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Mapping of a Further Malignant Hyperthermia Susceptibility Locus to Chromosome 3q13.1

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Summary

Malignant hyperthermia (MH) is a potentially lethal pharmacogenetic disease for which MH susceptibility (MHS) is transmitted as an autosomal dominant trait. A potentially life-threatening MH crisis is triggered by exposure to commonly used inhalational anesthetics and depolarizing muscle relaxants. The first malignant hyperthermia susceptibility locus (MHS1) was identified on human chromosome 19q13.1, and evidence has been obtained that defects in the gene for the calcium-release channel of skeletal muscle sarcoplasmic reticulum (ryanodine receptor; RYR1) can cause some forms of MH. However, MH has been shown to be genetically heterogeneous, and additional loci on chromosomes 17q and 7q have been suggested. In a collaborative search of the human genome with polymorphic microsatellite markers, we now found linkage of the MHS phenotype, as assessed by the European in vitro contracture test protocol, to markers defining a 1-cM interval on chromosome 3q13.1. A maximum multipoint lod score of 3.22 was obtained in a single German pedigree with classical MH, and none of the other pedigrees investigated in this study showed linkage to this region. Linkage to both MHS1/RYR1 and putative loci on chromosome 17q and 7q were excluded. This study supports the view that considerable genetic heterogeneity exists in MH.

Introduction

Malignant hyperthermia (MH) is a potentially lethal pharmacogenetic disease with autosomal dominant inheritance (Kalow 1987), which can be triggered in susceptible probands by exposure to commonly used inhalational anesthetics (e.g., halothane) or depolarizing muscle relaxants (e.g., succinyl choline). Patients react with acidosis, hypoxia, masseter or generalized muscle contracture, a rapid rise in body temperature, and rhabdomyolysis. To date, the only reliable way to determine susceptibility to MH is with an in vitro contracture test (IVCT) performed on a sample of freshly obtained muscle. A standardized protocol for this test has been established by the European Malignant Hyperthermia Group (EMHG) (EMHG 1984), and similar protocols have been implemented in North America (Larach 1989). According to the European protocol, patients are assigned the status of MH susceptible (MHS), MH normal (MHN), or MH equivocal (MHE). The latter category comprises individuals in whom the biopsy sample reacts positively to one, but not both, of the in vitro triggering agents, MHE(h) (halothane) or MHE(c) (caffeine). For practical reasons, these individuals are considered to be at risk for MH, and, for the sake of maximum diagnostic sensitivity, it is accepted that the MHE category will include some false-positive clinical diagnoses. For genetic studies, however, aimed at the etiology of the MHS reaction, differentiation of this category allows maximum specificity of assigned MHS and MHN phenotypes.

In some pedigrees, following the identification of the porcine locus, human MHS has been mapped to the homologous genetic linkage region on human chromosome 19q12-13.2 (MacLennan et al. 1990; McCarthy et al. 1990). The gene for the skeletal muscle calcium-release channel of the sarcoplasmic reticulum (ryanodine receptor; RYR1) is also found at this location. Biochemical and electrophysiological studies in swine and humans with MH pointed to the ryanodine receptor as a likely candidate for the molecular defect (reviewed by MacLennan and Phillips 1992). This has since been corroborated by the identification of base changes in the RYR1 gene, leading to amino acid substitutions in the gene product, which cosegregate with MHS in affected pigs (Fujii et al. 1991; Otsu et al. 1991) and a subset of human pedigrees with MHS and

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central core myopathy (Gillard et al. 1991; Deufel et al. 1992*a;* Quane et al. 1993, 1994; Rübsam et al. 1993; Zhang et al. 1993).

