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No evidence of mutations in the *CACNA1S* gene in the UK malignant hyperthermia population†

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Background. Malignant hyperthermia (MH) is an inherited, potentially fatal, pharmocogenetic disorder triggered by certain anaesthetic agents. In light of the reported genetic heterogeneity for the disorder and the recent introduction of DNA testing guidelines for the trait, we have assessed the role of the *CACNA1S* gene in MH susceptibility in UK patients. Linkage to this locus has previously been demonstrated in several European MH families.

Methods and results. We screened 200 unrelated MH-susceptible individuals for known *CACNA1S* mutations. With the aim to characterize further novel mutations at this locus, functionally relevant regions of the gene were also sequenced in 10 unrelated individuals from families where the involvement of other MH susceptibility loci was unlikely. No sequence variations were detected in any of the patients investigated.

Conclusions. Defects in *CACNA1S* are not a major cause of MH in the UK population. Diagnostic screening of this gene is unlikely to be of value to UK MH patients in the near future.

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Malignant hyperthermia (MH) is an autosomal dominant inherited disorder in which a serious disturbance in skeletal muscle calcium regulation occurs when an individual is exposed to previously demonstrated potent inhalational anaesthetics or depolarizing neuromuscular blocking drugs. Without rapid intervention, a patient suffering an MH crisis may die. For the last 30 years, the only reliable method to determine an individual's MH status has been by the *in vitro* contracture (IVC) test, whereby a muscle sample is biopsied and subsequently exposed to incremental doses of either caffeine or halothane and the relative contracture responses determined.[1] In 2001, the European MH group published guidelines for the introduction of genetic testing for the condition.[2]

Genetic analyses mapped the MH susceptibility trait to the ryanodine receptor locus (*RYR1*) on chromosome 19q12–13.2. This gene encodes the skeletal muscle sarcoplasmic reticulum calcium release channel, a key protein involved in the process of excitation–contraction coupling. To date, over 30 different mutations have been detected in the gene,[3] and 15 of these have been functionally characterized using *in vitro* cellular assays and are considered causative of MH.[2 4] It is important to demonstrate that these mutations have a 'pathogenic' effect in a functional assay, as the majority of mutations identified in MH patients are missense mutations (amino acid substitutions), which may have a minimal effect on normal calcium channel function. Approximately 25% of UK MH pedigrees

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Table 1 Primer sequences used for *CACNA1S* sequence analysis

CACNA1S exon	Primer sequence 5′–3′	
	Forward	**Reverse**
14	CTC CCT TCC ACC TAC AAC CA	GGC ATC ACG ACT GAG CAC T
15	TGG GAT CCT CTG AAG GTG TT	AGG TGC TCA CCT TGG CAG T
16	AAC GCT AAC AGG CTC TCC AG	CCC TAC TTC ACC CCA GCA C
17	AGC AAA GCA TGG GAA GAC AC	AGA TGA GAG CCG CAT CAA TC
18	CTC CAG CAG CTC ACA GCT C	CTG GGC ACC AGA CTG AGG
25	CCA CCC AGT CTG TCC TCT CT	CTC TGC CCT TCC ACC TCT G
26	TCT TGG TGC TGA CCT GTC CT	ATC CTG CCC TAC TCA TCC TG

carry one of the 15 causative MH mutations. Whilst other, currently undetected, mutations in *RYR1* may be responsible for a large percentage of MH cases (up to 50% show linkage), exclusion of linkage between MH susceptibility and *RYR1* has been reported on numerous occasions.[5] Linkage studies have implicated other candidate loci on chromosomes 1q, 3q, 5p, 7q and 17q. Therefore MH can be said to be a condition showing considerable allelic and locus heterogeneity.

