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Genetic Effects on the Variability of the Halothane and Caffeine Muscle Contracture Tests

Albert Urwyler, M.D., Kathrin Censier,† Mark A. Kaufmann, M.D.,‡ Jürgen Drewe, M.D.§*

Background: **The spectrum of the clinical presentation of malignant hyperthermia (MH) and the results of recent linkage studies suggest that there is a degree of heterogeneity in MH susceptibility. In the current study, we analyzed *in vitro* muscle contracture tests from members of large families with MH to evaluate if the results of these tests could be related to genetic influences.**

Methods: **Forty-seven subjects from four families with an MH-related death and with at least five clinically MH-susceptible individuals per family, as diagnosed by an *in vitro* muscle contracture test according to the protocol of the European MH Group, were included in the current analysis. We compared the strength of muscle contractures to challenges of halothane, caffeine, or both and the effect of these two drugs on twitch potentiation in response to supramaximal electrical stimulation among the families.**

Results: **Clinical MH susceptibility was confirmed in 36 individuals, and 11 individuals were diagnosed as MH-negative. In MH-susceptible individuals, muscle contractures to the 2% halothane challenge were significantly higher in family 1 (n = 15; 16.2 ± 2.9 mN, mean ± standard error of the mean) and in family 4 (n = 5; 16.4 ± 5 mN) than in family 2 (n = 9; 5.8 ± 1.5 mN) or family 3 (n = 7; 6.0 ± 1.1 mN). Muscle contractures to the caffeine challenge (2 mM) were significantly increased in family 1 (7.3 ± 1.4 mN) compared with those in family 3 (1.3 ± 1.0 mN). In addition, we found a dose-dependent twitch potentiation to the halothane challenge in family 2 ($P < 0.01$) and to the caffeine challenge in families 2 ($P < 0.001$) and 3 ($P < 0.01$), whereas there was no twitch potentiation in families 1 and 4.**

Conclusions: **The differences of *in vitro* muscle contracture tests among several families with MH provide evidence for genetic influences on the variability of this test procedure. However, it is not known if the observed differences are caused by heterogeneity of the MH gene mutation(s) or by other genetic factors that might modify muscle contractures *in vitro*.** **(Key words: Anesthetics, volatile: halothane. Hyperthermia: malignant. Hyperthermia, malignant: caffeine challenge; *in vitro* contracture test. Muscle: skeletal.)**

MOLECULAR genetic techniques are being used to identify the gene(s) causing malignant hyperthermia (MH) susceptibility.[1] Reports of recent linkage analyses suggest that more than one gene is responsible for MH susceptibility.[2,3] In several families, MH susceptibility has been linked to chromosome 19[4–7] and in other families to chromosome 17.[8] The experimental basis for the localization of MH gene(s) is the *in vitro* muscle contracture test (IVCT) performed on members of large families with MH. Currently, this is the only generally accepted method for presymptomatic MH diagnosis.[9] Muscle strips from MH-susceptible (MHS) individuals demonstrate abnormal contractures *in vitro* to lesser concentrations of caffeine or halothane compared with muscle strips from MH-negative (MHN) individuals.

The IVCT procedure and the interpretation of the results are performed in accordance with standardized protocols. Two test protocols are currently in use: those of the European[10] and North American[11] MH Groups.‖ These two protocols give different diagnostic results

* Staff Anesthesiologist.

† Medical Research Assistant.

‡ Staff Anesthesiologist.

§ Pharmacologist.

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Address reprint requests to Dr. Urwyler: Department of Anesthesia and Research, University of Basel/Kantonsspital, CH-4031 Basel, Switzerland.

‖ The major differences between the two test protocols are as follows: (1) In the North American protocol, six separate muscle strips are challenged (halothane: n = 3; caffeine: n = 3), whereas at least four separate muscle strips are tested in the European protocol (halothane: n = 2; caffeine: n = 2). (2) In the North American protocol, the caffeine test is started with 0.5, 1, and 2 mM caffeine. In addition to these concentrations, 1.5 mM is used in the European protocol. In both protocols, 0.2 g (2 mN) muscle contracture to ≤2 mM caffeine is considered to be abnormal. (3) The halothane challenge is performed using a single concentration (3%) in the North American protocol. In the European protocol, halothane is added in consecutive concentrations (0.5, 1, and 2%). In the North American protocol, each laboratory establishes its own cutoff for a normal response within a range of 0.2–0.7 g, whereas a contracture <0.2 g (2 mN) to ≤2% halothane is a normal response in the European protocol. (4) The responses to both caffeine and halothane need to be abnormal for an MHS diagnosis to be confirmed in the European protocol, but an abnormal response to either one is sufficient for the North American protocol.

in some individuals.[12] The protocol of the North American MH Group identifies two diagnostic groups: MHS and MHN. In addition to these two diagnostic groups, the protocol of the European MH Group includes an MH-equivocal (MHE) diagnosis for patients who have an abnormal response to either halothane or caffeine. Clinically, patients with an MHE diagnosis on the IVCT are regarded as susceptible to MH.

