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Evidence for Genetic Heterogeneity of Malignant Hyperthermia Susceptibility

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Summary

A locus for malignant hyperthermia susceptibility (MHS) has been localized on chromosome 19q12-13.2, while at the same time the gene encoding the skeletal muscle ryanodine receptor (RYR1) also has been mapped to this region and has been found to be tightly linked to MHS. RYR1 was consequently postulated as the candidate for the molecular defect causing MHS, and a point mutation in the gene has now been identified and is thought to be the cause of MH in at least some MHS patients. Here we report the results of a linkage study done with 19q12-13.2 markers, including the RYR1 cDNA, in two Bavarian families with MHS. In one of the families, three unambiguous recombination events between MHS and the RYR1 locus were found. In the second family only one informative meiosis was seen with RYR1. However, segregation analysis with markers for D19S75, D19S28, D19S47, CYP2A, BCL3, and APOC2 shows that the crossovers in the first family involve the entire haplotype defined by these markers flanking RYR1 and, furthermore, reveals multiple crossovers between these haplotypes and MHS in the second family. In these families, pairwise and multipoint lod scores below -2 exclude MHS from an interval spanning more than 26 cM and comprising the RYR1 and the previously described MHS locus. Our findings thus strongly suggest genetic heterogeneity of the MHS trait and prompt the search for another MHS locus.

Introduction

Malignant hyperthermia (MH) is a pharmacogenetic disease with autosomal dominant inheritance (Kalow 1987). The reported incidence is 1:12,000–1:40,000 (Britt and Kalow 1970; Ellis and Halsall 1980). Affected patients are at high risk for undergoing a fulminant MH crisis when exposed to certain inhalative anesthetics or muscle relaxants commonly used in anesthesia protocols. The MH crisis is characterized by a rapidly progressing increase in body temperature, acidosis, hypoxia, and muscular symptoms comprising masseter or generalized muscle spasm and rhabdomyolysis. If the crisis is not immediately recognized and treated with the drug dantrolene, the outcome is lethal in $\leqslant 70\%$ of cases. To date, noninvasive diagnostic tests providing sufficient diagnostic sensitivity and specificity are not available. However, patients at risk can be identified by the so-called in vitro muscle-contracture test, which is performed on a freshly obtained muscle biopsy sample. Protocols for this test have been standardized and evaluated by the European (European Malignant Hyperpyrexia Group 1984; Ørding 1988) and North American (Larach 1989) Study Groups for Malignant Hyperthermia, and they allow individuals to be defined as having MH susceptibility (MHS) or as being MH negative (MHN), or MH equivocal (MHE).

Biochemical data obtained in animal studies had pointed to a defect of the ryanodine receptor, a

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calcium-release channel expressed in the sarcoplasmic reticulum, in porcine MH (Mickelson et al. 1988). It was also in the pig model that MHS could be linked to the glucose phosphate isomerase (GPI) locus as part of a syntenic linkage group which has been well conserved through evolution and which has homologues in a variety of species (Cavanna et al. 1990). This directed the original linkage studies with MHS in humans to chromosome 19q12-13.2, where the human homologue for this group is located.

An MHS locus has been localized on chromosome 19q12-13.2 (fig. 1) in Irish families (McCarthy et al. 1990), and, at the same time, the gene for the skeletal muscle ryanodine receptor (RYR1) was mapped to the same region (MacKenzie et al. 1990) and was shown to be tightly linked to MHS in Canadian families (MacLennan et al. 1990). The ryanodine receptor gene was proposed as a candidate for the molecular defect in MH, and recently a point mutation in this gene was identified and described as the probable cause of malignant hyperthermia in MH-prone pigs (Fujii et al. 1991; Otsu et al. 1991). This mutation was also found in 1 of 35 human MH families where it segregates with MHS (Gillard et al. 1991).

In the present paper we present the results of linkage studies with markers from the chromosome 19q12-13.2 region in two Bavarian families with MHS defined by the in vitro muscle-contracture test according to the European protocol. The aim of these studies was to investigate possible genetic heterogeneity of the MHS trait.

