Web Supplement. Caffeine Halothane Contracture Test, Ryanodine Receptor Mutations, Variants and Polymorphisms, Clinical Grading Scale, and Personal and Family Medical Histories of Included Subjects

	Maximum CHCT contracture (g)		Exon Number	Amino Acid Substitution	Inclusion Indication	CGS	Personal Anesthetic and Medical History	Family History
Subject #	Caffeine (2mM)	Halothane 3%						
1	0.34	2.55	1	L13R	MH	-	-	-
2	0.83	2.7	2	G40A	FH	-	Had GA x 2, took atorvastatin without adverse effect Muscle at time of CHCT had rare atrophic fibers and diffusely distributed internal nuclei	Sibling had apparent MH episode 2 first-degree relatives had positive CHCT
3	1.9	6.1	6	R163C	FH	-	Had GA x 2 with no signs of MH	Apparent MH death in second-degree relative One first-degree relative had positive CHCT
4	0.9	4	6	R163C	FH	-	Had GA x 1 with no signs of MH	Perioperative death of sibling, etiology determined
5	1.3	5.6	9	G248R	FH	-	-	Mother died of apparent MH Mother's relatives had positive CHCTs

6	na	na	9	G248R	MH	43	Subject reported that in 1983 GA with halothane and succinylcholine was complicated by generalized rigidity, increase CK, acidosis ventricular fibrillation, and rapidly increase temperature CK increase was not counted in CGS because value was not provided	-
7	2.2	7.8	11	G341R	MH	-	Surgeon reported that this subject had an MH episode in an outpatient surgery clinic	-
8	na	na	11	G341R	MH FH	-	Subject reported that after < 30 minutes of halothane in 1977, he had lethargy and low grade fever lasted ~ 10 days. After GA in 1968 there was uncontrollable muscle shaking In 1980 CHCT was reported to be positive (pre-standardization) No quantitative CHCT data are available.	Subject reported apparent MH death in cousin
9	2.4	3.6	14	L487P	FH	-	Never had GA	Parent died of fulminant MH, the details of which were reviewed by the MH Diagnostic Center Director MH was listed on the death certificate.

10	1	1.3	14 86	V518A Y3933C	MH	5	Postoperatively renal failure occurred.[a]	-
11	1	6.1	17	R614C	FH	-	Subject was muscular, had heat stroke and one GA without signs of MH Muscle at time of CHCT had normal histology and histochemistry.	Sibling died of apparent MH Heatstroke was reported in the family history
12	0.97	7.35	17	R614C	MH	30	Had 4 GA x 4. Only in 1989 was MH suspected after enflurane and IV succinylcholine, severe MMR occurred with CK 16,000 IU/L, $ETCO_2$ 49 mm Hg Muscle at time of CHCT had a few fibers with small empty subsarcolemmal spaces, histology was normal	-

13	0.41	3.23	17	R614C	FH	-	Subject reported heat stroke and also muscle pain over entire body when training >15 min for long distance running Strabismus and 4 GA x 4 without MH were noted.	Subject's child had GA x 8 with one report of fulminant MH in 1987 after halothane & IV succinylcholine MMR, sinus tachycardia, and cyanosis occurred, pCO_2 was 49 mm Hg with controlled ventilation, arterial pH was 7.2, BE was -10 mE/L, and CK was 43,600 IU/L MH was successfully treated with dantrolene CHCT was positive Muscle at time of CHCT had focal decreases in oxidative activity suggestive of cores and normal histology.
14	0.9	4.9	17	R614C	FH	-	-	Elevated CK, postoperative fever, suspected MH
15	na	na	17	R614C	MH FH	48	GA with MH in 1981 when MMR occurred after succinylcholine complicated by tachycardia, PVCs BE-10 mEq/L, myoglobinuria CK 21,000 IU/L, and effective treatment with dantrolene	Parent had an abnormal prestandardization CHCT in which the muscle twitched even during dissection This muscle had fibers containing acid phosphatase Rare fibers had a decrease in oxidative activity There was a slight increase in fibrous connective tissue
16	0.6	0.7	24	*V974M*	-	-	-	-

