Original articles

Diagnosis of malignant hyperthermia: a comparison of the in vitro contracture test with the molecular genetic diagnosis in a large pedigree

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Abstract

Malignant hyperthermia (MH) is an inherited skeletal muscle disorder and is one of the major causes of death resulting from anaesthesia. MH is currently diagnosed by the in vitro contracture test performed on a muscle biopsy. Genetic linkage analysis on an Irish MH pedigree showed that when the thresholds for the standardised European protocol for MHS diagnosis was applied, linkage between the MHS phenotype and the RYR1 locus was excluded. When we raised the threshold values for assignment of MHS status and assumed MHN diagnosis in subjects where this threshold was not attained, tight linkage between MHS and RYR1 markers was observed, suggesting that MHS is linked to the RYR1 locus in this pedigree. Confirmation of these results was borne out by the fact that all of the MHS patients in the pedigree exceeding the raised threshold carried the known MHS Gly341Arg RYR1 mutation.

The results obtained could be explained (1) by false positive diagnosis of MHS in the recombinant subjects, (2) by the presence of a mutation in a predisposing gene other than RYR1, or (3) by the presence of mild subclinical myopathies. The implications of these results for heterogeneity studies is discussed.

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Key words: malignant hyperthermia; in vitro contracture test; ryanodine receptor.

Malignant hyperthermia (MH) is an inherited human skeletal muscle disorder and is one of the major causes of death resulting from anaesthesia.^{1,2} It is categorised as a metabolic disorder of skeletal muscle.^{1,2} It can be triggered in susceptible people by all commonly used inhalational anaesthetics such as halothane and by depolarising muscle relaxants such as succinylcholine.^{1,2} The classical presentation is a progressive rise in body temperature at a

rate of 2°C or more per hour, a profoundly accelerated muscle metabolism, contractures, metabolic and respiratory acidosis, and tachycardia.^{1,2} Once initiated, a futile metabolic cycle is established and a fulminant syndrome evolves in which the body temperature may exceed 43°C (109.4°F). Many susceptible patients present with some, but not all, of the classical signs with variable intensity when exposed to agents known to induce the condition. Some known susceptible patients have had previous triggering anaesthetics without any complications.^{1,2} Thus, the early clinical diagnosis of MH is often difficult. The reported incidence of MH ranges from approximately 1 per 10 000 to 1 per 50 000 anaesthetics with an apparently higher incidence in children.³

A standardised in vitro muscle contracture test (IVCT) has been established by the European Malignant Hyperthermia Group (EMHG) for investigation of MH susceptibility.⁴ This IVCT is based on the contracture tensions induced in muscle in vitro by caffeine and halothane and allows the following diagnosis to be made: MH susceptible (MHS), MH normal (MHN), and MH equivocal (MHE). A person is diagnosed as MHS if a threshold tension of 0.2 g or greater is generated at a caffeine concentration of 2 mmol/l and independently at halothane concentration of 2%. The person is diagnosed as MHN if this threshold tension is not reached at these caffeine and halothane concentrations. MHE denotes a person in whom the threshold tension is generated with caffeine (MHE(c)) but not with halothane or vice versa (MHE(h)). The tensions generated in the IVCT in MH probands is highly variable. In a study of 40 subjects who have survived a clinical episode of MH, 35 (88%) tested as MHS (0.2 g to >2.5 g at 2 mmol/l caffeine and 2% halothane) and five (12%) tested as MHE (12% of probands).⁵ For MHN, the tensions can range from a negative tension to 0.195 g. In one study, the range of tensions recorded for MHN subjects was -0.45 to 0.1 g at 2 mmol/l caffeine and -0.3 to 0.1 g at 2% halothane.⁶ Once a person is diagnosed as being susceptible to MH, the

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anaesthetics which trigger this syndrome can be avoided.

