

Exome Sequencing

One Small Step for Malignant Hyperthermia, One Giant Step for Our Specialty—Why Exome Sequencing Matters to All of Us, Not Just the Experts

ONE hundred years ago, the traditional world hitherto known to physicists ceased to exist. Within the time span of a generation, the foundations of classical mechanics were shattered by Einstein's theory of relativity and quantum mechanics. A new era and the Golden Age of New Physics began. One hundred years later—today—a comparable revolution is taking place and this time in medicine. Yet, few practicing physicians inside and outside our specialty are aware of this revolution. When future historians will look back at the first decades of the 21st century, they may refer to this time period as the era of genomic medicine. An era where for the first time the full power and information of the human genome became accessible and was harnessed to improve the lives and conditions of everyday patients. Two reports in this issue of *ANESTHESIOLOGY*^{1,2} represent a milestone for our specialty: for the first time exome sequencing has been used to identify novel mutations for malignant hyperthermia.

What Is Exome Sequencing and Why Is It Relevant for All of Us, Not Just Experts?

Exome sequencing is like the little brother of whole genome sequencing.³ For decades, a dream of geneticists, sequencing a whole human genome, has become a distinct possibility after the Human Genome Project was completed and a first draft released in the year 2000.⁴ At a cost of 3 billion dollars, with several hundreds of investigators involved and taking 10 yr of work to be completed, the Human Genome Project was without a doubt one of the most ambitious and costly biomedical research projects ever undertaken. Although many have questioned whether the investment in the Human Genome Project “was worth it” or “has paid off,” all scientific discoveries that jump-started the genomic medicine revolution in the last decade would have been impossible without the Human Genome Project, including exome sequencing. This is for two main reasons: first, the Human Genome Project provided a template of the human genome that all subsequently sequenced genomes could be compared with.⁵ Nowadays, geneticists can simply align the millions of little DNA pieces (“shotgun sequencing”) from a human genome on the backbone of a reference

genome. Back then, the researchers in the Human Genome Project had to assemble these millions of DNA pieces in the right order first—a painstaking and arduous process (fig. 1). Second, and this is the most dramatic development, the Human Genome Project resulted in a huge technological race toward cheaper and faster sequencing. Outpacing Moore's law, the law in the semiconductor field that states that the number of transistors on an integrated circuit doubles every 18 months, the cost of sequencing has decreased and the speed of sequencing has increased by several orders of magnitude. In the year 2013, the cost to sequence a whole human genome is approximately \$5,000 (not including analysis) and can be accomplished in less than 2 weeks. Yet, despite this dramatic reduction in cost and time to sequence a genome, both are still prohibitive for everyday clinical purposes, not even including the time and effort for the nontrivial analysis of the genome data. This is where exome sequencing comes in.

While whole genome sequencing truly sequences the whole human genome with its 3 billion base pairs (typically in 40–50× coverage to remove sequencing errors), exome sequencing represents a smart, efficient, and cost-effective approach to identify potentially disease-causing mutations. A mammalian gene is broken up into two parts: exons and introns (fig. 2). Exons are the stretches of DNA that are made (transcribed and translated) into protein (“coding DNA”). The vast majority of disease-causing mutations are located in exons. Introns are not made into protein and typically do not harbor disease-causing mutations. All the exons of the human genome combined (“exome”) comprise only a small fraction of the human genome, approximately 1.5%. So instead of sequencing the whole genome (3 billion bases), one could just target and sequence the exome (40 million bases) and still have a reasonably high probability of being able to identify a disease-causing mutation. This smart and efficient approach, originally labeled “targeted exome capture and sequencing” and now simply referred to as exome sequencing, was first used by Jay Shendure lab at

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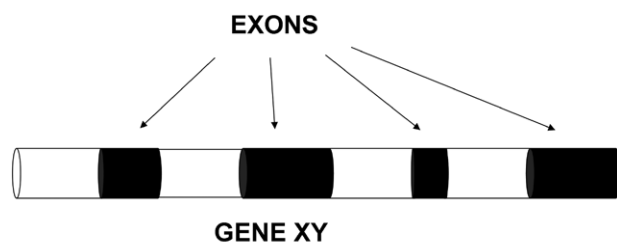
Fig. 1. “I think I found a corner piece!” Reprinted with permission from the cartoonist Drew Sheneman.

the University of Washington in Seattle.⁶ It was an instant landmark achievement first published in 2009 and has been cited more than 500 times since then. What is more important than the number of citations, however, is that the novel approach of exome sequencing has spearheaded a revolution in genomic medicine—and this brings us to the questions of relevance.

Why Is Exome Sequencing Relevant for More Than Just Experts?

Exome sequencing has become highly relevant and important in less than 5 yr since it was first introduced, because it makes genome sequencing attainable and useful in everyday clinical practice. Today, one can get an exome sequenced at a cost of less than \$750 and in just 1 day, and this has made exome sequencing very attractive for many clinicians (at present mostly pediatricians and oncologists). It

should be noted, however, that obtaining the exome data from sequencing requires 1 day, but not the analysis. The analysis of exome sequencing data is not trivial, requires an experienced bioinformatician, and often takes several weeks. Exome sequencing has resulted in some spectacular successes in recent years, particularly in the area of mental retardation and developmental delay,⁷ but also by uncovering the genetic basis of schizophrenia⁸ and familial amyotrophic lateral sclerosis,⁹ as well as by identifying the causal mutation of several hitherto unidentified rare inherited diseases. One of the most promising applications of exome sequencing is led by Evan Eichler group (also at the University of Washington/Howard Hughes Medical Institute) as they have identified several causal gene variants for autism spectrum disorder whose etiology has been enigmatic.^{10,11} Several university hospitals have begun to offer Clinical Laboratory Improvement Amendments (a federal laboratory standard)—certified clinical exome sequencing and it will be only a matter of time, before we will be confronted with our first patient whose exome has been sequenced and whose exome data are accessible on our electronic medical records. It probably will be our colleagues taking care of children with intellectual disabilities or cancer patients who will be confronted with clinical exome data. What we are going to do with them? What is the impact for the practicing anesthesiologist? At present and given the nearly uniform lack of guidelines on how to interpret clinical exome data, the impact is largely negligible. Over the course of the next years, however, as exome data interpretation will become more automated, one of the first areas of relevance for practicing anesthesiologists that should see an integration of exome data will be genotype-based perioperative drug therapy.



ALL EXONS in the HUMAN GENOME = EXOME

Fig. 2. Schematic organization of a human (mammalian) gene: exons (black blocks) are the protein coding elements of the gene. Introns (white) are noncoding elements. All exons in the human genome comprise the exome.

Although clinical integration of exome results is still in the future, at present we can marvel at the scientific breakthroughs exome sequencing has enabled. The two studies in this issue of *ANESTHESIOLOGY*,^{1,2} as well as two earlier reports,^{12,13} may represent only an incremental knowledge gain in malignant hyperthermia research. What sets them apart is that by using exome sequencing they shepherd our specialty into the era of genomic medicine and therefore they truly represent a milestone. What a wonderful coincidence that one of the studies¹ comes from the University of Washington, the very same place where the method of exome sequencing was originally invented.

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