17	na	na	24	R975W	CCD	-	GA with suspected MH event in late 1960s No details are available Subject reported weekly episodes of muscle cramps and weakness and heat and exercise intolerance due muscle pain and weakness	-
18	0.95	1.8	24	R1043H	FH	-	-	Possible MH episode in second-degree relative
19	2.11	4.17	25	*R1109L*	FH	-	Subject reported heat stroke or prostration and one GA without signs of MH Muscle at time of CHCT showed some internal nuclei Histochemistry was normal	Parent died of apparent MH 3 second- or third-degree relatives had positive CHCT
20	n/a	1.6	29	*K1393R*	-	-	-	-
21	1.18	5.74	29	*K1393R*	Elevated CK	-	-	-
22	0	1.2	29	*K1393R*	FH	-	Muscle cramps worse with exercise	Similar symptoms in family members
23	0.5	2.9	34 38	*P1787L* *G2060C*	-	-	-	-

24	0.3	0.9	38	*G2060C*	FH	-	-	-
25	na	na	38	*G2060C*	MH	38	In 2007, IV induction, desflurane and succinylcholine were followed by MMR, hypercarbia, sinus tachycardia, and resolution after dantrolene The subject reported months of headache, frequent sweating and inability to climb stairs after this event	-
26	1.6	5.3	39	R2163H	MH	-	Fulminant MH was reported during GA for surgery with a extremity tourniquet	-
27	na	na	39	V2168M	MH CCD	-	Muscle biopsy for histology found CCD GA in late 1970s suspected for MH Delayed motor milestones	-
28	1.3	2.7	40	T2206M	FH	-	Had GA x 3 without signs of MH	Fatal apparent MH reported in a nephew. 2 relatives had positive CHCT

29	2.72	6	40	T2206M	FH	-	Muscle cramps weekly GA x 4 without signs of MH CHCT muscle had diffusely distributed internal nuclei, a single fiber with multiple cytoplasmic inclusion bodies and increased subsarcolemmal oxidative enzyme activity	Sibling and 2 distant relatives were reported to have survived fulminant MH events
30	1	1.44	40	T2206M	MH	48	One GA complicated by tachycardia, MMR $PaCO_2$ 143 mm Hg, pH 6.90 ~3 hours after induction Resolved after dantrolene Normal histology and histochemistry of muscle at the time of CHCT	-
31	na	na	40	T2206M	MH	30	During GA with increased minute ventilation $ETCO_2$ was > 60 mm Hg, temperature reached 40° C CK was 6,000 IU/L Muscle histology was abnormal	-
32	0.3	2.3	41 44	R2248H I2358L	MH	15	CGS was reported to be 15	-
33	0.3	1.3	43	*I2321V*	MH	15	MMR	-

34	3.3	5.7	43 24	R2336H R1043C	MH FH	-	One GA with increased heart rate and temperature to 39.4° C, postoperative muscle pain and cramping	Parent had possible MH ~ 50 y ago Sibling has positive CHCT
35	0.2	1.5	43	N2342S	MH	15	MMR	-
36	1.36	5.9	44	del E 2347	FH	-	One GA with no signs of MH	MH with CGS 60
37	0.5	5	44	N2351H	MH	48	CGS was reported to be 48	-
38	1.25	3.4	44	V2354M	MH	28	Subject was muscular One GA with succinylcholine for emergency surgery with fulminant MH reported; pH 7.26 $ETCO_2$ 69 mm Hg, CK 6,000 IU/L, K^+ 5.6 mEq/L, temperature 38.9°C, sinus tachycardia	-
39	2.6	9.5	44	R2355W	MH	-	GA suspicious for MH	-
40	1.82	1.6	44	R2355W	MH	-	-	-
41	0.3	5.1	44	R2355W	MH	-	Postoperative MH suspected	-

42	1.9	1.1	45 34	R2435L *P1787L*	FH	-		Sibling died of apparent MH
43	1.17	4.01	45	G2434R	FH	-	Subject was muscular One GA without signs of MH Muscle histology and histochemistry at time of CHCT were abnormal with small tubular aggregates observed	Parent had possible MH event
44	1.7	8.08	45	G2434R	MH FH	-	2 possible MH events with rapidly increasing temperature as the only MH signs noted One GA with no signs of MH	3 generations in this family reported problems with GA Cousin had positive CHCT after his own suspected MH episode (CGS 15 with MMR as the only sign of MH)
45	0.74	7.71	45	G2434R	MH	30	Athletic, GA x 2 with MH in the 2nd GA in 1999 after volatile agent with succinylcholine, generalized rigidity, CK 70,000 IU/L, muscle weakness were noted Muscle at time of CHCT had occasional fibers with increased red subsarcolemmal staining	-
46	1	7.4	45	G2434R	FH	-	One GA with no signs of MH	Perioperative death of sibling, etiology undetermined