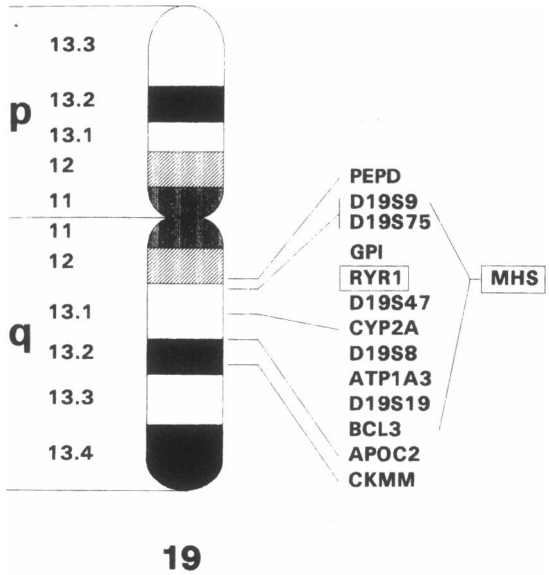


Figure 1 Chromosome 19, with markers for 19q12-13.2 in physical map order (Ropers and Pericak-Vance 1991) and including MHS(1) and RYR1 loci (McCarthy et al. 1990; MacLennan et al. 1990). Shown underlined are markers used in the present study: D19S75 (MFD13 [Weber et al. 1990]), D19S28 (p5818/*Taq*I [Smeets et al. 1989]), RYR1 (HRR3-1000/*Bam*HI [MacLennan et al. 1990]), D19S47 (MFD9 [Weber and May 1989]), CYP2A (pHP450(1)*Sst*I [Wainwright et al. 1985]), BCL3 (pα1.4/*Eco*RI/*Mlu*I [MacKenzie et al. 1989]), and APOC2 (MFD5 [Weber and May 1989] and NJ3.1/*Taq*I [Humphries et al. 1983]).

Subjects and Methods

Probands

Both family MH006 and family MH009 (fig. 2) were referred to our clinic for neurological examination and testing by the in vitro muscle-contracture test after one individual in each had suffered from a fulminant MH crisis during anesthesia. All probands were examined by clinicians experienced in the anesthesiological and neurological aspects of MH. The in vitro muscle-contracture test was performed on a fresh muscle sample obtained by biopsy. Part of each muscle sample obtained for the test was used for morphological evaluation. Two complete sets of venous blood samples for DNA extraction were obtained at different times from a total of 40 probands (16 in family MH006 and 24 in family MH009) including all 32 (12 in family MH006 and 20 in family MH009) of the members tested with the in vitro muscle-contracture test and 7 (1 in family MH006 and 6 in family MH009) untested parents of tested children. A total of 19 (11 in family MH006 and 8 in family MH009) individuals were typed as MHS, and 10 (1 in family MH006 and 9 in family MH009) were typed as MHN. Individuals 301 and 309 in pedigree MH009 were not tested, but individual 301 has suffered from a probable MH crisis, and both have affected sibs and children and were therefore assumed to be obligate MHS. Three individuals with MHE test results, all from family MH009, were also included in the study.

In Vitro Muscle-Contracture Test

The in vitro muscle-contracture test was performed according to the European standard protocol (European Malignant Hyperpyrexia Group 1984). Detailed descriptions of the procedure have been published by us previously (Klein et al. 1987; Lehmann-Horn and Iaizzo 1990).

DNA Analysis

Genomic DNA was prepared from approximately 10 ml of EDTA whole blood, according to methods

described elsewhere (Kunkel et al. 1977). For RFLP analysis, 5-μg aliquots of DNA were digested with the appropriate restriction enzyme (BRL Gibco). Digested DNA samples were separated on 0.8% agarose gels in Tris-borate buffer and were transferred to positively charged nylon membranes (Hybond N+; Amersham), according to standard protocols (Sambrook et al. 1989). Blots were hybridized at 65°C with DNA probes radiolabeled by the random primer technique (Feinberg and Vogelstein 1983). Following hybridization, they were washed to a final stringency of 0.015 M NaCl, 0.015 M sodium citrate (0.1 × SSC) at 65°C, and the bands were visualized by autoradiography at −70°C with double-intensifying screens on Fuji RX film. Primers and PCR conditions, labeling of primers, electrophoresis, and autoradiography for microsatellite repeat polymorphisms were as described elsewhere (Weber and May 1989; Weber et al. 1990) for the three markers used in the study.

Linkage Analysis

Pairwise and multipoint linkage analyses were performed using the LINKAGE program (Lathrop et al. 1984). Two liability classes were used for MHS probands: for the index patients with a documented classical MH crisis, penetrance was taken to be 1.00; for all other probands, typed as either MHN or MHS by the in vitro muscle-contracture test, and for obligate MHS individuals, a penetrance of .98 was used in the calculations. The phenocopy rate was set to .01. All untested and MHE-typed individuals were given the disease status "unknown." A frequency of .0001 was assumed for the MHS allele, and the mutation rate was taken to be .00001. In order to facilitate the computer analysis, the number of alleles for the microsatellite markers was reduced to include only those actually observed in the pedigree, and a correction was made for the allele frequencies. Pooled data from both pedigrees have been used for the multipoint analysis, but only individuals from the left-hand part of pedigree MH009 have been included, eliminating the loop through individual 204. Physical and genetic maps for the region have been reported (Brunner et al. 1989; Johnson et al. 1989; Korneluk et al. 1989; Schonk et al. 1989) and compiled at HGM 11 (Ropers and Pericak-Vance 1991) (fig. 1).

