of CaT1 and CaT2 with related proteins identified in the human databases. The ankyrin repeats (ANK), transmembrane domains (TM), and pores (PORE) for CaT1 and CaT2 are indicated using dotted lines, filled lines, and filled double lines, respectively. Gaps are indicated by dots. The overall amino acid sequence identity between CaT1 (GenBank acc. no. AF365927) and CaT2 (GenBank acc. no. AF304464) is 75.6%; sequence identities between CaT1 and CaT2, on the one hand, and VR1 (NM_018727), VRL-1 (AF129112), VRL-3, and OTRPC4 (AF258465), on the other, range from 30.5 to 33.3%, whereas sequence identities among the latter four proteins vary between 41.6 and 47.0%. VRL-3 is a hypothetical protein predicted using the GENSCAN program based on its genomic sequence.

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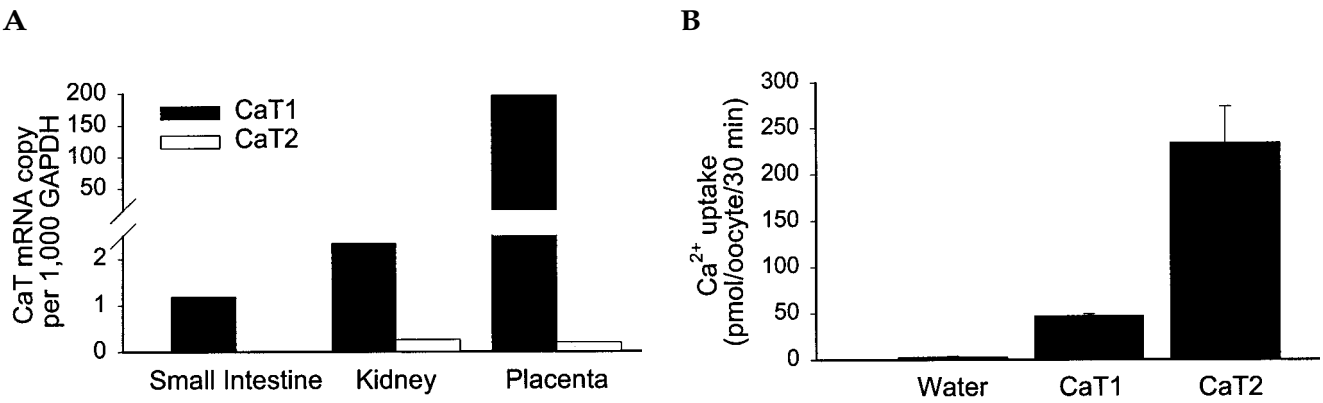

FIG. 2. Comparison of the abundances of *ECAC1* and *ECAC2* mRNAs for CaT1 and CaT2 in the major organs participating in transcellular Ca^{2+} transport and documentation of the Ca^{2+} transport activity of both proteins when expressed in *X. laevis* oocytes. (A) mRNA abundances as determined by quantitative PCR. The relative abundances of *ECAC1*, *ECAC2*, and *GAPDH* were assessed in the same samples in duplicate; data shown are mean values. (B) CaT1- or CaT2-mediated Ca^{2+} uptake when expressed in *X. laevis* oocytes. Data are from three independent experiments and are expressed as mean ±S.E.M.

RESULTS

Cloning of Both CaT1 and CaT2 from a Human Kidney cDNA Library

Low-stringency screening of a human kidney library resulted in the isolation of cDNAs encoding both CaT1 and CaT2 (GenBank acc. nos. AF365927 and AF304464). The respective encoded proteins are highly homologous to those encoded by the corresponding cDNAs for rat CaT1 and CaT2 [3,6], indicating that human CaT1 and rat CaT1 are orthologues, as are human CaT2 and rat CaT2. Figure 1 shows the alignment of the amino acid sequences of CaT1 and CaT2 as well as the related proteins VR1, VRL-1, OTPPC4, and a hypothetical protein designated here as "VRL-3, hypothetical." The overall amino acid sequence identity between CaT1 and CaT2 is 75.6% (based on the Gap program using the algorithm of Needleman and Wunsch, Wisconsin Package Version 10.1, GCG), whereas CaT1 and CaT2 share 30.5 to 33.3% identities with the other four proteins, and the latter four proteins share 41.6 to 47.0% identities among themselves. Therefore, based on their relative degrees of sequence identity and their functional properties, the six proteins can be divided into two subclasses: the Ca^{2+} selective channels, CaT1 and CaT2; and the nonselective cation channels VR1, VRL-1, OTRPC4, and, perhaps, VRL-3.

Comparison of Sequences of CaT2 and Human ECaC

CaT2 has 97% amino acid sequence identity with the previously reported human ECaC cDNA (GenBank acc. no. AJ271207; [31]). There are 20 amino acids that differ between human ECaC and human CaT2 (GenBank acc. no. AF304464) out of the 729 amino acids in each of the two full-length proteins. The CaT2 cDNA is also 97% identical to that of human ECaC in its nucleotide sequence in the coding region (64 nucleotide differences over 2187 bp). Alignment of the sequences of CaT1 (GenBank acc. no. AF365927) and CaT2 with that of human ECaC reveals long stretches of identity between CaT2 and ECaC, but there is at least one region in which the ECaC cDNA shows a stretch of sequence identity with CaT1 rather than with CaT2. The human expressed sequence tag (EST; GenBank acc. no. AA078617), which was used to design gene-specific primers for RACE of ECaC [31], actually corresponds to the sequence of human CaT1. The first four of these primers [31], therefore, match the human CaT1 sequence better than the CaT2/ECaC sequence. Consequently, the published human ECaC cDNA sequence both contains sequencing errors and includes stretches of sequence corresponding to those of human CaT1. Because the primers used to determine the gene expression pattern by RT-PCR were not gene-specific, the expression pattern shown by Müller *et al.* [31] almost certainly includes the expression patterns of both CaT1 and CaT2/ECaC. For instance, ECaC/CaT2 was shown to be expressed in intestine [31], but, when we used gene-specific primers for *ECAC2* and *ECAC1*, only *ECAC2* was detected in small intestine (Fig. 2A).

We also found that our CaT1 sequence, which was obtained from a cDNA cloned from a human kidney library, has differences in several nucleotides that produce changes in two amino acids, compared with the sequence that we reported previously, which was generated by PCR (GenBank acc. no. AF304463; [4]). Codons 37 and 74 encode asparagine and glutamine in the CaT1 sequence reported here instead of the aspartate and histidine at the corresponding positions that were reported earlier [4]. The CaT1 sequence reported here also has differences in four and one amino acid, respectively, compared with the sequences of htrp8A (GenBank acc. no. AJ243500) and htrp8B (GenBank acc. no. AJ243501), which were reported as CaT-like proteins [11]. These differences might be the result of polymorphisms of *ECAC2* or might represent sequencing errors or PCR artifact.