The European IVCT has been widely accepted by most European MH testing centres. The North American testing protocol and criteria for MHS diagnosis differ significantly from the European test and both tests are not directly comparable. For example, in contrast to the European MH group, the North American MH group propose a range of values for abnormal responses and each laboratory is responsible for deciding its cut off point.⁷ Furthermore, with the North American protocol, an abnormal response to either caffeine or halothane is regarded as a positive result and the person is diagnosed as MHS. There is no MHE status defined by the North American protocol.

Over a decade, the sensitivity of the IVCT using either the European or the North American protocol was considered to be high as it was reported that patients who previously tested negative for MH susceptibility did not develop a MH reaction on exposure to trigger agents.^{8,9} Therefore, the sensitivity of MHN diagnosis by the IVCT was thought to be high. Recently, however, Isaacs and Badenhorst¹⁰ presented four patients with negative IVCT results (as diagnosed by the European protocol) despite clinical evidence of MH susceptibility. The diagnosis of MHS is considered to err on the side of false positive diagnosis,¹¹ since the failure to detect an MHS person has a potentially fatal outcome. In agreement with this, a low specificity rate was shown for non-MHS control subjects diagnosed according to the North American IVCT protocol.¹² By contrast with the North American protocol, the diagnostic criteria of the European IVCT protocol are standardised, and in an investigation of 65 non-MHS control subjects we have failed to show a false positive diagnosis by the IVCT. Furthermore, there are no published reports of non-MHS control subjects registering an MHS or MHE reaction in other European MH testing centres. The accuracy of the European IVCT protocol has been validated by genetic linkage studies and the discovery of causative MH mutations in the RYR1 gene.^{13–17,21,22} Of the seven pedigrees which we investigated for the segregation of the Gly341Arg mutation, a total of 69 people were investigated by the IVCT.¹⁵ Of these, 63 were diagnosed as either MHS or MHN and the fact that the Gly341Arg mutation segregated precisely with MHS in this large sample substantiates the validity of MHS and MHN diagnosis by the IVCT. Within these seven pedigrees, six subjects were diagnosed as MHE(c) or MHE(h) and none of the MHEs except for one MHE(c) carried the Gly341Arg mutation. In addition to this mutation, which is the most common RYR1 mutation identified to date, accounting for 10% of white MHS cases, a further seven RYR1 point mutations have been identified in MHS and central core disease (an inherited myopathy closely associated with MH^{18–20}) pedigrees, namely, Arg163Cys,²¹ Gly248Arg,¹⁴ Ile403Met,²¹ Tyr522Ser,²² Arg614Cys,¹³ Gly-2433Arg,^{16,17} and Arg2434His.²³ The identi-

fication of these mutations firmly establishes the RYR1 gene as a susceptibility gene in MH and indicates that mutations are likely to be present in the RYR1 gene in all RYR1 linked families.

Genetic heterogeneity is considered to account for up to 50% of European MH pedigrees investigated²⁴ and to date a further three MHS loci have been reported. A MHS locus has been proposed to reside in the q11.2–24 region of chromosome 17 in North American pedigrees.²⁵ However, although European studies have also shown heterogeneity,²⁶ the possibility of a second MHS locus mapping to 17q11.2–24 has been excluded to date.^{27,28} Linkage has recently been established in a single large MH pedigree between MHS and markers on the q13.1 region of chromosome 3²⁹ and tentative linkage to the q11.23–q21.1 region of chromosome 7 has been reported in another single pedigree.³⁰

Linkage studies in large Irish pedigrees showed linkage between chromosome 19 and MHS in six families.^{15,16,31} We describe here linkage analysis in the seventh Irish MH pedigree using markers linked to the chromosome 19 MHS locus and the investigation of the pedigree for known RYR1 mutations.