We have previously reported two unrelated German pedigrees in whom the MHS trait was excluded from the MHS1 locus on chromosome 19q13.1 (Deufel et al. 1992*b*), and substantial evidence has been accumulated for genetic heterogeneity of the MHS trait (Levitt et al. 1991; Fagerlund et al. 1992; Iles et al. 1992; Rübsam et al. 1992). In the ensuing search for other MHS genes, a region on chromosome 17q has been proposed on the basis of linkage studies in North American and South African pedigrees typed with different protocols (Levitt et al. 1992) in which the gene for the adult muscle sodium channel (SCN4A) was suggested as a candidate (Olckers et al. 1992). No supporting evidence for this assumption is available so far. Three of the four genes encoding subunits of the skeletal muscle 1,4-dihydropyridine-sensitive calcium channel (dihydropyridine receptor)—i.e., the α1-subunit (CACNL1A3) on chromosome 1q (Gregg et al. 1993*a*) and the skeletal muscle β-subunit (CACNLB1) and γ-subunit (CACNLG), both localized on chromosome 17q11.2-q24 (Gregg et al. 1993*b;* Powers et al. 1993) in proximity to the proposed MHS2 and the SCN4A loci—have been excluded as candidates in German, British, and Belgian pedigrees (Iles et al. 1993; Sudbrak et al. 1993; authors' unpublished results from the European collaborative study). Recently, however, complete cosegregation of MHS with the marker D7S849 adjacent to the gene for the α2/δ subunit of the dihydropyridine receptor (CACNLA2) on chromosome 7q was reported (Iles et al. 1994) in one of the pedigrees where the disease gene had been excluded from the MHS1 locus on chromosome 19q as well as from the proposed 17q locus (Deufel et al. 1992*b;* Sudbrak et al. 1993).

In a collaborative initiative supported by the EMHG, pedigrees with MHS defined by the European standard IVCT protocol have been included in a systematic linkage study using a set of polymorphic microsatellite markers covering the entire human genome (Weissenbach et al. 1992; Gyapay et al. 1994). Pedigrees were included if (i) an unambiguous MH crisis had been documented in at least one index case, (ii) exclusion of the MHS1 locus was established by more than one recombinant individual, and (iii) the pedigree was large enough to allow for an individual lod score approaching 3.0 with positive linkage. The present report is on genetic linkage of the MHS trait in one of these pedigrees.

Subjects and Methods

Pedigree, Probands, and IVCT Results

A detailed description of clinical histories of index cases and IVCT results for typed individuals of the pedigree have been disclosed previously in a report describing exclusion of MHS from the MHS1 locus on chromosome 19q (pedigree MH009 in Deufel et al. 1992*b*). Further genetic data concerning the exclusion from the putative MHS2 locus on chromosome 17q have been reported (Sudbrak et al. 1993). The pedigree is shown in figure 1.

Genetic analysis in this pedigree is complicated by a marriage between second-degree cousins who are both MHS. However, the person through whom the loop is established (204) has been tested as MHN, and thus it must be presumed that two MHS mutations segregate independently in the left- and right-hand branches of the pedigree (Deufel et al. 1992*b*) and that the MHS alleles inherited by individuals 524 and 526 could have been transmitted by either parent or both. To avoid possible effects arising from the genetic heterogeneity of MH, only the left-hand part of the pedigree, complemented by three individuals (417, 419, and 421) who have been IVCT-typed since the pedigree was previously published, has been employed for the linkage analysis in the present study. The IVCT was performed (by F.L.-H.) according to the European standard protocol (EMHG 1984; Klein et al. 1987), and the results are summarized in table 1.

DNA Analysis of Microsatellite Markers

Genomic DNA was prepared from EDTA whole blood, according to standard methods (Kunkel et al. 1977; Miller et al. 1988) and amplified by PCR using primers for the microsatellite repeat markers specified (table 2). The primer sequences, allele sizes, and genetic marker order have been previously reported (Weissenbach et al. 1992; Gyapay et al. 1994), with the exception of markers D3S2496 (AFMa125yb1) and D3S2495 (AFM150ye7). Amplification of genomic DNA was performed according to standard protocols. Equal amounts of amplified DNA for up to 16 different markers were mixed and loaded on a sequencing gel. Separated products were blotted onto nylon membranes, and marker alleles were visualized by hybridization with ^{32}P end-labeled primer oligonucleotides, followed by autoradiography. Consecutive allele numbers were assigned, and the results were documented using CYRILLIC software (version 1.1) (Cherwell Scientific Publishing).