Results

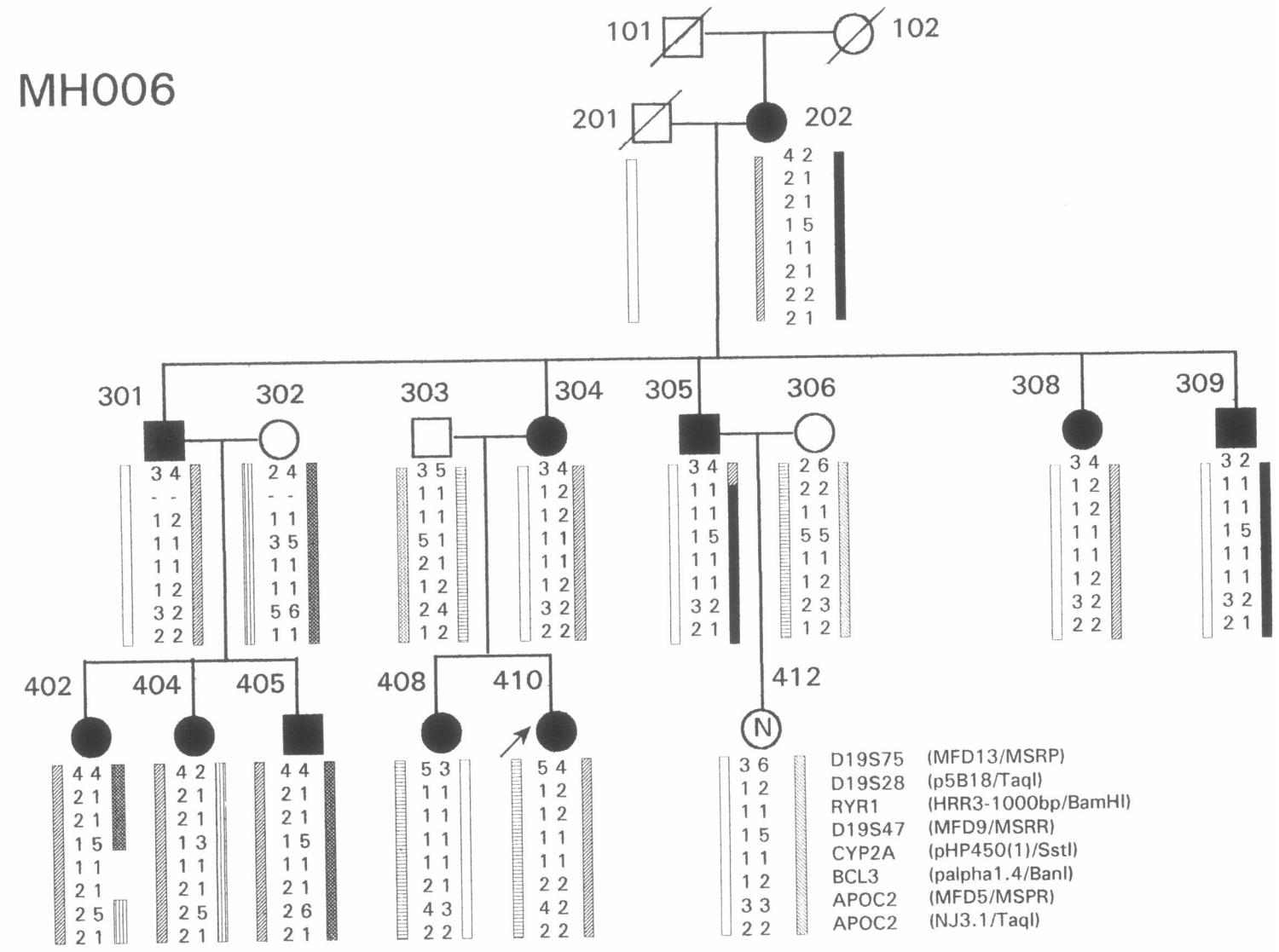
The pedigrees of families MH006 and MH009 are shown in figure 2. Both families originate from southeastern Bavaria.

The index patient in family MH006 (410), who had previously undergone one general anesthesia without complications, in 1986 suffered from a fulminant reaction under anesthesia with succinyl choline (100 mg) and isoflurane, during which her core temperature increased to 42°C. She survived under immediate treatment with dantrolene but had to be transferred to an intensive-care unit for several days. For some time following this episode she suffered from muscle pain, and she has felt a slight muscle weakness since. Five weeks after this episode she came to our clinic for clinical examination and the in vitro muscle-contracture test was performed.

