Supplemental Digital Content 4. Supplemental data for the publication titled "Using Exome Data to Identify Malignant Hyperthermia Susceptibility Mutations".

1. Supplemental Methods
2. Descriptions of variants identified in ClinSeq® and in databases, but determined to be of less than class 5 pathogenicity.

1. Supplemental Methods

The *RYR1* variants listed in this supplemental were annotated as described in the methods section of this paper and similar to that described by Johnston et al[1]. The *RYR1* nucleotide numbering is based on transcript variant NM_000540.2, according to the standards of the Human Genome Variation Society[*]. Variants with low genotype quality were designated as class 0; the remainder were scored on a pathogenicity scale of class 1-5 using an adaptation of published criteria[1-7] and Johnston et al[1]. Briefly, class 1 variants were definitely benign, class 2 probably benign, class 3 of uncertain pathogenicity, class 4 probably pathogenic, and class 5 definitely pathogenic. Further evaluation of the variants was performed using the Human Gene Mutation Database (HGMD)[†8] and the Locus-Specific database, Leiden Open Variation Database (LOVD)[9‡] and Universal Protein Resource[§] as well as citation trackers (Google Scholar 2012; Scopus, 2012 Elsevier B.V.; and Web of Science, 2012 Thomson Scientific). The criteria for

[*] The Human Genome Variation Society website. Nomenclature for the description of sequence variations. Available at: www.hgvs.org/mutnomen/. Accessed May 20, 2013.

[†] The Human Gene Mutation Database (HGMD), Professional 2012.2 from BIOBASE. Available at: www.hgmd.org. Accessed May 20, 2013.

[‡] Leiden Open Variation Database (LOVD), v.3.0. Available at www.lovd.nl/3.0/home. Accessed May 20, 2013.

[§] The Universal Protein Resource website, 2002-2012 UniProt Consortium. Resource for protein sequence and annotation data. Available at http://www.uniprot.org/. Accessed May 20, 2013.

determining pathogenicity were similar to those used previously in studies of other phenotypes (See Table 1, Variant Pathogenicity Classification System, from original article).

2. Descriptions of 14 variants that were identified both in ClinSeq® and in databases, but determined to be of less than class 5 pathogenicity.

The *RYR1,* c.7025A>G, p.Asn2342Ser Variant

The p.Asn2342Ser variant was listed in the Human Gene Mutation Database as pathogenic based on two publications[10,11], and in LOVD[12] with the variant pathogenicity in the cited publication as "pathogenic" by the authors (indicated with "+"), although the LOVD database curators concluded "effect unknown" (indicated with "?")[**]. Citation tracking of Malignant Hyperthermia Susceptibility (MHS) publications by author returned more listings[13,14]. The first described a 14 year old with documented MHS but who also had hypokalemic periodic paralysis (the latter not having been associated with *RYR1* mutations)[10]. The second report was a screen of 23 patients with MHS, one of which had p.Asn2342Ser, although no additional genetic data or *in vitro* contraction test (IVCT) were provided. Functional data of a metabolic protein release assay supported pathogenicity[11].

In a more recent study of families with patients selected for anesthetic reactions or for chronic, unexplained elevated blood levels of creatine kinase (hyperCKemia), the p.Asn2342Ser variant was identified in one patient with moderate hyperCKemia and confirmed MHS

[**] Variant pathogenicity, in the format Reported/Concluded; '+' indicating the variant is pathogenic, '+?' probably pathogenic, '-' no known pathogenicity, '-?' probably no pathogenicity, '?' effect unknown.

phenotype by positive IVCT[13]. The patient presented with another known RYR1 mutation p.Arg3903Gln in *trans* with p.Asn2342Ser. In the proband's family, the p.Asn2342Ser variant was carried by the patient's three sons diagnosed as malignant hyperthermia-nonsusceptible. The allele p.Arg3903Gln co-segregated with the MHS status in the family, whereas p.Asn2342Ser did not and was classified as a "variant allele without any pathological meaning". In addition, the study analyzed the skeletal muscle mRNA in two of the proband's sons and found no evidence for monoallelic silencing.

