

## CHRONIC MYOPATHY IN A PATIENT SUSPECTED OF CARRYING TWO MALIGNANT HYPERTHERMIA SUSCEPTIBILITY (MHS) MUTATIONS

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**Abstract**—Malignant hyperthermia (MH) is a pharmacogenetic myopathy triggered by a variety of anaesthetic agents and muscle relaxants. In humans, susceptibility to MH is inherited as an autosomal dominant trait, and susceptible patients do not show a clinically relevant myopathy unless having suffered from a MH crisis. Homozygosity for the MHS trait is thought to be an uncommon finding, and so far only a few cases of patients suggested to be homozygous for MH on the basis of pedigree information were reported and described as having a more severe form of this condition resulting in clinical symptoms also in the absence of triggering agents. We report clinical findings in a patient with chronic myopathy beginning at the age of 2 yr and associated with a number of unique features, the most important being a family history of MHS present in both parents. She became symptomatic with marked muscular weakness and elevated serum CK levels. A muscle biopsy showed a distinct enlargement and increase of muscle mitochondria. In the *in vitro* contracture test the patient's muscle responded with unusually high contractures already at basal levels of triggering agents indicating a particularly severe MHS condition. DNA markers for the MHS1 locus, described previously on chromosome 19q12–13.2 in Irish and Canadian pedigrees, could not be used to confirm her homozygous state because our molecular genetic studies had previously excluded the MHS trait in this pedigree from this locus. On the basis of clinical findings, results of the *in vitro* contracture test, and pedigree information presented, we put forth the hypothesis that the myopathy seen in the patient may represent a new clinical entity caused by her carrying two MHS mutations.

**Key words:** Malignant hyperthermia, myopathy, muscle disorders, mitochondria, *in vitro* contracture test.

### INTRODUCTION

Malignant hyperthermia (MH) is a pharmacogenetic myopathy triggered by a variety of anaesthetic agents (e.g. halothane) and muscle relaxants (e.g. succinyl choline). In humans, susceptibility to MH is mainly inherited as an autosomal dominant trait, with an estimated incidence in the range of 1:12,000–1:40,000 [1–3].

Patients who are heterozygous for the genetic defect can react with a MH crisis when exposed

to a triggering agent, but otherwise they usually do not show any clinically relevant signs of myopathy [3–6]. An *in vitro* muscle contracture test performed on fresh muscle obtained by biopsy is still the only way to detect susceptibility to MH in probands at risk. A standardized protocol for this test has been put forth and supported by the European Malignant Hyperthermia Group [7], according to which probands are assigned the status of MH susceptible (MHS), MH normal (MHN), and MH equivocal (MHE), respectively.

Due to the usual absence of clinical or morphological findings in susceptible individuals, unless exposed to a triggering agent, reliable epidemiological data are difficult to obtain. On the basis of published data [1–3], homozygosity for MH should be a rare event.

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Only in a few cases reported in the literature, patients have been suggested to be homozygous for MH susceptibility on the basis of pedigree information [8], and were described as having a more severe form of this condition with provocation of MH episodes by stress alone, without exposure to triggering agents [9]. These studies, however, did not provide direct evidence for MH homozygosity, and do not present detailed results of neurological examination, especially prior to the MH event.

A MHS locus has been localized on chromosome 19 [10], and a candidate gene for MH, the ryanodine receptor (RYR) was then shown to map to the same chromosomal region [11]. Recently, a mutation in this gene was identified and suggested to cause porcine [12, 13] and at least some cases of human MH [14]. With this progress made in elucidating the molecular basis of MH it has now become possible to study the genetics of MH susceptibility at the DNA level. However, genetic heterogeneity of the MHS trait was demonstrated by us [15, 16] and others [17], and restricts the use of chromosome 19 markers to those pedigrees where linkage to this region can be formally established. Here we report investigations in a large MH pedigree, where MHS is not linked to chromosome 19 [16], and where one patient, along with a family history of MHS in both parents, exhibits a number of unique clinical features. Presenting in detail results of: (i) neurological examination; (ii) *in vitro* contracture tests; and (iii) morphological analysis in skeletal muscle biopsies, and on the basis of extensive pedigree information, we put forth the hypothesis that the myopathy of this patient represents a unique clinical entity and is caused by her being homozygous or compound heterozygous for a mutation(s) causing typical MH susceptibility in her heterozygous relatives.

