SUPPLEMENTAL DATA

Expression of hVariants 2 and 3 in human tissues

End-point PCR was used to determine hVariant 3 expression in different human tissues **(Fig. S1)**. hVariant 3 and hVariant 2-specific sequences were amplified from cDNA made from the Human Total RNA Master Panel II and human DRG RNA purchased Clontech (Mountain View, CA). hVariant 3-specific transcripts were amplified from fetal brain, total brain, cerebellum, and DRG. Limited hVariant 3 was also observed in non-neuronal tissue including the thymus, trachea, colon, and small intestine. Very faint bands were also observed in uterus, testis, lung, prostate, and salivary gland. hVariant 2 was also found in all these tissues in addition to kidney, skeletal muscle, spleen, and thyroid. These results indicate that hVariant 3 is expressed in multiple human tissues in a pattern similar to hVariant 2.

A93S polymorphism in human ACCN2 transcript variant 3

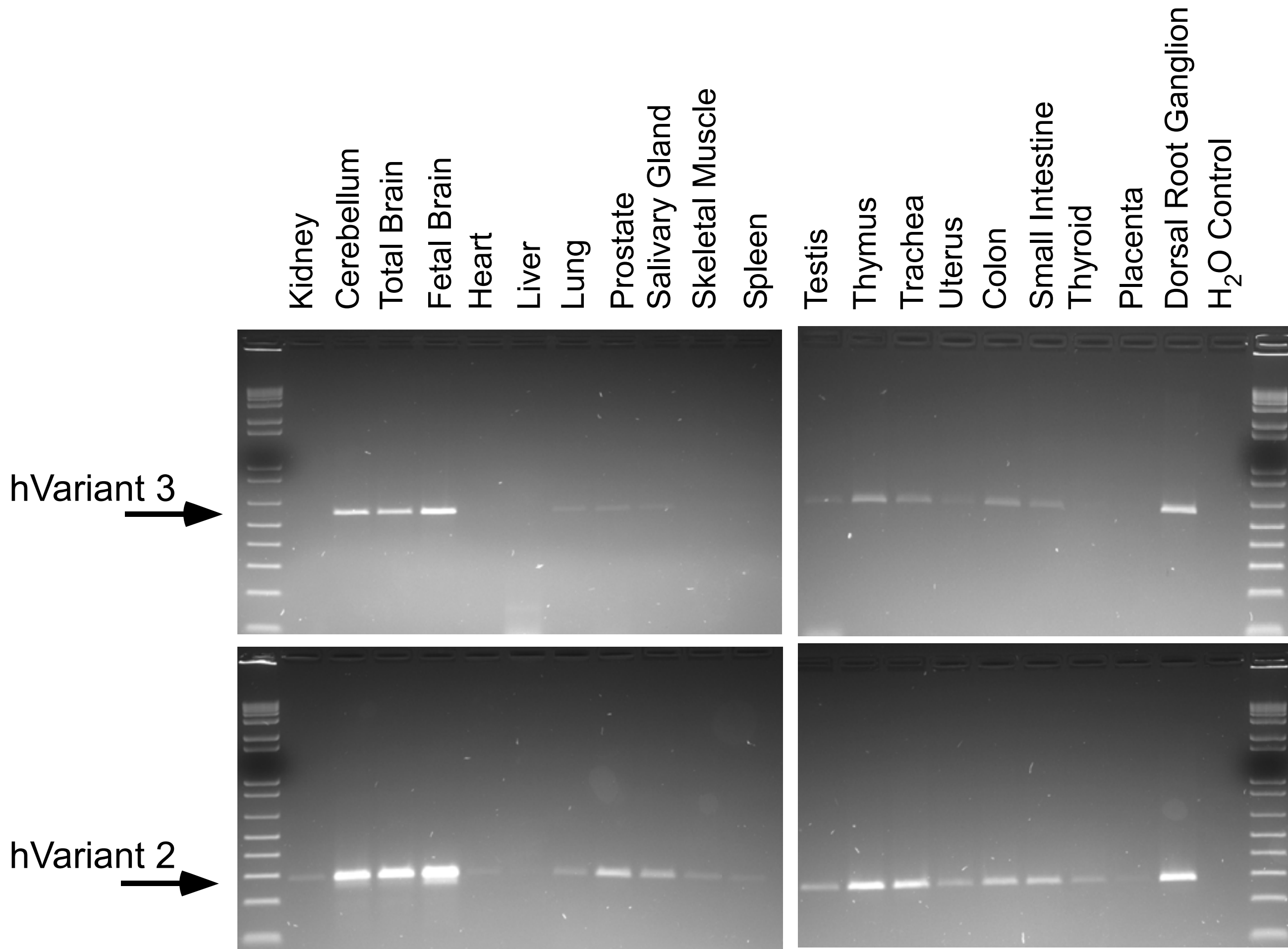
We searched the ENTREZ single nucleotide polymorphism (SNP) database to determine whether there are any polymorphisms that change the amino acid sequence of hVariant 3 within the human population. There is a common polymorphism that results in an amino acid change (A93S) in hVariant 3 (Genbank, dbSNP: rs706792, rs706793). The allele containing this polymorphism occurs in 75% of individuals of European descent, but occurs only rarely in other populations from Asia or Africa. Alanine 93 is located within transmembrane domain 1, in an area that may contribute to proper pore formation. This residue is relatively conserved among the ASICs, with only hydrophobic amino acids present at this location **(Fig. 1D, underlined)**. To determine whether the presence of a serine residue at location 93 affects channel properties, we used site-directed mutagenesis to generate hVariant 3 (A93S). We injected this construct into oocytes and tested the basic properties of the resulting channel. We determined that A93S hVariant 3 produces proton-gated currents in oocytes that are the same amplitude as normal hVariant 3 **(Fig. S2A, B)**. Further, hVariant 3 (A93S) currents in oocytes display the same rapid inactivation seen with hVariant 3 (hVariant 3 A93S $\tau_{inact} = 0.41 \pm 0.06$ seconds, $n = 16$; hVariant 3 = 0.42 ± 0.07 seconds, $n = 13$, $p = 0.79$ Students t test, data not shown). There is also the presence of a variable sustained current which is significantly different from human hVariant 2, but indistinguishable from the hVariant 3 channel (2.5 % ± 0.5 maximal current for A93S, $n = 20$, $p = 0.98$ compared to hVariant 3 and $p = 0.02$ compared to hVariant 2, data not shown). The proton-concentration dependence of activation and steady-state desensitization is identical between hVariant 3 and hVariant 3(A93S) **(Fig. S2C, D)**. In fact, the calculated $pH_{0.5act}$ and $pH_{0.5des}$ of A93S were not different from hVariant 3 ($pH_{0.5act}$ of A93S = 5.81 ± 0.08, $n = 7$, p = 0.16; $pH_{0.5des}$ A93S = 6.42 ± 0.02, $n = 10$, $p = 0.12$ compared to hVariant 3, Students' t test). Monovalent ion permeability was also indistinguishable between hVariant 3 (A93S) and hVariant 3 expressed in *Xenopus* oocytes (A93S $P_{Na}/P_K = 6.4 \pm 0.4$, $n = 4$, $p = 0.9$; $P_{Na}/P_{Li} = 1.00 \pm 0.06$, $n = 4$, p = 0.24 compared to hVariant 3) **(Fig. S2E)**. We also tested the properties of A93S in CHO cells using the whole-cell patch clamp technique. Again, the rate of inactivation and sustained current were indistinguishable between hVariant 3 and hVariant 3 (A93S) **(Fig. S2F, G)**. The relative calcium permeability of A93S was also identical to the wild-type channel **(Fig. S1H)**. Thus, the A93S polymorphism does not appear to alter the basic biophysical properties of *ACCN2* transcript variant 3.