The p.Asn2342Ser variant was most recently categorized as a *recessively* inherited missense mutation in a patient with a congenital myopathy and muscle biopsy finding compatible with the diagnosis of an *RYR1* related myopathy from a United Kingdom study of 92 patients from 71 families with *RYR1* mutations[14]. The patient was also reported to have the recessive RYR1 p.Arg3539His missense mutation (reported in ClinSeq®) in combination with a dominantly inherited mutation in the α-tropomyosin (*TPM3*) gene, (c.503G>A, p.Arg168His).The proband's daughter with the same phenotype, carried the *TPM3* mutation and the known recessive *RYR1* mutation c.10616G>A, p.Arg3539His.

This variant from a known hotspot MHS region II was identified in 12 of 10,746 alleles in the National Heart, Lung, and Blood Institute's[††], Exome Variant Server (EVS), which (assuming all are heterozygous), would indicate a carrier rate of 1/448, and an allele frequency of 0.1%, similar to the frequency in our data set (2/1,734). We conclude that this is a *Class 2* variant, probably benign.

The *RYR1*, c.10616G>A, p.Arg3539His Variant

The p.Arg3539His variant was identified in a single ClinSeq® participant. It was described as pathogenic in HGMD based on a single report of a patient with central core disease without segregation, histologic, or functional data[15]. The LOVD database returned results for a single report of the p.Arg3539His variant pathogenicity with an indication of "pathogenic" (+) by the authors, and concluded as "effect unknown" (?) by the curators[16]. In a study of nine families that met clinical and histological criteria for core congenital myopathies, one patient with generalized muscle weakness and severe skeletal complications had the p.Arg3539His missense variant along with another RYR1 mutation p.Gly4935Thr4957>AspfsX11 that affected the acceptor splice site of intron 102 and was thought to likely be the cause of the severe phenotype in the proband[16]. In the previously referenced United Kingdom study of 92 patients,

[††] The National Heart, Lung, and Blood Institute's, Exome Sequencing Project data browser. Available at: http://evs.gs.washington.edu/EVS/. Accessed May 20, 2013.

the p.Arg3539His variant was reported in two patients with congenital myopathies and muscle biopsy findings compatible with the diagnosis of an *RYR1*-related myopathy. The variant was classified as an *RYR1* mutation associated with an "uncertain inheritance pattern", for in one patient it was the only missense identified, and in another family it was seen with a clear recessive inheritance pattern[14]. This variant was identified in 17 of 10,741 alleles in the EVS for an allele frequency of ~0.2%. We concluded that this variant was a *Class 3* mutation of unknown significance.

The *RYR1*, c.2122G>A, p.Asp708Asn Variant

The p.Asp708Asn variant was detected in two ClinSeq® participants (2/870) and detected in 1/10,753 alleles in the EVS. It was described as *pathogenic* for congenital myopathy in HGMD[17] and found in two additional publications on *RYR1*-associated myopathies[14,18]. It was initially reported with the p.Arg2241X nonsense variant[18] and again in two patients with *RYR1* related myopathies of moderate severity, and in a third patient with a *RYR1* myopathy and a third recessive variant p.Arg2939Lys[17]. It was most recently categorized as a *recessively* inherited missense mutation with a high probability of pathogenicity in a patient with a congenital myopathy and muscle biopsy finding compatible with the diagnosis of an *RYR1* related myopathy from the United Kingdom study of 92 patients with *RYR1* mutations[14]. In this case, the

patient had two additional recessive pathogenic variants, and one variant of unknown significance (VUS), in addition to p.Asp708Asn: the nonsense p.Arg2241X variant, the recessive missense p.Met485Val, and VUS c.11547G>A(p(=)). The evidence of pathogenicity for the variants was in part based on *in silico* analysis. The p.Asp708Asn variant has been seen twice *without* p.Arg2241X—in one ClinSeq® participant and once in the EVS. We categorized it as a *Class 3* variant of unknown significance.

The *RYR1*, c.4178A>G, p.Lys1393Arg and c.5036G>A, p.Arg1679His Variants

The p.Lys1393Arg and p.Arg1679His variants (here described together, as two of the largest studies reported them jointly) were described as pathogenic in HGMD and first reported as novel *RYR1* missense variants in the same publication[19]. The p.Lys1393Arg and p.Arg1679His variants were found in a 5 year-old and 39 year-old Swedish male, MH-susceptible patients, respectively, known to be predisposed by prior clinical event—i.e., development of life-threatening Malignant Hyperthermic (MH) reactions during anesthesia—and positive IVCT. However, both variants were also found once in the study's 100 Swedish and 150 German control individuals. Segregation data available for the p.Arg1679His variant were inadequate to prove causality. The variant p.Arg1679His occurred in all MHS and MH-equivocal family members, based on the IVCT threshold concentrations, and in one MH-negative

individual. Although the missense variants occurred in conserved domains, they were initially acknowledged as "*may be 'neutral' polymorphisms*" by the study's authors[19].