Methods

SUBJECTS

Pedigree C1 is Irish by descent and was referred to the MH testing centre in Cork (fig 1A). Subject 23 is the proband and was anaesthetised for tonsillectomy at 11 years of age. Following anaesthesia using suxamethonium and isoflurane, she developed pronounced and prolonged masseter muscle spasm with associated metabolic acidosis developing after eight minutes (blood gas analysis showed a pCO₂ level of 7.7, a base excess of –9, and a pH of 7.2). CK levels of 10 050 IU/l were recorded 24 hours after the episode. Family members including the proband were investigated for MH susceptibility using the standardised European IVCT.⁴ Tests were performed on fresh muscle as follows: caffeine was added at increasing concentrations of 0.5, 1.0, and 2.0 mmol/l, with an additional test at 1.5 mmol/l in some cases; halothane was added at increasing concentrations of 0.5, 1.0, and 2.0% v/v, with an additional test at 1.5% v/v in some cases. The MH status of pedigree C1 is shown in fig 1A and the results of the IVCT are presented in fig 2A and B.

GENOTYPE ANALYSIS

Blood samples were collected from 25 members of the Irish MH pedigree C1 and stored at –70°C. DNA was extracted from approximately 10 ml of whole blood by the method of Old and Higgs.³² Oligonucleotide primer sequences for the markers D19S75, RYR1, WIS1 (a microsatellite marker within the RYR1 locus), and D19S178 have been published.^{14,33–35} Genotyping of the markers was performed by PCR amplification in a 50 µl reaction volume containing 500 ng of genomic

Figure 1 MH pedigree C1 using (A) standardised European thresholds for MH status assignment; (B) altered thresholds of 0.4 g and 0.8 g tension in response to 2 mmol/l caffeine and 2% halothane, respectively, for MHS diagnosis and the assignment of the MHN status to all subjects producing a response below these thresholds; and (C) altered thresholds of 0.4 g and 0.8 g tension in response to 2 mmol/l caffeine and 2% halothane, respectively, for MHS diagnosis and the standardised European thresholds for MHN diagnosis. Symbols: shaded, MHS; unshaded, MHN; half shaded, MHE(h); unshaded with question mark, disease status unknown. The proband (subject 23) is indicated by an arrow. The results of typing for the polymorphic markers D19S75, RYR1, WIS1, and D19S178 are shown in genetic map order. Segregation of the Gly341Arg RYR1 mutation is also indicated: +, presence of the mutant allele; -, presence of the normal allele.

Figure 2 Contracture data for members of pedigree C1. The standardised European MH threshold (0.2 g) is indicated by the line and arrow. (A) Contracture results in response to 2% halothane, (B) contracture results in response to 2 mmol/l caffeine.

DNA, 100 ng of each primer, 0.2 mmol/l of each dNTP, 50 mmol/l KCl, 10 mmol/l Tris-HCl, pH 9.0 at 25°C, 0.1% Triton X-100, 1.5 mmol/l MgCl₂, 2 µCi of α^{32} P dCTP (3000 Ci/mmol), and 1.0 U of *Taq* polymerase. Reaction mixtures were covered with an equal volume of mineral oil and amplification was carried out using the "hot start" technique in a LEP thermal cycler. Annealing temperatures were as published and samples were processed through 30 temperature cycles consisting of one minute denaturing, one minute annealing, and one minute extension. Marker alleles were resolved on 6% or 8% denaturing polyacrylamide gels as described³² and detected by autoradiography following exposure at -70°C

Table 1 Two point lod scores in MH pedigree C1 with four informative markers with subjects 17 and 27 scored as MHS and subjects 20, 22, and 25 as status unknown

	Recombination fraction (θ)						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
D19S75 <i>v</i> MHS	-5.29	-1.33	-0.09	0.32	0.52	0.44	0.25
RYR1 <i>v</i> MHS	-0.52	-0.37	-0.08	0.05	0.11	0.08	0.04
WIS1 <i>v</i> MHS	-4.72	-1.94	-0.74	-0.31	0.03	0.12	0.09
D19S178 <i>v</i> MHS	-5.98	-0.62	0.55	0.89	0.96	0.74	0.40

Table 2 Two point lod scores in MH pedigree C1 with four informative markers with subjects 17 and 27 scored as MHS and subjects 20, 22, and 25 scored as MHN

