BJA

British Journal of Anaesthesia, 121 (4): 953–961 (2018)

doi: 10.1016/j.bja.2018.07.008
Advance Access Publication Date: 10 August 2018
Translational Studies

Malignant hyperthermia, environmental heat stress, and intracellular calcium dysregulation in a mouse model expressing the p.G2435R variant of *RYR1*

J. R. Lopez[1,2,5], V. Kaura[3], C. P. Diggle[3], P. M. Hopkins[3,4] and P. D. Allen[1,2,3,*]

[1]Department of Anesthesia, Perioperative and Pain Medicine, Brigham and Women's Hospital, Boston, MA, USA, [2]Department of Molecular Biosciences, School of Veterinary Medicine, University of California at Davis, Davis, CA, USA, [3]Leeds Institute of Biomedical & Clinical Sciences, University of Leeds, Leeds, UK and [4]Malignant Hyperthermia Investigation Unit, St James' University Hospital, Leeds, UK

*Corresponding author. E-mail: p.d.allen@leeds.ac.uk

[5]Present address: Division of Neonatology, Mount Sinai Medical Center, Miami Beach, FL, USA.

Abstract

Background: The human p.G2434R variant of the *RYR1* gene is most frequently associated with malignant hyperthermia (MH) in the UK. We report the phenotype of a knock-in mouse that expresses the *RYR1* variant p.G2435R, which is isogenetic with the human variant.

Methods: We observed the general phenotype; determined the sensitivity of myotubes to caffeine-, KCl–, and halothane-induced Ca^{2+} release; determined the *in vivo* response to halothane or increased ambient temperature; and determined the *in vivo* myoplasmic intracellular Ca^{2+} concentration in skeletal muscle before and during exposure to volatile anaesthetics.

Results: *RYR1* pG2435R/MH normal (MHS-Heterozygous[Het]) or *RYR1* pG2435R/pG2435R (MHS-Homozygous[Hom]) mice were fully viable under typical rearing conditions, although some male MHS-Hom mice died spontaneously. The normalised half-maximal effective concentration (95% confidence interval) for intracellular Ca^{2+} release in myotubes in response to KCl [MH normal, MHN, 21.4 (19.8–23.1) mM; MHS-Het 16.2 (15.2–17.2) mM; MHS-Hom 11.2 (10.2–12.2) mM] and caffeine (MHN, 5.7 (5–6.3) mM; MHS-Het 4.5 (3.9–5.0) mM; MHS-Hom 1.77 (1.5–2.1) mM] exhibited a gene dose-dependent decrease, and there was a gene dose-dependent increase in halothane sensitivity. Intact animals show a gene dose-dependent susceptibility to MH with volatile anaesthetics or to heat stroke. *RYR1* p.G2435R mice had elevated skeletal muscle intracellular resting $[Ca^{2+}]_i$, (values are expressed as mean (SD)) (MHN 123 (3) nM; MHS-Het 156 (16) nM; MHS-Hom 265 (32) nM; $P<0.001$) and $[Na^{+}]_i$ (MHN 8 (0.1) mM; MHS-Het 10 (1) mM; MHS-Hom 14 (0.7) mM; $P<0.001$) that was further increased by exposure to volatile anaesthetics.

Conclusions: *RYR1* pG2435R mice demonstrated gene dose-dependent *in vitro* and *in vivo* responses to pharmacological and environmental stressors that parallel those seen in patients with the human *RYR1* variant p.G2434R.

Keywords: gene knock-in techniques; malignant hyperthermia; mouse; ryanodine receptor 1

Editorial decision: 16 July 2018; **Accepted:** 16 July 2018

Editor's key points

- The molecular mechanisms by which *RYR1* variants confer MH susceptibility are unknown.
- *RYR1* pG2435R knock-in mice, a mouse model of the most common human variant, were created and characterised phenotypically and biochemically.
- This novel mouse model recapitulates *in vitro* and *in vivo* responses to pharmacological and environmental stressors compared with the common human *RYR1* variant p.G2434R.

Malignant hyperthermia (MH) is a hypermetabolic condition triggered in genetically predisposed individuals by any of the potent volatile anaesthetics and by succinylcholine.[1] The MH crisis is characterised by hypermetabolism, hypercapnia, tachycardia, hypoxaemia, muscle rigidity, respiratory and metabolic acidosis, and hyperthermia. The great majority of MH-susceptible patients remains subclinical until challenged with pharmacological triggering agents.[1–4] If left untreated, the mortality of a fulminant MH episode is >70%, but improved understanding, better monitoring, and availability of dantrolene have reduced mortality to <8%.[1,5] The prevalence of MH susceptibility based on clinical incidence is estimated to range from as low as 1 in 250 000 to as high as 1 in 200, depending on the age and geographic location of the population, although accurate measures of MH susceptibility prevalence as a genetic predisposition remains difficult because of variable penetrance and the poor epidemiological data.[6–8] MH susceptibility can be diagnosed in patients with high *a priori* risk using the *in vitro* contracture test (IVCT) that measures contractile responses to halothane or caffeine of *vastus lateralis* or *vastus medialis* muscle biopsies.[6,9]