In their follow-up study, the authors tested the same Swedish MH-susceptible p.Lys1393Arg and p.Arg1679His variant patients—and three others—for MH susceptibility by using Epstein-Barr virus-transformed lymphoblasts cultured from the subject's lymphocytes[20]. The B lymphoblastoid cell lines were used to study resting cytoplasmic calcium concentration, and the dose-dependent calcium release induced by the ryanodine receptor agonist 4-chloro-m-cresol. The study showed that cell lines from the p.Lys1393Arg and p.Arg1679His variant patients, and other MH-susceptible individuals with distinct *RYR1* variants, responded to 4-chloro-m-cresol with statistically significant, half maximal concentration (EC50) than cells from normal individuals, and that the magnitude of the responses differed between individuals with different *RYR1* variants[20]. However, the size of the study was small, and the novel diagnostic test, experimental. Also, it has not been firmly established whether the response in the *RYR1* receptor from circulating B-cells are a valid model for the MHS phenotype in skeletal myocytes.

A recent published abstract of a mother and son presenting with a severe, slowly progressive adult-onset axial myopathy and cataracts implicated the RYR1 p.Lys1393Arg variant in both. Serum creatine kinase (CK) was slightly elevated in the son, and MRI showed

involvement of the lumbar, and less pronounced pelvic and posterior thigh muscles in both. The quadriceps muscle biopsy showed minor, non-specific changes in both[21]. No functional studies were done.

The p.Lys1393Arg was most recently identified in three patients with congenital *RYR1* myopathies—from the United Kingdom study of *RYR1* mutations—and described as a putative *dominant* missense mutation with a high probability of pathogenicity (i.e., high nucleotide and amino acid conservation)[14]. Two of the three p.Lys1393Arg patients also had additional synonymous variants (i.e., one patient with three *RYR1* VUS, and another patient with two VUS).

The p.Lys1393Arg variant was identified in four ClinSeq® participants (4/870), but detected in 47 of 10,703 alleles in the EVS, indicating an allele frequency of 0.4%. The p.Arg1679His was found in two ClinSeq® participants and detected in 11 of 10,741 alleles in the in the EVS for a carrier rate of 1/318, and an allele frequency of 0.1%. Based on the equivocal data presented in the published reports, we categorized these as *Class 2*, probably benign variants.

The *RYR1*, c.8327C>T, p.Ser2776Phe Variant

The p.Ser2776Phe variant was found in a single ClinSeq® participant and detected in 10 of 10,746 alleles in the in the EVS for an allele frequency of 0.09%. It was described as *pathogenic* for MHS in HGMD and reported in two publications on *RYR1*-associated

myopathies[22,23]. In the most recent study, a 16-year-old patient with suggestive features of King-Denborough syndrome—a rare syndrome characterized by the triad of dysmorphic features, a myopathy, and MHS—was found to have the heterozygous p.Ser2776Phe missense variant identified by sequencing of *RYR1* from muscle-derived complementary DNA[22]. The p.Ser2776Phe variant affected a highly conserved amino acid residue and was not found in 100 control chromosomes. There was no history of clinically manifest malignant hyperthermia reactions in the family. The sequence variant was considered not in itself sufficient to cause a pathological phenotype by the authors, as the same substitution was also identified in her asymptomatic father. Subsequent Western blot analysis of protein extracted from muscle tissue showed a marked reduction in RYR1 protein levels compared to an unaffected control, suggesting the presence of unidentified allelic *RYR1* mutations not detectable on routine sequencing.

In another study of MH-susceptible patients diagnosed by the IVCT, a male Swedish patient with a history of a life threatening MH reaction during surgery, and with a high positive score in the IVCT, was found to have the p.Ser2776Phe missense variant. However, the variant was also found in one German and in one Danish individual in controls[23]. Based on the

ambiguous data from the reports, the p.Ser2776Phe variant most likely must be considered a rare polymorphic variant and categorized as a *Class 2*, probably benign variant.

The *RYR1,* c.13513G>C, p.Asp4505His Variant

The p.Asp4505His variant was detected in four ClinSeq® participants (4/870) and detected in 36 of 10,710 alleles in the EVS for a carrier rate of 1/149 and an allele frequency of 0.3%. The variant was listed as pathogenic in HGMD based on one published report[24] and a published abstract[21]. Two additional reports were found in a literature search[14,25].

