Supporting Information

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SI Methods

Semiquantitative real-time PCR were determined by using the following method. Open muscle biopsies were obtained under femoral blockade from 8 HypoPP patients (4 with the Cav1.1-R1239H mutation, 3 with Cav1.1-R528H, and 1 with Nav1.4-R672G) and from 6 control subjects (individuals who had been screened for malignant hyperthermia but were found normal). RNA was extracted from ≈50 mg of muscle tissue by use of TRIzol (GIBCO BRL) according to the manufacturer's guidelines. Approximately 200 ng of RNA was reverse transcribed by use of an AMV enzyme kit (Roche) and Oligo dT primers. The integrity of the RNA was validated by visual inspection of the 18S and 28S bands on a 1.2% formaldehyde agarose gel stained with ethidium bromide. The obtained A260/A280 ratio was > 1.6. The RNA was reverse transcribed into cDNA using an AMV enzyme kit (Roche). Real-time PCR was carried out by use of the LightCycler instrument and the FastStart DNA Master SYBR Green I kit (Roche). Briefly, amplification was carried out by a TaqDNA polymerase enzyme, and SYBR Green I was used for fluorescent detection of the PCR product. All analyses for each subject were performed in duplicate in 1 run, to avoid variation between runs. Arbitrary concentrations of mRNA were calculated by the fit point method with arithmetic baseline adjustment and manual setting of the noise band (LightCycler software v. 3.5, Roche). From this analysis, a crossing-point (Cp) was determined, defined as the estimated cycle number where the fluorescence of the PCR product rises over baseline. Forward and reverse primers were designed to avoid amplification of genomic DNA (Table S2). The specificity of the primers was verified by a BLAST search, by standard agarose gel electrophoresis, and by melting curve analysis (Fig. S1). For analysis of genes with only 1 exon, we confirmed that the Cp determined for the reverse-transcribed RNA sample was at least 4 cycles lower (i.e., ≈16-fold higher) than for an RNA sample not subjected to reverse transcription. Expression of ß-actin ACTB mRNA was used as reference and was assumed to be constant. The ratio of target transcript concentration to the reference ACTB transcript concentration was calculated using external standard curves. All analyses were performed as duplicates. Possible differences between HypoPP subjects and control subjects were determined by a *t* test comparing the logarithm to the ratios obtained in the 2 groups.

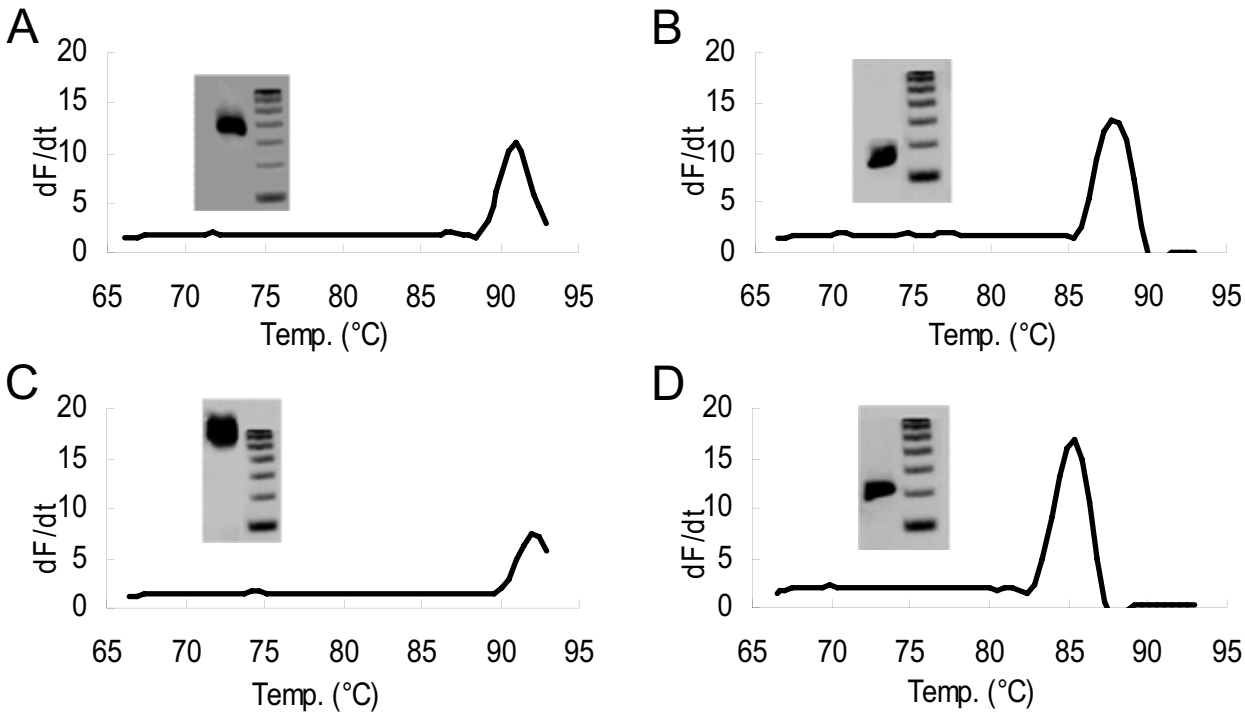

Fig. S1. ACTB (*A*), ATP1A1 (*B*), ATP1A2 (*C*), and ATP1B1 (*D*) show gel electrophoresis and melting curve analysis of product specificity.