47	3.9	10.5	45	G2434R	MH	45	MMR and total body rigidity $PaCO_2$ 57 mm Hg, temperature 37° C K^+ 7.6 mEq/L, CK 350,000 IU/L	
48	1.5	3.1	45	G2434R		-		-
49	na	na	45	G2434R	FH	-	Positive CHCT in 1983 (prestandardization, slight increase in connective tissue, more type 2 than type 1 fibers, very slight increase in lipid droplets in muscle fibers) One GA in 2004 without signs of MH	Sibling died of apparent MH during GA.
50	na	na	45	R2454H	FH	-	Muscle pain after GA in 1968	One apparent MH death reported during GA in 1947 at age 17 y and at least 1 more reported MH death and 1 positive CHCT in different relatives At time of CHCT CK was 950 IU and muscle had spontaneous contractures Reports of chronic muscle pain and intolerance to heat and exercise, chronic increase CK in relatives

51	na	na	47	R2508H	MH CC D	53	Subject reported that GA in 1979 was complicated by total body rigidity, rapidly increase temperature, increase heart rate, rapid reversal by dantrolene, CK > 100,000 IU Months were required for recovery of strength	Progressive CCD in 2 first degree relatives, diagnosed by the family's neurologist
52	0	0	66	R3238G	CC D	-	Subject presented with muscle weakness and fatigue	-
53	na	na	66	R3283Q	MH Deat h	76	Previous GA x 23, after several hours of volatile agent, total body rigidity pCO > 100 mm Hg, MV 3 times predicted, K^+ 7.5 mEq/L temperature 41.5°C, cardiac arrest, pH 6.9, BE -10 mEq/L, acidosis less after dantrolene *CACNA1S* variant, V875M, was also present	-

54	0.8	5.2	71	R3539H	MH	40	GA x 2. MH was suspected due to MMR and total body rigidity after succinylcholine and halothane anesthesia, inappropriate tachypnea, $PaCO_2$ 45 mm Hg during spontaneous ventilation, temperature 39°C, recovered without dantrolene.	-
55	1	2.6	72 101	E3583Q V4849I	MH FH	18	Suspected MH episode	Sibling died of apparent MH Relatives had presumed MH episodes
56	0.85	7.5	76	T3711R	FH	-	Muscular GA x 4 with no signs of MH CK was ~400 IU/L (normal 200 IU) at time of CHCT	Sibling and parent had apparent MH episodes during GA 3 relatives had chronic CK elevations of less than 1,000 IU/L Family history is complex because of coexistence of RYR1 R614C in some relatives
57	1.2	2.2	87	W3985R	MH	-	GA suspicious for MH in childhood	-
58	1.9	8.8	87 34	D3986E *P1787L*	*MH*	-	GA suspicious for MH	-

59	2.5	10.8	87	D3986E	**MH**	-	GA with MMR as a child Years later fulminant MH followed by daily cramps and exercise induced rhabdomyolysis without increased metabolism	-
60	1.77	6.95	90	G4178V	FH	-	2 GAs without signs of MH Muscle pain and increased CK after fluvastatin CK was 100 IU/L above normal and type 2 fiber atrophy were found at the time of CHCT	Parent and sibling died of apparent MH.
61	3.3	6.3	91	M4230R	MH FH	-	Abnormal skeleton therefore muscle was taken from the soleus for CHCT	GA with apparent MH death and parent reported to have had MMR
62	0.5	9.9	100	Q4837E	FH	-	-	-
63	na	na	101	V4847L	MH	23	GA suspicious for MH in childhood CGS reported by MH Diagnostic Center Director	-
64	1.9	9.8	101	V4849I	MH	-	GA suspicious for MH	-
65	0.3	3.6	101	V4849I	FH	-	-	Sibling died of apparent MH First-degree relative had positive CHCT
66	0.9	5	101	V4849I	MH FH	18	GA suspicious for MH in childhood	-

