Anesthesiology 2004; 100:1076–80

Molecular Genetic Testing for Malignant Hyperthermia Susceptibility

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Background: **For more than 30 yr, the *in vitro* contracture test (IVCT) was the only appropriate diagnostic tool for malignant hyperthermia (MH). After the introduction of molecular genetics into MH research, guidelines for molecular genetic diagnosis of MH susceptibility were published. The aim of this study was to establish applicability of the guidelines, sensitivity, and specificity of genetic testing in MH and advantages for studied patients.**

Methods: **The IVCT was performed following the guidelines of the European MH Group. Mutation analyses were performed by amplification of genomic DNA by polymerase chain reaction and restriction enzyme digestion.**

Results: **Two hundred eight individuals underwent MH testing between January 2001 and April 2003. In 32 of 67 initially genetic-tested patients, the familial mutation was identified, and they were diagnosed as MH susceptible. The IVCT followed negative genetic test results in 20 patients, and all but one had negative IVCT results. Three patients were scheduled to undergo elective surgery, and IVCT and genetic testing were performed simultaneously. All three had positive IVCT results and were carriers of their familial mutation.**

Conclusions: **In families with known MH mutations, there is a 50% chance of reliably confirming MH susceptibility by noninvasive testing. The authors found the negative predictive value of genetic testing to be 0.95 (95% confidence interval, 0.75–0.99), but for patient safety, they still recommend following the guidelines for genetic testing in MH and therefore performing an IVCT in case of negative genetic results.**

MALIGNANT hyperthermia (MH) is a pharmacogenetic disease with a dominant mode of inheritance. Clinical symptoms of an MH crisis are nonspecific, and the diagnosis can be difficult to establish. Nevertheless, MH must be rapidly recognized and adequately treated to prevent severe consequences.[1,2] Because MH is a subclinical myopathy, persons susceptible to MH (MHS) are asymptomatic during daily life. In the absence of the triggering agents (volatile anesthetics and succinylcholine), the diagnosis of MHS can only be established with specific testing. The knowledge of a correct MH diagnosis is important for patients who have survived a suspected MH episode because other family members may be affected by this dominantly inherited disorder. The major part of diagnostic investigations in MH is presymptomatic, *i.e.*, testing of relatives of MHS family members. Safe alternative anesthetic techniques are available for MHS individuals. Exclusion of MH is equally important because it can eliminate the threat of MH in the offspring and because MHS individuals might not be allowed to serve in military service, police departments, or fire departments and may experience difficulty obtaining health, disability, and life insurances.[1,3,4]

The accepted standard to test for MH susceptibility is the *in vitro* contracture test (IVCT). After an open muscle biopsy, muscle strips are challenged with halothane and caffeine. Molecular genetic investigations in MH have shown considerable locus and allelic heterogeneity.[5] More than 50% of MH families show genetic linkage to the skeletal muscle ryanodine receptor (RYR1) gene on chromosome 19q13.1,[6] and other loci have been identified as possible candidate loci on chromosomes 1q32, 7q11.23–21.1, 3q13.1, and 5p.[7] More than 30 mutations have been described in the RYR1 gene, with some of them detected at a frequency of 0.3–27%, whereas others are only identified in single families.[5,8–14]

According to recently published guidelines, the presence of a causative MH mutation allows for the diagnosis of MH susceptibility.[15] Because MH is heterogenetic and discordances between IVCT results and mutation analyses were reported,[5] a negative MH (MHN) diagnosis is not absolutely established by molecular genetic testing, and therefore, the IVCT must follow molecular genetic testing in patients in whom no MH mutation is detected.[15] Because the IVCT can only be performed in specialized laboratories and because of its invasiveness, research in MH has focused on the development of less invasive tests.[16] The guidelines for molecular genetic diagnosis of MH susceptibility are an important step toward less invasive MH diagnostics, but they were published as a consensus statement, and the process has not yet been validated.[17] The aim of this study was to analyze the application of these guidelines in our MH investigation unit with regard to sensitivity and specificity of genetic testing for MH and potential advantages for the tested patients.

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

Materials and Methods

Patient Selection

The study was approved by the ethics committee of the University of Basel hospital (Basel, Switzerland). All

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Received from the Departments of Anesthesia and Research, University of Basel, Basel, Switzerland. Submitted for publication September 12, 2003. Accepted for publication December 9, 2003. Supported by the Department of Anesthesia, Anästhesieverein, Basel, Switzerland, and grant No. 3200-063959.00 from the Swiss National Foundation, Bern, Switzerland.