Levels of Expression of *ECAC2* and *ECAC1* in Human Intestine, Kidney, and Placenta

To quantitatively estimate the relative levels of *ECAC2* and *ECAC1* mRNA in the three major organs participating in

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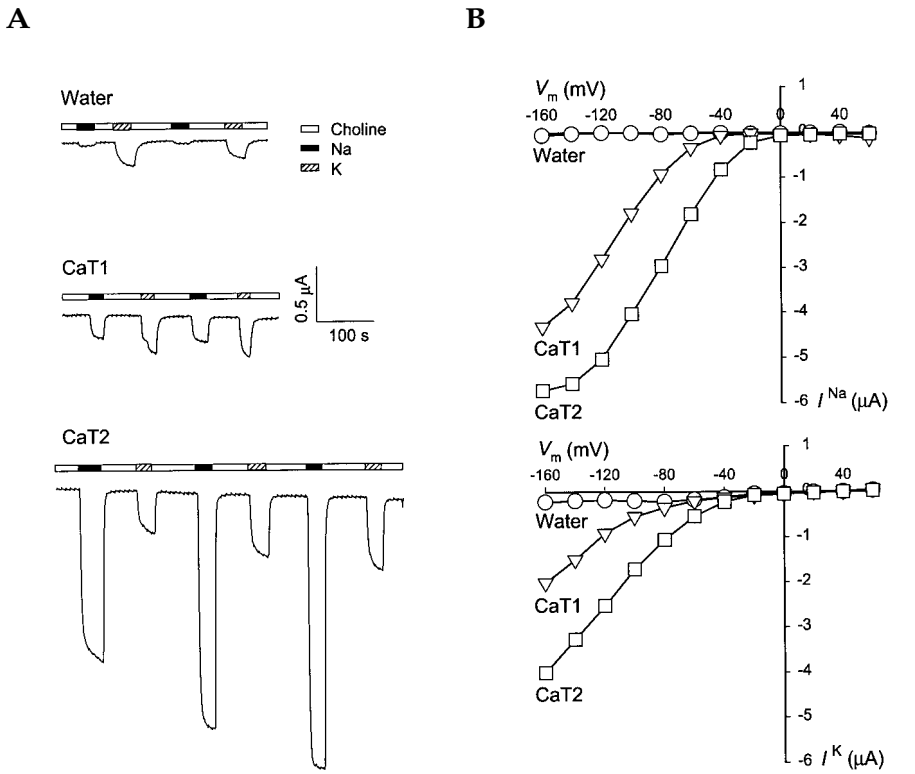

FIG. 3. Distinct properties of Na^+ and K^+ currents mediated by CaT1 and CaT2 when expressed in *X. laevis* oocytes. (A) Na^+ and K^+ currents in control oocytes or in CaT1- or CaT2-expressing oocytes. The holding potential was -50 mV. Na^+ and K^+ currents increased over time in the CaT2-expressing oocyte. (B) Current-voltage curves representing the Na^+ (top) or K^+ (bottom) currents at various test potentials in control oocytes or in CaT1- or CaT2-expressing oocytes. Data shown are the differences between the currents observed in the presence or absence (choline substitution) of 100 mM Na^+ and K^+. Shown are results observed with representative oocytes from a total of at least six oocytes that were tested in each group.

transcellular Ca^{2+} transport—small intestine, kidney, and placenta (which are known to express CaT1 and/or CaT2 in rat and rabbit)—we carried out real-time PCR using *ECAC2*- and *ECAC1*-specific primers. mRNA abundance was calculated based on the relative abundance of the respective cDNAs, which had been prepared from mRNA extracted from the three tissues and were used as templates for quantitative PCR. Given the high degrees of homology within extended regions of the *ECAC2* and *ECAC1* cDNAs, we took care to design primer pairs that would distinguish unequivocally between *ECAC2*- and *ECAC1*-derived transcripts. *ECAC2* is expressed in all three tissues, with the highest expression level in human placenta (Fig. 3B), as reported previously [4]. In contrast, *ECAC1* is not detected in human small intestine and the level of its mRNA is much lower in human kidney and placenta than that of *ECAC2*.

Functional Properties of CaT1 and CaT2 When Expressed in *Xenopus laevis* Oocytes

To confirm that CaT1 and CaT2 cDNAs encode functional proteins, we injected synthetic RNAs transcribed from their respective cDNAs into *Xenopus laevis* oocytes and characterized their capacities to mediate $^{45}Ca^{2+}$ uptake, as in our previous studies of the rat orthologues of these two proteins. CaT1 and CaT2 both mediated Ca^{2+} uptake, and the uptakes in oocytes injected with CaT1 and CaT2 cRNAs were substantially greater than the uptake observed in control, water-injected oocytes. CaT2-mediated Ca^{2+} uptake was about fourfold greater than that for CaT1 (Fig. 2B). Thus both clones encode functional proteins.

X. laevis oocytes possess Ca^{2+}-activated chloride channels. Therefore Ca^{2+}-evoked currents in oocytes expressing CaT1 or CaT2 are mostly due to Cl^- currents, which makes it difficult to analyze Ca^{2+} influx mediated by CaT1 or CaT2. In the absence of extracellular Ca^{2+} and Mg^{2+}, CaT1 and CaT2 were shown to be permeable to monovalent cations such as Na^+ and K^+ [7,28,29]. We therefore assessed differences in the electrophysiological properties of the two proteins by measuring Na^+ and K^+ currents mediated by CaT1 or CaT2 in *X. laevis* oocytes. When membrane potential was held at –50 mV, oocytes expressing either CaT1 or CaT2 had Na^+ and K^+ currents that were much greater than those observed in water-injected, control oocytes (Fig. 3A). However, the amplitudes of the Na^+ or K^+ currents observed in CaT2-expressing oocytes seemed to be much greater that those present in oocytes expressing CaT1. Furthermore, the currents in CaT2-expressing oocytes increased over time, whereas the same phenomenon was not observed in CaT1-expressing oocytes (Fig. 3A). The mechanism underlying this difference in the behavior of the two channels remains to be investigated. The current-voltage (*I-V*) relationships of the two channels (Fig. 3B) revealed that activation of CaT1 required more hyperpolarized potentials than did activation of CaT2. In addition, the K^+ currents mediated by both CaT1 and CaT2 were smaller than the corresponding Na^+ currents. Furthermore, the Na^+ currents showed saturation at very hyperpolarized potentials (–140 to –160 mV) in CaT2-expressing oocytes, regardless of the expression level of the protein. This saturation was not observed when K^+ was used as a charge carrier. The observation that CaT1 is activated at more hyperpolarized membrane potentials provides an explanation for the much smaller currents observed in CaT1- relative to CaT2-expressing oocytes held at –50 mV (Fig. 3A). cRNA-injected oocytes usually have resting potentials around -30 to -40 mV, and CaT1 exhibited much lower Ca^{2+} uptake activity than CaT2 (Fig. 2B). Although a difference in the

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expression levels of the two proteins cannot be ruled out, much of this difference can probably be attributed to the differences in the degree of activation of CaT1 and CaT2 at negative membrane potentials. In mammalian cells showing more negative membrane potentials, the activity of CaT1 could be much greater than that found in oocytes, which were studied at membrane potentials around –30 mV (Fig. 2B). Ca^{2+} uptake activity will also vary as a function of the expression levels of the two proteins.