67	1.15	6.4	101	R4861H	FH	-	Never had GA Prefers cool environment	First-degree relative and more distant relatives had positive CHCT after suspected MH event
68	0.4	2.1	102	A4906G	FH	-	-	Sibling died of apparent MH
69	2	11.3	103	D4939E	MH FH	15	MMR after succinylcholine	3 relatives with chronically elevated CK Grandparent had ADR to succinylcholine
70	0.84	8.42	103	A4940T	FH	-	GA x 8 with no signs of MH	3 suspected MH events 3 positive CHCT Rhabdomyolysis with atorvastatin
71	0.85	2.1	104	P4973L	-	-	-	-
72	0.67	1.47	-	nothing in all *RYR1*	FH	-	GA x 1 with no signs of MH	Positive CHCT in 4 relatives (3 siblings and 1 nephew) after sibling had suspected MH event (CGS 23)
73	0.3	1.5	-	nothing in all *RYR1*	MH	23	GA suspicious for MH at 2 years of age: increase temperature and tachycardia resolved with dantrolene	-
74	0.4	2	-	nothing in all *RYR1*	MH	10	Postoperative oxygen desaturation, temperature 41.1°C, BE -7 mEq/L, CK 920 IU/L	-
75	0.2	1.3	-	nothing in all *RYR1*	FH	-	-	Positive CHCT in 2 first-degree relatives

76	1.9	2.9	-	nothing in all *RYR1*	intraoperative rhabdomyolysis	-	CK 30,000 to 200,000 IU/L after GA with volatile agent	-
77	0.28	2.68	-	nothing in all *RYR1*	exercise induced rhabdomyolysis	-	Muscle weakness interferes with activity weekly Cola colored urine Heat prostration Never had GA Homozygous Arg50X mutation and McArdle's disease histochemistry	-
78	0.9	2.1	-	No finding in 103 *RYR1* exons	FH	-	-	-
79	0.3	1.1	-	No finding in 103 *RYR1* exons	FH	-	-	Son had MMR after succinylcholine with CK 35,000 IU/L
80	0.7	0.2	-	No finding in 103 *RYR1* exons	MH	-	-	-
81	0.8	2	-	No finding in 103 *RYR1* exons	FH	-	-	Positive CHCT in sibling
82	*0*	*1.5*	-	No finding in 103 *RYR1* exons	FH	-	-	Sibling had possible MH episode
83	1	2.3	-	No finding in 102 *RYR1* exons	FH	-	Severe rhabdomyolysis after succinylcholine as a child	One normal CHCT in 1st degree relative

84	0.59	1.64	-	No finding in 100 *RYR1* exons	MH	15	MMR after succinylcholine GA aborted in 1987, CK >15,000 IU/L	-
85	0.2	1	-	No finding in 100 *RYR1* exons	FH	-	-	Parent died during GA for superficial surgery with cardiac arrest at < 40 y of age, no known allergies
86	0.5	1.1	-	No finding in 100 *RYR1* exons	FH	-	Subject was muscular Never had GA	Subject's child is reported to have survived fulminant MH
87	0.1	1.2	-	No finding in 100 *RYR1* exons	MH	15	Severe MMR	-
88	1.1	2.3	-	No finding in 100 *RYR1* exons	-	-	-	-
89	0.4	0.9	-	No finding in 50 *RYR1* exons	FH	-	-	Muscle cramping GA in 1950s was reported to be complicated by increase temperature
90	1.2	1.4	-	No finding in 50 *RYR1* exons	MH	-	-	-
91	0.22	1.04	-	No finding in 36 *RYR1* exons	FH	-	One GA with no signs of MH	Son had cardiac arrest and died after short sevoflurane GA Son had dystrophinopathy
92	0	1.785	-	No finding in 34 *RYR1* exons	-	-	Spontaneous contractures of 0.68 g during CHCT	-
93	0	1.2	-	No finding in 35 *RYR1* exons	-	-	-	-