Data Analysis

Initial pairwise linkage analysis to determine sex-average lod scores during the genomic search was carried out on a personal computer with the MLINK option of the LINKAGE package of programs (Lathrop and Lalouel 1984) compiled to handle up to 10 alleles per locus, using pedigree files created by the CYRILLIC program. Two-point and multipoint linkage analyses (Lathrop and Lalouel 1988) were also performed using version 5.2 of the LINKAGE package of programs on a SUN 4/490 computer accessed at the Human Genome Mapping Project resource center of the Medical Research Council, England (Rysavy et al. 1992). Parameters were set as defined by the Genetics Section of the EMHG and reported elsewhere (Deufel et al. 1992*b;* Sudbrak et al. 1993): the disease allele frequency

Am. J. Hum. Genet. 56:684–691, 1995

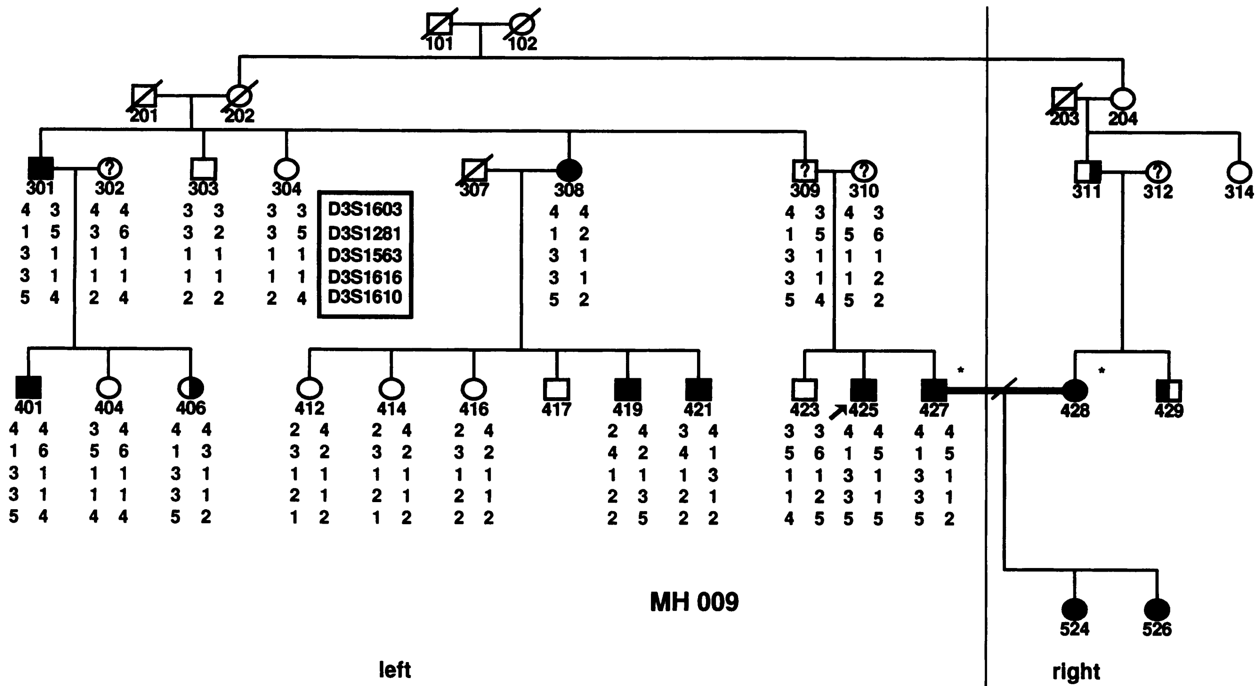

Figure 1 Pedigree MH009 with the MHS trait. Blackened symbols denote patients tested with the IVCT and typed as MHS (susceptible); unblackened symbols denote patients tested and typed as MHN (not susceptible); half-blackened symbols denote patients tested and typed as MHE(c) (left half) or MHE(h) (right half); and unblackened symbols with question mark denote untested family members (disease status unknown). For results of the IVCT, refer to table 1. The index patient (425), marked by an arrow, survived a fulminant MH crisis. Another patient (301) was found retrospectively to have experienced an MH crisis as well (for details see Deufel et al. 1992*b*). A second MHS mutation is supposed to occur in the right part of the pedigree. Thus, both parents of the individuals 524 and 526 are MHS, and the MHS trait in these children could be transmitted from either parent. The results of typing for the polymorphic microsatellite markers D3S1603, D3S1281, D3S1563, D3S1616, and D3S1610 are shown in genetic map order with the most proximal marker (D3S1603) on top and the most distal marker (D3S1610) at bottom. For details, see Subjects and Methods. One marker haplotype (4-1-3-3-5) cosegregates with the MHS phenotype throughout the entire left part of the pedigree, with the exception of individuals 419 and 421, in both of whom a recombination occurs. (For details see Results.)