	Recombination fraction (θ)						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
D19S75 <i>v</i> MHS	2.96	2.92	2.72	2.47	1.91	1.29	0.61
RYR1 <i>v</i> MHS	0.59	0.58	0.51	0.43	0.28	0.15	0.05
WIS1 <i>v</i> MHS	3.29	3.23	2.99	2.69	2.05	1.36	0.63
D19S178 <i>v</i> MHS	3.29	3.23	2.99	2.69	2.05	1.36	0.63

overnight with intensifying screens. Screening for the known RYR1 mutations were performed as previously described.^{13-16 21-23}

LINKAGE ANALYSIS

For linkage analysis, two point lod scores were calculated with the LINKAGE program.³⁶ MHS penetrance was taken to be 0.98. The frequency of the MHS allele was assumed to be 0.0001. Linkage analysis was performed using three different criteria for MH diagnosis. In the first instance, the criteria for diagnosis were according to the standardised EMHG protocol and an unknown disease status was assigned to MHE subjects. In the second instance, the threshold for diagnosis was raised to 0.4 g tension at a caffeine concentration of 2 mmol/l and to 0.8 g tension at a halothane concentration of 2% v/v. People registering a tension greater than these thresholds were scored as MHS while those registering a tension below these thresholds were scored as MHN. In the third instance, a threshold of 0.4 g tension at 2 mmol/l caffeine and 0.8 g tension at 2% halothane was used for assignment of MHS status and a threshold of below 0.2 g tension at 2 mmol/l caffeine and 2% halothane was used for MHN diagnosis (the accepted European criteria for MHN diagnosis). An unknown disease status was assigned to those who fell between these two thresholds.

Results

The large Irish MH pedigree C1 investigated here is presented in fig 1A. The markers D19S75, RYR1, WIS1, and D19S178 were genotyped in the members of the pedigree (fig 1A). Linkage analysis was initially performed according to the criteria agreed by the Genetics Section of the European Malignant Hyperthermia Group.²⁶ Two point lod scores calculated with the LINKAGE package are presented in table 1. The lod scores generated at $\theta=0.00$ for the markers D19S75, RYR1, WIS1, and D19S178 were -5.29, -0.52, -4.72, and -5.98 respectively. The RYR1 marker is a PCR RFLP and as such is not as informative as the other microsatellite markers.¹⁴ The WIS1 marker is a microsatellite within the RYR1 locus.³⁴ These results clearly show that MHS is not linked to the RYR1 locus in this pedigree. This may indicate true heterogeneity in this pedigree or, alternatively, could be explained by false positive or false negative diagnosis of MHS or MHN respectively. To investigate the possibility of the former, we again performed linkage analysis and applied altered sets of parameters where subjects generating a tension of equal to, or greater than 0.4 g, 0.6 g, 0.8 g in response to 2 mmol/l caffeine and a tension of equal to, or greater than 0.4 g, 0.6 g, 0.8 g in response to 2% halothane, respectively, were scored as MHS while all subjects producing a response below these thresholds were scored as MHN. Using a threshold value of 0.4 g in response to 2 mmol/l caffeine and of 0.8 g in response to 2% halothane for MHS diagnosis, the linkage analysis

Table 3 Two point lod scores in MH pedigree C1 with four informative markers with subjects 17 and 27, and 20, 22, and 25 scored as status unknown

	Recombination fraction (θ)						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
D19S75 <i>v</i> MHS	2.28	2.24	2.09	1.88	1.44	0.95	0.44
RYR1 <i>v</i> MHS	0.50	0.49	0.44	0.38	0.26	0.15	0.06
WIS1 <i>v</i> MHS	2.40	2.36	2.18	1.96	1.47	0.95	0.42
D19S178 <i>v</i> MHS	2.40	2.36	2.20	1.98	1.52	1.01	0.47