The medical history of this family revealed that the mother of individual 202 had unexpectedly died after an intravenous injection in 1960. No further information is available on this case. A cousin of the proband (daughter of individual 308) suffers from seizures and is mentally handicapped. There is no history of muscle disease in the family.

Clinical examination of the index patient in family MH006 gave no abnormal findings except for a slight weakness of her proximal muscles. Serum creatine kinase (CK) levels were persistently elevated to >500 U/liters. No indications of muscle disease were found in any of the other family members examined, except for elevated serum CK levels in some of the affected individuals (table 1).

In family MH009, individual 425 suffered, in 1986, from a fulminant MH crisis following general anesthesia with a volatile anesthetic. Hours after the operation was completed, he developed hyperthermia (41.9°C), tachycardia (160 beats/min), hypoxia (P_{O_2} 51 mm Hg), and hypercapnia (P_{CO_2} 50 mm Hg). Serum potassium was elevated to 7.1 mmol/liter, and serum CK increased to 99,000 U/liter. He was transferred to an intensive-care unit and was treated with dantrolene, which normalized his body temperature and pulse within 20 min. He was discharged from the intensive-care unit 4 d later, in good health and with only his serum CK still elevated to 13,400 U/liter. It was then found that another member of the family, individual 301, also had a medical history suggestive of MH. He had survived a crisis which had occurred during his third exposure to volatile anesthetics in a general anesthesia including the use of halothane, enflurane, and succinyl choline. It was also noted that one individual, 526, suffered from a chronic myopathy which had been diagnosed as mitochondrial myopathy at the age of 18 mo, and before anything was known about the occurrence of MH in the family. Both her parents, who are second cousins, have MHS,


MH006
101
102
201
202
301
302
303
304
305
306
308
309
402
404
405
408
410
412
N
D19S75 (MFD13/MSRP)
D19S28 (p5B18/TaqI)
RYR1 (HRR3-1000bp/BamHI)
D19S47 (MFD9/MSRR)
CYP2A (pHP450(1)/SstI)
BCL3 (palpha1.4/BanI)
APOC2 (MFD5/MSPR)
APOC2 (NJ3.1/TaqI)