Structures of *ECAC2* and *ECAC1*

The sequence of *ECAC2* was assembled from genomic fragments in the Celera human genome database using the sequence of the human CaT1 cDNA. Intronic sequences were assembled by walking along portions of the genome where contiguous sequences were available in different genomic fragments using known sequence to search for adjacent sequences. A gap in the genomic sequence, which included portions of exon 1 and intron 1, was filled by long-range PCR using human genomic DNA as a template (sequences obtained have been deposited in GenBank under acc. nos. AY030305 and AY030306). *ECAC1* was identified in a bacterial artificial chromosome (BAC) genomic clone (GenBank acc no. AC073079) and its entire coding sequence was found to match that of the CaT2 cDNA perfectly. As with *ECAC2*, gaps in the sequence of *ECAC1* were filled using sequences available in the Celera database. Using a combination of GENSCAN and two-sequence BLAST, the structures of *ECAC2* and *ECAC1* were assembled in their entireties (Table 1). We compared the organizations of *ECAC2* and *ECAC1* and their corresponding cDNAs (Fig. 4). Because the exonic structures of *ECAC2* and *ECAC1* are identical, we generated a model of the overall topology of the CaT1/2 proteins (Fig. 5) to illustrate the domains encoded by the individual exons in the two proteins.

The structure of *ECAC1* reported here is consistent with the majority of that reported by Müller, *et al*. [32]; however, there are numerous differences in the two sets of data. According to our data (Table 1), all of the intron–exon junctions obey the GT/AG rule for splice junctions. In contrast, according to Müller, *et al.* [32], there are six intron–exon junctions of *ECAC1* (excluding the first and the last) that do not follow that rule. We found *ECAC1* to have 15 exons, whereas *ECAC1* was reported to have 16 [32]. Based on our data, exons 4 and 5 of *ECAC1* are included in a single exon. Exon 12 of *ECAC1* should be 67 bp according to our data. Furthermore, a portion of the promoter region of *ECAC1* reported by Müller, *et al*. [32] is actually included within exon 1 in our data. Finally, the total length of the *ECAC1* exons [32] is inconsistent with the length of its cDNA [31].

Gene Structures of Other Proteins Sharing Similar Structures with CaT1/2

A complete search of both the NCBI GenBank database and the Celera human fragment database revealed no additional genes encoding proteins sharing greater than 50% amino acid sequence identity with CaT1/2. Only four other genes were identified in these databases that share lower but significant degrees of overall sequence similarity with CaT1 and CaT2 (for example, ~30% overall sequence identity). These are the genes encoding VR1/SIC [12,13], VRL-1 [14] or GRC [15], OTRPC4 [16–19], and another hypothetical gene whose protein product has not yet been reported (currently named the VRL-3 gene). The VR1 gene resides on chromosome 17q13 [33], and the VRL-3 gene is located about 50 kb away. For the VRL-1 gene, we could only predict its exon structure by comparing the sequence of its cDNA with that of a genomic DNA fragment in the Celera database. Table 2 summarizes the sizes of the exons of CaT1 and CaT2, and those of these four additional related genes in the human genome.

Exon 13 Codes for a Stretch in CaT1 Sharing Similarity with Other Channels

Exon 13 encodes a stretch of 90 amino acids coding for part of the TM 5, pore, and TM 6, and a

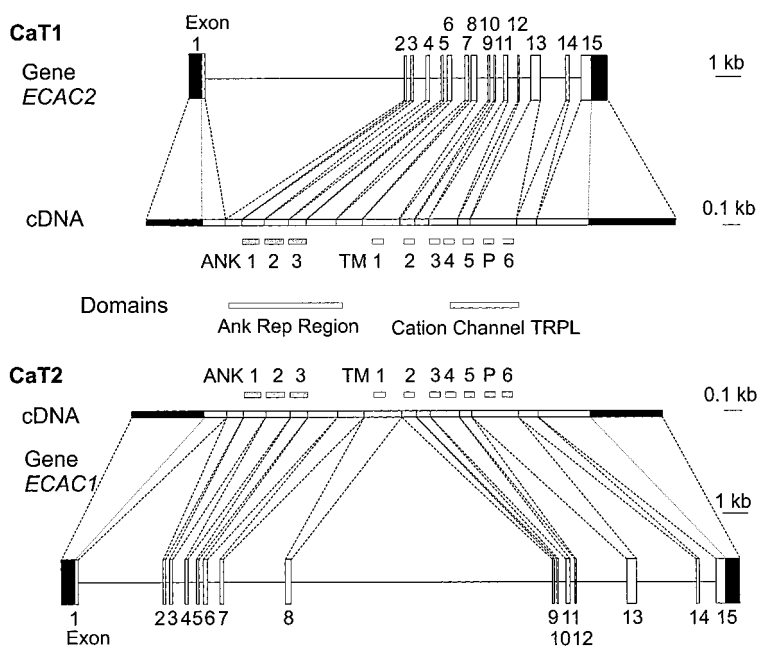

FIG. 4. Genomic structures of *ECAC1* and *ECAC2*. Corresponding regions of genomic DNA for *ECAC1* and *ECAC2* are aligned with the respective cDNAs. The coding sequences and untranslated regions of the cDNAs are shown by open and filled bars, respectively. The regions of the genes corresponding to the exons (bars) of the two genes are shown in the same manner and the introns are shown as lines. The corresponding regions of the cDNAs and genomic DNA for *ECAC1* and *ECAC2* are indicated with dotted lines. The regions encoding the ankyrin repeats (ANK), transmembrane domains (TM), and pore region (P) are also indicated. The portions of the cDNAs encoding the ankyrin repeats and the regions of *ECAC1* and *ECAC2* that are homologous to the corresponding regions of the TRPL cation channel proteins are also indicated.

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TABLE 1: Exon–intron organization of ECAC1 and ECAC2[a]

Exon no.	Exon (bp)		Intron (kb)		5′ Donor sequence 3′ Acceptor sequence	
	ECAC2	*ECAC1*	*ECAC2*	*ECAC1*	*ECAC2*	*ECAC1*
1	≥344	≥477	~7.3	2.9	AGCAGAAGAGgtaaggcccc	AGCAGAAGAGgtaggcttcc
2	98	98	0.15	0.17	cccttcatagGATCTGGGAG	ctttccccagGATTCTAGAG
					CACCAGAGAGgtaagaagca	CGACAAAGAGgtgggatctg
3	123	123	0.37	0.49	taaatgccagGAGCCATGGG	tccaatccagGAGCCCTGGG
					CTCTATGAGGgtgagggccc	GCTTTTGCAGgtaaggagcc
4	138	138	0.30	0.31	tctgggccagGTCAGACTGC	tctgggccagGTCAGACTGC
					ATCTACTTTGgtgagagcag	ATCTACTTTGgtgagagcag
5	99	99	0.16	0.16	gtgtccacagGGGAGCACCC	gtgtccacagGGGAGCACCC
					GACTCCCTGGgtaagagctg	GACTCCCTGGgtaagagctg
6	176	176	0.50	0.46	tcctccccagGAAACACAGT	tcctccccagGAAACACAGT
					TAACACTGTGgtgagagacg	TAACACTGTGgtaagagacg
7	147	147	0.08	2.3	tttcccttagATGTTTCAGC	ggtctctcagATGTTCCAGC
					GAAGCGGGAGgtatggctca	TAAACGAGAGgtatggatgc
8	213	213	0.30	9.9	tctgttgcagGCTCGCCAGA	tccatgccagGCTCGCCAAA
					GCTACTTCAGgtgacaccct	ACTACTACAGgtgattctcc
9	87	87	0.10	0.10	cccccattagGAAGCCTACA	ccccacatagGAGGCCTATG
					GCTGGTAGAGgtgaggtgtg	GCTCCTAGAGgtgaggggca
10	77	77	0.25	0.26	acctcttcagGTTCCAGACA	ctctccccagATTCCAGACA
					ATGTCCTCATgtgagtttcc	ATGTCATCATgtgagtatcc
11	166	166	0.35	0.17	gctcccccagCATCACCTAT	gctcccccagCATCACCTAT
					GATTCAGAAGgtcagtcctc	GATCCAGAAGgtcagtgctt
12	67	67	0.36	1.9	ctccttctagATGATTTTTG	tttcctgcagATGATTTTTG
					TTTGCTTCAGgtaatcatct	TTTGCCTCCGgtaaggcacc
13	269	269	0.97	2.3	ggtggagcagCCTTCTATAT	ggcggacgagCGTTCTATAT
					GAGGGCCCAGgtgagccccc	GAGGGCCCAGgtgagtttat
14	107	107	0.38	0.68	ccatacgcagATTGTGGCCA	ctccttccagGTCGTGGCCA
					GGTTCCTGCGgtgagtgata	GGTTCCTGCGgtgagtgatg
15	783	>709			cattccacagGGTGGAAGAC	cccttatcagGGTTGAGAAC
					CGGATCCCTGcccagctgcc	