clearly showed that MHS in this pedigree is linked to the RYR1 locus (table 2) with a maximum lod score of 3.29 being recorded in favour of linkage of MHS to WIS1 at $\theta=0.00$. These altered diagnostic parameters identified subjects 17 and 27 as MHN. The MH status of the pedigree using these altered thresholds is shown in fig 1B. A third analysis was performed where the threshold of 0.4 g tension at 2 mmol/l caffeine and 0.8 g tension at 2% halothane was used for the assignment of MHS status and a threshold of below 0.2 g tension at 2 mmol/l caffeine and 2% halothane was used for MHN diagnosis (the accepted European criteria for MHN diagnosis). In this case the status of subjects 20, 22, and 25 (diagnosed as MHE by the European protocol) and subjects 17 and 27 (diagnosed as MHS by the European

protocol) were scored as unknown. The MH status of the pedigree using these altered thresholds is shown in fig 1C. In this case, a maximum lod score of 2.4 was recorded in favour of linkage of MHS to WIS1 at $\theta=0.00$ (table 3).

In light of the findings of the linkage analysis, we analysed the contracture data of the members of this pedigree in two ways. Firstly, we graphed the tensions recorded in the IVCT at 2 mmol/l caffeine and 2% halothane for each person (fig 2A and B). As can be seen, subjects 17 and 27 produce the weakest response of the MHS patients to the test agents. Secondly, analysis of the tensions generated in each MHS patient at different doses of caffeine and halothane (fig 3A and B) show that subjects 17 and 27 produced the weakest response at all concentrations of the test agents used in the IVCT. Therefore these two people could reasonably be considered in a different category from the other MHS patients in the family and could represent false positives from a genetic analysis viewpoint.

The pedigree was screened for the presence of the eight RYR1 mutations identified to date. The Gly341Arg mutation was identified in all MHS subjects in the pedigree with the exception of subjects 17 and 27 (fig 1A). Thus, the Gly341Arg mutation segregated with the MHS phenotype using the altered diagnostic criteria. Furthermore, the MHN and MHE (as defined by the standardised European MH protocol) subjects in this pedigree were negative for the presence of the Gly341Arg mutation. Of the nine people who had not yet been investigated by the IVCT, two (subjects 16 and 19) were positive for the presence of the mutation while seven (subjects 4, 9, 13, 15, 18, 29, and 30) were negative for the presence of the mutation.

Discussion

Genetic linkage studies on MH have shown linkage to the RYR1 locus in six Irish pedigrees investigated.^{15,16,31} The results of the linkage analysis on a seventh pedigree are shown here and clearly exclude linkage to the RYR1 locus when the standardised European protocol for MHS diagnosis is applied. In this pedigree, there are two key MHS subjects (17 and 27) who were recombinant for the RYR1 and RYR1 linked markers (fig 1A). Analysis of the contracture data showed that the recombinant MHS subjects displayed a significantly weaker response than the other affected members (figs 2 and 3). To investigate the possibility of genetic heterogeneity in this pedigree, we chose to perform the linkage analysis using altered diagnostic parameters. When the threshold for MHS diagnosis was raised to 0.4 g tension at 2 mmol/l caffeine and 0.8 g tension at 2% halothane and subjects exhibiting tensions below these values were classified as MHN, a maximum lod score ($Z=3.29$ at $\theta=0.00$) in favour of linkage of MHS to the markers WIS1 and D19S178 (fig 1B, table 2) was generated. This clearly shows linkage of MHS to the RYR1 locus in this pedigree. Using the same threshold for MHS diagnosis while maintaining the

Figure 3 Dose response curve of members of MHS pedigree C1 during the IVCT. (A) Tension generated in response to increasing concentration of halothane, (B) tension generated in response to increasing concentration of caffeine.

standardised threshold for MHN diagnosis (fig 1C, table 3), a maximum lod score ($Z=2.4$ at $\theta=0.00$ for the markers WIS1 and D19S178) was generated. These results strongly suggest that MHS is linked to the RYR1 locus in this pedigree. Confirmation of these results was borne out by the fact that all of the MHS subjects in the pedigree, with the exception of the recombinant subjects 17 and 27, carried the Gly341Arg RYR1 mutation, which is the most common MHS mutation known to date.¹⁵ The alteration of the diagnostic criteria applied here is a valid and rational approach from a linkage analysis point of view, since raising the diagnostic threshold does not impose a bias on the analysis.