The p.Asp4505His variant was first described in a 29-year old asymptomatic male with an elevated serum CK level (407 UI/I) - slight variation in fiber size found on muscle biopsy, and MHS susceptible by IVCT results[25]. However, the patient had an additional *RYR1* novel nucleotide change c.7085A>G of exon 44 predicted to cause p.Glu2362Gly. No segregation data were included in this analysis of 37 Italian subjects with elevated CK levels, but without significant weakness or other neurological symptoms[25].

A more recent study included a functional analysis of the p.Asp4505His variant and introduction into a full-length rabbit *RYR1* complementary DNA, described two children who had died from of non-anesthetic MH-like episodes—described as drug-free fatal "awake" events—triggered by either exposure to environmental heat or infection[24]. A 6 year-old girl with

the maternally inherited p.Asp4505His variant, and an additional RYR1 variant, p.Arg3983Cys, on another allele, showed marked increased bulk of the leg muscles and recurrent cramping with rigid gait. The girl had a previous awake episode of high fever (>105 °F) and total body rigidity at 4 years of age. There was no family history of MHS or other neuromuscular disorders[24].

In this study, the p.Asp4505His variant was *not* found in the 100 previously reported MHS individuals from North America, or in 100 controls representing the U.S. Caucasian population. In the girl's family, the novel p.Asp4505His variant was identified on a separate haplotype from the p.Arg3983Cys variant, and was present in both the patient's mother and brother. Segregation analysis within the *RYR1* gene in the second family showed association of the p.Asp4505His variant with the maternal haplotype. The p.Asp4505His variant was reported to result in approximately two-fold increase in the sensitivity to activation by caffeine. Importantly, this increase in caffeine sensitivity occurred when the p.Asp4505His variant was co-expressed with wild-type RYR1, consistent with the known autosomal dominant pattern of inheritance of MH. They reported that *allelic segregation* could be a significant pathogenic factor in individuals with MHS, and that the unusually high caffeine sensitivity when the two variants localize to the same subunit demonstrates an allele-dependent synergism of two novel *RYR1* gene variants[24].

The c.13513G>C;p.Asp4505His variant was reported in a United Kingdom study with *RYR1* mutations, together in four patients with congenital myopathies and muscle biopsy findings compatible with the diagnosis of an *RYR1* related myopathy[14]. The p.Asp4505His variant was described as a putative dominant mutation in two patients with a relatively severe and a mild phenotype, and in two other patients with an apparently recessive inheritance. The two patients with recessive inheritance each had an additional missense variant [14]. No information was provided if those variations were in *trans* or *cis*, and family members were not tested in the two patients with recessive inheritance.

A recent case report of single 80 year-old patient harboring the p.Asp4505His variant with severe, slowly progressive adult-onset axial myopathy, marked head-drop, respiratory impairment, cataracts, and increased fiber size and cores on muscle biopsy, expanded the spectrum of the phenotype seen with this variant[21]. Based on the equivocal data presented in the multiple published reports and the low frequency in the EVS, we categorized the p.Asp4505His variant as *Class 3* variants of unknown significance.

The *RYR1*, c.4999C>T, p.Arg1667Cys Variant

The c.4999C>T,p.Arg1667Cys variant was not listed in HGMD, but referenced in LOVD with a citation to two studies[26,27]. LOVD listed the variant pathogenicity for the first study

(reported/concluded) as effect unknown and effect unknown by the publication's authors and site curators, respectively (?/?)[26], and for the second as probably no pathogenicity and effect unknown (-?/?) [26,27]. One study sequenced the entire *RYR1* coding region from genomic DNA of unrelated Japanese patients diagnosed as MH-susceptible by the calcium-induced calcium release test—the Japanese equivalent of the IVCT test. The tests subjects were recruited after experiencing an MH episode, or for having an MH relative, or an increase in CK. The p.Arg1667Cys variant in exon 34 was present in five patients. However, since the variant was also seen in two control samples, the researchers presumed the variant to be benign[26]. No segregation data were included in the study.

