

**Supplemental Digital Content 3. RYR1 Participant Description Table**

Characteristics of ClinSeq <sup>®</sup> Volunteers with RYR1 Variants						
Nucleotide Change; Predicted Protein Change	Subject No.	Race/ Ethnicity/Age/ Gender	Family History MHS, Heat Illness or Myopathy	Serum CK <sup>**</sup> level (U/L)	LDH (U/L)	Medical/ Surgical/ Procedures History
c.89A>T;p.Glu30Val	135473	C/N/63/F	Negative	103*	166	Intermittent hypokalemia (on K+ supplements)
c.1099C>T;p.Arg367Trp	190330	C/N/58/F	Negative	76	174	Psoriasis
c.1384G>A;p.Glu462Lys	145545	A/N/57/M	Negative	214*	180	Surgeries: CABG
c.1453A>G;p.Met485Val	134364	C/N/57/M	Negative	69	146	Negative
	144294	C/N/59/F	Negative	36	147	Negative
c.1474C>T;p.Arg492Cys	177065	A/N/65/M	Negative	80*	122	Surgeries: CABG 50 y
c.1753A>G;p.Ile585Val	157045	C/N/61/F	Negative	81*	123	Surgeries: cardiac stenting 59 y, mastectomy and breast implants 30 y. Alcohol over consumption: self-reports $\geq 4$ glasses of wine daily
c.1840C>T;p.Arg614Cys	131685	C/N/59/F	No proximal muscle weakness in family, no complications from anesthesia in any family member.	194 <sup>^</sup>	194	Surgeries: (1) nasal turbinates, (2) left thigh mass removal, and (3) cholecystectomy 58 y. Scarlet fever (5 y)
c.2075C>T;p.Thr692Ile	117126 <del>7</del>	C/N/63/M	Negative	143	126	Negative
c.2122G>A;p.Asp708Asn	181028+	C/N/63/F	Negative	50* <sup>^</sup>	148	Negative
	115542	C/N/61/M	Negative	53	---	Colonoscopy
c.2173G>A;p.Val725Met	163600	C/N/58/M	Negative	103*	174	Cardiac catheterization 57y
c.2255C>T;p.Pro752Leu	107068	C/N/50/M	Negative	182	162	Negative
c.2275A>G;p.Asn759Asp	118968	C/N/58/M	Negative	363	188	Surgeries: prostate (TURP) 52y. Colonoscopy 57 y. Rheumatic fever 7 y, Hypothyroidism on THR

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c.2461C>A;p.His821Asn	194655	C/N/63/F	Negative	29	151	Surgeries: cholecystectomy 40s; Statin intolerance
c.2533C>T;p.Leu845Phe	189882	C/N/56/M	Negative	<b>727*</b>	207	Surgeries: cardiac stenting 56 y
c.2697C>A;p.Asn899Lys	132267+	C/N/48/M	Negative	279*	189	Negative
c.2812G>A;p.Val938Met	159946	AA/N/56/M	Negative	<b>487*</b>	175	Negative
	142923	AA/N/59/F	Negative	60	124	Hypothyroidism on THR
c.2824G>A;p.Asp942Asn	132306	C/N/57/M	Negative	60	137	Negative
c.2956C>T;p.Arg986Cys	154982	C/N/58/F	Negative	48	137	Hypothyroidism on THR
c.3431C>T;p.Pro1144Leu	128062	A/N/63/M	Negative	79*	129	Surgeries: CABG 61 y, then cardiac stenting 61 y
c.4024A>G;p.Ser1342Gly	121670	O/N/55/F	Negative	196	184	Surgeries: total abdominal hysterectomy, unilateral salpingo-oophorectomy
c.4178A>G;p.Lys1393Arg	106963	C/N/55/M	Negative	37	144	Colonoscopies x2 since 40s. Gilbert's disease
	115065+[]	C/N/59/M	Negative	120	179	Negative
	185991	C/N/65/M	Negative	332	153	Hypothyroidism on THR Colonoscopies
	136706	C/N/52/F	Negative	77^	111	Negative
c.4999C>T;p.Arg1667Cys	112443	C/N/54/F	Negative	63	179	Surgeries: thyroidectomy 46 y, total abdominal hysterectomy 45 y. Thyroid cancer 46 y. Hypothyroidism on THR
	193390	C/N/59/F	Negative	92	140	Negative
	167185	C/N/63/F	Negative	149*	132	Ehlers-Danlos Syndrome Surgeries: umbilical hernia repair 8 y, cholecystectomy 42 y, right inguinal hernia repair and total abdominal hysterectomy 48 y, repeat bladder resuspension and right inguinal hernia repair