MHS diagnosis, indicative of predisposition to MH, in the standardised European protocol has been considered to have a high specificity and has been validated by the results of several linkage and mutation analyses.^{13-17 21-23 31} Apart from the report of Isaacs and Badenhorst,¹⁰ the specificity of MHN diagnosis, indicative of the normal phenotype, is also considered high and has also been validated by the molecular genetic analyses.^{13-17 21-23 31} The MHE category represents those subjects who cannot be assigned to the MHN or MHS group and probably represents predisposed and non-predisposed people,¹⁵ although all MHE patients must be considered susceptible to MH from a clinical viewpoint. The existence of the MHE category accounts for the high specificity of the MHS and MHN diagnosis. By contrast, the North American protocol does not have the equivalent of the MHE category and, as such, diagnosis of susceptibility is considered to err significantly on the false positive side in order to achieve high sensitivity at the expense of reduced specificity.¹¹

In one study of a North American MH pedigree, MacKenzie *et al*³⁷ excluded linkage between MHS and the RYR1 locus using the North American protocol. However, when they altered the diagnostic parameters, linkage between RYR1 and MHS was demonstrated. The altered parameters used cannot be applied to pedigrees tested by the standardised European protocol because of the differences in the testing procedures and, unlike the pedigree investigated in our work, a MH causative mutation was not identified in the North American pedigree.

The contracture response of the two MHS recombinants in pedigree C1 to the test agents is significantly lower than those of the other MHS patients in this pedigree. It is unclear why these people registered an MHS reaction. The abnormal contractures could be explained (1) by false positive diagnosis of MHS in the recombinant subjects, (2) by the presence of a mutation in a predisposing gene other than RYR1 (examination of the haplotypes excludes the presence of a second RYR1 mutation segregating in the pedigree), or (3) by the presence of subclinical myopathies which alters their response. Histological examination of the muscle biopsies of these people showed the presence of occasional cores in some type II

fibres in subject 27 while subject 17 appeared normal.

Unlike most other genetic disorders, the MH clinical phenotype is usually only observed in one or two patients per pedigree. The reason for this is that once a person exhibits a MH episode, all members of his/her pedigree are considered to be MHS and avoid the use of triggering anaesthetic agents unless diagnosed as MHN by the IVCT. The current thresholds for MHS assignment have been derived from IVCT investigations of probands. Apart from the four cases reported by Isaacs and Badenhorst,¹⁰ no proband has generated tensions below these thresholds. As the clinical phenotype is rarely seen in MHS patients (apart from probands), it is difficult to calculate a false positive rate for the IVCT. Molecular genetic analysis offers an opportunity to determine the false positive rate. In the case reported here, we have observed two patients diagnosed as MHS in the European IVCT who are negative for the segregating Gly341Arg mutation. Assuming that the frequency of MHS in the population is low, the simplest and most likely explanation is that these people are false positives. To date, the Gly341Arg mutation has been reported in all 35 MHS subjects from seven MH pedigrees diagnosed by the European IVCT.¹⁵ In the pedigree presented here, the mutation is present in five of the seven MHS subjects. Taken together, this indicates a false positive rate of $2/42=5\%$. Statistical analysis of the IVCT tensions in subjects in a significant number of MH pedigrees where known MHS mutations are segregating may allow the assignment of a probability score for false positive diagnosis based on the tensions recorded in the IVCT. We are currently in the process of performing such an analysis. Such a score would be very useful for linkage studies but would have little use clinically.

Heterogeneity has been reported in MH and some authors have estimated that about 50% of cases are not RYR1 linked.²⁴ Interestingly, in a systematic linkage study using a set of polymorphic microsatellite markers covering the entire genome performed on MH pedigrees where exclusion of the RYR1 locus had been established, a convincing second MHS locus was only identified in a single family on the q13.1 region of chromosome 3.²⁹ This result, taken together with the results presented here, indicates that the incidence of genetic heterogeneity in the MH population may be lower than predicted to date. Therefore, we suggest that where MH families show non-linkage to the RYR1 locus with the standardised thresholds, linkage analysis should be performed with altered diagnostic thresholds to improve the specificity of the diagnosis.

