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Molecular Genetics and Malignant Hyperthermia

The laboratory diagnosis of malignant hyperthermia (MH) is, at present, cumbersome, expensive, and controversial. However, when properly conducted, the caffeine–halothane contracture (CHC) test can predict susceptibility to MH with an acceptable sensitivity and specificity.[1] Two different approaches that are now converging may overcome the limitations of the CHC test, the only accepted diagnostic test for MH. First, those performing muscle biopsies for MH diagnosis have been meeting on a regular basis to standardize the testing procedure and to define the sensitivity and specificity of the test by comparing contracture responses in muscle from normal surgical patients with those from patients with a definitive clinical diagnosis of MH.[2] Second, linkage analysis using molecular genetic techniques are being used to identify the location of the gene(s) responsible for MH.

The latter approach has tentatively localized the MH defect to a region on chromosome 19. The ryanodine receptor (RYDR) and several other genes[3,4] are encoded within this region. The RYDR is a calcium-release channel located between the T tubule and the terminal cisternae of the sarcoplasmic reticulum in skeletal muscle. This proposal has much appeal, since it is known that intracellular free calcium is increased during an MH crisis and since there is some biochemical evidence suggesting that an abnormality exists in the RYDR in MH swine. Also, if a gene defect is identified, it would be possible to isolate the deoxyribonucleic acid (DNA) from peripheral blood cells to diagnose MH susceptibility directly. Hence, a less invasive diagnostic test for MH is a possibility for the future.

In this issue of ANESTHESIOLOGY, MacKenzie and colleagues[5] compare the outcome of the CHC test with inheritance of DNA markers in RYDR and arrive at some rather interesting and challenging conclusions. Their study is the first, to date, to present specific contracture responses in each patient for whom linkage analysis for RYDR was conducted. An appreciation of the significance of the work of these investigators requires an examination of the controversies surrounding the RYDR gene and MH susceptibility as well as of the limitations of the methodologies used.

The molecular techniques are simple in concept. DNA markers immediately surrounding the gene(s) for MH will be inherited with the abnormal phenotype (here, an abnormal CHC test). If we know the location of the DNA markers, we know where to begin searching for the defective gene(s). Two previous reports mapped the MH susceptibility phenotype (as determined by an abnormal CHC test) to chromosome 19, near the RYDR gene by "linking" the inheritance of the MH phenotype to inheritance of DNA markers on the long arm of chromosome 19.[3,4]

What does this really mean? Crossing-over of homologous chromosomes can occur at meiosis, which recombines maternal and paternal chromosomes. In general, the closer one interval of chromosome lies to another on the same chromosome, the more likely they will be inherited together, or "linked." Consequently, the further one interval of chromosome is from another, the more likely recombination will occur between them and they will not be co-inherited. The rate of recombination between genes can be used to place them in a linear order along a chromosome (a linkage map). In this manner, genes further from the MH susceptibility gene will show higher recombination rates. Since chromosomes are inherited independently at meiosis, so will genes that have

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Address reprint requests to Dr. Rosenberg: Department of Anesthesiology, Hahnemann University, Broad and Vine, 9th Floor North Tower, Philadelphia, Pennsylvania 19102-1192.

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been separated by recombination. If MH can be inherited independently from a marker for the RYDR gene (*i.e.*, if recombination occurs between them), then it is likely that a defect in this gene does not cause MH.

Human linkage is evaluated by the method of likelihood ratios. We compare the probability that the observed pedigree data would arise under one hypothesis (for instance, that an RYDR gene marker is linked to an abnormal CHC test with no recombination [$\theta = 0$]) with the probability that it would arise under an alternative hypothesis (nonlinkage). The ratio of these probabilities is called the odds ratio. For convenience, human geneticists prefer the log of the odds ratio (lod score), which can be added together from independent families. Convention dictates that when the odds become overwhelming (lod = 3, or 1000:1 in favor of linkage), linkage is considered proven.

By evaluating whether an abnormal CHC test is inherited with one RYDR marker in a large family pedigree in which many individuals have had clinical episodes of or have been tested for MH, MacKenzie *et al.*[5] have found disparate results between the outcome of the CHC test and analysis for linkage to RYDR. They observe that by changing the CHC test contracture cutoffs, they can demonstrate linkage (in this family). However, if eventually it is found that the CHC test data are true and the linkage data are faulty, it is possible that some MH-positive outcomes will have been mislabeled as normal. The positive value of their suggested alterations of thresholds for susceptibility is that the biopsy testing groups may wish to test these modifications against the CHC test data obtained from surgical controls and those with known susceptibility. The cutoff values for susceptibility, as determined at the initial meeting of the North American Malignant Hyperthermia Group, were arrived at by consensus; it was recognized that they would need verification. They were to be considered suggested values against which each laboratory should compare its own results. Indeed, it now does seem likely that the caffeine specific concentration cutoff of 4 mM is too high and is associated with false positive results. The halothane–caffeine specific concentration test is already considered an optional test because of the high incidence of false positive responses using the cutoff of 1 mM.[6]

Although false negative classification by the CHC test is probably close to zero,[7] the false positive rate is not known with certainty. Therefore, using the CHC test, the linkage studies can only estimate θ between the RYDR gene and this diagnostic test. An error of only 1% in estimating θ would place an MH gene approximately 1,000,000 base pairs from RYDR. The significance of such an error can be appreciated if one considers that as many as 30 genes or more may lie in this interval of chromosome.

It remains unclear whether the data of MacKenzie *et al.*[5] represent evidence of misclassification by the CHC test or confirm that recombination occurs between MH susceptibility and the RYDR gene. If we knew what genetic mutation(s) produce MH susceptibility, then we could evaluate how often the CHC test misclassifies a patient with certainty. Recent evidence from our laboratories demonstrates recombination between an abnormal CHC test and genetic markers on chromosome 19q13.1–13.2, including the RYDR gene.[8,9] In addition, the only persuasive biochemical evidence for a defect in the RYDR *per se* has been gathered in swine.[10] The function of the human RYDR appears to be different from that of porcine RYDR.[11] Although we agree that the function of the RYDR is altered in MH, we have been unable to confirm any biochemical evidence for a defect in the RYR protein itself.[11,12] Rather, there is a strong possibility that an altered second messenger system, such as fatty acids[11] or inositol 1,4,5-trisphosphate,[13] may lead to alteration of the RYDR function.

Undoubtedly, MH is a heterogeneous disorder. For example, it may be triggered by succinylcholine in some patients but not in others, and the severity of signs and symptoms differ among patients. Altered RYDR receptor function may play a role in MH in some families. Although we may have a long way to go in understanding this disorder, the information from the study by MacKenzie *et al.* point the way for further studies in the diagnosis and pathophysiology of MH. Once the gene(s) for MH is (are) defined, then other techniques may be used to determine which base pairs are abnormal in the DNA coding for that gene. It is encouraging that the talents of geneticists, molecular biologists, biochemists, and anesthesiologists are being applied to unraveling the mysteries of this unique disorder.

ROY C. LEVITT, M.D.
Assistant Professor of Anesthesiology and Critical Care Medicine
Department of Anesthesiology

DEBORAH MEYERS, PH.D.
Associate Professor of Medicine
Department of Medicine, Division of Genetics
Johns Hopkins University
Baltimore, Maryland

JEFFREY E. FLETCHER, PH.D.
Associate Professor of Anesthesiology

HENRY ROSENBERG, M.D.
Professor and Chairman
Department of Anesthesiology
Hahnemann University
Philadelphia, Pennsylvania

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