Molecular Medicine and Malignant Hyperthermia

A Step Ahead

Those of us who are practicing anesthesiologists have roughly even odds of encountering a single patient with malignant hyperthermia (MH) over the full course of our careers. The likelihood that we’ve left our training with deep knowledge of human molecular biology is somewhat more remote. For these reasons, the report by Lynch et al. may escape attention in a quick glance at this issue’s contents. Yet, as a benchmark of how far molecular medicine has already taken us, and as an indication of the direction our specialty will be heading in the future, this article repays a closer look.

Lynch et al. begin by first defining the MH phenotype (history, physical examination, and laboratory diagnosis) in a patient and 17 family members. Next, using DNA isolated from the white blood cells (genomic DNA) of the phenotyped individuals, they used linkage analysis to test their suspicion that an alteration in the skeletal muscle ryanodine receptor gene (RYR1) is responsible for inheritance of MH in this pedigree. Small sequence variations (alleles) in regions (loci) of noncoding DNA on either side of the RYR1 gene were identified and numbered (e.g., alleles 1, 2, 3, . . . 15 at locus 19S191). From the assortment of possible alleles, one may be found at identical loci along an individual’s paired chromosomes. If loci are in close enough proximity that the odds of crossing over between sister chromatids (recombination) during meiosis are remote, a cluster of alleles from two or more loci will be inherited as a unit (haplotype) “linked” to the gene of interest. 

In four loci bracketing the RYR1 gene, Lynch et al. found that MH-susceptible (MHS) individuals shared the same haplotype (alleles ‘13, 8, RYR1, 2, 9’ at loci 19S191, 19S220, RYR1, 19S190, and 19S223, respectively), whereas healthy individuals inherited a variety of distinct haplotypes. Therefore, each MHS individual inherited the same RYR1 allele, providing strong presumptive evidence that RYR1 is a candidate gene for MH in this kindred.

Finding none of the previously published MH-associated RYR1 mutations, the authors undertook a mutation search using single strand conformation polymorphism analysis. Because the casual mutation is unlikely to reside in noncoding regions (introns) of RYR1, they started by assembling DNA in vitro to be complementary (cDNA) to messenger RNA (mRNA) isolated directly from the proband’s muscle, in which introns have been deleted before translation of the RYR1 mRNA into the RYR1 protein. By altering the three-dimensional shape of cDNA fragments denatured to single strands, changes as small as a single nucleotide substitution produce detectable differences in mobility through an electrophoretic gel. Single strand conformation polymorphism analysis is efficient in screening large quantities of DNA (e.g., RYR1 cDNA with more than 15,000 base pairs), but it suffers a small miss rate in the identification of new mutations, and, in this instance, failed to pick up a difference between the RYR1 of the MHS proband and normal.

Undaunted by their negative result, and buoyed by tight linkage of the MHS trait and RYR1 haplotype, the authors turned to direct sequencing of amplified and cloned fragments of the proband’s RYR1 cDNA. Starting at the 5’ terminus of the RYR1 gene (corresponding to the amino terminus of the RYR1 peptide), they identified a previously unknown mutation (T130) that results in substitution of an arginine for cysteine at the 35th of more than 5,000 amino acids that compose the RYR1 protein. Knowing the normal and mutant sequence in this region, the authors were now able to design primers for polymerase chain reaction amplification of genomic DNA from family members that would enable rapid detection of the mutation by digestion of the polymerase chain reaction product with a restriction endonuclease (AcI), which cuts only at the mutant DNA sequence. Not only did they demonstrate co-inheritance of the mutation with dominantly inherited MHS in the family, but they found that two individuals in the pedigree (the proband and a sister) were homozygous for the mutation, each carrying two copies of the mutant RYR1 gene. This result might have been suspected in the past on the basis of consanguinity in the family, and of greater sensitivity to caffeine in the contracture test of these two individuals, but it could not be tested. Confirmation of MH homozygosity by genotype is a novel discovery, representing a profound example of the parsimony
of molecular medicine in bundling observations made at the laboratory bench with those at the bedside.