#### PATIENTS AND METHODS

The patient (526, Fig. 1) is the younger of two daughters from a marriage of second cousins (427 and 428). Before anything was known about the presence of MH susceptibility in her family she was presented, at the age of 2 yr, with retarded motor development, hypotonia and weakness in her proximal limbs. She could not stand alone until the age of 14 months, and only started to walk independently at 18 months. On examination her gait was unsteady, she walked plantar-flexed and had

difficulties climbing stairs, the Gower sign could be elicited. Her serum CK level was elevated to values exceeding  $200 \text{ U l}^{-1}$ . An EMG gave no significant pathological result, nerve conductivity was normal. Morphological examination of a muscle biopsy, described below in more detail, revealed abnormal findings related to mitochondria, but activities of complexes I, II and IV of the mitochondrial respiratory chain, and carnitine palmitoyl transferase, as well as carnitine content measured at the time in muscle stored frozen at  $-80^\circ\text{C}$ , were all normal.

About 2 yr later, her uncle (425) suffered from a fulminant MH reaction during an operation under general anaesthesia with a volatile anaesthetic. In retrospect, it was then found that another member of the family (301) also had a history consistent with a diagnosis of MH. This led to investigations of family members at risk with the *in vitro* contracture test.

At that time, the patient (526) whose myopathy had been slowly progressing over the years was again seen at our clinic and presented with significant motor retardation. Then aged 4, she had difficulties climbing and descending stairs independently, and walked plantar-flexed. Only recently had she become able to raise herself from a sitting position without the use of her hands. On neurological examination, cranial nerves, sensory and motor nerve functions were found to be normal. Proximal muscles, especially of the hip, were found hypotonic and weak, Gowers' sign was positive, and her reflexes could hardly be elicited or were absent except for the ankle jerks.

Her weakness and walking difficulties had further increased at age 7, when she was most recently examined. She could climb stairs only one at a time, Gowers' sign was still positive. She could not climb onto a chair without using her hands, nor rise from a lying position. Muscle reflexes were now all absent. She only walked on her toes and both heel cords were shortened with no other contractures present. Serum CK levels were persistently elevated. Co-ordination of her movements was normal, and her psychological development and intellectual functions were appropriate for her age. One yr later, another muscle biopsy was obtained in order to perform the *in vitro* muscle contracture test. By that time, surgery had become necessary to correct the permanent shortening of her heel cords and was performed under general anaesthesia and protection with dantrolene.