Since misdiagnosis of MHS subjects as MHN is potentially fatal, the alteration of the IVCT diagnostic criteria should obviously only be performed to assist genetic analysis. As the recombinant subjects 17 and 27 may actually be susceptible to MH for reasons other than the Gly341Arg RYR1 mutation, we stress that all MHS and MHE patients as diagnosed by the standardised European protocol should al-

ways be considered MHS clinically to avoid the possibility of them reacting to triggering agents during anaesthesia.

It is unclear why some people within a family where a known MHS mutation is present exhibit an MHE or MHS response in the standardised European IVCT in the absence of the mutation. Further investigation of such subjects is warranted to establish the factors responsible for a MHE or MHS response in apparently normal people.

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- Ellis FR, Heffron JJA. Clinical and biochemical aspects of malignant hyperthermia. In: Atkinson RS, Adams AP, eds. *Recent advances in anaesthesia and analgesia*. Vol 15. New York: Churchill-Livingstone, 1985:173–207.
- Gronert GA. Malignant hyperthermia. In: Engel AG, Banker BQ, eds. *Myology*. Vol II. New York: McGraw-Hill Press, 1986:1763–84.
- Britt BA. Dantrolene, an update. In: Britt BA, ed. *Malignant hyperthermia*. Amsterdam: Martinus-Nijhoff Press, 1987:325–67.
- European Malignant Hyperthermia Group. A protocol for investigation of malignant hyperpyrexia susceptibility. *Br J Anaesth* 1984;56:1267–9.
- Ording H. In: Britt, BA, ed. *Malignant hyperthermia*. Amsterdam: Martinus-Nijhoff Press, 1987:269–77.
- Ording H, Bendixen D. Sources of variability in halothane and caffeine contracture tests for susceptibility to malignant hyperthermia. *Eur J Anaesthesiol* 1992;9:367–76.
- Larach MG. Standardised of the caffeine halothane contracture test. *Anesth Analg* 1989;69:511–5.
- Ording H, Hedengran AM, Skovgaard LT. Evaluation of 119 anaesthetics received after investigation for susceptibility to malignant hyperthermia. *Acta Anaesthesiol Scand* 1991;35:711–16.
- Allen GC, Rosenberg H, Fletcher JE. Safety of general anaesthesia in patients previously tested negative for malignant hyperthermia susceptibility. *Anesthesiology* 1990;72:619–22.
- Isaacs H, Badenhorst ME. False-negative results with muscle caffeine halothane contracture testing for malignant hyperthermia. *Anesthesiology* 1993;79:5–9.
- Larach MG. Should we use muscle biopsy to diagnose malignant hyperthermia susceptibility. *Anesthesiology* 1993;79:1–4.
- Larach MG, Landis JR. False positive diagnosis of malignant hyperthermia susceptibility on control subjects using the North American caffeine halothane contracture test. *Anesthesiology* 1990;73:A1013.
- Gillard EF, Ostu K, Fujii J, et al. A substitution of cysteine for arginine 614 in the ryanodine receptor is potentially causative of human malignant hyperthermia. *Genomics* 1991;11:751–5.
- Gillard EF, Ostu K, Fujii J, et al. 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.
- Quane KA, Keating KE, Manning BM, et al. Detection of a novel common mutation in the ryanodine receptor gene in malignant hyperthermia: implications for diagnosis and heterogeneity studies. *Hum Mol Genet* 1994;3:471–6.
- Keating KE, Quane KA, Manning BM, et al. Detection of a novel RYR1 mutation in four malignant hyperthermia pedigrees. *Hum Mol Genet* 1994;3:1855–8.