was 1/10,000 (corresponding to an incidence of 1/5,000); the penetrance for the index case was taken as 1.0; the penetrance of the disease heterozygote was set at .98; the phenocopy rate was .02; and MHE individuals were assigned unknown status (i.e., they do not contribute to the lod score in this family). In the absence of specific data, allele frequencies for the hypervariable microsatellite markers were set as equal and thus dependent on the maximum number of alleles reported for each marker. To decrease memory requirements and computation time, genotypes were recoded to a maximum of 10 alleles per locus for two-point analysis and 3 alleles per locus for map-specific multipoint analysis, by reducing alleles to those observed in the root family and adjusting those of individuals joining the pedigree by marriage, to preserve informativity. Frequencies of the segregating alleles were fully preserved in the two-point analyses, and in the multipoint analyses allele frequencies of the informative alleles were preserved. Accordingly, the two-point lod scores remained unchanged by the recoding.

The genetic distances (in centimorgans) between markers have been published (Weissenbach et al. 1992; Gyapay et al. 1994), except for D3S2496 (AFMa125yb1) and D3S2495 (AFM150ye7) (J. Weissenbach, unpublished results). D3S2496 was mapped between D3S1616 and D3S1302; D3S2495 showed no recombination with D3S1281, D3S1563, or D3S1616. These three markers, originally reported at a distance of 0 cM, are now separated by recombination in this family, with D3S1616 distal from the remaining markers in this group (see below). In the map-specific multipoint analysis, only fully informative markers were included, and the order and distances (in centimorgans) between these markers were taken as (D3S1281/D3S1563)–1–D3S1616–6–D3S1610. However, the maximum lod score within the smallest region of overlap between D3S1281/D3S1563 and D3S1616 (see below) does not vary, if these distances are altered within the overall map distance for the region.

Results

Segregation of Markers on Chromosome 3 with the MHS Phenotype

Along with the results of the IVCT, figure 1 includes the alleles found for the fully informative PCR-based microsatellite markers on the proximal long arm of chromosome

Table 1

Results of In Vitro Contracture Test

	Threshold Concentration[b]		
Individual[a]	Caffeine (mmol/liter)	Halothane (% vol)	Assigned Phenotype[c]
204	>4.0	>4.0	MHN
301	ND	ND	Obligate MHS, MH crisis
303	3.0	>4.0	MHN
304	3.0	>4.0	MHN
308	2.0	1.0	MHS
309	ND	ND	Obligate MHS
311	3.0	2.0	MHE(h)
314	4.0	>4.0	MHN
401	2.0	1.5	MHS
404	3.0	>4.0	MHN
406	4.0	1.5	MHE(h)
412	4.0	>4.0	MHN
414	3.0	>4.0	MHN
416	4.0	>4.0	MHN
417	>4.0	>4.0	MHN
419	1.0	2.0	MHS
421	2.0	1.0	MHS
423	3.0	3.0	MHN
425	ND	ND	MHS (MH crisis)
427	1.0	.5	MHS
428	1.5	1.0	MHS
429	2.0	>4.0	MHE(c)
524	1.5	1.5	MHS
526	.5	.5	MHS

[a] Corresponding to fig. 1.

[b] At which a contraction >20 mN was obtained; ND = no data.

[c] MHS defined as threshold ⩽2.0% vol halothane and ⩽2.0 mmol caffeine/liter; MHE(h) or MHE(c) is assigned if only one of the thresholds for either halothane (MHE(h)) or caffeine (MHE(c)) was lowered.

3. One haplotype cosegregates with the MHS phenotype throughout the entire pedigree, with the exception of individuals 419 and 421, in both of whom a recombination occurs. The minimal overlapping region comprises a small interval between D3S1281/D3S1563 and D3S1616. D3S1616 is separated from the other two markers by recombination in this pedigree, but this has not previously been reported. The haplotypes clearly suggest that D3S1616 is distal to D3S1281 and D3S1563, since double recombinants are unlikely (fig. 1). This interval is presumably on the order of 1 cM (fig. 2). Marker D3S2496 was not informative in the key female (308) and thus cannot be placed precisely in this map, with respect to D3S1281/D3S1563 and D3S1616. Currently, there are no markers available that have been unequivocally localized between D3S1281/D3S1563 and D3S1616 and that might be directly informative for linkage within this interval.