A crucial aspect of the IVCT is the definition of an abnormal muscle contracture. Recently, it has been suggested that the currently used threshold forces of contracture for the IVCT are too low[13]; this may be the case, because it is more important to avoid false-negative than false-positive diagnoses. It is not known if the threshold forces of abnormal muscle contractures are different for each family with MH, depending on the genetic defect(s). Additional information concerning the IVCT may help to differentiate among different MH defects, *e.g.,* drug effects on muscle contractions (twitches) *in vitro* in response to electrical stimulation also may be of interest, because halothane and caffeine potentiate twitches[14] and an alteration of excitation–contraction coupling may be involved in MH susceptibility.[15]

Because the clinical variability of MH has been interpreted as an effect of heterogeneity of MH susceptibility,[8] one can speculate that muscle contractures and twitch potentiations *in vitro* in different families with MH may have a different molecular biologic basis. If this is true, one could expect similarity in muscle contractures and twitch potentiations *in vitro* to challenges of halothane, caffeine, or both in MHS individuals within the same family but not between families. The aim of the current study was to investigate this possibility.

Materials and Methods

Patient Selection and Diagnosis of Malignant Hyperthermia Susceptibility

Forty-seven patients from four large families with MH-related deaths and at least five clinically MHS individuals per family diagnosed by IVCT were included in this study. In the selected families with MH, there was no evidence for neuromuscular disorders from the history and histologic findings. All investigations were performed between 1986 and 1992 in this laboratory in accordance with the published protocol of the European MH Group.[10,16] All biopsy samples were taken from the vastus medialis of the quadriceps muscle. Patients were anesthetized with either a femoral nerve block or an epidural block using mepivacaine 1–2% or bupivacaine 0.5% or both. All IVCT were performed with identical equipment.[16] Pure caffeine base was obtained from Merck (Darmstadt, Germany) and halothane from Halocarbon Laboratories (Hackensack, NJ). Individuals with muscle contractures $\geq$ 2 mN (corresponding to $\geq$ 0.2 g in the original version of the protocol) to both halothane ($\leq$ 2%) and caffeine ($\leq$ 2 mM), were diagnosed as MHS. Patients with muscle contractures < 2 mN in both tests were diagnosed as MHN. Individuals with an abnormal response ($\geq$ 2 mN) only to $\leq$ 2% halothane were designated as MHE with respect to halothane (MHEh).

Data Collection and Management

In all families, data from each individual were labeled throughout the study according to the individual's position in the pedigree. The incidence of MHS and MHEh diagnoses was compared among the four families. The concentrations of caffeine and halothane that were necessary to produce a sustained increase $\geq$ 2 mN in baseline tension (contracture) were determined for each individual. The maximum muscle contractures to 2% halothane and 2 mM caffeine were recorded for each patient. The twitch height to supramaximal electrical stimulation (0.2 Hz, 1 ms) was measured before and after challenge with each concentration of halothane (0.5, 1, and 2%) or caffeine (0.5, 1, 1.5, and 2 mM). Mean heights of the last five twitches were calculated just before the concentrations of the test drugs were increased.

Data from individuals with an MHS or MHEh diagnosis were pooled in one clinically MHS group for each family. The maximum muscle contractures of the clinically MHS groups to the halothane challenges and to the caffeine challenges are presented as mean ($\pm$ standard error of the mean), range, and 95% confidence interval. The percentage of muscle bundles exhibiting a positive response to halothane or caffeine and the mean predrug tension of muscle specimens were determined for each family group of clinically MHS individuals. The effects on twitch potentiation were expressed as the percentage of change from the predrug control. To study the effect of halothane and caffeine challenges on twitch potentiation, the 36 clinically MHS individuals were divided into three equally sized groups according to the extent of muscle contracture.