In another study, a representative cohort of 36 unrelated Canadian individuals positive for the caffeine-halothane contracture (*in vitro*) test for MHS or with a history of an MH event, were screened for *RYR1* mutations, and selected regions of *CACNA1S* transcripts[27]. After analyzing the correlation of the caffeine-halothane contracture results to the *RYR1* genotypes within MHS families, the p.Arg1667Cys variant was found in one individual with a positive the caffeine-halothane contracture test for MHS, but absent in two of the proband's tested relatives with negative test results. In the remaining families with variant, the absence of *in vitro* caffeine-halothane contracture testing results for additional family members prevented segregation

analysis[27]. The p.Arg1667Cys variant was detected in four ClinSeq® participants (4/870) and in 21/10,727 alleles for a carrier frequency of 1/255 and an allele frequency of 0.2% in the EVS. Based on the data presented and the relatively low allele frequency of 0.2% we categorized this as a *Class 3* variant.

The *RYR1,* c.6301A>G, p.Met2101Val Variant

The rare p.Met2101Val variant was found in a single ClinSeq® participant (1/870) but was not listed in the EVS, HGMD or LOVD, but referenced in the Universal Protein Resource database for a single publication[28]. The p.Met2101Val variant was identified in one family—in members positive for MHS by IVCT test—in a study of 52 Italian families (one to 12 individuals in each family) recruited for *RYR1* DNA analysis that included at least one family member identified by increased serum CK concentrations or selected for episodes of hyperthermia associated with an anesthetic. The variant was determined to be nonpathogenic based on the results from the study's functional test—denaturing high performance liquid chromatography. The affected families were only screened for 27 *RYR1* exons from critical regions[28].

The p.Met2101Val variant was recently reported again by the same authors in a study of 75 families with patients selected for anesthetic reactions or chronic unexplained hyperCKemia. Fifty-four families were confirmed for the MHS phenotype by positive IVCT. The

p.Met2101Val variant, located within putative hotspot MH region II, was identified in one patient with MHS and classified as a change of "unknown pathological meaning"[13]. Based on the data presented from the limited citations with incomplete confirmation, and the low allele and population frequency, we categorized this as a *Class 3* variant of unknown significance.

The *RYR1,* c.12553G>A, p.Ala4185Thr Variant

The p.Ala4185Thr variant was not listed in HGMD, but found in LOVD with a reference to one study with a reported/concluded variant pathogenicity as effect unknown (?/?)[27]. In this study of 36 unrelated Canadian MHS individuals screened for *RYR1* mutations by sequencing *RYR1* transcripts, one patient carried two novel mutations, c.12553G>A, p.Ala4185Thr variant (exon 90) along with c.14524G>A, p.Val4842Met (exon 101). No information was provided if those variations were in *trans* or *cis*. The p.Ala4185Thr variant was predicted to be neutral by bioinformatic programs (i.e., SIFT, Pmut, and PolyPhen2) and was absent in 200 controls. The p.Ala4185Thr variant was detected in two ClinSeq® participants (2/870) and in 6/10,752 alleles for an allele frequency of 0.06% in the EVS. We categorized this as a *Class 3* variant of unknown significance.

The *RYR1,* c.1453A>G, p.Met485Val Variant

The p.Met485Val variant from a known hotspot MHS region I was detected in two ClinSeq® participants (2/870) and in 1/10,757 alleles in EVS. It is described as pathogenic in HGMD based on a single report without segregation, histologic, or functional data[15], in LOVD with reference to a single study[29], and a literature search found additional references[14,30,31]. In an early study of 11 affected individuals from five families with Multi-minicore disease and external ophthalmoplegia where *RYR1* haplotyping and mutational analysis were carried out, the p.Met485Val variant was identified on the paternal allele of the asymptomatic parent in one United Kingdom family, along with the RYR1 p.Gly2060Cys variant, and classified as a polymorphism[30]. In another study of four patients from three families with core myopathies, two affected siblings carried the p.Met485Val substitution along with p.Arg109Trp in *cis*, inherited from the unaffected father[29]. The two affected siblings only transcribed the mutated paternal allele in skeletal muscle, whereas the maternal allele was silent. Functional measurements showed that the mutant *RYR1* channels—carrying both substitutions—lost the ability to conduct calcium. However, the study classified the p.Met485Val variant as "*probably a polymorphism"* due to the variant not being a conserved residue, and p.Met485 shows as a leucine in all other species[29]. In the most recent study, involving patients with *RYR1* related congenital myopathies, the p.Met485Val variant was seen in two unrelated patients. Both patients, in addition to

p.Met485Val**,** had an additional nonsense and missense variant[14]. We categorized this as a *Class 2*, probably benign variant.