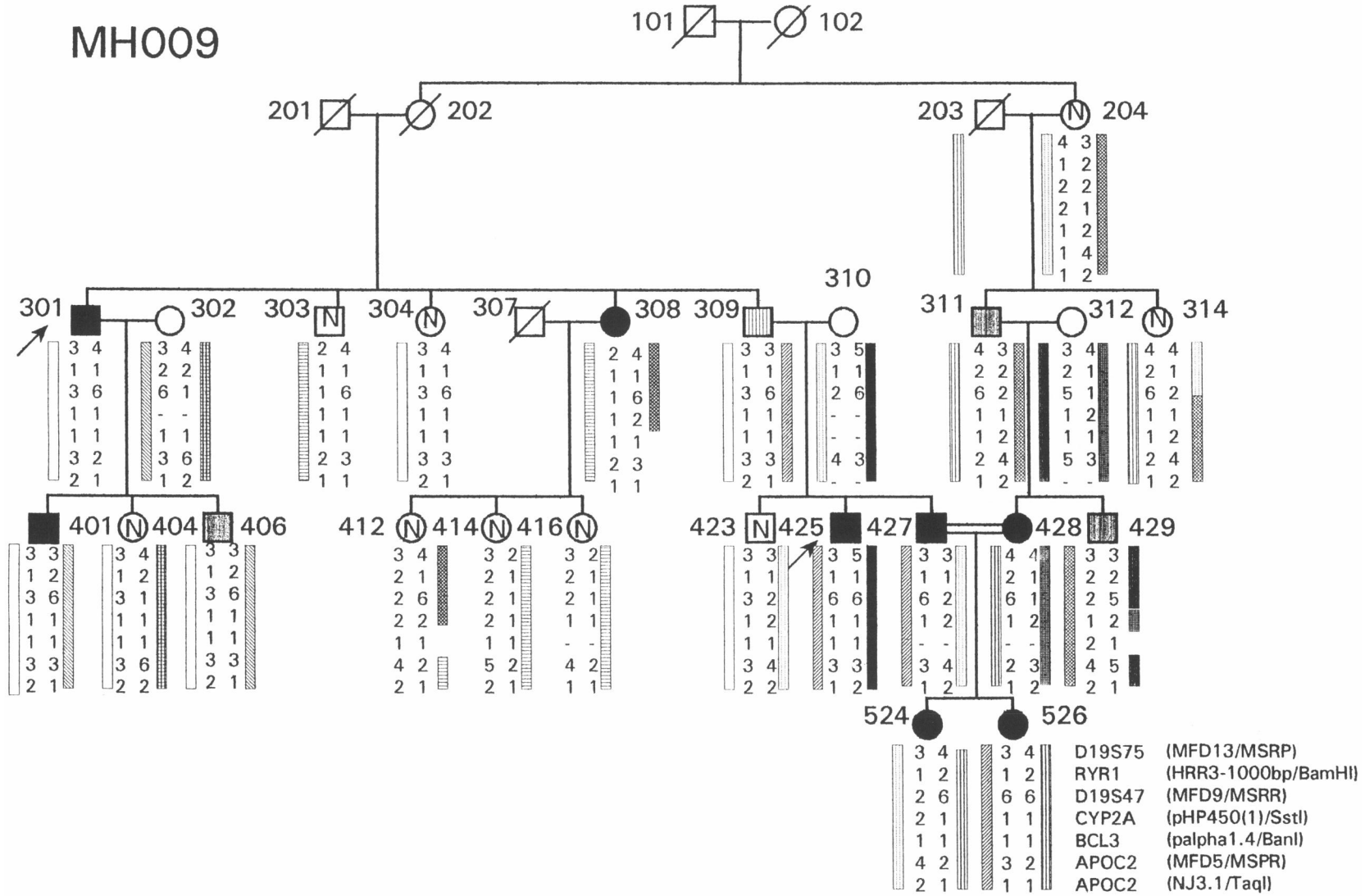


Figure 2 Pedigree of families MH006 and MH009, which have MHS. Blackened symbols denote patients tested with the in vitro contracture test and typed as MHS (susceptible); hatched symbol denotes patient untested but obligate MHS; unblackened symbol with "N" denotes patients tested and typed as MHN; shaded symbols denote MHE-typed probands; and empty unblackened symbols denote patients not tested. Index patients with an MH crisis are indicated by arrows. In pedigree MH006, patient 410 suffered from a fulminant MH crisis and subsequently has been tested MHS, and the mother of 202 reportedly died suddenly following an injection. In pedigree MH009, patient 425 has suffered from an MH crisis and is considered MHS although not tested, and 301 survived an event suggestive of an MH crisis. Both 301 and 309 have not been tested but, because they have affected sibs and offspring, are assumed to be obligate MHS. Both individual 524 and individual 526 in this pedigree have two MHS-typed parents and therefore may carry two MHS chromosomes, and 526 also suffers from a chronic myopathy and shows significantly lower thresholds in the in vitro contracture test, which makes her a likely homozygous or compound heterozygous MHS patient. Note, however, that she would most likely be carrying two independent MHS chromosomes, since individual 204 is MHN. The results of typing with RFLP and microsatellite repeat markers for the loci D19S75, D19S28, RYR1, D19S47, CYP2A, BCL3, and APOC2 are shown. (For details of the markers, see legend to fig. 1.) When defined haplotypes could be constructed, they have been marked by bars with different shading. Two single crossovers are found in pedigree MH006, in 305 (between D19S75 and D19S28) and in 402 (between D19S47 and APOC2); and two single crossovers are found in pedigree MH009, in 314 (between D19S47 and CYP2A) and in 412 (between CYP2A and APOC2), as is one presumed double crossover, in 429 (between D19S47 and APOC2). Recombination events for the entire haplotypes defined by the markers flanking RYR1 are seen in both families, as described in detail in the Results section, and suggest exclusion of MHS from this region.

Table 1

Results of In Vitro Muscle-Contracture Test

Family and Proband[a]	Serum CK (U/liter)	Threshold Concentration[b]		Status[c]
		Caffeine (mmol/liter)	Halothane (v %)	
MH006:				
202	Normal	2.0	1.0	MHS
301	94	1.0	1.0	MHS
304	90	1.0	1.0	MHS
305	244	2.0	2.0	MHS
308	Normal	1.5	2.0	MHS
309	122	2.0	2.0	MHS
402	Normal	2.0	2.0	MHS
404	Normal	2.0	2.0	MHS
405	276	1.5	2.0	MHS
408	Normal	1.5	.5	MHS
410	531	.5	.5	MHS (crisis)
412	Normal	4.0	>4.0	MHN
MH009:				
204	ND	>4.0	>4.0	MHN
301	Normal	ND	ND	Obligate MHS (crisis)
303	Normal	3.0	>4.0	MHN
304	Normal	3.0	>4.0	MHN
308	Normal	2.0	1.0	MHS
309	ND	ND	ND	Obligate MHS
311	Normal	3.0	2.0	MHE(h)
314	Normal	4.0	>4.0	MHN
401	Normal	2.0	1.5	MHS
404	Normal	3.0	>4.0	MHN
406	Normal	4.0	1.5	MHE(h)
412	ND	4.0	>4.0	MHN
414	Normal	3.0	>4.0	MHN
416	Normal	4.0	>4.0	MHN
423	Normal	3.0	3.0	MHN
425	ND	ND	ND	MHS (crisis)
427	595	1.0	.5	MHS
428	Normal	1.5	1.0	MHS
429	Normal	2.0	>4.0	MHE(c)
524	122	1.5	1.5	MHS
526	200	.5	.5	MHS (myopathy)[d]

[a] Probands at risk of MH were tested according to the European standard protocol.