Linkage Analysis

Two-point lod scores between genetic markers from 3q13 and the MHS trait in this family are given in table 3. None of the pairwise lod scores exceeds the significance threshold of $\geq +3$. Markers outside the smallest region of overlap all have at least one recombinant (either 419 or 421). No marker that is informative in the key female (308) is available within this region, to date. Map-specific multipoint linkage analysis, however, results in a maximum lod score for the disease trait of 3.22 (corresponding to a location score of 14.8) between markers D3S1281/D3S1563 and D3S1616 (fig. 2). This compares with a theoretical maximum two-point lod score of ~3.8 expected from the typed individuals if a fully informative marker were found. The multipoint lod score does not otherwise rise above 2.2 (equivalent to a fully informative two-point analysis at $\theta = 0$, with one recombinant individual, depending on the precise allele frequencies), reflecting the flanking recombination events, so that this localization within chromosome 3 is well supported.

Discussion

The results obtained in one single large pedigree with the MHS trait defined according to the European IVCT protocol provide strong evidence for the existence of a

Am. J. Hum. Genet. 56:684–691, 1995

Table 2

Microsatellite Markers

Marker	Locus	Primer Sequences[a]	Allele Size (bp)	Heterozygosity
AFM311vh1	D3S1603	5′-CCCTAACTCCACTTGAAAGC-3′ 5′-TCAGCGAACAGCAACAAAT-3′	159–177	.71
AFM177xh8	D3S1281	5′-TAGGCCAAAGTTTACATTTC-3′ 5′-ACAGATTTACATGCAAGGTG-3′	114–132	.66
AFM224xc9	D3S1563	5′-CAAAGCAGACACACAATTC-3′ 5′-CAGGTAAGTAAAAATAACTGGG-3′	216–226	.27
AFM150ye7	D3S2495	5′-ATCTGTAAGBTCTGCTTTCTGAAT-3′ 5′-AAATATTGTATGCGATGTGATTTA-3′	...	.64
AFM348te9	D3S1616	5′-CTCCCTACCTGAAAAGATGC-3′ 5′-TCGGCTTAAAGACTCAGTTTATT-3′	101–107	.64
AFM225xb12	D3S1302	5′-CTGAGTTTCAGTTTCCTTATCT 5′-ACCTACTAAGTCCCCAGC-3′	127–143	.64
AFMa125yb1	D3S2496	5′-TAGTATTCACTAAATCATTTGGG-3′ 5′-GATAATCAGAGCAAGTAATGCAG-3′	...	.77
AFM259va9	D3S1572	5′-GTCCAGAACAAGCAACAG-3′ 5′-CCACTCCATCCTTAATTGA-3′	244–266	.69
AFM321xf5	D3S1610	5′-CTTACATACCTAAGTTTGCGAG-3′ 5′-AAAGTGGTGAAGAAGTTCCTC-3′	171–189	.73
AFM242xh2	D3S1310	5′-CATTATTGTCAAAGCAACACAC-3′ 5′-AATGAGTTTTGGCAGAGGGT-3′	137–147	.67
AFM284xf9	D3S1586	5′-AGGGTTCTCTGACTGGGTAG-3′ 5′-TGTGTCCTTCAATCCAATCA-3′	291–309	.61

[a] CA strand (top) and GT strand (bottom) primer sequences are shown. Data are taken from Gyapay et al. (1994); for details of PCR conditions and analysis see Subjects and Methods.