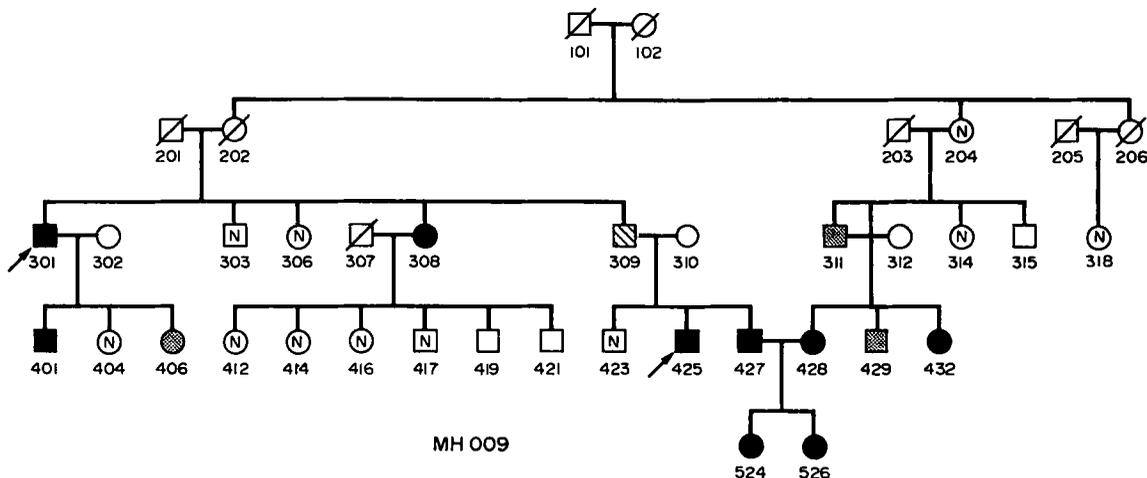


Fig. 1. Pedigree. A marriage between second cousins has occurred (427, 428), and their younger daughter (526) has a chronic myopathy. Index patients with MH crises were 425 (proven), and 301 (retrospective) as indicated by arrows; filled symbols denote probands tested and typed MHS (susceptible), or affected with MH crisis (301, 425); hatched symbols: obligate affected (309), shaded symbols: tested and typed MHE (equivocal), empty symbols with (N): tested and typed MHN (negative), empty symbols: not tested. Note that individual 204 is MHN, suggesting that a second MHS allele has entered the pedigree.

Her elder sister (524), now 11 yr old, had similarly elevated serum CK levels. However, in contrast to patient 526 she has never had any clinical signs of myopathy and weakness, nor any other pathological findings on neurological examination. The same was true for all the other members of the pedigree subjected to neurological examination. Muscle biopsies were performed for morphological investigation and the *in vitro* contracture test in order to assess their MH risk status.

Muscle samples were obtained from the m. vastus lateralis under local anaesthesia with mepivacaine. One specimen was used for the *in vitro* contracture test according to the standard protocol of the European Malignant Hyperpyrexia Group [5, 7]. A second one was fixed for light and electron microscopy as described in ref. [18]. A third sample was immediately frozen in liquid nitrogen and stored frozen at  $-80^{\circ}\text{C}$ . From this sample cryostat sections were prepared for dystrophin analysis by immunofluorescence with polyclonal antiserum anti-60 kDa (kindly made available by E.P. Hoffman, Pittsburgh, PA, U.S.A.) and commercially available monoclonal antibodies Dys1 and Dys2 (Novo Castra, Newcastle, U.K.).

## RESULTS

### *In vitro* contracture test, serum CK measurements

The pedigree of the family shown in Fig. 1 provides the MH risk status of members who

had suffered from a MH crisis or were tested by the *in vitro* contracture test. The specific threshold concentrations determined for the patient, both of her parents and her sister, as well as all the other tested members of the pedigree are listed in Table 1. Individuals 301 and 309 were not tested but considered obligate MHS because they both have affected siblings and children, and 301 has furthermore survived a MH crisis. Three members of the pedigree (311, 406 and 429) were by definition assigned the status MHE(h) or MHE(c) because lower than normal thresholds were determined only for one of the two triggering agents used in the test protocol. Surprisingly, individual 204 is clearly MHN and thus unlikely to have transferred the MHS allele which is present in the mother of the patient, individual 428.

Threshold concentrations for both parents and the sister of the patient were lowered in the range typically seen in probands susceptible for MH, which defines them as MHS according to the European MH protocol [7]. Muscle from patient 526, however, showed vigorous contractures significantly above the threshold force of 0.2p already at the basal concentrations used in the protocol for both triggering agents.

### *Histopathological findings in muscle*

The muscle biopsy from the patient, taken at the time of the first examination, showed moderate myopathic changes with interstitial fibrosis and increased variation of fibre diameters (Fig. 2a). NADH reductase staining was in-

creased in all type I fibres with subsarcolemmal deposits of mitochondrial enzyme activity (Fig. 2b). Electron microscopy revealed a distinct enlargement and an increase in the number of muscle mitochondria which were structurally normal (Fig. 2c). Similar findings were described in the second biopsy taken at the time when the *in vitro* contracture test was performed. Dystrophin analysis with antibodies against the N-terminal, the rod-like and the C-terminal domains showed a normal distribution pattern and normal quantity of the protein.

Biopsies taken from the elder sister (524) as well as from all the other tested members of the family at the time when the *in vitro* contracture test was performed were structurally normal except for discrete accumulations of subsarcolemmal mitochondria which have been previously described in MHS patients [6].