Table S1. Clinical features

Mutation	Sex	Age	Aids	Prox. MRC	Vastus histology	Calf MRC	Calf fat	Calf edema	First attack	Attack frequency	Torque reduction (%}	Reaction to AZ	Remarks
Na-R675Q	F	33	–	4	Vac. myop.†	4	+	+	1	7/year	26	+	Ictal dyspnea
Na-R675Q	F	63	–	5	n.t.	5	–	–	16	1/year	6	n.t.	–
Na-R675Q	M	39	–	4	Tub. aggr.	5	–	–	15	1/year	6	n.t.	–
Na-R672G	F	16	–	5	n.t.	5	–	–	11	1/week	n.t.	+	–
Na-R672G	M	44	–	4	Vac. myop.†	4	–	–	9	1/day	25	+	–
Na-R672H	M	35	–	5	n.t.	5	–	+	15	1/year	20	n.t.	Myalgia
Na-R672H	M	28	–	5	n.t.	5	–	–	23	1/year	20	n.t.	–
Na-R672H	M	62	–	4	Vac. myop.†	4	+	–	16	1/year	6	n.t.	Type 2 atrophy
Ca-R528G	M	28	–	5	n.t.	5	–	–	17	1/year	7	+	Myalgia
Ca-R528H	F	44	Railing	3	Vac. myop.	4	++	++	14	1/month	18	+	Group atrophy
Ca-R528H	F	32	–	5	n.t.	5	–	++	22	1/year	20	n.t.	–
Ca-R528H	F	52	–	5	Normal	4	+	–	–	none	n.t.	n.t.	–
Ca-R528H	F	42	–	4	Myopathy	4	+	+++	36	1/year	7	+*	–
Ca-R528H	F	46	–	4	Vac. myop.	4	+	–	20	10/year	19	+	–
Ca-R528H	M	60	Chair	3	Myopathy	3	+++	++	15	2/month	33	+*	Group atrophy
Ca-R528H	M	36	–	5	Vacuoles	5	–	–	13	2/month	20	n.t.	–
Ca-R528H	M	39	–	5	n.t.	5	–	+	15	1/quarter	30	n.t.	–
Ca-R528H	M	68	–	5	n.t.	5	+	–	16	1/month	20	n.t.	–
Ca-R528H	M	60	Railing	4	n.t.	4	++	–	1	6/month	n.t.	–	Myalgia
Ca-R528H	M	38	–	4	Vac. myop.†	5	–	–	16	1/month	n.t.	+	–
Ca-R528H	M	39	–	5	n.t.	5	–	–	15	2/week	26	+	–
Ca-R528H	M	31	–	4	Vac. myop.	4	+	–	18	2/year	20	–	–
Ca-R1239H	F	37	Railing	3	Vac. myop†	3	++	++	13	1/month	39	+*	–
Ca-R1239H	F	33	–	4	Vac. Myop.†	4	+	+++	11	1/month	25	+*	Myalgia
Ca-R1239H	F	27	–	4	Vac. myop.†	4	–	++	10	1/week	18	+	Myalgia
Ca-R1239H	F	23	–	4	n.t.	4	–	++	4	1/day	22	–	Myalgia
Ca-R1239H	F	45	Railing	4	n.t.	4	+	+	11	1/day	33	+	Myalgia
Ca-R1239H	F	20	Railing	3	n.t.	4	+	++	1	1/day	15	–	Myalgia
Ca-R1239H	M	62	Railing	3	n.t.	4	++	–	9	1/week	8	+	–
Ca-R1239H	M	55	Cane	3	n.t.	3	+	++	4	1/week	29	+*	Myalgia
Ca-R1239H	M	58	Chair	3	Vac. myop.	4	+++	–	12	1/day	9	+	Myalgia
Ca-R1239H	M	46	Cane	3	n.t.	3	++	++	1	1/day	8	+	–
Ca-R1239H	M	48	Cane	3	n.t.	4	+	++	12	1/day	42	+*	–
Ca-R1239H	M	57	Railing	3	n.t.	3	+++	++	10	1/day	38	n.t.	–
Ca-R1239G	F	23	Chair	2	Myopathy	3	+	++	–	none	10	n.t.	Weak at age 3 years
Ca-R1239G	F	37	–	4	Vac.myop.	5	–	++	6	1/day	26	–	Ictal dyspnea

The 36 HypoPP patients were recruited between 2005 and 2008. No subject was excluded. Abbreviations: AZ = acetazolamide; Chair = wheelchair; Edema = muscle edema; Fat = fatty degeneration; First attack = age in years at first attack; Myopathy = myopathy without vacuoles; n.t. = not tested; Prox. MRC = strength of weakest proximal muscle; Tub. aggr. = T-tubular aggregates; Vac. myop = vacuolar myopathy.

*Patients in AZ study.

†Specimens used for E_m recordings.

Table S2. Primer sequences for semi-quantitative real-time PCR

Gene	Forward primer	Reverse primer
ACTB	GGGCATGGAGTCCTGTGG	TGCGCAAGTTAGGTTTTGTCA
ATP1A1	CAGCCCAGAAATCCCAAAACA	AGCGGTCATCCCAGTCCA
ATP1A2	GGTCTCCTTCTTCGTGCTCTCCC	CACCTTGGGGTTTCTGTCTCTCAT
ATP1B1	CGAGGAGAGCGAAAGGTCTGC	CTTCATCTCGCTTGCCAGTGC