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	152415	C/N/57/F	Negative	86	199	49 y, left inguinal hernia repair 53 y Negative
c.5036G>A:p.Arg1679His	106945	C/N/58/M	Negative	<b>516*</b>	176	Hypothyroidism on THR; Alcohol over consumption: self-reports ≥3 glasses of wine daily
	148840	C/N/55/M	Negative	143	187	Negative
c.5183C>T;p.Ser1728Phe	134994	C/N/47/F	Negative	97	149	Negative
c.5317C>T;p.Pro1773Ser	191186	A/N/46/F	Negative	160	175	Surgeries: total abdominal hysterectomy 43 y
c.5360C>T:p.Pro1787Leu	N =39					
c.5980C>T;p.Arg1994Cys	155247	C/N/48/M	Negative	130	152	Negative
c.6178G>T:p.Gly2060Cys	N =116					
c.6191T>G:p.Met2064Arg	195344	C/N/60/F	Negative	128	146	Surgeries: cholecystectomy.
c.6301A>Gp.Met2101Val	118954	C/N/45/F	Negative	<b>288</b>	177	Hyperuricemia (8.9 mg/dL) Colonoscopies x3 starting 35 y
c.6607A>G;p.Met2203Val	182262	C/N/56/M	Negative	248*	160	Surgeries: right radical nephrectomy 55 y
c.6670C>T;p.Arg2224Cys	151637	C/N/57/F	Negative	70	135	Negative
c.6688A>T;p.Thr2230Ser	115065+//	C/N/59/M	Negative	120	179	Negative
c.6700C>T;p.Arg2234Cys	116576	C/N/51/F	Negative	73	145	Surgeries: total abdominal hysterectomy 49 y Colonoscopy
c.6721C>T;p.Arg2241X	154209	C/N/58/F	Negative	58*	190	Negative
	181028+	C/N/63/F	Negative	50* <sup>^</sup>	148	Negative
c.6961A>G:p.Ile2321Val	100288	C/N/58/M	Negative	108* <sup>^</sup>	167	Surgeries: cardiac stenting 48 y
c.7025A>G:p.Asn2342Ser	187842	C/N/52/M	Adopted	170	152	Negative
	106838	C/N/45/M	Negative	181	208	Negative
c.7487C>T;p.Pro2496Leu	132267+	C/N/48/M	Negative	279*	189	Negative
c.7902C>A;p.Asn2634Lys	135629	C/N/62/M	Negative	82	106	Negative
c.7958A>G:p.Lys2653Arg	114689	C/N/56/F	Negative	72 <sup>^</sup>	140	Colonoscopy

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c.8113G>A;p.Ala2705Thr	120721	C/N/63/F	Negative	82 <sup>^</sup>	141	Subclinical hypothyroidism (TSH 4.16 mIU/mL / FT4 1.6 ng/dL)
c.8155T>C;p.Tyr2719His	190433	--/HL/59/M	Negative	142	127	Negative
c.8305G>A;p.Asp2769Asn	195358	A/N/61/F	Negative	48*	161	Negative
c.8327C>T;p.Ser2776Phe	104866	C/N/58/M	Negative	106	164	Negative
c.8360C>G;p.Thr2787Ser	N =6					
c.9242T>C;p.Met3081Thr	177243	AA/N/58/F	Negative	247	223	Negative
	179265	AA/N/52/F	Negative	143	161	Negative
	110716	AA/N/64/F	Negative	143	154	Negative
c.9347C>T;p.Ser3116Leu	139926	C/N/65/F	Negative	66	151	Myositis 2 <sup>nd</sup> to autoimmune disease. Surgeries: cerebral aneurysm; repair total abdominal hysterectomy, unilateral salpingo-oophorectomy 55 y
c.9800C>T;p.Pro3267Leu	119835	C/N/61/F	Negative	102*	171	Negative
	195394	C/N/60/M	Negative	90*	145	Surgeries: cardiac stenting 59 y
	169830	C/N/53/M	Negative	119	143	Negative
c.10046C>G;p.Ala3349Gly	100818	C/N/54/F	Negative	93*	160	Negative
c.10183C>T;p.Arg3395Trp	132356	C/N/50/F	Negative	107	162	Negative
c.10492C>G;p.Arg3498Gly	133237	C/N/51/M	3 Gen History of MH (MH events, (+) CHCT	151	138	MHS, (+) CHCT
c.10616G>A;p.Arg3539His	178697	C/N/61/F	Negative	85	164	Negative
c.10664A>T;p.Asn3555Ile	117201	A/N/45/M	Negative	159	129	Colonoscopy
c.10747G>C;p.Glu3583Gln	N =14					
c.11061_11063del;p.Glu3689del	108006	C/N/65/F	Negative	148*	180	Surgeries: colectomy 62 y. Colonoscopies
	162256	C/N/57/M	Negative	94*	159	Surgeries: cardiac stenting 57 y