Figure S1. Expression of hVariant 3 in human tissue. PCR primers specific to hVariant 3 (5'-cccatccagatcttctgctc-3' and 5'-taaagggctccccagagaag-3') and hVariant 2 (5'-

atggaactgaaggccgaggaggag-3' and 5'-cgtgacagcagggaaggtaagctg-3') were used to amplify transcript sequences from cDNA made from the Human Total RNA Master Panel II and human DRG RNA purchased Clontech (Mountain View, CA) using Applied Biosystems' SuperScript® VILO™ cDNA Synthesis Kit (Applied Biosystems, Carlsbad, CA). RNA was treated with DNase (Ambion DNA-free Kit, Ambion, Austin, TX) according to manufacturer's instructions prior to cDNA synthesis. PCR was performed with a hot start at 94°C for 2 min followed by 33 cycles of 94°C for 30s, 57°C 30s, and 72°C 1min to amplify the 555 base pair hVariant 3 product and 272 base pair hVariant 2 product. A H_2O control was done in which water was added instead of cDNA. PCR reactions were run onto ethidium bromide stained 1.5% agarose gels.

Figure S2. ***ACCN2*** **transcript variant 2 polymorphism (A93S). A**. Representative traces of hVariant 3 wild-type and the A93S polymorphism expressed in *Xenopus* oocytes. **B.** Peak current amplitude of pH 5.0-activated currents in oocytes expressing hVariant 3 and A93S. Apparent proton sensitivity of activation **(C)** and steady-state desensitization **(D)** of hVariant 3 alleles. Data was obtained as described in Fig. 4. Representative traces of hVariant 3 A93S are shown above. **E**. I/V plots of A93S in sodium, lithium, or potassium extracellular solutions (as in Fig. 3). **F-H** Inactivation kinetics **(F)**, sustained current **(G)**, and P_{Na}/P_{Ca} ratio **(H)** of hVariant 3 and A93S expressed in CHO cells (as in Fig. 6). Inactivation kinetics and sustained current were analyzed similarly to oocyte data. Ion permeability experiments were performed and analyzed as before (Fig. 6).

Fig. S1



hVariant 3 (A93S)

Fig. S2