[a]Intron–exon organization and the boundary sequences of ECAC1 were derived from genomic sequence with GenBank acc. no. AC073079; those for ECAC2 were determined by comparing the sequence of the CaT1 cDNA with the genomic sequences of Celera human genomic databases, and the gap was filled by PCR.

portion of the C terminus of CaT1 or CaT2. Using these sequences to search the GenBank database revealed a number of proteins sharing similarity with portions of this 90-amino-acid stretch of sequence. The respective amino acid sequences were aligned and a phylogenetic tree for the encoded proteins was generated (Fig. 6). Of note, the regions of greatest similarity among the various proteins reside in TM 6, whereas the sequences encoding the putative pore regions of the respective proteins are less well conserved. This phylogenetic tree suggests the existence of four groups of proteins: group 1 comprises CaT1, CaT2, OTRPC4, VRL-3, VR1 or SIC and VRL-1 or GRC; group 2 contains TRPC1 [34], TRPC6 [35], TRPC3 [36], HTRP7 (GenBank acc. no. AJ272034), TRPC5 [37], and TRP4 [38]; group 3 comprises TRPC7 [39], MTR1 [40], a protein with GenBank acc. no. AK026281, melastatin [41], and KIAA1616 [42]; and group 4 includes polycystin-2 (PC2) [23], PC2L (PCL) [24,25], and PC2L2 [26,43].

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DISCUSSION

The purpose of our studies was to extend to the human species our earlier work showing the presence of two highly homologous, calcium transport proteins in rat intestine and kidney, CaT1 and CaT2, and to describe the human orthologues of these gene products, taking advantage of the human genome sequence. We generated full-length, functional cDNA clones of both CaT1 and CaT2 from a human kidney library and determined the relative abundance of their mRNAs in three major tissues involved in transcellular Ca^{2+} transport: intestine, kidney, and placenta. In addition, we identified and analyzed the structures of the corresponding human genes and compared them with those of four related channel proteins identified in the human databases.

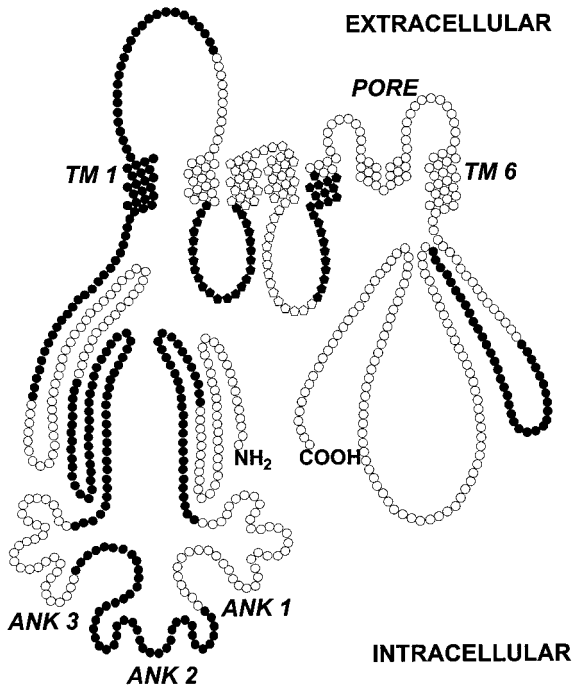

FIG. 5. Domains encoded by individual exons in a unified model of the overall topologies of CaT1 and CaT2. The amino acid sequences encoded by the individual exons of the two genes are shown as alternating black and white segments. CaT2 is 4 amino-acid residues longer than CaT1 (not shown). Exons that are conserved in size among the 6 genes (Table 2) are shown in pentagons.

A Family of Six Genes Encodes Distinct Cation Channels

A search of both the NCBI and Celera databases revealed only six genes encoding proteins sharing more than ~30% overall amino acid sequence identity with CaT1 and CaT2. Taking into consideration that the Human Genome Project is in its final stages and that Celera has finished their shotgun sequencing of the human genome, it is likely these are the only six genes within this family that exist in the human genome. Thus we conclude that the previously reported ECaC [5] and CaT2 [6] are products of the same gene. There are notable differences, however, between the previously reported sequences of the human ECaC cDNA and its gene [31,32] and the corresponding sequences of CaT2 and its gene reported here. We cloned the CaT2 cDNA directly from a human kidney library, and its cDNA sequence matches perfectly with the genomic sequence that is available as part of a BAC in GenBank. In contrast, the human ECaC sequence was assembled using a PCR approach [31]. Therefore, we believe that the mRNAs encoding CaT2 and ECaC are derived from one and the same gene, but that the use of PCR with primers capable of amplifying sequences from either CaT1 or CaT2 may have lead to the inadvertent generation of a "chimeric" ECaC cDNA (and subsequent construction of its gene) that contains sequences corresponding to those of both CaT1 and CaT2. These errors in the human ECaC sequence would also affect its pattern of gene expression [31] and the analysis of its gene structure [32].

Within this family of cation-permeable channels, CaT1 and CaT2 share 75.6% overall amino acid identity with one another, whereas they share from 30.5 to 33.3% identity with the other four proteins. The degree of sequence identity among these latter four proteins varies from 41.6 to 47.0%. Therefore, this protein family can be further divided into two subfamilies: CaT1/2 and the VR1-related proteins. A distinct feature of this family of proteins is their functional diversity. In terms of their gating mechanisms, these proteins can be divided into several groups: VR1 is a ligand-gated channel, SIC is a mechanosensitive channel, and VR-OAC (or OTRPC4, Trp12 and VRL-2) is an osmolarity-gated channel, and VR1 and VRL-1 are also heat-activated channels, whereas CaT1 and CaT2 are activated by hyperpolarization. CaT1 is activated by Ca^{2+}-store depletion in specific cell types through an as-yet-unknown cellular mechanism [7]. In terms of their ion selectivity, CaT1 and CaT2 are more Ca^{2+} selective than the other members of this family, which can be characterized as nonselective cation channels. The six genes within this family presumably arose from a common ancestral gene, with *ECAC2* and *ECAC1* having likely arisen from a relatively recent gene duplication event, and the various gene products have subsequently acquired their functional diversities over the course of evolution.

