Be Wary of Genes Governing Awareness

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On October 16, 1846 William Morton demonstrated the successful administration of diethyl ether allowing the removal of a neck tumor from a quiescent and pain-free patient. With the advent of muscle relaxants, the significance of immobility as evidence of adequate anesthesia was diminished. However, it was not until 1950 that the first case report of insufficient anesthesia appeared.[1] Recently, a great deal of interest has surfaced in improving our ability to eliminate this catastrophic occurrence.[2]

Aranake *et al.*[3] demonstrated a fivefold increased risk of awareness with recall in patients with a previous history, but whether this increase reflects a genetic predisposition, or is attributable to environmental factors, remains unknown. The study by Sleigh *et al.*[4] in this issue of Anesthesiology represents a bold first attempt to determine a potential genetic vulnerability. Assuming that there are a small number of important molecular targets for anesthetics, alleles of those targets may exist which cause an individual to be resistant to general anesthesia. Although we limit our discussion to molecular targets, the reader should realize that similar arguments apply to developmental genes controlling neuronal circuits underlying anesthetic effects; changes at the circuit level could also shift anesthetic sensitivity.

Several molecular targets have been hypothesized to form the site(s) of action of volatile anesthetics although, as yet, no single protein or channel has been identified as the unique anesthetic target to the exclusion of all others. If multiple genes contribute to the behavioral response of anesthesia then the relative importance of each gene product cannot be easily predicted. Regardless of the number of anesthetic targets, the genetic blueprint specifies proteins that determine the nature of all components of a cell; any response to a neuroactive drug must also depend on this genetic information. A natural extension of this logic indicates that specific alterations in appropriate genes will alter specific components of a cell (be they protein, lipid, or other) and in turn the responses to volatile anesthetics. After excluding cases attributable to inadequate drug delivery, awareness under anesthesia should reflect a decreased sensitivity to anesthetics and therefore potentially herald mutations in crucial target genes. Strategies to look for resistance to volatile anesthetics could identify the responsible genes, determine their products, and therefore identify the cellular components responsible for awareness with recall.

"[G]enetic data…do not identify a single protein that is uniquely responsible for awareness under anesthesia."

Sleigh *et al.*[4] report an important first step in identifying any genetic bases for awareness under general anesthesia. The authors are aided by almost unbelievable progress in genomic sequencing compared with even a decade ago. It is now feasible to identify specific changes in the DNA of patients that correlate to a shared behavioral change. This makes possible the following experimental approach used by the authors.

1. Identify a group of individuals with the phenotype of interest (documented awareness with recall despite appropriate anesthetic exposure)
2. Identify a second group who do not have the phenotype
3. Do a full sequencing of the genetic material that codes for all proteins in each group
4. Determine consistent genetic differences between the two groups
5. Deduce important coding changes that differentially affect the awareness "phenotype"

Although this approach sounds straightforward conceptually, there are a number of complicating factors. First, one must be sure that the test group actually had some type of awareness under adequate anesthesia concentrations with or without recall. Second, one must be sure that all members

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in the control group would be adequately anesthetized at the same adequate doses. These are incredibly difficult points to ensure, making any study of awareness with (or without) recall open to concern.

Our discussion is limited to the genetic approach and its ensuing caveats. Complications generally arise from the fact that the human genome contains about 30,000 genes and 3 billion base pairs. Therefore, a large number of likely noncontributory differences will be identified, which must somehow be filtered out. One method to address this problem is to take a very large group of normal sequences to use as controls. Because 99.8% of people do not experience awareness with recall, should they exist, important DNA changes must be rare and unlikely to be found in the control population. Any variation that occurs above some cutoff frequency in the general population is automatically assigned as noncontributory. This requires sequencing control genomes of a sufficient quantity to determine the normal distribution. It is difficult to say how many such normal genomes should be sequenced, but typically 5 to 10 times the number of affected genomes is standard.[5] Similarly, known mutations that cause overt disease are eliminated from consideration because there is no known correlation of awareness under anesthesia with known diseases.

One will still be left with a large number of base pair variants in the affected data set, so two further assumptions were made in the present study. The first assumption was that there are probably a small number of such target genes. In that case, one would expect that in a reasonable number of patients experiencing awareness, mutations in the same genes (or at least pathways) should occur multiple times. Consider genetic experiments designed to determine how many genes are in a developmental pathway. One collects mutants until a large percentage of identified genes have been identified by multiple different mutations (although only one per patient, generally); then researchers know that they have sampled the genome adequately so that all genes likely to have a major effect have been identified. In the present study, the authors have only one such duplication. This indicates that complete coverage of the genes controlling sensitivity is unlikely. It is difficult to expect repeated identification when only 12 affected individuals and 12 controls are sequenced, unless the list of possible causative genes is extremely small.

The final assumption was that the variations must be in genes that make sense biologically. However, the targets for volatile anesthetics are not clear, meaning that filtering may eliminate important molecular targets. For example, mitochondrial targets, synaptic proteins, developmental genes, and molecular motors (kinesins, microtubules) are not considered in the screen. If one is going to rule out monogenic causes, one probably needs to look at all possible genes, a monumental task when no consensus exists as to the likely anesthetic targets. By restricting their genes of interest, the authors may have biased their results. Hence, it seems premature to conclude that awareness is not caused by changes in one or a few genes. In fact, most of the genes identified by Sleigh *et al*. have relatively minor contributors to volatile anesthetic sensitivity. The authors point out this serious limitation, and appropriately state that their study must be extended with a wider net.

Nevertheless, it is interesting to note what genetic data fail to do—they do not identify a single protein that is uniquely responsible for awareness under anesthesia. Unfortunately, the necessarily small study cohort does not carry the power to boldly interpret the sequencing data. Such conclusions will require a much larger group of patients with documented awareness under anesthesia and several-fold more negative controls. Thus, although Sleigh *et al*.'s results are consistent with a lack of monogenetic causes of awareness under anesthesia, they do not yet force that conclusion. Most importantly, the authors have beautifully mapped out an approach to identifying whether a genetic component exists to awareness under general anesthesia. All that remains is to extend the approach to a much larger cohort.

Competing Interests

The authors are not supported by, nor maintain any financial interest in, any commercial activity that may be associated with the topic of this article.

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