Monnier and colleagues[6] determined linkage to the *CACNA1S* gene on chromosome 1q with a high degree of probability (two-point LOD score of +4.38) in a large French MH pedigree. *CACNA1S* encodes the α1 subunit of the dihydropyridine receptor (DHPR), a transverse tubule calcium channel that is tightly coupled to the ryanodine receptor.[7] The DHPR functions as the voltage sensor in excitation–contraction coupling. Upon further sequence analysis of MH-susceptible individuals within the pedigree, a G-to-A change at nucleotide 3333 in exon 25 of the *CACNA1S* gene, substituting arginine for histidine at amino acid 1086, was discovered, which segregated completely with the MH phenotype. This represented the first direct molecular evidence of MH locus heterogeneity. Subsequently Jurkatt-Rott and colleagues[8] have found a similar amino acid substitution (Arg1086Cys) in exon 25 that displays partial segregation with the MH phenotype in a German pedigree. Both exons 25 and 26 of *CACNA1S* encode the intracellular loop that links domains III and IV within the DHPR α1 subunit. Although the functional significance of this region remains unclear, one model suggests that it in some way participates in signalling with the ryanodine receptor.

The aim of this study was to determine whether mutations in the *CACNA1S* gene predispose the UK population to MH susceptibility. Results of such analyses could have implications for the genetic diagnosis of MH in the future.

Methods and results

A cohort of 200 unrelated IVC-tested MH-susceptible individuals representative of the UK MH population was tested to determine the presence or absence of the G3333A mutation (exon 25 of DHPR α1s) using a restriction enzyme digest assay.[6] This sample size was sufficient to detect a mutation with <1% prevalence. Final analysis provided us with no evidence of this mutation existing within the UK MH population, giving an estimated frequency of <0.25% (upper 95% confidence interval: 0.75%) in this group. We concluded that alongside further investigation of this domain (III–IV linking region), other regions of the DHPR α1s that interact with the ryanodine receptor protein should be analysed for possible MH associated mutations. The II–III cytoplasmic loop, an important determinant in excitation–contraction coupling,[9] is one such region encoded by the *CACNA1S* exons 14 to 18.[10]

A group of MH-susceptible individuals representing 10 UK MH pedigrees was selected. Three exhibited discordance between the *RYR1* genotype and MH-susceptibility phenotype, implying the possible presence of a second/alternative causative mutation, or linkage to another susceptibility locus. One represented a pedigree in which a known *RYR1* mutation (G7300A) completely segregated with the MH phenotype and therefore served as a *CACNA1S* mutation negative control. The remaining seven represented pedigrees in which known mutations were excluded and evidence of linkage to chromosome 19 was inconclusive. Genomic DNA samples from all representative MH-susceptible individuals were amplified by the polymerase chain reaction using exon-specific primers for exons 14–18 and 25–26 (Table 1) and sequenced.

No sequence variations were identified within this cohort for the regions investigated. Therefore mutations in *CACNA1S*, or at least these specific regions, do not appear to be a significant cause of MH in the UK MH population. It would also suggest that exons 14–18 and 25–26 are highly conserved regions of the gene and are therefore crucial to the normal function of the DHPR.

Comment

As our investigation has provided no evidence of mutations in the *CACNA1S* gene in the UK MH population, there is currently no basis to propose adding mutation screening of *CACNA1S* to that of *RYR1* in guidelines for the DNA-based diagnosis of MH for UK patients.[2] Screening for mutations in this gene may, however, be beneficial in the assessment of

MH status in other populations. Further studies examining physical or functional interactions between the DHPR and the ryanodine receptor may highlight other regions in the α1 subunit that may harbour mutations causative of MH. Alternatively, other genes of the DHPR heterotetramer complex – β and γ subunit genes on chromosome 17q, and the α2/δ subunit gene on chromosome 7q – may warrant investigation. Numerous potential candidate genes have been proposed for MH on the basis that they code for proteins involved in skeletal muscle calcium homeostasis, the disruption of which appears to be the primary physiological defect in patients suffering an MH reaction. Examples include genes coding for triadin, calsequestrin and the tacrolimus-binding protein, FKBP12. Genetic analysis to date would indicate that, if involved, such candidates are likely to be rare susceptibility loci. Evidence indicates that defects in the *RYR1* gene account for the majority of MH cases. Future research may therefore be better prioritized towards determining the presence of novel mutations within this locus.

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