How Carefully Can We Phenotype Patients Suspected of Malignant Hyperthermia Susceptibility?

IN this issue of Anesthesiology, Fletcher et al.\(^1\) compare the European and North American protocols for laboratory diagnosis of malignant hyperthermia (MH) using halothane contracture. This carefully executed study concludes that muscle biopsy results, by either protocol, correlate sufficiently well to permit the use of either test for determining a patient’s phenotype (clinical expression of an inherited disorder) for molecular genetic study. Even though genetic research demands accurate phenotyping, does it matter whether clinicians accurately phenotype or label patients as MH susceptible in their clinical practices?

Yes. When we fail to diagnose MH susceptibility accurately, we end up labeling significant segments of our population MH susceptible. Because MH susceptibility follows autosomal dominant inheritance, diagnosis of MH susceptibility in a patient necessarily affects the family. MH-susceptible patients and their unevaluated but labeled “blood” relatives will not benefit from many routine anesthetic techniques and will require prolonged postanesthetic observation times. Those labeled as MH susceptible will be prevented from enlisting in the military forces and may not be allowed to serve in police or fire departments. In the United States, many MH-susceptible people and their families will experience difficulty obtaining adequate health, disability, and life insurance. Once labeled MH susceptible, patients and their incompletely evaluated relatives must notify all of their health care providers (including emergency medical technicians, emergency physicians, intensivists, respiratory therapists, perfusionists, oral surgeons, nurse anesthetists, and anesthesiologists) of their diagnosis and appropriate treatment. Once a patient is labeled MH susceptible, it becomes difficult to remove the label. Before a recent study by Bachand et al.\(^2\) 1.2% of the population of a region in Northwestern Quebec were labeled MH susceptible. As a result of their extraordinary efforts, which included retrieval of more than 2,000 medical charts and evaluation of nearly 1,600 general anesthetic records, Bachand et al.\(^2\) removed the MH-susceptible label from more than 1,000 patients. Now, only 0.47% of that population needs to be managed as MH susceptible. The US population would benefit from a similar reassessment of MH clinical status.\(^3\)

In the past decade, four significant advances have improved the accuracy of MH diagnosis. First, a comprehensive clinical case definition for MH has been accepted and, through the use of the “MH clinical grading scale,” the clinical likelihood of MH susceptibility can be estimated.\(^4\) To ensure its proper application, clinicians are urged to measure temperature, expired CO\(_2\), blood gases, creatine kinase, and potassium levels in all suspected MH episodes. Second, the European and North American MH diagnostic protocols have been standardized.\(^5,6\) Third, sensitivity and specificity for phenotype determination by these diagnostic tests have been determined.\(^7,8\) Sensitivities, defined as the ability of the test to detect disease when disease is present, are 97–99%. This means that false-negative results are rare, so clinicians can safely administer MH-triggering anesthetic agents to a patient who has had a negative MH muscle biopsy. However, false-positive MH muscle biopsy results are more frequent: reported specificities, defined as the ability of the same test to rule out disease when disease is absent, are 78% for the North American protocol and 94% for the European protocol. Thus, some patients with positive MH muscle biopsies may not be truly MH susceptible. Fourth, MH mutations in two gene loci have been identified,\(^9,10\) accounting for the inheritance of MH in 20–40% of MH families.\(^11\) For families carrying known mutations, inheritance can be followed, with the proviso that no second MH mutation has appeared in the family under study.

Dr. Fletcher et al.\(^1\) conclude that although either protocol can be applied to genetic studies, the European protocol might yield slightly better results. Unfortunately, as the authors acknowledge, the use of either the European or North American protocols leads to difficulty
in genetic analysis because both tests achieve high sensitivity at the cost of reduced specificity. Both false-negative and false-positive diagnoses will lead to discordance (lack of linkage) with the presence or absence of a defined mutation. This conclusion does not alter the clinical value of both tests, wherein high sensitivity is compatible with sound clinical practice.

Genetic linkage analyses published to date support only about 50% linkage to either of the two MH loci where mutations have been found that result in MH or central core disease (CCD) as the primary disease. These loci are the RYR1 gene on chromosome 19q13.1\(^2\) and the CACNL1A3 gene on chromosome 1q32.\(^3\) The RYR1 gene encodes the skeletal muscle calcium-release channel (or “ryanodine receptor”); the CACNL1A3 gene encodes the \(a_1\) subunit of the skeletal muscle L-type, voltage-dependent calcium channel (or “dihydropyridine receptor”). Abnormalities in the products of either of these genes lead to abnormal regulation of calcium in skeletal muscle. Possible explanations for lack of linkage to either of these two loci are that there are additional MH loci,\(^4\) that phenotyping has limited accuracy, or that multiple mutations segregate in some MH families.\(^5\)

Figure 1 illustrates data selected from several European and North American laboratories\(^6\) using the European protocol, observed linkage to chromosome 19q13.1 in 9 of 20 families studied (families 1–20). Linkage was excluded in three families on the basis of two or more recombinant events, manifested as discordance between the presence of MH and presence of a specific marker for chromosome 19q, and in the remaining eight families, a single recombinant event was observed. Phillips et al.,\(^7\) using the North American protocol, studied segregation of the RYR1 mutation, R2435G, with MH in four families (families 23–26), finding concordance in two families (9/9 and 1/1) and discordance in two other families (3 discordant out of 20 and 1 discordant out of 2). Both of these studies illustrate linkage of MH to RYR1 in about 50% of MH families.