#### DISCUSSION

Myopathy in MH patients has been a controversial issue especially as clinical data and reports on neurological findings in patients prior to an MH crisis are scarce. Harriman [4]

has pointed out that even in a large series of MHS patients and their MHN relatives, histopathological signs of myopathy that could be attributed to a specific MH myopathy were found only in a very small number of cases. It has to be further stressed that this relates to minute changes in muscle morphology, and none of these patients have been described as showing clinical signs of muscle disease. Summarizing these reports [3, 19] together with our own previously reported findings [6] it seems justified to state that MHS patients most probably do not present clinically relevant signs of myopathy prior to MH episodes.

On the other hand, MH-like episodes, as discussed by Brownell [20], are also seen in a number of patients with specific myopathies, such as Duchenne muscular dystrophy or myotonic dystrophy. It has therefore been argued whether MH was, in fact, a genetically homogeneous disorder in its own right. The recent elucidation of the molecular genetics of some MH cases [10–14], however, provides strong evidence for MH being a distinct disease entity rather than an unspecific symptom associated with a heterogeneous group of myopathies [21].

Table 1. Serum CK levels and results of the *in vitro* muscle contracture tests. Listed are results for serum CK (in  $U l^{-1}$ ), and *in vitro* contracture test threshold concentrations and assigned MH risk status for the patient (526), her sister (524), and her parents (427 and 428), and other members of her family at risk of MH (see Fig. 1). The threshold concentrations for halothane and caffeine represent the concentrations of both triggering agents which cause contracture ( $>0.2p$ ) of the muscle sample. For experimental details see the protocol of the EMH group [7]. Patient (526) showed vigorous reactions significantly above the threshold force of 0.2p already at the lowest concentrations for both triggering agents employed in the protocol

Proband	Serum CK $U l^{-1}$	Threshold concentration		Status
		Caffeine ( $mmol l^{-1}$ )	Halothane (vol %)	
204	n.d.	$>4.0$	$>4.0$	MHN
301	normal	n.d.	n.d.	obligate MHS (crisis)
303	normal	3.0	$>4.0$	MHN
306	normal	3.0	$>4.0$	MHN
308	normal	2.0	1.0	MHS
309	n.d.	n.d.	n.d.	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	n.d.	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	n.d.	n.d.	n.d.	MHS (crisis)
427	595	1.0	$\geq 0.5$	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	$\geq 0.5$	$\geq 0.5$	MHS (myopathy)

However, it is also genetically heterogeneous, as we have recently shown for some pedigrees, including the one discussed in this report, where the MHS trait can clearly be excluded from the locus on chromosome 19q12–13.2 [15, 16]. This stresses the need to formally establish linkage to chromosome 19 in families to be included in genetic studies, and prior to any attempt to use genetic markers in the preclinical diagnosis of the MHS status. In the case presented here, MHS does not map to chromosome 19q12–13.2 and therefore DNA markers cannot be used to prove homozygosity at a specific MHS locus at the molecular level. The presence and origin of the MHS mutation(s) in this pedigree can therefore only be discussed on the grounds of clinical and *in vitro* contracture test data which for the patient 526 suggest that she is significantly more severely affected than all the other members of her family tested as MHS. On the basis of pedigree information, she is quite likely to be carrying two MHS mutations, her a priori risk as an MHS tested individual for the constellation being 0.33.

From the results of the *in vitro* contracture tests presented here, it seems, however, that two independent mutations segregate in the pedigree. The consanguineous loop is established

through individual 204. As shown in Table 1, she is unambiguously MHN. This suggests that a second MHS allele has been introduced into the pedigree, either through individuals 203 or 312 who have not been available for testing. The patient 526, even though she is probably homozygous for the MHS trait, still does not necessarily carry two identical MHS chromosomes, as might be suggested by the consanguinity in the family. Whether she is therefore a compound heterozygote rather than a true homozygote for one mutation at both MH loci cannot be decided at this point, because the chromosomal localization of MH susceptibility in this family is not yet known and studies at a molecular level are thus not possible.

There are only a few reports on presumably homozygous MH patients [8, 9]. In none of these cases was neurological data presented prior to anaesthesia and MH crisis. As in the present case, both reports cannot provide definite proof for homozygosity. Furthermore, in the pedigree described by Britt there remain some doubts as to the ascertainment of the MHS status, because the diagnosis of MH susceptibility in some of the cases was based on anamnestic evidence and serum CK levels only.