[b] At which a contraction >20 mN in muscle fiber was obtained.

[c] MHS halothane threshold ≤2.0 v % and caffeine threshold ≤ 2.0 mmol/liter; probands in whom only one of the thresholds was lowered are designated as either MHE(h) (halothane threshold lowered [Lehmann-Horn and Iaizzo 1990]) or MHE(c) (caffeine threshold lowered [Lehmann-Horn and Iaizzo 1990]).

[d] Muscle-contraction amplitudes already high at the lowest caffeine and halothane concentrations routinely employed in the test.

which raised the possibility of her carrying two MHS genes (Deufel et al. 1990, and submitted). Individual 204, however, has since been tested and typed MHN, and it must therefore be assumed both that a second MHS chromosome was introduced into the pedigree, probably through individual 203 or individual 312, and that individual 526, despite the consanguineous constellation, therefore most probably has inherited

two independent—and thus not necessarily identical—MHS mutations. None of the other family members have been found to suffer from muscle disease.

Histological examination revealed unstructured central cores in the muscle biopsy of the index patient (410) in family MH006. Central cores were not found in the muscle biopsies from the other members of this pedigree, including those of the proband's mother (304) and her sister (408), who both had positive (MHS) muscle-contracture test results. In family MH009, no morphological signs of myopathy were seen in any of the probands except for individual 526, for whom an increased number of structurally normal mitochondria were found in the muscle biopsy.

The results of in vitro muscle-contracture tests for the members of both families are listed in table 1. It should be noted that in pedigree MH006 both the index patient (410) and her sister (408) have similarly low thresholds for caffeine and halothane and that proband 412, as an MHN individual, has thresholds for both halothane and caffeine that are much higher than those found in any of the other family members typed as MHS. In pedigree MH009, patient 526 was found to have high muscle-contracture amplitudes already at the lowest caffeine and halothane concentrations routinely used in the test protocol. Her sister and both parents show thresholds typically found in MHS patients. Individual 204 is unambiguously MHN, thus making it highly unlikely that the MHS chromosome transmitted in this part of the pedigree is the same as that in the other part, from which the father of patient 526 is descended.

The allele patterns obtained for the markers of the loci D19S75, D19S28 (family MH006 only), RYR1, D19S47, CYP2A, BCL3, and APOC2, described in the legend to figure 1, are shown in figure 2. In family MH006 (fig. 2, *top*) at least three crossovers can be demonstrated between MHS and the RYR locus: individual 202, who is heterozygous at this locus, has two affected children (305 and 309) homozygous for allele 1 and has three affected children (301, 304, and 308) who are heterozygous like herself. If it is assumed that MHS is segregating with RYR1 allele 2, then crossovers must have occurred in individuals 305 and 309, and a third crossover would be necessary to explain the homozygous RYR1 marker constellation in individual 408. In the case of cosegregation of MHS with allele 1, at least four crossovers would have to have occurred (in individuals 402, 404, 405, and 410).

Combined pairwise lod scores for both pedigrees with informative markers are listed in table 2. The −1.87 lod score obtained for RYR1 (1.86 for family MH006 alone) strongly suggests exclusion of MHS from this locus. None of the other loci tested from the region previously reported to comprise the MHS locus gives positive lod scores to suggest linkage in any of the two families. In fact, in family MH006 the entire haplotypes defined by these loci (fig. 2, *top*), except for single recombination events (in individuals 305 and 402) follow the same segregation pattern as described for RYR1 and recombine with MHS in the individuals pointed out above. In pedigree MH009 (fig. 2, *bottom*), multiple recombinations of entire haplotypes of markers flanking RYR1 with MHS can be seen, as, e.g., in individuals 401 and 404, who are MHS and MHN, respectively, but who have inherited the same chromosome from the affected father (301), or in individuals 308 and 309, who are both MHS but share different chromosomes with their affected brother (301).