A caveat, however, is that accurate linkage depends on accurate phenotypes, and the sensitivities and specificities of both the European and North American diagnostic protocols do not guarantee accuracy. Furthermore, the lack of perfect concordance between the European and North American phenotypic tests, reported in the paper by Fletcher et al.,\(^1\) supports the conclusion that MH diagnostic testing does not provide unambiguous phenotyping for use in genetic tests. Figure 1 also illustrates that CACNL1A3 mutations have shown linkage (discordance) for 19/19 members of one MH family (family 22),\(^2\) but lack of linkage (discordance) for 4/51 members of another MH family (family 21),\(^3\) posing a parallel problem to that observed in linking MH to RYR1. To date, there has been full concordance between central core disease associated with MH (CCD/MH) and RYR1.\(^4,5\) This is probably because of greater accuracy in phenotyping CCD patients on the basis of their characteristic clinical presentation and muscle histochemistry, in addition to their reaction to MH-triggering agents.

What research directions are likely to improve the phenotypic diagnosis of MH? First, the search for new contracture tests might lead to greater accuracy. Recent evaluations of the ryanodine contracture test, however, show overlap between contractures of MH-negative and MH-susceptible patients, that are consistent with the same sensitivity and specificity as caffeine halothane contracture tests.\(^6\) Investigators are testing α-chloro-m-cresol, a succinylcholine solvent that modulates calcium release channel gating, to determine whether it will help improve contracture test specificity.\(^7,8\)
Second, we must understand the reasons underlying the limitations in phenotypic accuracy. One possibility is compensation within MH muscle. A recent study measured the sensitivity to caffeine and halothane of 15 MH or CCD/MH mutations, expressed in HEK-293 cells. Each of these mutations had higher sensitivity compared with wild-type, which is in line with the assertion that these mutations are potentially capable of causing MH even though their presence is not invariably associated with MH crises or with positive MH diagnostic tests. Of additional interest was the observation that HEK-293 cells transfected with MH and, particularly CCD mutations, had elevated resting Ca\(^{2+}\) concentrations. The synthesis of Ca\(^{2+}\) pumps in these transfected cells was increased by up to twofold, apparently in an effort to compensate for the excess release of Ca\(^{2+}\) through mutant release channels. Varying degrees of compensatory protein synthesis could account for the problems in phenotypic analysis of MH and explain its gender- and age-dependent expression.

A third research direction should attempt to rationalize the numerical similarities that exist between the type of data illustrated in figure 1 and the data published for the sensitivity and specificity of the European and North American phenotypic tests. Although linkage of RYR1 or CACNL1A3 to MH with either the European or the North American tests occurs in only about 50% of MH families, the number of discordant patients is low, typically in the range of 1 discordant individual per 10 tests. It is intriguing to note that this number of discordant persons is in the range that would be predicted if the specificity of the phenotypic test were in the 78–94% accuracy range and if the sensitivity were 97–99%.

Current limitations of linkage analysis, however, do not support the proposal that linkage really does exist in all of the cases presented in figure 1.

Fourth, because genotyping has the potential to lead to 100% accurate diagnosis, it is clear that identification of new MH loci and new MH mutations should receive priority. However, inaccurate phenotypic diagnosis can create problems in both of these areas. The search for new loci will be frustrated if apparent linkage is based on inaccurate phenotyping. Similarly, the discovery of potentially causal new mutations that may not always link to MH in the extended family confuses the issue of whether they are truly causal mutations. Fortunately, functional analysis of mutant proteins can help to prove their potential causal nature.

In the next decade, we predict substantial progress in improving MH genotyping, including the identification of additional causal genes and mutations within these genes that are causal of MH. A major goal will be to understand, at cellular and organ levels, the pathophysiological disturbances responsible for the clinical syndrome we currently call MH, with the prospect of improving phenotyping. More effective therapies are likely to follow.

**References**


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Memory Function during Anesthesia

WHAT happens to the thinking brain during “general anesthesia”? More specifically, are the higher processes of cognition turned off? Two articles in this issue of Anesthesiology show that some form of memory can be formed during what every clinician would call adequate anesthesia.1,2 These findings are in line with many previous investigations in this area. Anesthesia represents a iatrogenic state in which drug-induced “sleep” and memory interact, usually in the setting of the liberal use of muscle relaxants. During clinically adequate anesthesia, the brain is capable of receiving auditory stimuli and of processing them at a fairly complex level, at least in certain cases.3–7 Therefore, the brain, although anesthetized, is functional in the higher cognitive sense. Because we still do not know why the brain sleeps, how anesthesia works, or even the workings of memory, it is no surprise that we cannot figure out what happens to memory and cognition during anesthesia. As with sleep,

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