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c.11266C>G:p.Gln3756Glu	N =34					
c.10747G>C;p.Glu3853Lys	179251	C/N/60/F	Negative	149 <sup>^</sup>	169	Hypothyroidism on THR (TSH 6.1 mIU/mL / FT4 1.2 ng/dL)
c.11599C>T:p.Arg3867Cys	101329	C/N/60/M	Negative	121	189	Negative
c.11731A>G:p.Thr3911Ala	164979	C/N/47/ M	Negative	152	135	Hypothyroidism on THR
c.11958C>G;p.Asp3986Glu	194459	C/N/45/M	Proband with history of fulminant MH events and (+) CHCT; 3 siblings with myotonia	<b>1,271</b>	<b>238</b>	MHS, (+) CHCT Myopathy - myotonia with dysphagia and proximal muscle weakness; 3 fulminant MH events: 1) after surgery 1972, 2) after exercise in 2004 and 3) 2011 with rhabdomyolysis. Current medications: Dantrolene sodium, 100mg, daily in AM, by mouth; Morphine control release, 15mg, 2 times a day, by mouth
c.12553G>A:p.Ala4185Thr	104905	C/N/52/F	Negative	51	128	Negative
	140929	C/N/56/M	Negative	<b>702</b>	200	Negative
c.13513G>C:p.Asp4505His	102488	C/N/63/M	Negative	111	132	Negative
	137940	C/N/51/M	Negative	186*	160	Negative
	174073	C/N/62/M	Negative	96*	202	Surgeries: inguinal hernia repair 41 y; nephrectomy - benign tumor 40s; cardiac stenting x2 (52 y and 62 y) Colonoscopies x3
	119120	C/N/52/F	Negative	100	143	Negative
c.14167C>T:p.Arg4723Cys	100199	C/N/60/M	Negative	175	165	Negative
c.14189G>T;p.Gly4730Val	160480	C/N/53/F	Negative	54	138	Negative
c.14901C>G:p.Asp4967Glu	142361+ <del>7</del>	C/N/63/M	Negative	84	131	Surgeries: appendectomy 10 y

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c.14915C>T:p.Thr4972Ile	142361+//	“	“	“	“	“
c.14929G>C:p.Glu4977Gln	116259	C/N/52/M	Negative	208	134	Negative
c.15098A>G:p.Tyr5033Cys	157401	C/N/60/F	Negative	149	160	Negative
			Positive Family History =2			

Participant information for *RYR1* variants identified in the 870 ClinSeq<sup>®</sup> cohort. Medical histories were reviewed for surgeries/ procedures and clinical conditions indicative of myopathy or that elevate serum creatine kinase (CK). Participants were evaluated for: any myopathy, MHS, and/or exertional heat illness; history of elevated CK; hypothyroidism and/or elevated thyroid stimulating hormone (TSH) > 4.00 mIU/mL; hypokalemia <3.3 mmol/L; hyperuricemia > 8.6 mg/dL (uric acid normal range: 3.7-8.6 mg/dL); Statin and Nicotinic Acid use; self-report alcohol use (≥3 bottles/glasses/shots per days); autoimmune conditions: rheumatoid arthritis, psoriasis, lupus, Sjogren's syndrome, scleroderma; and renal disease. Participants race and ethnicity were determined by self report.

Age =age at enrollment. CABG =coronary artery bypass graft. CHCT =caffeine halothane contracture test. CK =creatin kinase, range: Female =38-252 U/L; Male =52-386 U/L. Ethnicity: N =Not Hispanic or Latino; HL =Hispanic or Latino. LDH =lactate dehydrogenase, range = 113-226 U/L. K+, potassium, range = 3.3-5.1 mmol/L. MHS =Malignant Hyperthermia Susceptible. Nucleotide change =NM\_000540.2. Race: C =Caucasian, A =Asian, AA =African American, 0 =other, “—“ =Not reported. THR =thyroid replacement hormone. TSH range: 0.40–4.00 mIU/mL. Thyroxin, FT4 range =0.9 - 1.5 ng/dL. TURP =transurethral resection of the prostate. “+” =participant has a second *RYR1* variant. “★” =participant with *CANA1S* variant. “\*” =on Statin. “^” =elevated serum TSH level. “v ” =low serum TSH level. “---” =sample hemolyzed.