94	0	1.2	-	No finding in 32 *RYR1* exons	-	-	-	-
95	0.1	1.3	-	No finding in 30 *RYR1* exons	FH	-	-	Suspected MH in 2 first-degree relatives Cousin died of apparent MH
96	0.15	1.8	-	No finding in 30 *RYR1* exons	rhabdomyolysis	-	Muscle cramps at least once weekly, cola colored urine idiopathic, elevated temperature at least 6 time yearly GA x 9 with no signs of MH	-
97	0.35	1.4	-	No finding in 30 *RYR1* exons	FH	-	GA x 5 with no signs of MH	2 first-degree relatives had suspected MH, one of whom had an equivocal CHCT
98	0.5	1	-	No finding in 30 *RYR1* exons	FH	-	Never had GA	One cousin is reported to have survived a fulminant MH event 6 relatives had equivocal CHCT 2 relatives had normal CHCT
99	0.7	4	-	No finding in 30 *RYR1* exons	FH	-	Never had GA	Second-degree relative had suspected MH
100	1.9	3.4	-	No finding in 30 *RYR1* exons	FH	-	One GA with no signs of MH	First-degree relative and cousin had suspected MH
101	1.1	2.6	-	No finding in 30 *RYR1* exons	FH	-	One GA with no signs of MH	Sibling had suspected MH episode
102	0.3	2.4	-	No finding in 30 *RYR1* exons	-	-	-	-

103	0.5	5.61	-	No finding in 30 *RYR1* exons	-	-	-	-
104	1	5.14	-	No finding in 30 *RYR1* exons	-	-	-	-
105	0	1.56	-	No finding in 30 *RYR1* exons	-	-	-	-
106	1.5	6.3	-	No finding in 30 *RYR1* exons	-	-	-	-
107	0.3	0.9	-	No finding in 30 *RYR1* exons	-	-	-	-
108	1.3	3	-	No finding in 30 *RYR1* exons	-	-	-	-
109	0	1.4	-	No finding in 30 *RYR1* exons	-	-	-	-
110	1.1	2.6	-	No finding in 30 *RYR1* exons	-	-	-	-
111	0.45	2.3	-	No finding in 30 *RYR1* exons	-	-	-	-
112	0.15	1.5	-	No finding in 30 *RYR1* exons	-	-	-	-
113	0.55	0.8	-	No finding in 30 *RYR1* exons	-	-	-	-

114	0.2	1.4	-	No finding in 30 *RYR1* exons	-	-	-	-
115	0	2	-	No finding in 30 *RYR1* exons	-	-	-	-
116	0.1	1.3	-	No finding in 30 *RYR1* exons	-	-	-	-
117	n/a	4.5	-	No finding in 30 *RYR1* exons	-	-	-	-
118	1.9	1.7	-	No finding in 30 *RYR1* exons	-	-	-	-
119	n/a	1.6	-	No finding in 30 *RYR1* exons	-	-	-	-
120	1.35	2.7	-	No finding in 30 *RYR1* exons	MH	18	CGS was reported to be 18	-

Descriptions of personal and familial medical history were reported by the MH Diagnostic Center Directors to the North American Malignant Hyperthermia Registry on the Malignant Hyperthermia Biopsy and Diagnostic Consultation Report unless another source is noted. When MH was an indication for CHCT the subject had experienced a suspected MH episode in the judgment of the MH Diagnostic Testing Center Director. The drugs given as part of this anesthetic are noted when this information was available. Variants of uncertain significance (VUS) which have been reported before are noted in black, novel variants are noted in blue font,

known MH mutations are noted in red font, amino acid substitutions in *green italics* indicate known polymorphisms. Beige highlighting indicates that this subject had no findings in previous studies at Uniformed Services University of the Health Sciences. A dash (-) indicates there is no information.

ADR is adverse drug reaction, BE is base excess, CCD is central core disease, CGS is the Clinical Grading Scale, also known as the Larach Scale, CHCT is caffeine halothane contracture test, CK is creatine kinase, $ETCO_2$ is end-tidal carbon dioxide, FH is family history of MH susceptibility, GA is general anesthesia (GA x 2, or another number, reflects the number of general anesthetics the subject has had), IV is intravenous, MH is malignant hyperthermia, MMR is masseter muscle rigidity, MV is minute ventilation, PVCs are premature ventricular conductions, na is not applicable (not done).

[a]This case was reported in McKenney KA, Holman SJ. Delayed postoperative rhabdomyolysis in a patient subsequently diagnosed as malignant hyperthermia susceptible. Anesthesiology 2002; 96:764-5.