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patients referred to the Swiss MH investigation unit (Department of Anesthesia, Kantonsspital Basel, Switzerland) for MH testing between January 2001 and April 2003 were included in this study after giving their written informed consent. According to the guidelines for molecular genetic detection of MH susceptibility, genetic investigations were performed in patients from families with known MH-associated mutations.[15] MHS individuals from families without previous genetic investigations were screened for the 15 mutations included in the guidelines and 8 additional mutations in the RYR1 gene. Details are given in our previous publication.[10]

Molecular Genetic Investigations

Mutation detection was performed in our anesthesia research laboratory by amplification of genomic DNA using the polymerase chain reaction followed by restriction enzyme digestion and polyacrylamide gel electrophoresis. Genomic DNA was isolated from whole blood. Conditions for polymerase chain reactions and oligonucleotides were as previously described.[10]

IVCT

During regional anesthesia, an open muscle biopsy was taken from the vastus medialis or lateralis muscle. The IVCT was performed according to the protocol of the European MH Group (EMHG).[18] Individuals were diagnosed as MHS if a contracture of 0.2 g or greater occurred at 2 mM caffeine or less and 2% halothane or less. An MHN diagnosis was established if a contracture of 0.2 g was not reached by 2 mM caffeine and 2% halothane. An MH equivocal (MHE) diagnosis was made if a contracture of 0.2 g or greater occurred only at 2 mM caffeine or less (MHEc) or 2% halothane or less (MHEh) but not with both testing substances. If surplus muscle strips were available, a 3% halothane bolus test corresponding to the halothane test defined by the protocol of the North American MH Group was performed.[19]

Results

At the Swiss MH diagnostic center, 208 patients from 62 different families underwent diagnostic procedures for MH between January 2001 and April 2003. In 11 of the 62 families (17.7%), the following MH associated mutations were known: R614C, V2168M, and G2434R. For the remaining 51 families, we screened for 23 MH mutations in 17 families, whereas 34 families were not screened for MH-associated mutations because the investigated patient was the first individual of his or her family to undergo MH diagnostic procedures (n = 26) or because the only MH-positive family members were diagnosed MHEh and not MHS (n = 8). We require at least one MHS individual within a family to start the screening program for MH mutations.

Table 1. Results of IVCT and Mutation Analysis in Three Patients Who Underwent IVCT during General Anesthesia for Elective Surgery

			Threshold Concentration to Reach ≥ 0.2 g		Contracture Force	
Surgery	Mutation	Twitch	Halothane	Caffeine	2% Halothane	2 mM Caffeine
Spinal	V2168M	2.1	0.5%	1 mM	1.9 g	0.9 g
CABG	R614C	1.0	1%	1.5 mM	1.4 g	0.7 g
Dental	R2458C	2.2	1%	2 mM	2.4 g	0.7 g

Three patients from families with known malignant hyperthermia mutations were tested by *in vitro* contracture test (IVCT) and molecular genetic investigations. IVCT was chosen because all three patients underwent elective surgery requiring general anesthesia. Malignant hyperthermia susceptibility was diagnosed by IVCT in all three patients, and all three were carriers of their familial mutation.

CABG = coronary artery bypass grafting.

Seventy-seven individuals were from families with known MH mutations. Sixty-seven of these underwent molecular genetic testing before being scheduled to undergo IVCT. Of the other 10 patients, three were scheduled for elective surgery during general anesthesia, and IVCT was performed at the same time, and seven patients from different families were not selected for molecular genetic investigations because the pedigree information excluded inheritance of the familial mutation, *e.g.*, a parent being diagnosed as MHEh and not carrying the familial mutation. All three patients scheduled to undergo elective surgery were diagnosed as MHS by IVCT and carried their familial mutation (table 1).

Of the 67 individuals who were initially evaluated using molecular genetics, the familial mutation was identified in 32 (48%). The mutations identified were V2168M (n = 22), G2434R (n = 6), and R614C (n = 4). In 35 individuals (52%), the familial mutation could not be identified (table 2).

Of the 35 individuals who did not carry the mutation of their family, 20 underwent open muscle biopsy and IVCT after molecular genetic investigations. The interval between molecular genetic investigations and IVCT was 0.5–21 months (median, 3.1 months). The diagnoses obtained by IVCT were MHN in 19 patients and MHEh in 1 patient, resulting in a negative predictive value of 0.95 (95% confidence interval, 0.75–0.99). In every patient, ICVT was performed according to the protocol of the

Table 2. Mutation Analysis of Patients Initially Tested by Molecular Genetic Analysis

Mutation	Positive, No.	Negative, No.	Total
R614C	4	2	6
V2168M	22	29	51
G2434R	6	4	10
Total	32 (48%)	35 (52%)	67

All patients with genetic testing as the first-line investigation for malignant hyperthermia susceptibility.