Table 2

Pairwise *Z* Values for Linkage between MHS and Markers from Chromosome 19q12-13.2 Region

Locus (probe)	θ_{max}	Z_{max}	Z AT θ =						
			.00	.01	.05	.10	.20	.30	.40
D19S75 (MFD13)	.31	.340	−2.59	−1.94	−.88	−.35	−.17	.34	.26
D19S28 (p5B18[a])	.48	.001	−2.33	−1.51	−.89	−.60	−.28	−.11	−.02
RYR1 (HRR3-1)	.33	.200	−1.87	−1.74	−1.00	−.45	.04	.19	.16
D19S47 (MFD9)	.35	.080	−3.97	−3.19	−1.74	−.91	−.19	.05	.06
APOC2 (MFD5)	.26	.410	−2.24	−1.64	−.06	−.04	.36	.40	.25

NOTE.—Only the results for those markers informative in several meioses are given, and data have been pooled for both pedigrees (except for D19S28, which was only used in pedigree MH006). Two liability classes were used—one for the index patients with a fulminant MH crisis (penetrance 1.00) and one for the family members either typed by the in vitro contracture test or assumed to be obligate affected (penetrance .98), with .01 phenocopy rate. Gene frequency for MHS was assumed to be .0001, and mutation rate was .00001. (For details on the markers, see legend to fig. 1.)

[a] Pedigree MH006 only.

The results of a multipoint analysis with markers for the loci D19S75, RYR1, D19S47, and APOC2 are shown in figure 3. With lod scores ≤ -2.0, they exclude MHS from an interval spanning at least 26 cM and defined by those four markers. The obtained values depend strongly on the MHS penetrance value, at the given gene frequency, used in the program; and, if individual 204 in MH009 is assigned penetrance 1.00 for MHN instead of .98, there is a further drop of the lod scores, below the negative values shown in figure 3 and obtained as described in the Methods section. This would seem justified if one takes into account that penetrance data for the in vitro contracture test have been defined on the basis of only one observed false-positive MHS test result in a relatively small sample size of about 90 probands and that, so far, no false-negative results have been reported for the European test protocol.

Discussion

Our results in two Bavarian families strongly suggest the exclusion of the MHS mutation from the chromosome 19q region where MHS has been mapped previously in Irish and Canadian families (McCarthy et al. 1990; MacLennan et al. 1990). We interpret this as evidence for genetic heterogeneity in MHS as defined by the in vitro muscle-contracture test performed according to the European standard protocol. The clinical features of the index patient in each of the two pedigrees and the results of the in vitro muscle-contracture test are indistinguishable from those of other cases of MH and define these two index patients as classical MHS-risk patients.

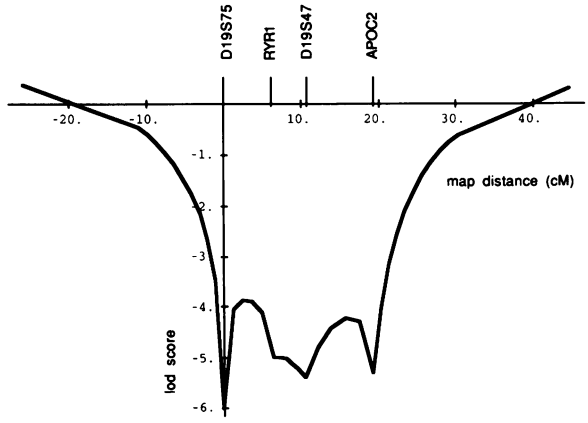


Figure 3 Multipoint analysis of MHS and 19q markers. The location map indicates composite lod scores for MHS at various map positions in a fixed marker map comprising markers D19S75, RYR1, D19S47, and APOC2 (MFD5). (For details of the markers, see legend to fig. 1.) D19S75 is arbitrarily placed at 0 cM, with the rest of the markers at the distances shown, which are based on genetic maps published for this region and calculated on the basis of sex-average recombination fractions. Haldane's mapping function was used to convert recombination fractions to genetic distances (in cM). Note that the lod score is ≤ -2.0 in a 26-cM interval around the RYR1 locus.

While the numbers of MHS and MHN individuals are balanced in pedigree MH009, the high proportion of MHS individuals seen in pedigree MH006 remains puzzling. Hypothetically it could be explained by the presence of a second MHS chromosome introduced by either individual 201, who was not available for study, or individual 202, who would then have to be assumed to be either homozygous or compound heterozygous for MHS. In the first case, one crossover is still left in MHN proband 412, who inherits the allele from individual 201 through her affected father 305. In the latter case one is still left with one recombination event for individual 408, who is MHS like her sister 410 but who has inherited a different chromosome from her affected mother, 304. Taking into consideration both the absence of any evidence for consanguinity and the fact that neither of the two hypothetical constellations could account for all the observed crossovers in the pedigree, these assumptions do not lend strength to an argument against recombination and thus support genetic heterogeneity of MH. Furthermore, linkage analysis as performed by the LINKAGE program already takes account of these possibilities. This can be demonstrated by the variation in the lod scores obtained for pedigree MH006; for example, for the RYR1 locus the lod score is -1.86 under the conditions described in the Methods section, i.e., with individual 201 assigned the status "unknown," but decreases even further, to -2.88, if this status is changed to MHN.