further MHS locus on chromosome 3q13.1 and thus firmly establish a significant degree of genetic heterogeneity in this disorder. The structure of pedigree MH009 provides some complications in the interpretation of linkage data, because of a consanguineous marriage of second-degree cousins, both typed MHS, with two MHS children. The individual establishing the consanguinity loop (204 in fig. 1), however, has been typed as MHN. As has been discussed previously (Deufel et al. 1992*b*), it is most likely that two different MHS mutations segregate independently in this pedigree. This might seem unusual, but at least three other families of this kind have so far been reported to the genetics section of the EMHG. To avoid the false elimination of loci tested during the genomic search due to this feature of the pedigree structure, especially in a situation of known genetic heterogeneity, the linkage analysis was confined to the left-hand branch of the pedigree (fig. 1). The right-hand branch is too small to establish linkage to any of the previously reported MHS loci, by itself. Markers flanking the MHS1 locus on chromosome 19q13.1 are rather uninformative in this branch, and none of the reported RYR1 mutations was found (data not shown). Thus, there is at present no way to define the genetic basis of MHS in this part of the pedigree. The available mapping data for other MHS loci (chromosomes 7q and 3q; the present report) have so far not shown linkage (lod score $\geq +3.0$) to any location for more than a single family, with the notable exception of MHS1 on chromosome 19q. This makes it quite likely that the second MHS mutation in pedigree MH009 is different from the one present in the larger left-hand branch where we now report linkage to chromosome 3q13.1. The identification of this novel MHS locus, however, now allows us to reject an earlier hypothesis that a chronic myopathy present in one child of the two affected parents (524) might be due to homozygosity for the MHS trait (Deufel et al. 1992*c*), as the patient 524 has clearly not inherited the chromosome 3 haplotype (data not shown) segregating with the disease in the paternal branch of the pedigree. This also means that this child is unlikely to be a compound heterozygote for MHS mutations at two loci.

In general, linkage studies in MH are complicated by the difficulty of ascertaining the MHS phenotype. Its diagnosis relies solely on the IVCT, for which various protocols have been employed in the past to detect patients at risk. The results are known to vary, depending on which IVCT protocol is used. Differences between protocols must be expected in the number of false-positive and false-negative test results. Protocols that do not define a category of equiv-

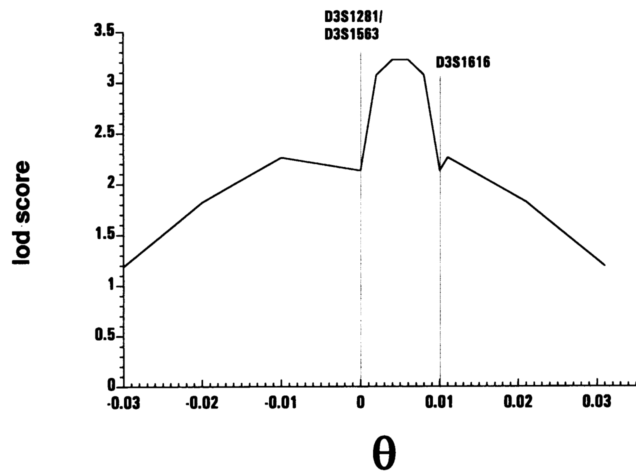

Figure 2 Multipoint analysis of MHS and markers for the proximal long arm of chromosome 3. The location map indicates composite lod scores for MHS at various map positions in a fixed-marker map comprising markers D3S1281, D3S1563, and D3S1616. (For details of the markers see table 2.) D3S1281/D3S1563 is arbitrarily placed at 0 cM. The marker order is that of the genetic map described elsewhere (Weissenbach et al. 1992; Gyapay et al. 1994). D3S1616 is separated from the other two markers by recombination in this pedigree, but this has not previously been reported. The haplotypes clearly suggest that D3S1616 is distal to D31281 and D3S1563, since double recombinants are unlikely. The genetic distance was assumed as 1 cM. A maximum lod score for the disease trait of 3.22 (corresponding to a location score of 14.8) between markers D3S1281/D3S1563 and D3S1616 is obtained.