Statistics

The incidence of MHEh *versus* MHS diagnoses and the numbers of individuals with the same values of the halothane and caffeine threshold concentrations that produced muscle contractures ≥ 2 mN were compared among the families by using Fisher's exact test. Predrug tensions of the muscle bundles were compared among the families by using one-way analysis of variance. Mean muscle contractures to the halothane challenges and to the caffeine challenges were compared with Duncan's test among the families and with two-sided Student's *t* tests within the families. Statistical analyses of halothane and caffeine effects on twitch potentiation were performed with analysis of variance and subsequent multiple *t* tests with Bonferroni's correction. The overall statistical significance level was taken to be 5%.

Results

The pedigrees of the four families included in this analysis and their MH status evaluated by IVCT are shown in figure 1. The families originated from different parts of Switzerland and were not related. Thirty-six subjects were diagnosed as clinically MHS (25 MHS and 11 MHEh), and 11 subjects were MHN.

In all four presented fatal outcomes, halothane and succinylcholine had been used as anesthetics. In family 1, a 20-yr-old man (II-11) had died in 1970 after an MH episode during anesthesia given for the osteosynthesis of a tibia fracture. About 55 min after the uneventful induction of anesthesia, spontaneous ventilation increased suddenly and was followed by tachycardia and extrasystoles. About 15 min later, the diagnosis of MH was made. The patient was hyperthermic at 42°C and presented with generalized rigidity and a mottling skin. Cardiac arrest occurred 2 h after the induction of anesthesia. Despite therapeutic interventions the patient died about 2 h later. In family 3, a 6-yr-old girl (III-2) had died in 1971 during anesthesia for dental surgery. Ninety minutes after induction of anesthesia, arrhythmias occurred and were followed by a cardiac arrest that could not be treated. The MH diagnosis was made by the anesthesiologist in charge.

In two of the four families, a clinically suspected MH crisis in addition to an MH death had been observed. In family 2, the MH death of the 3-month-old boy (III-2) had occurred in 1972 during a hernia repair, but no clinical data were available. Despite this fatal event, in 1978 his brother (III-3) had been anesthetized with halothane and succinylcholine for a tonsillectomy. After a masseter spasm, halothane was immediately stopped, and he was treated with dantrolene. He had myoglobinuria, and his plasma creatine kinase concentration increased to a maximum of 6,630 U/l. This patient was diagnosed as MHS with the IVCT (table 1 and fig. 1). In family 4, a 27-yr-old man (II-5) had died in 1975 during anesthesia given for wound treatment. The MH diagnosis was made after a rapid increase in temperature to 42°C. The patient died within a short time despite surface cooling and hyperventilation of the lungs. In the same year, his 5-yr-old niece (III-1) was anesthetized for tonsillectomy with halothane and succinylcholine. She had a fulminant MH crisis with generalized rigidity, cyanosis, and tachycardia. Her temperature increased from 38.4 to 40°C within 5 min. Dantrolene was not available. The patient's lungs were hyperventilated with oxygen, and surface cooling was performed. Plasma creatine kinase concentration increased to 22,520 U/l. She survived this episode but so far has refused muscle biopsy for IVCT. Her mother (II-2) was diagnosed as MHS with the IVCT (table 1 and fig. 1).

Subjects with an MHEh diagnosis were observed in all four families. There was a significantly increased incidence of MHS diagnosis in family 1 (14 MHS and 1 MHEh) compared with family 2 (4 MHS and 5 MHEh) ($P < 0.05$) and family 3 (3 MHS and 4 MHEh) ($P < 0.05$). There were no differences in the incidence of MHS *versus* MHEh between families 2 and 3 and between family 4 (4 MHS and 1 MHEh) and all other families.

In the group of clinically MHS individuals, the percentage of muscle bundles exhibiting an abnormal contracture for the halothane and the caffeine tests, respectively, was 100% and 90% in family 1, 67% and 29% in family 2, 75% and 73% in family 3, and 89% and 71% in family 4. The predrug tension was not significantly different among the families (for the halothane and the caffeine tests, respectively, family 1: 12.4 ± 1.6 and 13.3 ± 0.9 mN; family 2: 16.2 ± 2.6 and 11.8 ± 1.7 mN; family 3: 20.5 ± 2.9 and 16 ± 1.3 mN; family 4: 15.7 ± 1.4 and 12.4 ± 1.3 mN, [mean ± standard error of the mean]).