The *RYR1,* c.8360C>G, p.Thr2787Ser Variant

The p.Thr2787Ser variant was seen in six ClinSeq® participants (6/870) and in 113/10,645 alleles (109 African American alleles, four European American Alleles) for an allele frequency of 1% in EVS. It is described as pathogenic in HGMD based on a single entry[32], and in LOVD for a single entry[33]. We categorized this as a *Class 2*, probably benign variant, based on its high allele frequency in both our cohort and in the EVS.

The *RYR1,* c.6961 A>G, p.Ile2321Val Variant

The p.Ile2321Val variant was detected in one ClinSeq® participant (1/870) and in 10/10,748 alleles for an allele frequency of 0.1% in EVS. It is described as pathogenic in HGMD based on a single review entry[12] and in LOVD for an additional entry[34]. The HGMD entry reported the p.Ile2321Val variant in association with MHS in one family. In a study of 36 unrelated MHS patients five members of one family harbored both the p.Ile2321Val and p.Leu13Arg variants. However, the p.Ile2321Val variant was recorded as one of eight non-causative variants due to a previous description as polymorphism in the National Center

Biotechnology Information, Database of Single Nucleotide Polymorphisms[‡‡34]. We categorized this as a *Class 2*, benign polymorphism.

‡‡ The National Center Biotechnology Information, Database of Single Nucleotide Polymorphisms. Available at: www.ncbi.nlm.nih.gov/projects/SNP. Accessed May 20, 2013.

References

1. Johnston JJ, Rubinstein WS, Facio FM, Ng D, Singh LN, Teer JK, Mullikin JC, Biesecker LG: Secondary variants in individuals undergoing exome sequencing: Screening of 572 individuals identifies high-penetrance mutations in cancer-susceptibility genes. Am J Hum Genet 2012; 91: 97-108

2. Plon SE, Eccles DM, Easton D, Foulkes WD, Genuardi M, Greenblatt MS, Hogervorst FB, Hoogerbrugge N, Spurdle AB, Tavtigian SV: Sequence variant classification and reporting: Recommendations for improving the interpretation of cancer susceptibility genetic test results. Hum Mutat 2008; 29: 1282-91

3. Akbari MR, Zhang S, Fan I, Royer R, Li S, Risch H, McLaughlin J, Rosen B, Sun P, Narod SA: Clinical impact of unclassified variants of the BRCA1 and BRCA2 genes. J Med Genet 2011; 48: 783-6

4. Bergman JE, Janssen N, van der Sloot AM, de Walle HE, Schoots J, Rendtorff ND, Tranebjaerg L, Hoefsloot LH, van Ravenswaaij-Arts CM, Hofstra RM: A novel classification system to predict the pathogenic effects of CHD7 missense variants in CHARGE syndrome. Hum Mutat 2012; 33: 1251-60

5. Greenblatt MS, Brody LC, Foulkes WD, Genuardi M, Hofstra RM, Olivier M, Plon SE, Sijmons RH, Sinilnikova O, Spurdle AB: Locus-specific databases and recommendations to strengthen their contribution to the classification of variants in cancer susceptibility genes. Hum Mutat 2008; 29: 1273-81

6. Jordan DM, Kiezun A, Baxter SM, Agarwala V, Green RC, Murray MF, Pugh T, Lebo MS, Rehm HL, Funke BH, Sunyaev SR: Development and validation of a computational method for assessment of missense variants in hypertrophic cardiomyopathy. Am J Hum Genet 2011; 88: 183-92

7. Thompson BA, Greenblatt MS, Vallee MP, Herkert JC, Tessereau C, Young EL, Adzhubey IA, Li B, Bell R, Feng B, Mooney SD, Radivojac P, Sunyaev SR, Frebourg T, Hofstra RM, Sijmons RH, Boucher K, Thomas A, Goldgar DE, Spurdle AB, Tavtigian SV: Calibration of multiple in silico tools for predicting pathogenicity of mismatch repair gene missense substitutions. Hum Mutat 2013; 34: 255-65

8. Stenson PD, Ball EV, Mort M, Phillips AD, Shiel JA, Thomas NS, Abeysinghe S, Krawczak M, Cooper DN: Human Gene Mutation Database (HGMD): 2003 update. Hum Mutat 2003; 21: 577-81