ocal test results must be presumed either to lack sensitivity or to detect a higher number of positive probands, confounding the validity of the results when these families are used in genetic studies (MacKenzie et al. 1991). A certain degree of standardization, along with reasonable diagnostic specificity, has been achieved with the protocol that is followed by the majority of IVCT centers in Europe (EMHG 1984; Ørding 1987, 1988). The exclusion of individuals typed as MHE (who clinically are still treated as "at risk" patients) from the genetic analysis slightly reduces the potential statistical power of families where such individuals are present but greatly improves the specificity of the test and thus the validity of assigned phenotypes. It is interesting to note that individual 406 in our study, who is typed as MHE(h), has clearly inherited the high-risk haplotype in the present study. For families linked to 19q, previous studies have shown that some MHE individuals have inherited high-risk haplotypes and some have inherited low-risk haplotypes (Quane et al. 1994; D. Heilinger, W. Hackl, W. Mauritz, K. Steinbereithner, A. De Vries, A. Golla, and T. Deufel, unpublished results). For linkage analysis, as opposed to clinical diagnosis, it is therefore clear that the policy of excluding MHE individuals from the analysis is preferable to the danger of reaching false conclusions when uncertain data are taken as valid. A number of pedigrees with MHS defined according to this protocol are now available throughout Europe that are large enough individually to yield significant lod scores, thus avoiding the pitfalls inherent in the use of cumulative linkage data in a disease that is clearly as heterogeneous as MH. The validity of the European protocol in defining MH status is further supported by the results from linkage studies at the well-established MHS1 locus where convincing evidence for linkage or exclusion has been reported for a number of families (McCarthy et al. 1990; Healy et al. 1991; Levitt et al. 1991; Deufel et al. 1992*b;* Fagerlund et al. 1992; Iles et al. 1992; Rübsam et al. 1992; Ball et al. 1993). Thus, the European IVCT protocol has so far appeared adequate to define the complex MH phenotype for genetic analysis.

In contrast to MH in swine, human MH is characterized by substantial genetic heterogeneity (reviewed in MacLennan and Phillips 1992; Ball and Johnson 1993; Johnson 1993). Elsewhere, we have excluded several candidate genes encoding subunits of the skeletal muscle dihydropyridine receptor in families where linkage to the RYR1 locus on chromosome 19q13.1 had been excluded (Iles et al. 1993; Sudbrak et al. 1993; EMHG, unpublished data). Recently, however, cosegregation of markers for the α2/δ-subunit gene with the MHS phenotype was reported in one pedigree (Iles et al. 1994). For the putative MHS locus on chromosome 17q, which was suggested by cumulating small lod scores from pedigrees typed with different protocol (Levitt et al. 1991), the gene for the muscle sodium channel (SCN4A) has been proposed as a candidate (Olckers et al. 1992). This should be discussed bearing in mind that mutations in this gene have been identified elsewhere as causing hyperkalemic periodic paralysis, paramyotonia congenita, and other forms of nondystrophic myotonias (Fontaine et al. 1990; Koch et al. 1991; Heine et al. 1993; Ricker et al. 1994). The paramount importance of a consistent phenotype in genetic studies of MHS is further emphasized by the observation that anesthesia-related events

Table 3

Two-Point Lod Scores

	Lod Score at θ =		
Marker[a]	.00	.05	.10
D3S1603	2.05	1.83	1.60
D3S1281	2.13	2.35	2.26
D3S1563	2.05	2.28	2.19
D3S2495	1.76	1.56	1.36
D3S1616	2.08	2.30	2.21
D3S2496	−.19	−.18	−.15
D3S1302	.57	.50	.43
D3S1572	1.77	1.59	1.41
D3S1610	2.13	2.35	2.26
D3S1310	−.16	.09	.22
D3S1586	.82	.71	.60

[a] Markers are listed in genetic order, with D3S1281/D3S1563 and D3S1310/D3S1586 reported at 0 cM. D3S2495 was found at 0 cM with D3S1281/D3S1563/D3S1616. It is not informative in the key female (308) and cannot be positioned with respect to these three markers.

clearly distinct from MH—as far as triggering agents, clinical features, and treatment are concerned—may occur in these myotonic patients. In our hands, and on the basis of the phenotype defined by the European IVCT protocol, published linkage data on chromosome 17q (Iles et al. 1993; Sudbrak et al. 1993), as well as the data from the genomic search project by members of the EMHG, do not support a locus on chromosome 17q. Since several of the pedigrees included in the genomic search project do not show linkage to chromosomes 19q, 17q, 7q, or 3q, the existence of at least one additional MH locus is strongly inferred. Thus, an even greater degree of genetic heterogeneity than hitherto expected must be assumed for the MHS trait, which is phenotypically homogeneous. Unfortunately, this means that genetic diagnosis of the MH risk, which was a goal when these studies were initiated, is still not a feasible prospect. The presence, however, of a multitude of mutations in different genes resulting in the MHS phenotype as a disorder of muscle calcium homeostasis might provide a tool to dissect, at a molecular level, this key function in excitation contraction coupling.

Acknowledgments

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