False-positive results for the in vitro muscle-contracture test could be another explanation for observed crossovers. This has been studied for the North American protocol, in which a relatively high ratio of MHS results versus MHN results is obtained. Apparent crossovers between MHS according to the North American protocol with chromosome 19 markers could be eliminated by changing the threshold values for halothane and caffeine in the test (MacKenzie et al. 1991). However, the European protocol differs from the North American in a way that produces significantly fewer MHS results, and, consequently, the false-positive rate is considered to be low (Ørding 1988). We have shown an overall distribution of

MHN and MHS results in 21 European families tested according to the protocol which, in contrast to North American figures, is fully compatible with autosomal dominant inheritance (McCarthy et al. 1990). Accordingly, the overall frequency of MHS in 10 families tested in our own center is 38 MHS, 31 MHN, and 10 MHE and thus does not exceed the numbers expected for an autosomal dominant trait. Segregation analysis including approximately 90 MHS individuals in several large pedigrees defined according to the European protocol, where linkage to chromosome 19 was established, gave no indications of false-positive results (Healy et al., in press; T. Deufel, A. Golla, W. Mauritz, and W. Hackl, unpublished data). Few positive results have been reported only in patients with well-defined muscle diseases other than MH (Lehmann-Horn and Iaizzo 1990). Finally, at least three false-positive MHS results in pedigree MH006 (individuals 305, 309, and 408), together with three false-negative MHN results in pedigree MH009 (individuals 304, 404, and 423) would have to be assumed to explain the crossovers found with the chromosome 19 markers. Not only is this high number of mistypings unlikely to have occurred with a protocol which otherwise produces results perfectly fitting the genetic model and mapping results for MH, but it could only be considered for cases with borderline threshold values in the test. As shown in table 1, however, there are recombinant individuals, e.g., patients 408 and 410 in pedigree MH006, who show virtually identical test results. Summarizing published evidence and our own data, we have no reason to believe that results of the in vitro muscle-contracture tests in these two families reflect any defect other than typical MHS.

The presence of other muscle diseases is ruled out in members of both pedigrees, by both clinical and morphological criteria—with the exception of one patient (526) in family MH009, who suffers from a chronic myopathy but who is also possibly carrying two MHS chromosomes (Deufel et al. 1990, and submitted). Central core disease (CCD), a neuromuscular disease for which there is good evidence for an association with MHS, has been mapped to the same region on chromosome 19q12-13.2 as has MHS in two previously published, independent reports (Haan et al. 1990; Kausch et al. 1991). It is therefore interesting to note that the index patient in pedigree MH006, where MHS clearly does not map to this locus, also had central cores in her muscle biopsy. However, none of the other MHS members of her family have shown central cores, and, as her biopsy was obtained fairly soon after the acute MH crisis had taken place, this finding could represent an unspecific alteration as a consequence of the MH crisis. Thus, there is no consistent basis for a diagnosis of familial central core myopathy in her family. The pathogenetic connection between MHS and the presence of central cores in skeletal muscle, however, remains to be elucidated.

The recombinations with the RYR1 locus reported in the present study obviously cannot rule out the possibility that the ryanodine receptor is a true candidate for MHS in those families where linkage to chromosome 19q12-13.2 can be shown. In the light of (*a*) the recent identification of a point mutation in the ryanodine receptor gene in porcine MH (Fujii et al. 1991, (*b*) its linkage to MHS in 338 informative meioses, with a lod score of 102 at a recombination fraction of 0 (Otsu et al. 1991), and (*c*) the identification of the corresponding mutation, across species barriers, in a human family where it segregates with MHS (Gillard et al. 1991), it must be assumed, at least for some cases, that mutations in this gene cause MH.

There was hope that DNA analysis could eventually provide a new and noninvasive way to diagnose MHS in at-risk families. However, we have shown that the disease is most probably genetically heterogeneous, and therefore this approach is only feasible in (*a*) probands in whom linkage of the MHS phenotype to chromosome 19 can be proved within the same family or (*b*) situations in which a known mutation can be demonstrated in the index patient. Further studies must be aimed at mapping and characterizing a second MHS locus suggested by our data, as well as at investigating the possibility of even greater genetic heterogeneity in other ethnic backgrounds. This should involve the investigation of the largest possible pedigrees fulfilling the criteria of the European Malignant Hyperthermia Study Group for the diagnosis of MH.

Note added in proof.—Since this article was submitted, Levitt et al. (1991) reported recombination of chromosome 19q markers with MHS, as defined by the North American protocol, in three American families. No details of index cases and in vitro muscle-contracture test data are given; however, it can be considered as corroborating our finding of genetic heterogeneity in MHS.

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