Molecular genetic studies have established the type 1 ryanodine receptor gene (*RYR1*) encoding the skeletal muscle sarcoplasmic reticulum (SR) Ca^{2+} release channel (RyR1 protein) as the primary locus for both MH susceptibility and central core disease (CCD).[4,6,7,10] More than 200 *RYR1* variants have been associated with MH, CCD, or both.[7] Although MH-related *RYR1* variants can be found in all regions of the gene, most have been described in three clusters corresponding to the: N-terminal (C35-R614, MH region 1), central (D2129-R2458, MH region 2), and C-terminal (I3916-A4942, MH region 3) regions of the RyR1 protein. To date, a porcine and three knock-in murine models that express *RYR1* variants analogous to variants associated with human MH have been described. Two murine, p.R163C[11] and p.Y522S,[12] and the porcine p.R615C[13–15] models have mutations in MH region 1 of the N-terminal domain of RyR1. The third currently available murine mutant RyR1 MH model, p.T4826I,[16] involves MH region 3 in the putative cytoplasmic linker between transmembrane segments S4 and S5.[17] All four models reported exhibit anaesthetic-triggered fulminant MH episodes and environmental heat stress, with varying gene–dose relationships. The molecular mechanisms by which *RYR1* variants confer MH susceptibility are unknown. A common characteristic of all animal models with MH-RyR1 mutations to date is an increased resting skeletal muscle intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$)[14,18,19] compared with non-susceptible muscle. In porcine and murine models, we have shown that exposure to halothane or isoflurane at clinically relevant concentrations causes $[Ca^{2+}]_i$ to rise several fold in MH susceptible (MHS) muscle, whereas exposure to the same concentrations of halothane or isoflurane has no effect in non-susceptible (MHN) muscle.[15,19,20]

Although interesting observations have been made using these models involving MH regions 1 and 3, the most prevalent *RYR1* variant associated with human MH is NM_000540.2(RYR1_i001):p.(Gly2434Arg (G2434R)), which is caused by a missense point mutation NM_000540.2:c.7300G>A in exon 44 involving MH region 2 of the RyR1 protein.[21] Because of its prevalence, the p.G2434R variant has been used as the comparator variant for describing differences in human phenotypes associated with different *RYR1* genotypes.[22] For example, MHS patients with the p.G2434R variant have a relatively weak IVCT phenotype and are less likely to have an elevated serum creatine kinase concentration compared with humans with either the p.T4826I or p.R163C RyR1 variants.[22] Furthermore, p.G2434R is not associated with CCD and is the most frequent variant associated with MH in the UK to be implicated in familial genotype–phenotype discordance.[7,21] The genotype–phenotype discordance is present in almost 22% of families in which segregation analysis has been done, and includes individuals who are carriers of the p.G2434R variant but have a normal IVCT and individuals who do not carry the familial p.G2434R variant but have an abnormal IVCT.[21]

Detailed study of the p.G2434R variant is crucial to identify fundamental molecular mechanisms that are generically implicated in MH. Furthermore, a model of p.G2434R is necessary if we are to have confidence that human *RYR1* genotype–phenotype relationships are recapitulated in isogenic murine models. The aim of the present study was to develop a new knock-in murine model of MH with a mutation in RyR1 MH region 2 (p.G2435R), and to characterise whole animal and cellular phenotypes of heterozygous and homozygous mice.

Methods

Animal care and maintenance

All experiments on animals from the creation of knock-in mice to establishment of their physiological and biochemical phenotypes were conducted using protocols approved by the institutional animal care and use committees (IACUCs) at Harvard Medical School, University of California at Davis, MRC Harwell, and University of Leeds; the latter two both through project licenses approved by the UK Home office. Animals were housed in specific pathogen-free conditions with free access to food and water and a 12 h light and dark cycle.

Creation of p.G2435R knock-in MH mice

Site-directed mutagenesis (QuickChange Multi-Site-Directed Mutagenesis Kit; Stratagene, La Jolla, CA, USA) was used to mutate the glycine at codon 2435 in the murine RyR1 to arginine (p.G2435R). This mutation is equivalent to amino acid position 2434 in human RyR1. A 5.862 kb *Not*I fragment harbouring *RYR1* exons 40–47 (Supplementary Fig. S1A) was isolated from a 129Sv/J mouse genomic library and used to construct the targeting vector. A bacterial locus of crossover in P1 (LoxP) recombination site flanked neomycin (G418) cassette was inserted between the 3.9 kb upstream arm and the 1.9 kb downstream arm. 129Sv embryonic stem cells were electroporated with the linearised vector and subjected to positive