The maximum muscle contractures and the halothane and caffeine threshold concentrations that initiated a contracture *in vitro* of ≥ 2 mN for all individuals are shown in table 1. Threshold concentrations for halothane and caffeine that initiated a muscle contracture *in vitro* of ≥ 2 mN were significantly lower in family 1 compared with family 2 ($P < 0.01$ and $P < 0.05$,

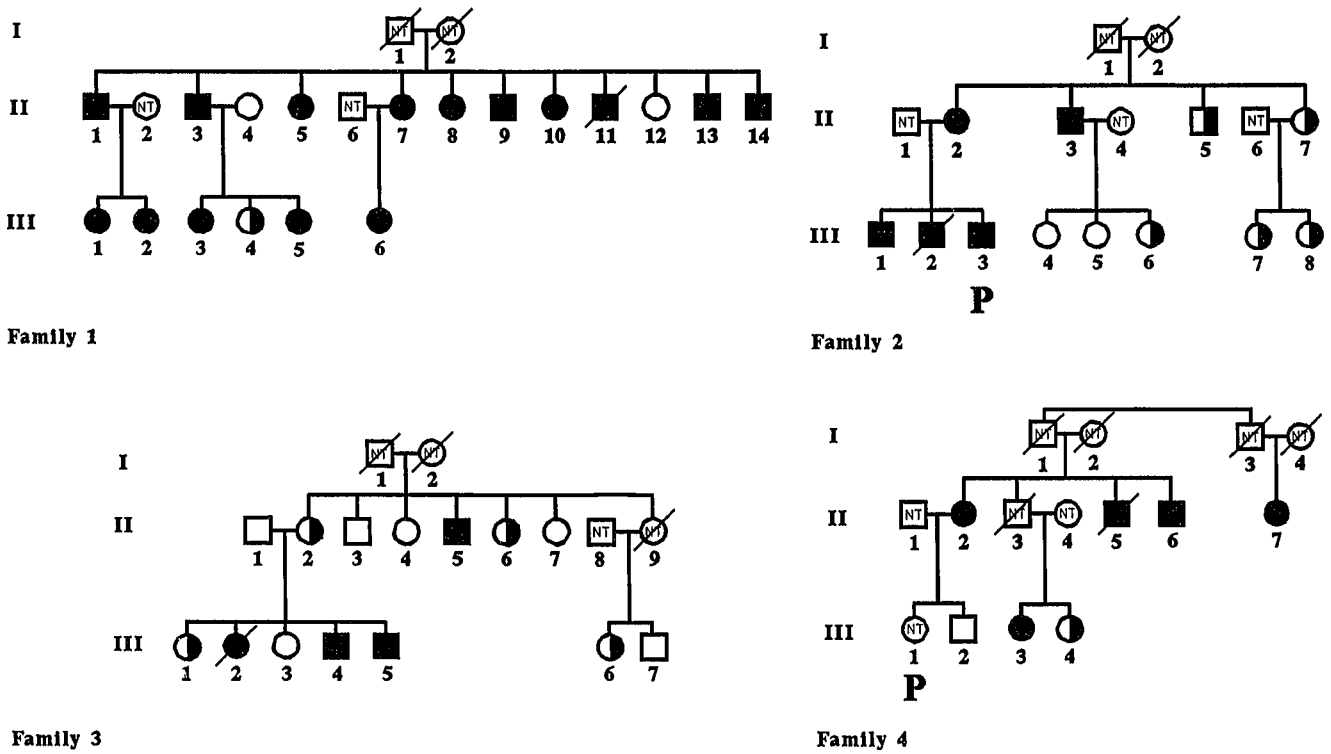

Fig. 1. Pedigrees of the four investigated families. In families 2 and 4, there were reported clinical malignant hyperthermia episodes (P) in addition to the malignant hyperthermia–related death (filled symbols with slash). Individual diagnoses to the *in vitro* contracture test: filled symbols = malignant hyperthermia–susceptible; open symbols = malignant hyperthermia–negative; half-filled symbols = malignant hyperthermia equivocal abnormal response (≥ 2 mN) only to ≤ 2% halothane. Circles = female; squares = male; NT = not tested; slash = deceased.

respectively) and in family 1 compared with family 3 ($P < 0.001$ and $P < 0.05$, respectively). In addition, there were significant differences between families 2 and 4 ($P < 0.05$) and between families 3 and 4 ($P < 0.01$) for the halothane threshold concentrations (table 2).

We found statistically significant differences of mean muscle contractures to the halothane and caffeine challenges among the family groups of clinically MHS individuals. Mean muscle contractures of the MHS individuals to the halothane challenge were increased compared with mean muscle contractures to the caffeine challenge in all families. These differences were statistically significant in families 1 and 3 ($P < 0.01$) (table 3).