Table 3. MH Diagnoses of the 208 Patients Referred to our Center between January 2001 and April 2003

Diagnosis	IVCT Only	Genetics Only	Genetics and IVCT	Total
MHS	23	32	0	55
MHE	30	0	1	31
MHN	88	0	19	107
Unknown	0	15	0	15

All patients referred to our malignant hyperthermia (MH) investigation unit from January 2001 to April 2003 for MH diagnostic investigations. Diagnosis by *in vitro* contracture test (IVCT) only (IVCT only), molecular genetic investigations only (genetics only), and molecular genetics followed by IVCT (genetics and IVCT).

MHE = malignant hyperthermia equivocal; MHN = malignant hyperthermia negative; MHS = malignant hyperthermia susceptible.

EMHG, *i.e.*, two halothane and two caffeine tests performed on separate muscle bundles.[18] In the patient without his familial mutation diagnosed as MHEh, both halothane test results were positive, and both caffeine test results were negative. In this patient, threshold concentrations for a contracture of 0.2 g or greater were 0.5% for halothane and 4 mM for caffeine. The maximal contracture at 2% halothane was 1.3 g. Using a surplus muscle bundle, a bolus dose of 3% halothane was applied, similar to the NAMHG testing procedure, and it induced a contracture of 0.6 g, which corresponded to a positive test result (MHE) according to the NAMHG protocol.[19,20]

Fifteen patients with negative molecular genetic results have not yet undergone an open muscle biopsy and an IVCT. In nine patients, IVCT was not performed because of the patients' age: six were younger than 15 yr, and three were aged 80 yr or older. Six individuals were aged between 15 and 50 yr and will be scheduled to undergo IVCT according to our testing schedule availability.

A total of 141 individuals underwent IVCT without previous molecular genetic investigations. The resulting MH diagnoses were 23 MHS, 88 MHN, and 30 MHEh (table 3). In the 23 patients diagnosed as MHS, our mutation-screening program identified three mutations (one V2168M, two R614C).

Discussion

In this study, 67 individuals from MH families with known RYR1 gene mutations were tested by molecular genetic methods for MH susceptibility. Thirty-two were mutation carriers and were diagnosed as MHS. In 20 patients with negative genetic results, an IVCT was performed and resulted in a negative diagnosis in 19. The remaining patient was diagnosed with MHEh by IVCT. The negative predictive value of molecular genetic testing in MH families with known MH associated mutations was found to be 0.95.

With the publication of the guidelines for molecular genetic detection of MH susceptibility in 2001,[15] we initiated our program for genetic testing in our MH investigation unit. Although mutation V2168M is not yet included as a causative mutation in the European guidelines for molecular genetic detection of MH susceptibility, we decided to include this mutation in our diagnostic procedure for several reasons: (1) V2168M is the most prevalent mutation in the Swiss MH population[10]; (2) for 76 individuals carrying this mutation, none was diagnosed MHN by IVCT (unpublished data from our MH database, June 2003); (3) cultured human skeletal muscle cells[21] and B lymphocytes[22,23] from patients carrying mutation V2168M were significantly more sensitive to RYR agonists than controls; and (4) mutation V2168M is currently in the process of being included in the guidelines of the EMHG, as discussed at the 22nd Annual Meeting of the European MH Group, June 11–14, 2003.

From January 2001 to April 2003, 208 individuals were referred to our institution to be tested for MH susceptibility. After collection of family data and pedigree analysis, every person applying for an MH diagnostic procedure is automatically assessed if genetic testing is possible in the family. Accordingly, we developed a computerized relational database linking patient data, family information, IVCT results, and molecular genetic data. If the pedigree information does not exclude the possibility of inheritance of the familial mutation, a muscle biopsy and IVCT are not performed without previous genetic testing for the familial mutation. In 32 (48%) of 67 patients who were initially genetically tested, the familial mutation was identified, and an open muscle biopsy was avoided. The finding that 48% of genetically tested patients had the familial mutation is close to the expected 50% for a dominantly inherited disease and reflects careful selection of patients for genetic testing. With positive mutation analysis results, a simple blood test eliminated the need of ambulatory surgery, open muscle biopsy, and subsequent IVCT.

The integration of family information, IVCT results, and molecular genetic investigations into a single database made recently identified MH mutations readily available for genetic testing in other family members. In one family, the genetic screening program identified an MH mutation 2 months after a positive IVCT result. Two weeks later, two additional family members were genetically tested. One patient was found to carry the familial mutation and was therefore diagnosed as MHS without the need for further investigations. The other patient did not have the familial mutation and was diagnosed as MHN by IVCT 14 days after the genetic result.