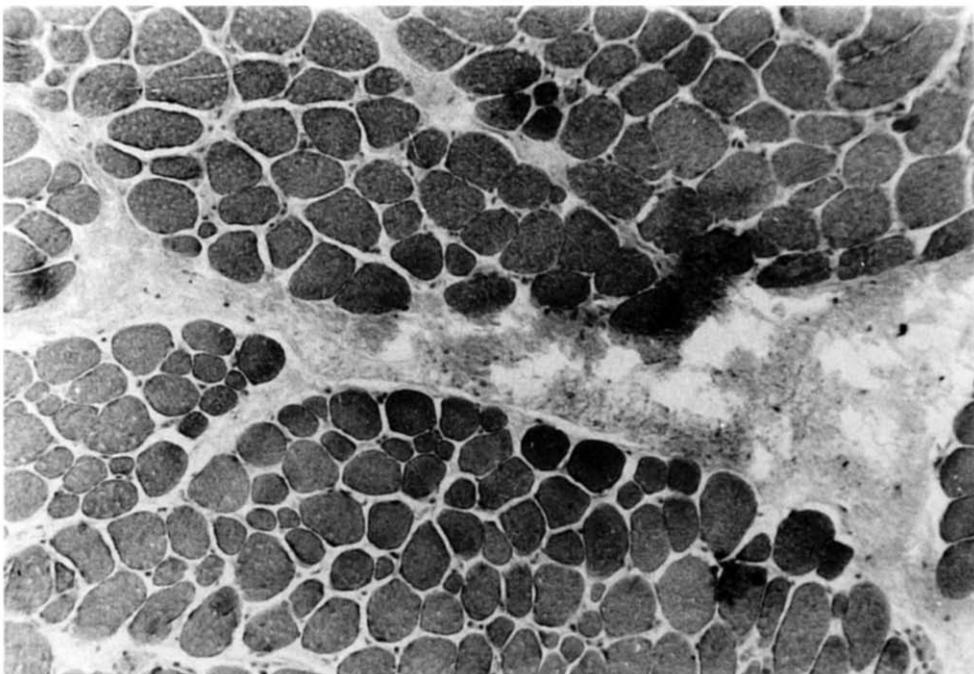


Fig. 2. Muscle biopsy from the patient (526). 2a. HE stain. ( $\times 100$ ) showing myopathic changes with moderate interstitial fibrosis and increased variation in fibre diameters. 2b. NADH reductase reaction ( $\times 400$ ) showing increased mitochondrial enzyme activity in all types of fibres with subsarcolemmal deposits of mitochondria. 2c. Electron micrograph ( $\times 10,000$ ) showing accumulation of enlarged mitochondria.



FIG. 2b.