- Phillips MS, Khanna VK, de Leon S, et al. The substitution of Arg for Gly2433 in the human skeletal muscle ryanodine receptor is associated with malignant hyperthermia. *Hum Mol Genet* 1994;3:2181–6.
- Dubowitz V, Roy S. Central core disease in muscle: clinical, histochemical and electron microscopic studies of an affected mother and child. *Brain* 1970;93:138–46.
- Denborough MA, Dennett X, Anderson RM. Central core disease and malignant hyperthermia. *BMJ* 1973;i:272–6.
- Eng GD, Epstein BS, Engel WK, McKay DW, McKay R. Malignant hyperthermia and central core disease in a child with congenital dislocating hips: case presentation and review. *Arch Neurol* 1978;35:189–97.
- Quane KA, Healy JMS, Keating KE, et al. Mutations in the ryanodine receptor gene in central core disease and malignant hyperthermia. *Nature Genet* 1993;5:51–5.
- Quane KA, Keating KE, Healy JMS, et al. Mutation screening of the RYR1 gene in malignant hyperthermia: detection of a novel Tyr to Ser mutation in a pedigree with associated central cores. *Genomics* 1994;23:236–9.
- Zhang Y, Chen HS, Khanna VK, et al. A mutation in the human ryanodine receptor gene associated with central core disease. *Nature Genet* 1993;5:46–9.
- Ball SP, Johnson KJ. The genetics of malignant hyperthermia. *J Med Genet* 1993;30:89–93.
- Levitt RC, Olckers A, Meyers S, et al. Evidence for the localisation of a malignant hyperthermia susceptibility locus (MHS 2) to human chromosome 17q. *Genomics* 1992;14:562–6.
- Deufel T, Golla A, Iles D, et al. Evidence for genetic heterogeneity of malignant hyperthermia susceptibility. *Am J Hum Genet* 1992;50:1153–61.
- Iles DE, Segers B, Sengers CA, et al. Genetic mapping of the β_1 - and γ -subunits of the human skeletal muscle L-type voltage-dependent calcium channel on chromosome 17q and exclusion as candidate genes for malignant hyperthermia susceptibility. *Hum Mol Genet* 1993;2:863–8.
- Sudbrak R, Golla A, Hogan K, et al. Exclusion of malignant hyperthermia susceptibility (MHS) from a putative MHS2 locus on chromosome 17q and of the α_1 , β_1 , and γ subunits of the dihydropyridine receptor calcium channel as candidates for the molecular defect. *Hum Mol Genet* 1993;2:857–62.
- Sudbrak R, Procaccio V, Klausnitzer M, et al. Mapping of a further malignant hyperthermia susceptibility locus to chromosome 3q13.1. *Am J Hum Genet* 1995;56:684–91.
- Iles DE, Lehmann-Horn F, Scherer SW, et al. Localisation of the gene encoding the $\alpha_2\delta$ -subunits of the L-type voltage-dependent calcium channel to chromosome 7q and analysis of the segregation of flanking markers in malignant hyperthermia susceptible families. *Hum Mol Genet* 1994;3:969–75.
- McCarthy TV, Healy JMS, Heffron JJA, et al. Localisation of the malignant hyperthermia susceptibility locus to human chromosome 19q12–13.2. *Nature* 1990;343:559–61.
- Old JM, Higgs DR. In: Weatherall D, ed. *Methods in enzymology*. Edinburgh: Churchill-Livingstone 1983:74.
- Weber JL, Kappel C, May PE, Kwitek AE. Dinucleotide repeat polymorphism at the D19S75 locus. *Nucleic Acids Res* 1990;18:4639.
- Couch FJ, Hogan K, McCarthy TV, Gregg RG. Dinucleotide repeat polymorphism at the RYR1 locus (19q13.1). *Nucleic Acids Res* 1991;19:5094.
- Weber JL. Marshfield Markers Database 1991, Release 5.
- Lathrop GM, Lalouel HM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 1984;81:3442–6.
- MacKenzie AE, Allen G, Lahey D, et al. A comparison of the caffeine halothane muscle contracture test with the molecular genetic diagnosis of malignant hyperthermia. *Anesthesiology* 1991;75:4–8.