9. Fokkema IF, Taschner PE, Schaafsma GC, Celli J, Laros JF, den Dunnen JT: LOVD v.2.0: The next generation in gene variant databases. Hum Mutat 2011; 32: 557-63

10. Marchant CL, Ellis FR, Halsall PJ, Hopkins PM, Robinson RL: Mutation analysis of two patients with hypokalemic periodic paralysis and suspected malignant hyperthermia. Muscle Nerve 2004; 30: 114-7

11. Zullo A, Klingler W, De Sarno C, Ferrara M, Fortunato G, Perrotta G, Gravino E, Di Noto R, Lehmann-Horn F, Melzer W, Salvatore F, Carsana A: Functional characterization of ryanodine receptor (RYR1) sequence variants using a metabolic assay in immortalized B-lymphocytes. Hum Mutat 2009; 30: E575-90

12. Robinson R, Carpenter D, Shaw MA, Halsall J, Hopkins P: Mutations in RYR1 in malignant hyperthermia and central core disease. Hum Mutat 2006; 27: 977-89

13. Tammaro A, Di Martino A, Bracco A, Cozzolino S, Savoia G, Andria B, Cannavo A, Spagnuolo M, Piluso G, Aurino S, Nigro V: Novel missense mutations and unexpected multiple changes of RYR1 gene in 75 malignant hyperthermia families. Clin Genet 2011; 79: 438-47

14. Klein A, Lillis S, Munteanu I, Scoto M, Zhou H, Quinlivan R, Straub V, Manzur AY, Roper H, Jeannet PY, Rakowicz W, Jones DH, Jensen UB, Wraige E, Trump N, Schara U, Lochmuller H, Sarkozy A, Kingston H, Norwood F, Damian M, Kirschner J, Longman C,

Roberts M, Auer-Grumbach M, Hughes I, Bushby K, Sewry C, Robb S, Abbs S, Jungbluth H, Muntoni F: Clinical and genetic findings in a large cohort of patients with ryanodine receptor 1 gene-associated myopathies. Hum Mutat 2012; 33: 981-8

15. Dekomien G, Gencik M, Gencikova A, Klenk Y, Epplen JT: Gene symbol: RYR1. Disease: Malignant hyperthermia. Hum Genet 2005; 118: 543

16. Monnier N, Marty I, Faure J, Castiglioni C, Desnuelle C, Sacconi S, Estournet B, Ferreiro A, Romero N, Laquerriere A, Lazaro L, Martin JJ, Morava E, Rossi A, Van der Kooi A, de Visser M, Verschuuren C, Lunardi J: Null mutations causing depletion of the type 1 ryanodine receptor (RYR1) are commonly associated with recessive structural congenital myopathies with cores. Hum Mutat 2008; 29: 670-8

17. Klein A, Jungbluth H, Clement E, Lillis S, Abbs S, Munot P, Pane M, Wraige E, Schara U, Straub V, Mercuri E, Muntoni F: Muscle magnetic resonance imaging in congenital myopathies due to ryanodine receptor type 1 gene mutations. Arch Neurol 2011; 68: 1171-9

18. Zhou H, Lillis S, Loy RE, Ghassemi F, Rose MR, Norwood F, Mills K, Al-Sarraj S, Lane RJ, Feng L, Matthews E, Sewry CA, Abbs S, Buk S, Hanna M, Treves S, Dirksen RT, Meissner G, Muntoni F, Jungbluth H: Multi-minicore disease and atypical periodic paralysis associated

with novel mutations in the skeletal muscle ryanodine receptor (RYR1) gene. Neuromuscul Disord 2010; 20: 166-73

19. Broman M, Gehrig A, Islander G, Bodelsson M, Ranklev-Twetman E, Ruffert H, Muller CR: Mutation screening of the RYR1-cDNA from peripheral B-lymphocytes in 15 Swedish malignant hyperthermia index cases. Br J Anaesth 2009; 102: 642-9

20. Vukcevic M, Broman M, Islander G, Bodelsson M, Ranklev-Twetman E, Muller CR, Treves S: Functional properties of RYR1 mutations identified in Swedish patients with malignant hyperthermia and central core disease. Anesth Analg 2010; 111: 185-90

21. Torbergsena T, Karimeb B, Løseth S: P3.42 Mutation in the ryanodine receptor (RYR1) gene in a family with slowly progressive axial muscular dystrophy. Neuromuscul Disord 2011; 21: 694-695