There was a significant, dose-dependent twitch potentiation in the halothane test in family 2 and in MHN individuals (fig. 2), whereas twitch potentiation to the caffeine challenge was significant and dose dependent in families 2 and 3, and in MHN individuals (fig. 3). Halothane and caffeine exposures caused smaller twitch potentiations in muscle strips from clinically MHS individuals who had higher contractures than in muscle strips from clinically MHS individuals who had lower contractures *in vitro*. Patients with muscle contractures > 11 mN showed a decrease of the initial twitch height to the halothane challenge (fig. 4).

Discussion

The aim of molecular biologic MH research is to develop a method of detecting noninvasively before anesthesia the presence of any genetic abnormality that might predispose an individual to an MH episode. Currently, research is concentrated on individuals with clinical MH episodes who have large families investigated by IVCT. The interpretation of clinical data is often difficult, either because important clinical information and laboratory results are not available or because the MH crisis has been successfully treated at a

Table 1. Individual *In Vitro* Muscle Contractures (mN) and Threshold Concentrations of a Muscle Contracture ≥2 mN to Halothane or Caffeine Exposure

Family	Individual	Halothane (2%)	Caffeine (2 mM)	Halothane Threshold (%)	Caffeine Threshold (mM)	Diagnosis
1	II-1	28	5	0.5	1.5	MHS
	II-3	10	12	0.5	1	MHS
	II-4	−1	0	>3	>4	MHN
	II-5	20	16	0.5	2	MHS
	II-7	18	2	0.5	2	MHS
	II-8	20	8	0.5	1	MHS
	II-9	28	9	0.5	1.5	MHS
	II-10	11	2	0.5	2	MHS
	II-12	−2	0	>3	>4	MHN
	II-13	6	9	0.5	2	MHS
	II-14	7	6	0.5	1	MHS
	III-1	5	8	0.5	0.5	MHS
	III-2	18	4	1	1.5	MHS
	III-3	13	2	0.5	2	MHS
	III-4	6	1	0.5	4	MHEh
	III-5	8	5	1	2	MHS
	III-6	45	20	0.5	1.5	MHS
2	II-2	5	5	1	2	MHS
	II-3	3	8	1	1	MHS
	II-5	3	−2	2	4	MHEh
	II-7	6	0	1	4	MHEh
	III-1	5	4	0.5	1.5	MHS
	III-3	17	9	0.5	0.5	MHS
	III-4	−1	−1	>3	>4	MHN
	III-5	0	−1	>3	>4	MHN
	III-6	4	1	1	4	MHEh
	III-7	3	−1	2	>4	MHEh
	III-8	6	−1	1	>4	MHEh
3	II-1	−1	−4	>3	4	MHN
	II-2	5	−3	1	4	MHEh
	II-3	0	−2	>3	>4	MHN
	II-4	1	0	>3	>4	MHN
	II-5	8	4	2	2	MHS
	II-6	4	1	1	4	MHEh
	II-7	1	−2	>3	>4	MHN
	III-1	4	0	1	3	MHEh
	III-3	−2	−5	>3	>4	MHN
	III-4	11	5	1	1.5	MHS
	III-5	7	2	1	2	MHS
	III-6	3	0	2	>4	MHEh
	III-7	1	−2	>3	>4	MHN
4	II-2	16	11	0.5	0.5	MHS
	II-6	26	6	0.5	1	MHS
	II-7	29	5	0.5	1.5	MHS
	III-2	1	−2	3	4	MHN
	III-3	8	3	0.5	1	MHS
	III-4	3	−2	0.5	>4	MHEh

very early stage. It thus remains unclear whether or not the patient has a predisposition to MH. The accuracy of MH diagnosis by IVCT is difficult in patients presenting with borderline results. Therefore, progress in standardization of the IVCT and improvement in its predictive value are needed.[17]

In the current study, we have selected families with MH-related deaths. All fatalities occurred between 1970

Table 2. Distribution of Clinically MH-Susceptible Individuals (MHS and MHEh diagnoses) in Four Families According to the Threshold Concentrations of Halothane and Caffeine That Initiated an *In Vitro* Muscle Contracture ≥2 mN

	Individual Threshold Concentrations			
	Halothane (%)		Caffeine (mM)	
Family (n)	0.5	1–2	0.5–2	>2
1 (15)	13	2	14	1
2 (9)	2	7	4	5
3 (7)	0	7	3	4
4 (5)	5	0	4	1
		*P**		
Statistical Differences between Families				
1 *vs.* 2	0.01		0.05	
1 *vs.* 3	0.001		0.05	
1 *vs.* 4	NS		NS	
2 *vs.* 3	NS		NS	
2 *vs.* 4	0.05		NS	
3 *vs.* 4	0.01		NS	

* Fisher's exact test.

and 1975 when dantrolene was not yet available. Based on the clinical course, we believe that there is a high probability of MH susceptibility in the four selected families. Additional arguments support this view. First, in two of the four families, additional clinical MH episodes have occurred and the subject with a reported clinical MH episode in family 2 has clearly tested MHS in the IVCT. Second, there were clear MHS diagnoses in the IVCT in at least two siblings in all four families. Assuming that MH susceptibility is actually present in all four families, our data analyses are of interest, because all individuals have been investigated in the same laboratory with identical equipment and personnel in accordance with a standardized protocol.

