◇ This Month in

ANESTHESIOLOGY

■ Screening of Tissue from Malignant Hyperthermia–susceptible Patients Reveals Novel Genetic Mutations. Sambuughin *et al.* (page 594)

More than 20 ryanodine receptor 1 (RyR1) mutations have been identified in families with an inherited susceptibility to malignant hyperthermia (MH). Sambuughin *et al.* obtained skeletal muscle samples from 73 unrelated people with diagnoses of MH susceptibility by using the caffeine–halothane contracture test of the North American Malignant Hyperthermia Group. The patients had been referred for the test because of either a positive family history of MH or development of signs of MH during anesthesia.

The muscle samples were collected during a period of 3 yr and were stored at −70°C. Genomic DNA was extracted from stored muscle tissue or peripheral blood and was amplified using standard polymerase chain reaction–based restriction fragment length polymorphism, single-strand conformation polymorphism, and sequencing analysis. Most of the known RyR1 mutations were analyzed using the restriction fragment length polymorphism method, whereas new mutations were searched by single-strand conformation polymorphism in exons 12, 15, 39, 40, 44, 45, and 46 of the gene. Seven known RyR1 mutations were detected at frequencies ranging from 1.4% to 5.5%. The research team also detected three novel amino acid substitutions, at frequencies of 1.4% each. All 10 mutations were in 16 unrelated individuals and account for 21.9% of the screened North American MH-susceptible population. Statistical correlation between caffeine–halothane contracture test phenotypes and RyR1 was not studied because of the small number of subjects carrying the same mutation and lack of family members for the study. The frequency and distribution of RyR1 gene mutations detected in this North American MH-susceptible population were different from those previously identified in Western Europe. Larger-scale studies are needed, say the authors, to assess the type and frequency of mutations in RyR1 associated with MH in the North American population.

■ Extensively Long Recovery after Mivacurium Caused by Rare "Silent" Gene Mutations. Gätke *et al.* (page 600)

In patients who have the butyrylcholinesterase gene (*BChE*), mivacurium is rapidly hydrolyzed in plasma, and duration of action is short. An estimated 24% of the white population carries a genetic variant allele of the BChE enzyme, which can result in slow hydrolysis of mivacurium and prolonged neuromuscular block. In this article, Gätke *et al.* describe the anesthetic course of a healthy 30-yr-old man undergoing a correction of the inner nose who received a single dose of 10 mg mivacurium to facilitate tracheal intubation.

Ninety minutes after administration of mivacurium, at completion of surgery, the patient had no response either to train-of-four or to tetanic stimulation. The patient was transferred to the intensive care unit, where neuromuscular blockade was continuously monitored until a train-of-four ratio of 0.75 was obtained—nearly 8 h (469 min) after injection of mivacurium. While the patient was in the intensive care unit, venous blood samples were collected at 12 regular intervals (from 134 to 494 min after mivacurium administration). A blood sample drawn when the patient arrived in the intensive care unit was analyzed to determine BChE activity, phenotype, and genotype. The patient's BChE activity was zero, indicating that he was homozygous for silent mutations. Subsequent to the patient's discharge from the hospital, the researchers obtained permission to perform complete nucleotide sequencing of the BchE gene of not only the patient, but also of his parents and siblings.

DNA analysis revealed two point mutations, the known S7 variant and an undescribed mutation, which introduced a stop codon at amino acid residue 172 of the 574 amino acid residues of normal BChE. Biochemical data supported the finding that the patient was compound heterozygous for two silent mutations. Pedigree analysis showed that S7 was inherited from the patient's mother and the novel mutation was inherited from his father, proving that both alleles are affected in the patient. Screening for abnormal BChE genotypes is not practical or cost-effective in daily clinical practice, but it could be used, according to the authors, to supplement equivocal results obtained by biochemical methods and in clinical situations such as the one described in this article.

■ Is the Basolateral Amygdala Complex Involved in Mediating Propofol-induced Amnesia? Alkire *et al.* (page 708)

Alkire *et al.* designed an experiment to test whether the basolateral amygdala complex (BLAC) is a brain site

involved with mediation of propofol-induced amnesia. The team assigned 85 male Sprague-Dawley rats to one of two surgery groups: sham-operated controls or those to receive bilateral *N*-methyl-D-aspartate lesions of the BLAC. The rats were then allowed 6 or 7 days of recovery time before beginning inhibitory avoidance training.

On the training day, either 25 mg/kg propofol or saline was administered to the rats 5 min before the training procedure began. The training apparatus featured a light-safe compartment and a dark-shock compartment. Rats instinctively favor a dark environment. When the animals stepped into the dark compartment with all four paws, they received a foot shock (0.4 mA) until they escaped back to the light compartment. The door to the dark compartment was left open; learning was considered to have occurred when animals avoided the dark side for more than 60 consecutive seconds. Memory retention was tested 24 h after the training session. No shock was delivered during the memory testing, but longer latencies to cross into the dark side of the apparatus were considered to be indicative of better retention of training. At the end of the experiments, the rats were killed, and their brains were removed for histologic categorization of the BLAC lesions.

Rats in the sham-operated group that were given saline injections had a median memory latency of 300 s, whereas sham-propofol rats had significant amnesia, with a median latency of only 63 s. Rats with BLAC lesions showed robust memory latency despite administration of propofol: those in the saline group had a median memory latency of 300 s, and those in the propofol-injected group had a median memory latency of 323 s. In these experiments, discrete BLAC lesions blocked the amnestic effect of propofol, suggesting that the BLAC is a key brain site mediating anesthetic-induced amnesia. The results may help explain the clinical phenomenon of intraoperative recall and the enhanced retention of negative emotional material, and they point out the need to understand anesthetic-induced amnesia more fully.

■ Genetic Mutation Characterized in Malignant Hyperthermia Canine Population. Roberts *et al.* (page 716)

In pigs and in up to 50% of humans who experience malignant hyperthermia (MH) events, the disorder has been linked to mutations in the calcium release channel of the sarcoplasmic reticulum, also known as the ryanodine receptor 1 (RyR1). To determine the molecular basis of canine MH, Roberts *et al.* established a breeding colony by mating a male mixed-breed dog that was MH-susceptible (MHS) and had survived an *in vivo* halothane–succinylcholine challenge to three unaffected females, producing four litters. Two additional litters were produced by back-crossing to an unaffected daughter and by mating an MHS son to an unaffected female. Of the total of 47 dogs produced from this breeding program, 31 were subjected to a halothane–succinylcholine challenge in an effort to trigger an MH event. The researchers also biopsied specimens of gracilis muscle obtained from all 47 dogs to determine whether they were MHS or MH normal (MHN) according to the North American Malignant Hyperthermia Group's test protocol for establishing MH (the *in vitro* contracture test with halothane and caffeine).

During the *in vivo* challenge, tachycardia, hyperthermia, and hypercapnia developed in 24 dogs, establishing them as MHS. (In contrast to pigs and humans with MH, dogs do not exhibit lactic acidemia, metabolic acidosis, or extensor rigidity.) With the *in vitro* contracture test, 23 dogs were MHS, 19 were MHN, and 5 were indeterminate. Animals were then tested for linkage to the MHS phenotype. Results of the mutational analysis showed that MH susceptibility in this study colony was transmitted as an autosomal dominant trait. All MHS dogs were heterozygous for the new T1640C mutation found on chromosome 1. None of the MHN dogs carried this mutation. Detection of this mutant allele by clinical challenge and *in vitro* contracture testing suggests that a dog model may be useful for developing improved phenotypic detection of heterozygotes needed for human diagnosis and genetic investigations.

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