Nine RYR1 MHS mutations have been published to date, and an additional seven have been identified. At least three other chromosomal loci (3, 7, 17) have been proposed as candidates for MH on the basis of linkage analysis. Leaving out MH-like events associated with other inherited myopathies, and summing the frequencies of each candidate mutation and locus in databases screened thus far, the search for human MH-associated mutations and candidate genes is now perhaps halfway to completion, a remarkable record for investigations begun within the past decade.

How do we know that any of these genotypic alterations truly cause MH, and are not co-inherited with MHS by coincidence? This question, which is fundamental to informed use of the data for anesthetic management and counseling of family members, may be addressed by both statistical and biologic criteria. In the family reported by Lynch et al., inheritance of the mutation is highly correlated with the MH status of the individual. In addition, the mutation is not found in samples (100 individuals, therefore 200 chromosomes) from the healthy population. Presence of the mutation in two or more unrelated families with MH would strengthen the statistical argument for causality somewhat, but may be confounded by the small number of well-phenotyped families available to be tested.

Biologic criteria for genetic causality are more compelling, but far more challenging to meet. Lynch et al. show that the mutation produces a change in the composition of the RYR1 peptide, and, insofar as this protein is integral to the regulation of calcium in skeletal muscle, the mutation is biologically plausible. The case for causality would be buttressed if an identical mutation produced a comparable phenotype in two distinct species, as is the case for the RYR1 R615C mutation found in both porcine and human MH. However, no animal models have been identified mirroring any of the other human MHS genotypes. Conferring the mutant phenotype in a second species or tissue by gene transfer, using mutant cDNA as a reagent, represents the strongest possible evidence favoring causality. Functional expression of normal and mutant human RYR1 genes in cell lines such as that created by Nakai et al. to be homozygous for a disrupted RYR1 gene,\(^5\) has enormous potential to illuminate the cellular pathophysiology of MH, particularly if differences are detected between heterozygous and homozygous transfections, and in the presence of volatile anesthetic agents.

How far are we from relying on MH molecular genetic data for clinical decision making? A geneticist will answer this question with another: What degree of risk is acceptable? If no MH risk is acceptable, we are obligated to discontinue the use of triggering agents, conserving resources currently spent on dantrolene, contracture testing, and molecular genetics for other purposes. Yet we have tacitly decided, as a profession, that the benefits conferred by ongoing use of triggering agents (or the disasters averted) outweigh the rare catastrophe when they are administered to a patient who has lost the genetic lottery. However, as overall anesthetic morbidity and mortality approaches or dips below MH risk, as the incidence of MH-associated alleles in the population becomes known with precision, and as alternative agents and techniques carrying no MH risk enter our practice, routine and widespread use of triggering agents must be reconsidered periodically.

If, however, some measure of MH risk is acceptable, to what use can we put molecular genetic techniques now? For diagnosis of members of families such as that reported by Lynch et al., with close accord between the MHS phenotype and a genotype believed to be causal, an individual with the normal allele has slightly less risk for an MH event than someone selected at random from the general population, albeit the risk is still greater than zero. Conversely, as reports of families accumulate in which MH-associated mutations are found, but the correlation between genotype and phenotype is partial, the risk that either (or both) the genetic and contracture tests are incorrect must be estimated by testing the concordance between genotype and phenotype in members of intervening generations. In absence of this data, or if the concordance in obligate MH carriers is incomplete, MH diagnosis using genetic tests alone is premature.

Most MH-associated mutations and candidate loci are “orphans” occurring in only a single family, as found in the pedigree described herein. Coupled with the genetic heterogeneity underlying human MH, hopes for routine preoperative population screening using DNA-based methods currently appear to be frustrated. Although existing technologies are not up to the task, in the near future, it will be possible to perform cost-effective, simultaneous analysis of hundreds of samples of genomic DNA for a large number of known mutations in different genes.\(^6\) As the enabling technologies converge with our expanding pharmacogenetic knowl-

\(^{*}\) T. McCarthy (personal communication).
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edge, the ability of the anesthesiologist in clinical practice to interpret and evaluate investigations such as that by Lynch et al. will become the rate-limiting step in tailoring the selection of anesthetic agent and technique to each patient’s unique genotype.

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References