CaT1 is Expressed More Widely and at Higher Levels in Human Tissues Than CaT2

Intestine, kidney, and placenta are three major organs participating in transcellular Ca^{2+} transport. Dietary Ca^{2+} is taken up into the body primarily by means of small intestinal absorption, which represents the sole means of assimilating calcium from the outside environment. In terms of its egress from the body, Ca^{2+} in the blood and extracellular fluids is filtered and variably reabsorbed in the kidney depending on need for this ion. In addition, in pregnant women, Ca^{2+} is supplied to the fetus for the growth of the skeleton and other tissues through the placenta. In all three tissues (intestine, kidney, and placenta), CaT1 mRNA is more abundant than that of CaT2, even in the kidney; where in the rat, mRNA for

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TABLE 2: Conservation of the organization of genes encoding CaT1, CaT2, and other related channel proteins[a]

Exon No.	CaT1 27q33–q34	CaT2 17q35	VR1 17p13	VRL-3 17p13	VRL-1	OTRPC4 12q24
1	≥344	≥477	≥127	≥105		
2	98	98	317	87	218	1255
3	123	123	167	155	134	173
4	138	138	153	177	291	153
5	99	99	141	141		141
6	176	176	299	281	299	299
7	147	147	180	177	171	180
8	213	213	159	159	156	159
9	87	87	93	102	99	93
10	77	77	71	74	71	74
11	166	166	166	166	166	166
12	67	67	67	67	67	67
13	269	269	323	275	335	317
14	107	107	128	117	128	128
15	783	709	116		80	
16		758		>168		

[a]Listed are sizes of the exons of the genes for the six related proteins. Gene loci are as reported: ECAC2 [4]; ECAC1 [32]; VR1 [33]; OTRPC4 [17,19].

CaT2, but not CaT1, was detected [6]. CaT2 mRNA is localized in the distal convoluted tubule and particularly in the connecting segment, sites of regulated calcium reabsorption. It remains to be determined where CaT1 is expressed in the human kidney and what function(s) it subserves in human renal physiology and mineral ion metabolism.

It is also not surprising that CaT1 is highly expressed in human placenta, because transplacental Ca^{2+} transport is critical for normal fetal growth and development. The high level of expression of CaT1 in human placenta may indicate that the transcellular, rather than the paracellular, pathway of transepithelial calcium transport (in which the CaT proteins do not participate) may be dominant in placenta. In intestine and kidney, in contrast, there are substantial components of paracellular calcium transport, which may contribute more to overall calcium transport in these tissues relative to that in placenta. The apparently lower abundance of CaT1 in intestine may also have resulted from the presence of ileum, where CaT1 is not expressed [3,4], in the small intestine samples from which RNA was prepared.

In addition to its presence in kidney and intestine, we previously detected human CaT1 mRNA expression in pancreas, placenta, prostate, salivary gland, liver, and testis [4]. It has been reported that human ECaC (or CaT2) has a tissue distribution similar to that reported by us for CaT1 when assessed using RT-PCR [31]. However, as noted above, the cDNA sequences of CaT1 and CaT2 (or ECaC) are very similar. In addition, the reported human ECaC sequence seems to contain CaT1 sequences, perhaps because of the use of PCR primers capable of amplifying products from both CaT1 and CaT2/ECaC. Therefore, it seems that the expression pattern reported for ECaC [31] may actually correspond to the expression pattern for CaT1. Of note, BLAST searches of the EST database using the sequences of the cDNAs for CaT1 and CaT2 revealed the presence of CaT1 sequence in numerous tissues, including fetal liver and spleen, prostate, placenta, breast, cervix, prostatic intraepithelial neoplasia-2 (PIN2) cells, colon adenocarcinoma, and pooled germ cell tumors. In contrast, the CaT2 sequence is only reported in one EST (in lung). Thus it seems that the major role of CaT2 is the reabsorption of calcium in the kidney, whereas CaT1 may have absorptive (and/or other) roles in a broader range of tissues.

Conservation of Exons and Introns in *ECAC2* and *ECAC1*

The sizes of the exons in *ECAC2* and *ECAC1* are identical in their coding regions. Furthermore, in the regions of the DNA encoding the ankyrin repeats, this conservation extends to introns as well (Table 1; sequence data not shown). Among the six related genes of the family of cation channels evaluated here, the intron–exon organizations are similar (Table 2); however, none of the genes other than *ECAC2* and *ECAC1* show complete conservation of their exon sizes. The four exons that encode sequences from TM 2 to TM 5, however, are quite similar in size among the six genes (Table 2 and Fig. 5).

Structures of *ECAC2* and *ECAC1* and Other Genes Suggest Gene Duplication Events

ECAC2 and *ECAC1* reside close together on chromosome 7 in the regions 7q33–q34 [4] and 7q35 [32]. The genes encoding VR1 and VRL-3 reside in the region 17p13.2, about 50 kb away

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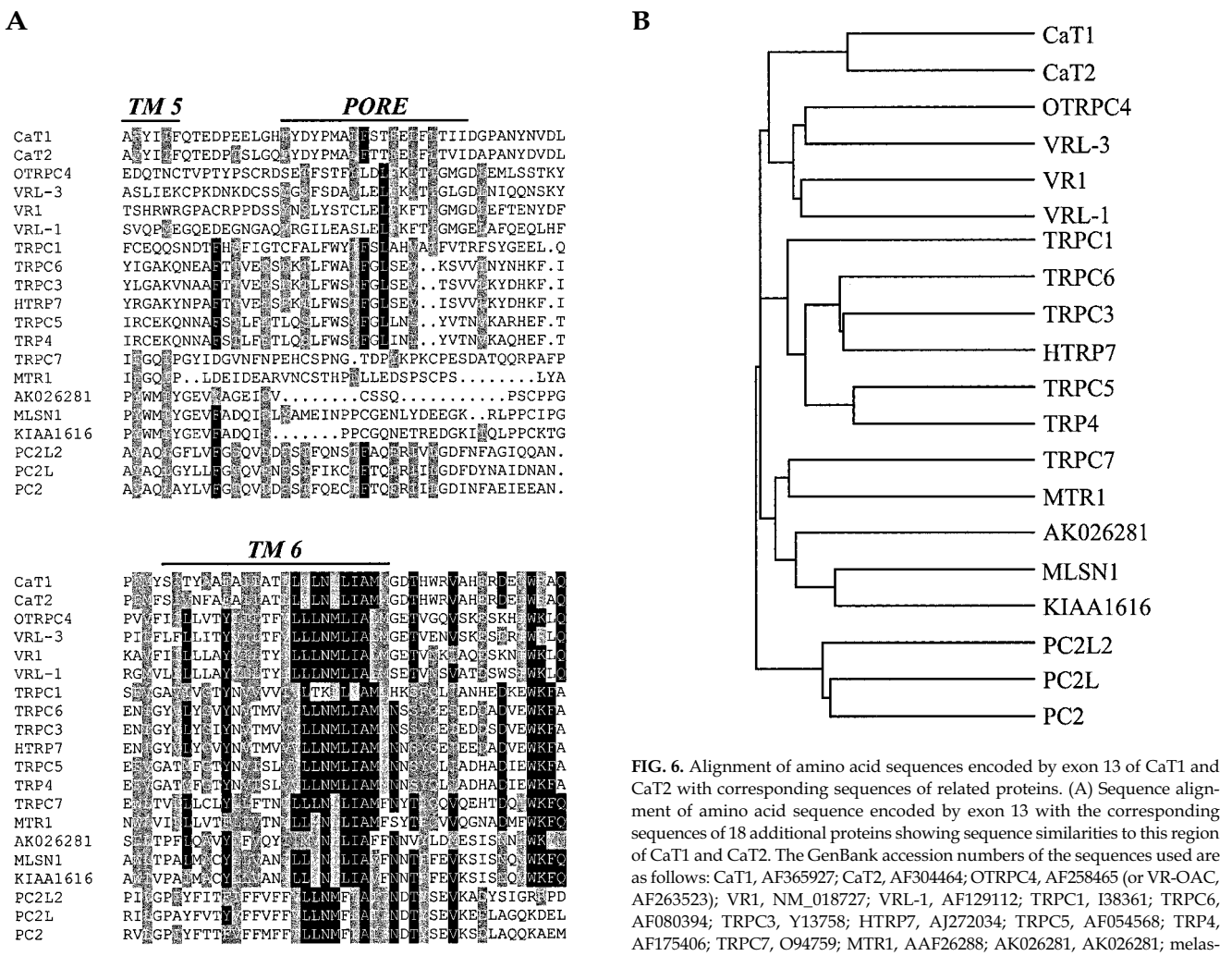