The inherited predisposition to MH has been confirmed to be an autosomal dominant in many families. This pattern of inheritance is consistent with results in families 2, 3, and 4. In family 1, however, it is very unlikely that an autosomal dominant mode of inheritance of the MH gene explains the IVCT results, but it is still possible. One could speculate, however, that in family 1 MH susceptibility was present in the mother and father of the first generation. An alternative explanation is an extremely low threshold force of contractures in the test protocol making individuals with borderline results false-positive in the IVCT. But muscle contractures to both halothane and caffeine were clearly in the abnormal range in all MHS individuals in family 1. The only MHEh patient in this family had a muscle contracture of 6 mN at 2% halothane and a halothane threshold at 0.5%, suggesting positive MHS. Assuming a predisposition to MH in both parents, some individuals of the second generation could be homozygous. Yet it is not known whether homozygous carriers have increased contractures in the IVCT, this possibility should not be overlooked although the highest muscle contractures were not found in the second generation but rather in an individual of the third generation (III-6) with 45 mN in the halothane test and 20 mN in the caffeine test.

Because of the concern of false-negative diagnosis of MH susceptibility the threshold values have been set in such a manner that there is an incidence of false-positive diagnosis in pooled data.[18] It is not surprising, therefore, that misclassification with the IVCT through the use of inadequate muscle contracture thresholds has been previously suggested.[13] Because MH susceptibility must be present in patient II-2 of family 3, assuming MH susceptibility as a hereditary predisposition, our results indicate that the threshold force contracture for the halothane test could be eventually

Table 3. *In Vitro* Muscle Contractures (mN) to Halothane (2%) and Caffeine (2 mM) Challenges in Clinically MH-Susceptible Individuals (MHS and MHEh Diagnoses)

Family (n)	Halothane	Caffeine	*P* (Halothane *vs.* Caffeine Test, *t* test)
1 (15)			
Mean (SEM)	16.2 (2.9)*	7.3 (1.4)†	0.01
Range	5–45	1–16	
95% CI	10.1–22.3	4.3–10.3	
2 (9)			
Mean (SEM)	5.8 (1.5)	2.6 (1.4)	NS
Range	3–17	−2–9	
95% CI	2.4–9.1	0–5.7	
3 (7)			
Mean (SEM)	6.0 (1.1)	1.3 (1.0)	0.01
Range	3–11	−3–5	
95% CI	3.4–8.6	−1.3–3.8	
4 (5)			
Mean (SEM)	16.4 (5.0)*	4.6 (2.1)	NS
Range	3–29	−2–11	
95% CI	2.5–30.3	−1.2–10.5	

* $P < 0.05$ (family 1 and family 4 *vs.* family 2 and family 3, Duncan's test).
† $P < 0.05$ (family 1 *vs.* family 3, Duncan's test).

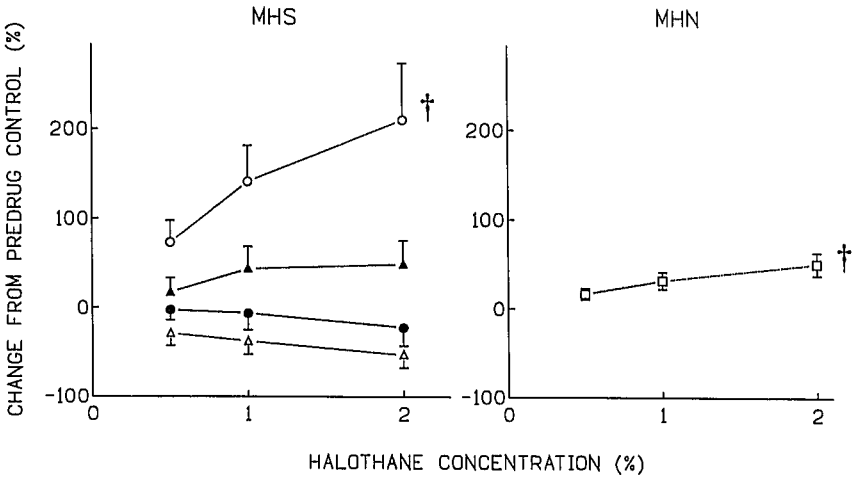

Fig. 2. Effects of halothane exposure on twitch potentiation (mean ± standard error of the mean) in the four family groups of clinically malignant hyperthermia–susceptible individuals and in malignant hyperthermia–negative individuals from all four families. Filled circles = family 1 (n = 15); open circles = family 2 (n = 9); filled triangles = family 3 (n = 7); open triangles = family 4 (n = 5); open squares = malignant hyperthermia–negative group (n = 11). †$P < 0.01$ (multiple *t* tests with Bonferroni's correction)