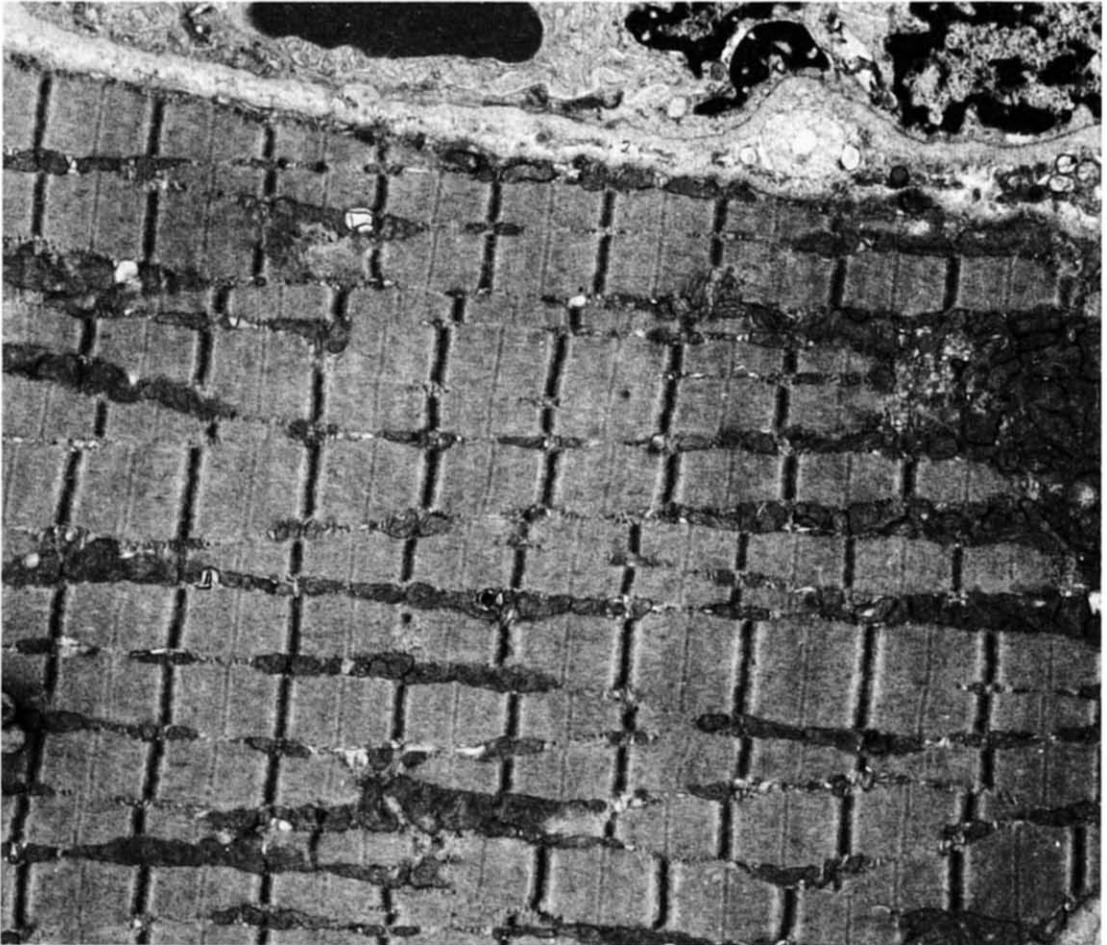


FIG. 2c.

In the case described here, the family history of MHS is well established and based on investigations with the *in vitro* muscle contracture test, according to the European standard protocol. Test results for the patient indicate a more severe reaction to triggering agents than seen for the rest of the pedigree and in unrelated MHS cases. The patient differs significantly further from typical MHS patients in that she shows muscular pathology, both by clinical and morphological criteria, even in the absence of any triggering agent. Rather than assuming a chance association of two equally rare muscular disorders, such as MH and a chronic myopathy with abnormal mitochondria and otherwise unknown biochemical etiology, we suggest that these clinical signs might in fact be connected and both due to her carrying two mutations causing MH susceptibility in her heterozygous relatives.

In conclusion, we suggest our findings are compatible with the hypothesis that MH susceptibility can be described as a condition where, in the heterozygote, the biochemical function of a gene product, in some cases the ryanodine receptor calcium release channel [11–14], is only slightly impaired. It may not be until the cellular environment of this gene product is altered further, e.g. by binding of a volatile anaesthetic, or affected directly by a drug, e.g. caffeine, that any abnormality becomes clinically significant. In the homozygous state, however, the defect cannot be compensated for and, as in our patient, results in a primary myopathy. This is mirrored in the animal model of porcine malignant hyperthermia where in homozygous animals symptoms can be induced by stress alone, and this so-called porcine stress syndrome [22] has consequently been considered a recessive trait. However, when tested with the standardized muscle contracture test, heterozygous animals are found to be MH susceptible, as shown recently [23], and, as in man, this MHS status in clinically unaffected animals follows an autosomal dominant pattern of inheritance.

Our findings might therefore represent an unusual example of an individual presumed to be homozygous for a dominant disease mutation showing a significantly more severe, but still not lethal, phenotype. This was not seen in another dominant disease, Huntington's Disease, where homozygotes are not distinguishable, phenotypically, from the heterozygote [24], and in this case could be due to the involvement

of two distinct loci, as MH does not map to the MHS locus on chromosome 19q12–13.2 in at least one branch of the pedigree, and the patient, if indeed homozygous for the MHS trait, is most likely to carry two independent MHS mutations. Further studies will be directed at identifying the other genes involved in MH susceptibility. However, the establishment of an MHS myopathy as a distinct clinical entity awaits further studies in individuals who are homozygous or compound heterozygous for the MHS trait, and will be greatly facilitated once the molecular basis of MH susceptibility is further elucidated.

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