FIG. 6. Alignment of amino acid sequences encoded by exon 13 of CaT1 and CaT2 with corresponding sequences of related proteins. (A) Sequence alignment of amino acid sequence encoded by exon 13 with the corresponding sequences of 18 additional proteins showing sequence similarities to this region of CaT1 and CaT2. The GenBank accession numbers of the sequences used are as follows: CaT1, AF365927; CaT2, AF304464; OTRPC4, AF258465 (or VR-OAC, AF263523); VR1, NM_018727; VRL-1, AF129112; TRPC1, I38361; TRPC6, AF080394; TRPC3, Y13758; HTRP7, AJ272034; TRPC5, AF054568; TRP4, AF175406; TRPC7, O94759; MTR1, AAF26288; AK026281, AK026281; melastatin (MLSN1), AF071787; KIAA1616, AB046836; PC2L2, AF182034 (AF118125); PC2L, AF094827 (AF073481); PC2, Q13563. (B) Phylogenetic tree derived from the alignment shown in (A).

from one another. Therefore, it is quite possible that gene duplication events occurred during evolution that gave rise to these two highly homologous pairs of genes. However, *ECAC2* and *ECAC1* are more conserved in the sizes of their exons and even in some of their introns than are the genes for VR1 and VRL-3, which are, in fact, less similar to one another than they are to the OTRPC4 gene, which resides on another chromosome. At the protein level, CaT1 and CaT2 share 75.6% sequence identity, whereas VR1 and VRL-3 share only 44.4% identity. Compared with VR1 and VRL-3, therefore, it seems that either the conservation of the functions of CaT1 and CaT2 has led to the high degree of conservation of their structures, or that the duplication event that generated them occurred more recently than for the former two genes, or both.

Gene duplication seems to occur commonly in membrane transporter genes and likely represents a mechanism for generating diversity of function, tissue distribution, and regulation. For example, genes encoding four sodium phosphate cotransporters reside in the region of chromosome 6p23–p21.3 [44–46], and two urea transporter genes reside in 18q11–q21.1 [47,48]. Moreover, the latter two genes subsequently fused to generate a single larger gene composed of two internally homologous portions [49]. Furthermore, the α-subunit of the voltage-dependent Ca^{2+} channels contains four homologous segments, each with six membrane-spanning segments, and was likely generated by two sets of duplications of genes each containing six transmembrane domains (for example, the family of cation channels to which CaT1/2 belong).

Conserved TM 6, Less Conserved Pore

Many cation channels, including members of the TRP family as well as polycystin-2 and the related proteins, share structural similarities in their overall topologies, including, for instance, the presence of six membrane-spanning segments containing a putative pore region. The largest exon of *ECAC2* and *ECAC1* is the region within the TM domains that encodes part of TM 5, the pore region, and TM 6. Within the sequence

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encoded by this exon, TM 6 is highly conserved in 20 different proteins belonging to various families of channels of divergent structure and function. However, the pore regions of CaT1 and CaT2 are quite distinct from those of these other channels. The conservation of TM 6 may suggest a role for it as a key structural element for this diverse group of TM proteins. The less-conserved pore region, in contrast, may be one determinant of the characteristic Ca^{2+}-selectivity of CaT1 and CaT2, on the one hand, and the varying ion selectivity of the other channels, on the other hand.

MATERIALS AND METHODS

Cloning of CaT1 and CaT2 cDNAs from human kidney library. A human kidney λ cDNA library (Stratagene, La Jolla, CA) was constructed by the manufacturer using the Uni-ZAP XR vector and cDNAs prepared from whole kidneys pooled from a total of 8 male and female Caucasians ranging in age from 24 to 55 years. Screening of the library was performed as described [6] using the CaT1 cDNA [4] as a probe. The resultant positive clones were sequenced in the Brigham and Women's Hospital Sequencing facility.

Quantitative detection of ECAC2 and ECAC1 expression by real-time PCR. To determine the number of cDNA molecules encoding CaT1 and CaT2 that had been reverse-transcribed from RNA isolated from human small intestine, kidney, and placenta (Clontech Laboratories, Inc., Palo Alto, CA), real-time PCR was carried out using the LightCycler system (Roche Molecular Biochemicals, Indianapolis, IN) and the respective Marathon ready cDNAs as templates (Clontech). PCR was carried out using 2 μl of LightCycler DNA master SYBR Green I (Roche), 0.25 μM of each 5′ and 3′ primer pair, 2 μl cDNA samples, and 2 μl H_2O in a total volume of 20 μl. The $MgCl_2$ concentration was adjusted to 3 mM. Samples were denatured at 95°C for 20 s with a temperature transition rate of 20°C/s. Amplifications and fluorescence determinations were carried out as follows: (1) denaturation at 95°C for 0 s with a temperature transition rate of 20°C/s; (2) annealing at 60°C for 5 s with a temperature transition rate of 8°C/s; (3) extension at 72°C for 20 s (except for the GAPDH cDNA, where 25 s was used) with a temperature transition rate of 4°C/s; and (4) detection of SYBR Green fluorescence, which reflects the amount of double-stranded DNA, at 85°C for 3 s. The amplification cycle number was 40 for CaT1 and CaT2 and 35 for GAPDH. To discriminate between specific and nonspecific products, a melting curve was performed at the end of each run. Products were denatured at 95°C for 3 s, then the temperature was reduced to 65°C for 15 s and raised slowly from 65°C to 95°C at 0.1°C/s.