Because a negative genetic investigation result does not unequivocally provide an MH diagnosis, it is important to explain to a patient the need for IVCT in case of a negative genetic test result. We prefer to discuss this issue and to obtain consent for an IVCT before initiating

genetic testing, to minimize the number of persons tested by molecular genetic methods who decline an IVCT. This would lead to inconclusive information regarding their MH status. In the 35 patients who did not have their familial mutation, the majority (75%) agreed to undergo an IVCT. The others were either too young or too old. The three patients aged older than 80 yr were investigated by genetic methods, although they did not agree to have an IVCT. We decided to go ahead with genetic testing because these patients were first-degree relatives of MHS individuals with known mutations, and a positive result in mutation analysis would have been the basis for genetic testing of their offspring.

In 20 individuals with negative genetic results, an IVCT was performed. With a single exception, they all tested MHN. The only positive IVCT result was from a patient who tested MHEh and came from a family carrying the mutation R614C. A second blood sample was taken, and the absence of mutation R614C was confirmed. Comparisons of the EMHG protocol and the NAMHG protocol for contracture testing have yielded slightly different results, with either the North American[24] or the European[25] protocol producing more positive results; it is important to note that this patient was MH positive (*i.e.*, MHE) by both protocols. Although the MHEh diagnosis in our patient could represent a false-positive IVCT result, he must still be considered as clinically MH positive, and any triggering agents must be avoided. This result confirms the importance of the guidelines that call for performing an IVCT in case of negative mutation analysis results. The finding of 5% (1 of 20) mutation-negative individuals being positive by IVCT is close to the results of a European study reported by Robinson *et al.*[5] in which IVCT and genetic data of more than 500 unrelated individuals from 11 European MH investigation units were analyzed. They found that 2.6% of families, or approximately 10% of individuals, had negative mutation analysis results, although the IVCT results were positive.

Three patients had to undergo general anesthesia for elective surgery because they were from families with known MH mutations, but we decided to perform an IVCT during the scheduled surgery rather than genetic testing. One reason was that the IVCT reveals a definite diagnosis of MH status, and the patient would have had to be scheduled for another surgery for open muscle biopsy in case of a negative genetic result. A second reason was quality control for both IVCT and molecular genetic investigations. Although IVCT is considered the accepted standard of MH diagnosis, it lacks the possibility of unambiguous quality control. Before molecular genetics were introduced into research and causative mutations of MH were identified, the sensitivity and specificity of the contracture test were determined by testing "normal" controls and "high risk" individuals. Positive results in controls were considered to represent false-positive results, while negative results in high-risk individuals were considered false negative. Although the probability is low, there is a chance of including true MH-positive individuals in the group of healthy controls. The selection of high-risk subjects is made by ranking their clinical symptoms of a suspected MH event according to the clinical grading scale.[26] Because the clinical presentation of MH is highly variable[3] and clinical data are frequently incomplete, the proportion of true MH-positive individuals within a group of patients with high clinical grading scales is unknown. The estimated specificities of the IVCT following the EMHG protocol and the caffeine halothane contracture test following the NAMHG protocol were 94% and 78%, respectively.[20,27] A new possibility for quality control of the IVCT was created with the identification of MH-associated mutations and molecular genetic methods. Confirmation of a positive IVCT result by detection of a familial mutation or absence of such a mutation, as in the case of a negative IVCT result, provides a new tool for quality control of IVCT. With the implementation of molecular genetic testing, the proportion of positive IVCT results is dramatically decreasing in families with known MH mutations because most MHS individuals will have been diagnosed by genetic testing. Therefore, concomitant IVCT and genetic testing in patients scheduled to undergo elective surgery provides important opportunities for quality control in MH testing.

As suggested by Rosenberg *et al.*,[28] molecular genetic analysis should be used in selected families when possible and appropriate, and it is becoming an important supplement of IVCT. As a prerequisite for genetic testing, mutation frequencies in the geographic region served by the investigation center must be known. Such frequency investigations have already been published for Germany, Italy, North America, Switzerland, and the United Kingdom, but data for other countries are still missing.[8,10,13,14,29] Screening for MH mutations without this knowledge is costly and is not recommended. The integration of molecular genetic results with pedigree information as well as IVCT data avoids open muscle biopsies and IVCT in a person whose family is known to have an MH mutation.

In families with known MH mutations, there is nearly a 50% chance to reliably confirm MH susceptibility by a noninvasive test, thus reducing health care costs and improving anesthesia risk management.[16] We found the negative predictive value of genetic testing to be 0.95, but for patient safety, we still recommend following the guidelines for genetic testing in MH and therefore performing an IVCT in case of negative genetic results.

The authors thank Joan Etlinger, B.A. (Scientific Secretary, Department of Anesthesia, Kantonsspital, University of Basel, Basel, Switzerland), for expert editorial assistance.

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