22. Dowling JJ, Lillis S, Amburgey K, Zhou H, Al-Sarraj S, Buk SJ, Wraige E, Chow G, Abbs S, Leber S, Lachlan K, Baralle D, Taylor A, Sewry C, Muntoni F, Jungbluth H: King-Denborough syndrome with and without mutations in the skeletal muscle ryanodine receptor (RYR1) gene. Neuromuscul Disord 2011; 21: 420-7

23. Broman M, Heinecke K, Islander G, Schuster F, Glahn K, Bodelsson M, Treves S, Muller C: Screening of the ryanodine 1 gene for malignant hyperthermia causative mutations by high resolution melt curve analysis. Anesth Analg 2011; 113: 1120-8

24. Groom L, Muldoon SM, Tang ZZ, Brandom BW, Bayarsaikhan M, Bina S, Lee HS, Qiu X, Sambuughin N, Dirksen RT: Identical *de novo* mutation in the type 1 ryanodine receptor gene associated with fatal, stress-induced malignant hyperthermia in two unrelated families. Anesthesiology 2011; 115: 938-45

25. Malandrini A, Orrico A, Gaudiano C, Gambelli S, Galli L, Berti G, Tegazzin V, Dotti MT, Federico A, Sorrentino V: Muscle biopsy and in vitro contracture test in subjects with idiopathic HyperCKemia. Anesthesiology 2008; 109: 625-8

26. Ibarra MC, Wu S, Murayama K, Minami N, Ichihara Y, Kikuchi H, Noguchi S, Hayashi YK, Ochiai R, Nishino I: Malignant hyperthermia in Japan: Mutation screening of the entire ryanodine receptor type 1 gene coding region by direct sequencing. Anesthesiology 2006; 104: 1146-54

27. Kraeva N, Riazi S, Loke J, Frodis W, Crossan ML, Nolan K, Kraev A, Maclennan DH: Ryanodine receptor type 1 gene mutations found in the Canadian malignant hyperthermia population. Can J Anaesth 2011; 58: 504-13

28. Tammaro A, Bracco A, Cozzolino S, Esposito M, Di Martino A, Savoia G, Zeuli L, Piluso G, Aurino S, Nigro V: Scanning for mutations of the ryanodine receptor (RYR1) gene by denaturing HPLC: Detection of three novel malignant hyperthermia alleles. Clin Chem 2003; 49: 761-8

29. Zhou H, Yamaguchi N, Xu L, Wang Y, Sewry C, Jungbluth H, Zorzato F, Bertini E, Muntoni F, Meissner G, Treves S: Characterization of recessive RYR1 mutations in core myopathies. Hum Mol Genet 2006; 15: 2791-803

30. Jungbluth H, Zhou H, Hartley L, Halliger-Keller B, Messina S, Longman C, Brockington M, Robb SA, Straub V, Voit T, Swash M, Ferreiro A, Bydder G, Sewry CA, Muller C, Muntoni F: Minicore myopathy with ophthalmoplegia caused by mutations in the ryanodine receptor type 1 gene. Neurology 2005; 65: 1930-5

31. Gillard EF, Otsu K, Fujii J, Duff C, de Leon S, Khanna VK, Britt BA, Worton RG, MacLennan DH: Polymorphisms and deduced amino acid substitutions in the coding sequence of the ryanodine receptor (RYR1) gene in individuals with malignant hyperthermia. Genomics 1992; 13: 1247-54

32. Guis S, Figarella-Branger D, Monnier N, Bendahan D, Kozak-Ribbens G, Mattei JP, Lunardi J, Cozzone PJ, Pellissier JF: Multiminicore disease in a family susceptible to malignant

hyperthermia: Histology, *in vitro* contracture tests, and genetic characterization. Arch Neurol 2004; 61: 106-13

33. Monnier N, Kozak-Ribbens G, Krivosic-Horber R, Nivoche Y, Qi D, Kraev N, Loke J, Sharma P, Tegazzin V, Figarella-Branger D, Romero N, Mezin P, Bendahan D, Payen JF, Depret T, Maclennan DH, Lunardi J: Correlations between genotype and pharmacological, histological, functional, and clinical phenotypes in malignant hyperthermia susceptibility. Hum Mutat 2005; 26: 413-25

34. Levano S, Vukcevic M, Singer M, Matter A, Treves S, Urwyler A, Girard T: Increasing the number of diagnostic mutations in malignant hyperthermia. Hum Mutat 2009; 30: 590-8