To determine the copy numbers of the targeted cDNAs in the samples, serially diluted, purified PCR products of known concentrations were included in each experiment as external standards. The instrument plotted the standard curves using the data obtained during the portion of the PCR in which the fluorescence readings increased in a log-linear fashion. By comparing results obtained using the unknown samples with those for the standards, the instrument generated quantitative estimates of the starting copy number of the target cDNAs in the unknowns. For each sample, the amount of target cDNA was determined twice and the mean value was used to estimate copy number. The primer sequences used for PCR were as follows:

CaT1 forward primer (nt 1125–1147 from the start codon), 5′-AGCCTACATGACCCCTAAGGACG-3′; CaT1 reverse primer (1572–1550), 5′-GTAGAAGTGGCCTAGCTCCTCGG-3′; CaT2 forward primer (1125–1147), 5′-GGCCTATGAGACACGTGAAGATATC-3′; CaT2 reverse primer (1572–1550), 5′-ATAGAATTGCCCCAGACTGGTTG-3′; GAPDH forward primer, 5′-TCACCATCTTCCAGGAGCG-3′; and GAPDH reverse primer, 5′-CTGCTTCACCACCTTCTTGA-3′.

Expression of CaT1 and CaT2 in X. laevis oocytes and $^{45}Ca^{2+}$ uptake assay. The CaT1 and CaT2 cDNAs were subcloned into a vector (pNWP) suitable for *in vitro* transcription and expression in *X. laevis* oocytes. *In vitro* transcription, injection of the resultant synthetic RNAs (cRNAs) for CaT1 and CaT2 into oocytes, and the Ca^{2+} uptake assay in oocytes were conducted as described [3,4]. Ca^{2+} uptake data are presented as mean values from at least 3 experiments with 7 to 10 oocytes per group, using the standard error of the mean (SEM) as the index of dispersion.

Two-microelectrode voltage clamp. Experiments using the two-microelectrode voltage-clamp technique were performed as described [50]. The resistance of microelectrodes filled with 3 M KCl was 0.5–2 MΩ. In experiments involving voltage jumping or holding, currents and voltages were digitized at 0.3 or 200 ms/sample, respectively. After ~3 min of stabilization of membrane potential following impalement with the microelectrode, the oocyte was clamped at the holding potential (V_h) of –50 mV. We than applied 100-ms voltage pulses between –160 and +60 mV, in increments of 20 mV, and obtained steady-state currents as the average values in the interval from 80 to 95 ms after the initiation of the voltage pulses. The standard perfusion solution used contained 100 mM choline-Cl, 2 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES, pH 7.5 (adjusted using Trisbase and HCl). Choline-Cl was substituted with either NaCl or KCl when testing Na^+ or K^+ evoked current.

Sequence analyses. Sequence analyses of *ECAC1*, *ECAC2*, and other genes were carried out using the following databases and resources. BLAST searches were performed using the National Center for Biotechnology Information (NCBI) web site (http://www.ncbi.nlm.nih.gov/BLAST/). BLAST searches in human genome were performed using the web site http://www.ncbi.nlm.nih.gov/genome/seq/HsBlast.html. Sequence searches using the Celera Genomics Group (Rockville, MD) database were obtained at http://www.celera.com/celerascience/. Sequence alignments and phylogenetic trees were carried out using Wisconsin Package Version 10.1 of the Genetics Computer Group (GCG; Madison, WI). GENSCAN web server at Massachusetts Institute of Technology (http://genes.mit.edu/GENSCAN.html) was used to predict hypothetical protein VRL-3.

Determination of the missing portion of the gene for CaT1. To isolate exon 1 and intron 1 of *ECAC2*, which were missing in the Celera databases, primers were designed based on available, partial sequences of exon 1 and intron 1. A 4.4-kb fragment was amplified using the expand long template PCR system (Roche Molecular Biochemicals, Mannheim, Germany), human genomic DNA as template, and the following primers: 5′-GGGAGACTCCCAAGGAACTCGTCAGG-3′ and 5′-CTAGCTCACCTGCTTGGGAGTAAGGCTG-3′. Sequencing of the fragments obtained from 3 reactions confirmed that this fragment contains all of the missing portions of exon 1 and intron 1.

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REFERENCES

1. Brown, E. M. (1991). Extracellular Ca^{2+} sensing, regulation of parathyroid cell function, and role of Ca^{2+} and other ions as extracellular (first) messengers. *Physiol. Rev.* **71:** 371–411.
2. Clapham, D. E. (1995). Calcium signaling. *Cell* **80:** 259–268.
3. Peng, J.-B., *et al.* (1999). Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. *J. Biol. Chem.* **274:** 22739–22746.
4. Peng, J.-B., *et al.* (2000). Human calcium transport protein CaT1. *Biochem. Biophys. Res. Commun.* **278:** 326–332, doi:10.1006/bbrc.2000.3716.
5. Hoenderop, J. G., *et al.* (1999). Molecular identification of the apical Ca^{2+} channel in 1,25-dihydroxyvitamin D_3-responsive epithelia. *J. Biol. Chem.* **274:** 8375–8378.
6. Peng, J.-B., *et al.* (2000). A rat kidney-specific calcium transporter in the distal nephron. *J. Biol. Chem.* **275:** 28186–28194, doi:10.1074/jbc.M909686199.
7. Yue, L., Peng, J.-B., Hediger, M. A., and Clapham, D. E. (2001). CaT1 manifests the pore properties of the calcium-release-activated calcium channel. *Nature* **410:** 705–709.
8. Putney, J. W., Jr. (1997). *Capacitative Calcium Entry*. Landes Biomedical Publishing, Austin, TX.
9. Parekh, A. B., and Penner, R. (1997). Store depletion and calcium influx. *Physiol. Rev.* **77:** 901–930.
10. Peng, J.-B., *et al.* (2001). CaT1 expression correlates with tumor grade in prostate cancer. *Biochem. Biophys. Res. Commun.* **282:** 729–734, doi:10.1006/bbrc.2001.4638.
11. Wissenbach, U., *et al.*, (2001). Expression of CaT-like, a novel calcium selective channel,

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correlates with the malignancy of prostate cancer. *J. Biol. Chem.* **276:** 19461–19468, doi:10.1074/jbc.M009895200.

12. Caterina, M. J., *et al.* (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* **389:** 816–824.

13. Suzuki, M., Sato, J., Kutsuwada, K., Ooki, G., and Imai, M. (1999). Cloning of a stretch-inhibitable nonselective cation channel. *J. Biol. Chem.* **274:** 6330–6335.

14. Caterina, M. J., Rosen, T. A., Tominaga, M., Brake, A. J., and Julius, D. (1999). A capsaicin-receptor homologue with a high threshold for noxious heat. *Nature* **398:** 436–441.

15. Kanzaki, M., *et al.* (1999). Translocation of a calcium-permeable cation channel induced by insulin-like growth factor-I. *Nat. Cell Biol.* **1:** 165–170.

16. Strotmann, R., Harteneck, C., Nunnenmacher, K., Schultz, G., and Plant, T. D. (2000). OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. *Nat. Cell Biol.* **2:** 695–702.

17. Liedtke, W., *et al.* (2000). Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell* **103:** 525–535.

18. Wissenbach, U., Bodding, M., Freichel, M., and Flockerzi, V. (2000). Trp12, a novel Trp related protein from kidney. *FEBS Lett.* **485:** 127–134.