increased to ≥5 mN in this family. Before such an increase can be generally used as diagnostic, many individuals with clinical MH episodes need to be evaluated. Although false-positive MHS diagnoses are possible with the currently used contracture thresholds, it is not advisable to alter these values on the basis of genetic linkage analysis, before the specific genetic alteration(s) responsible for MHS is(are) known. Such linkage interpretations are speculative, because recombination(s) could have occurred between the MHS gene and the genetic markers.[1]

The validation and standardization of the currently used IVCT protocols were based on test results obtained from normal control individuals and from patients having presented with a clinical MH episode.[18,19] However, because the clinical presentation of MH varies and depends on the time of therapeutic interventions,[20,21] the selection of patients for a test validation is difficult. Further work to validate IVCT has been performed on muscle biopsy samples from normal and MHS pigs.[22,23] Generally, extrapolation of the results from a pig model to human beings may not be justified. In MHS pigs a single mutation of the *RYR* gene has been identified,[24] but in an analysis of 35 human families predisposed to MH the corresponding substitution was only found in a single family.[6]

In nearly all MHS individuals, we found increased muscle contractures to the halothane challenge compared with muscle contractures to the caffeine challenge. It is not surprising, therefore, that in patients with lower halothane contractures, no abnormal caffeine contractures could be observed. In other laboratories, increased muscle contractures to the caffeine challenge were found.[13] Although it has been observed

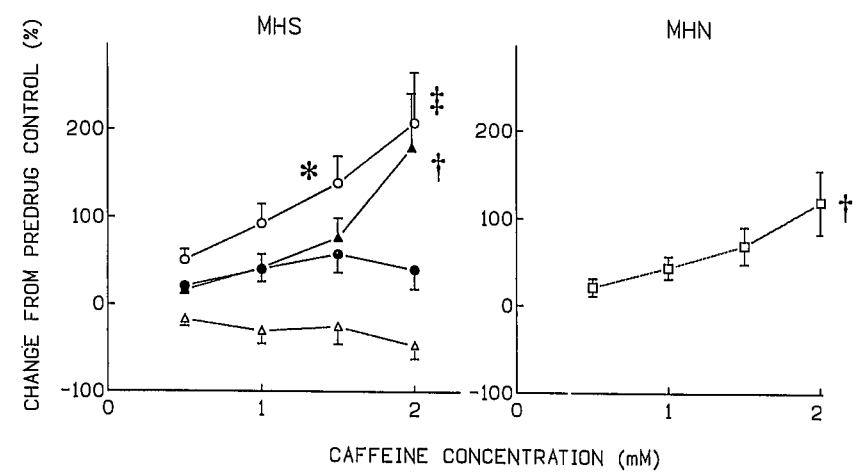

Fig. 3. Effects of caffeine exposure on twitch potentiation (mean ± standard error of the mean) in the four family groups of clinically malignant hyperthermia–susceptible individuals and in malignant hyperthermia–negative individuals from all four families. Symbols are identical to those in figure 2. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ (multiple *t* tests with Bonferroni's correction).