19. Delany, N. S., *et al.* (2001). Identification and characterization of a novel human vanilloid receptor-like protein, VRL-2. *Physiol. Genomics* **4:** 165–174.

20. Montell, C., and Rubin, G. M. (1989). Molecular characterization of the Drosophila trp locus: a putative integral membrane protein required for phototransduction. *Neuron* **2:** 1313–1323.

21. Phillips, A. M., Bull, A., and Kelly, L. E. (1992). Identification of a Drosophila gene encoding a calmodulin-binding protein with homology to the trp phototransduction gene. *Neuron* **8:** 631–642.

22. Zhu, X., *et al.* (1996). trp, a novel mammalian gene family essential for agonist-activated capacitative Ca^{2+} entry. *Cell* **85:** 661–671.

23. Mochizuki, T., *et al.* (1996). PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* **272:** 1339–1342.

24. Nomura, H., *et al.* (1998). Identification of PKDL, a novel polycystic kidney disease 2-like gene whose murine homologue is deleted in mice with kidney and retinal defects. *J. Biol. Chem.* **273:** 25967–25973.

25. Wu, G., *et al.* (1998). Identification of PKD2L, a human PKD2-related gene: tissue-specific expression and mapping to chromosome 10q25. *Genomics* **54:** 564–568.

26. Guo, L., *et al.* (2000). Identification and characterization of a novel polycystin family member, polycystin-L2, in mouse and human: sequence, expression, alternative splicing, and chromosomal localization. *Genomics* **64:** 241–251, doi:10.1006/geno.2000.6131.

27. Chen, X.-Z., *et al.* (1999). Polycystin-L is a calcium-regulated cation channel permeable to calcium ions. *Nature* **401:** 383–386.

28. Nilius, B., *et al.* (2000). Whole-cell and single channel monovalent cation currents through the novel rabbit epithelial Ca^{2+} channel ECaC. *J. Physiol.* **527:** 239–248.

29. Vassilev, P. M., Peng, J.-B., Johnson, J., Hediger, M. A., and Brown, E. M. (2001). Inhibition of CaT1 channel activity by a non-competitive IP_3 antagonist. *Biochem. Biophys. Res. Commun.* **280:** 145–150, doi:10.1006/bbrc.2000.4110.

30. Niemeyer, B. A., Bergs, C., Wissenbach, U., Flockerzi, V., and Trost, C. (2001). Competitive regulation of CaT-like-mediated Ca^{2+} entry by protein kinase C and calmodulin. *Proc. Natl. Acad. Sci. USA* **98:** 3600–3605, doi:10.1073/pnas.051511398.

31. Müller, D., *et al.* (2000). Molecular cloning, tissue distribution, and chromosomal mapping of the human epithelial Ca^{2+} channel (ECAC1). *Genomics* **67:** 48–53, doi:10.1006/geno.2000.6203.

32. Müller, D., Hoenderop, J. G., Merkx, G. F., Van Os, C. H., and Bindels, R. J. (2000). Gene structure and chromosomal mapping of human epithelial calcium channel. *Biochem. Biophys. Res. Commun.* **275:** 47–52, doi:10.1006/bbrc.2000.3227.

33. Touchman, J. W., *et al.* (2000). The genomic region encompassing the nephropathic cystinosis gene (CTNS): complete sequencing of a 200-kb segment and discovery of a novel gene within the common cystinosis-causing deletion. *Genome Res.* **10:** 165–173.

34. Wes, P. D., *et al.* (1995). TRPC1, a human homolog of a Drosophila store-operated channel. *Proc. Natl. Acad. Sci. USA* **92:** 9652–9656.

35. Hofmann, T., *et al.* (1999). Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. *Nature* **397:** 259–263.

36. Xu, X. Z., Li, H. S., Guggino, W. B., and Montell, C. (1997). Coassembly of TRP and TRPL produces a distinct store-operated conductance. *Cell* **89:** 1155–1164.

37. Sossey-Alaoui, K., *et al.* (1999). Molecular cloning and characterization of TRPC5 (HTRP5), the human homologue of a mouse brain receptor-activated capacitative Ca^{2+} entry channel. *Genomics* **60:** 330–340, doi:10.1006/geno.1999.5924.

38. McKay, R. R., *et al.* (2000). Cloning and expression of the human transient receptor potential 4 (TRP4) gene: localization and functional expression of human TRP4 and TRP3. *Biochem. J.* **351:** 735–746.

39. Nagamine, K., *et al.* (1998). Molecular cloning of a novel putative Ca^{2+} channel protein (TRPC7) highly expressed in brain. *Genomics* **54:** 124–131, doi:10.1006/geno.1998.5551.

40. Prawitt, D., *et al.* (2000). Identification and characterization of MTR1, a novel gene with homology to melastatin (MLSN1) and the trp gene family located in the BWS-WT2 critical region on chromosome 11p15.5 and showing allele-specific expression. *Hum. Mol. Genet.* **9:** 203–216.

41. Hunter, J. J., *et al.* (1998). Chromosomal localization and genomic characterization of the mouse melastatin gene (Mlsn1). *Genomics* **54:** 116–123, doi:10.1006/geno.1998.5549.

42. Nagase, T., Kikuno, R., Nakayama, M., Hirosawa, M., and Ohara, O. (2000). Prediction of the coding sequences of unidentified human genes. XVIII. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. *DNA Res.* **7:** 273–281.

43. Veldhuisen, B., Spruit, L., Dauwerse, H. G., Breuning, M. H., and Peters, D. J. (1999). Genes homologous to the autosomal dominant polycystic kidney disease genes (PKD1 and PKD2). *Eur. J. Hum. Genet.* **7:** 860–872.

44. Chong, S. S., Kristjansson, K., Zoghbi, H. Y., and Hughes, M. R. (1993). Molecular cloning of the cDNA encoding a human renal sodium phosphate transport protein and its assignment to chromosome 6p21.3-p23. *Genomics* **18:** 355–359, doi:10.1006/geno.1993.1476.

45. Ruddy, D. A., *et al.* (1997). A 1.1-Mb transcript map of the hereditary hemochromatosis locus. *Genome Res.* **7:** 441–456.

46. Shibui, A., *et al.* (1999). Isolation and chromosomal mapping of a novel human gene showing homology to Na^+/PO_4 cotransporter. *J. Hum. Genet.* **44:** 190–192.

47. Olives, B., *et al.* (1995). Kidd blood group and urea transport function of human erythrocytes are carried by the same protein. *J. Biol. Chem.* **270:** 15607–15610.

48. Olives, B., *et al.* (1996). Molecular characterization of a new urea transporter in the human kidney. *FEBS Lett.* **386:** 156–160.

49. Shayakul, C., Steel, A., and Hediger, M. A. (1996). Molecular cloning and characterization of the vasopressin-regulated urea transporter of rat kidney collecting ducts. *J. Clin. Invest.* **98:** 2580–2587.

50. Chen, X.-Z., *et al.* (1999). Yeast SMF1 mediates H^+-coupled iron uptake with concomitant uncoupled cation currents. *J. Biol. Chem.* **274:** 35089–35094.

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under accession numbers AF365927, AF304464, AY030305, and AY030306.

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