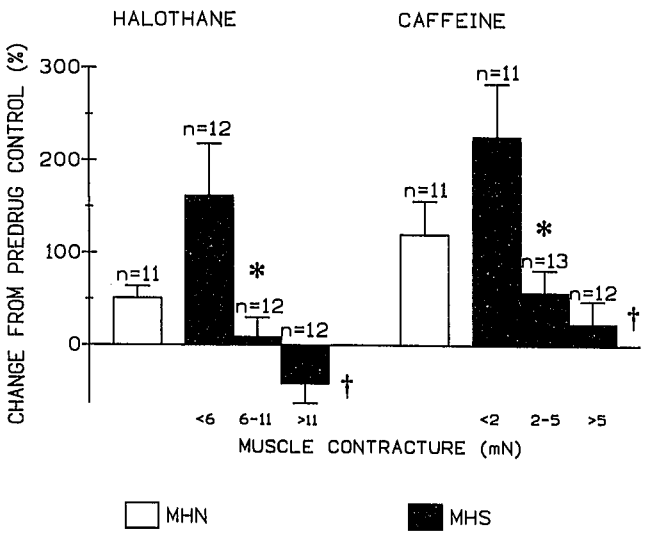

Fig. 4. Effect of muscle contractures on twitch potentiation (mean ± standard error of the mean) to halothane or caffeine exposure. Individuals with clinical malignant hyperthermia susceptibility (n = 36) were separated into three groups of approximately the same group size for the 2% halothane or 2 mM caffeine challenge. Twitch potentiation in the malignant hyperthermia–negative group (n = 11) was not statistically different from the other groups, neither for halothane nor caffeine exposure. *$P < 0.05$; †$P < 0.01$ (multiple *t* tests with Bonferroni's correction)

that the sensitivity of muscle strips to caffeine may decrease with time,[25] we can hardly accept this as a cause for decreased contractures to caffeine in our laboratory, because the IVCTs were performed simultaneously immediately after biopsy. In addition, we have observed an abnormal response only to caffeine in four individuals from other families (unpublished observation). We speculate that these differences between halothane and caffeine could be explained by either a different test procedure[12] or by a variety of genetically determined effects on IVCT. The different incidence of MHS *versus* MHEh diagnosis among the four families is reflected by significant differences of the force of contracture in the IVCT. Furthermore, the effects of halothane and caffeine on twitch potentiation of electrically stimulated skeletal muscle were significantly different among the families. Both muscle contracture and twitch potentiation are dependent on myoplasmic calcium concentration.[14,26] In families with lower muscle contractures and in MHN individuals, there was a significant dose-dependent twitch potentiation to halothane and caffeine (figs. 2 and 3). The decrease of twitch potentiation in individuals with higher muscle contractures (fig. 4) could potentially be explained by a faster loss of myoplasmic calcium control in these muscle bundles following halothane or caffeine exposure in contrast to muscle bundles presenting lower muscle contractures.

Although the predrug tension of muscle bundles in the current study was not statistically different, there are environmental influences on the results of IVCT. The variability in the IVCT in a study performed by Fletcher *et al.* on homozygous MHS pig muscle suggests a role for environmental effects on the test results.[23] The maximum muscle contracture to a 2% halothane challenge in the group of MHS pigs (n = 10) was 7.3 ± 1.3 mN (mean ± standard error of the mean). The 95% confidence interval was 4.3–10.3 mN. These results are comparable to those obtained in families 2 and 3, whereas halothane-induced muscle contractures were increased in family 1. The extent of variation in the IVCT results between this group of pigs and the family groups of clinically MHS individuals in our study (table 3) was not statistically different (F ratio test: $P > 0.05$). There is no basis to compare the caffeine IVCTs, because detailed results were not presented in the study by Fletcher *et al.*[23] Although we are unable to prove differences in the MH gene(s) among the four families presented, we speculate that the significant differences of the IVCT results among the families are caused by genetic rather than environmental influences because there was a degree of consistency in the test results within the families (incidence of MHS *versus* MHEh diagnosis, muscle contracture, percentage of muscle bundles exhibiting an abnormal contracture, twitch potentiation). It is not known, however, if the speculated genetic influences on the IVCT could have been caused by heterogeneity of the MH gene(s) itself or by heterogeneity of potential modifiers of muscle contractures to the halothane and caffeine challenges.

Our results underline the importance of detailed analysis of IVCT in families used for genetic studies. A "spectrum of susceptibility" to MH among the human population has been suggested for more than a decade.[27] The apparent heterogeneity of the predisposition to MH suggests more than a single genetic basis. Thus it may be some time before genetic testing provides preoperative diagnosis of all MHS individuals.[17] However, in certain selected families, it may be possible to diagnosis MH susceptibility with molecular biologic techniques if a large number of individuals has been previously investigated by IVCT.[1,28]

In summary, we found evidence for genetic differences in the results obtained by IVCT. These differences

might have occurred by heterogeneity of the MH gene(s) itself or by heterogeneity of various modifiers of muscle contractures. Our results underline the importance of a careful interpretation of IVCT findings in families used for linkage analysis as recently suggested.[7] IVCT data and properly documented clinical MH episodes are essential components for future MH research. Until such time that the molecular biologic aspects of MH susceptibility are clarified and a noninvasive diagnosis is available, we continue to recommend the evaluation of potentially MHS individuals by IVCT with proper test protocols.

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