Game Changer

Genetics and Genomics: Making the Invisible Visible

By Howard E. Jarvis, E. Paige Sensenbrenner, and Laura K. Whitmore

Sophisticated genomics soon will raise specific causation proof standards to a new level, making it possible to present competent evidence to prove or to disprove exposure-related injury.

In the spring of 2012, defense counsel was planning strategy and case presentation for a trial scheduled to begin in a few short months. The plaintiff claimed that she had developed thyroid cancer from exposure to radioactive material contained in and on used oilfield pipes owned by the defendant. Counsel posed the question to one of the defendant's medical experts, "Can the plaintiff's tissue specimens be tested to determine if she had a genetic predisposition to thyroid cancer?" Not only was the answer to that question "yes," but recent developments in the fields of genetics and genomics offered much more. And those developments may be a game changer in certain toxic tort and product liability cases.

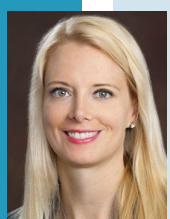
In October 2000, an article titled "The Defendant's Right to Compel Genetic Testing" appeared in this publication. In discussing a litigant's right to compel genetic testing, the author explained how advances in genetics and biotechnology could have future utility in criminal and civil litigation. The author predicted that

> [t]he study of genetics and biotechnology, while a relatively new topic to non-scientists, is having and will continue to have an increasingly profound effect on our society. Applications of the growing body of knowledge have already revolutionized principles of evidence in certain types of criminal prosecutions, and they will undoubtedly have similar influences on civil litigation in the near future.

With equal foresight, the author suggested that

> [i]n civil litigation, the results of genetic tests can be used as evidence in the quest to determine the cause of certain medical problems. It is foreseeable that such results will be used to indicate that some person's problems are caused by their genetic "map," as opposed to a medicine manufactured by a defendant corporation.

Over 14 years later, the future is here, and it is clear that scientific advances in genetics, genomics, and biotechnology have in-



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deed profoundly affected all aspects of our society. In April 2003, the Human Genome Project was completed, allowing scientists to read the complete genetic blueprint for a human being—the human genome. With the completion of the Human Genome Project, genetic variations between people now can be identified. This, in turn, has given rise to advances in newer fields of science known as "genomics" and "toxicogenomics." We now live in a world where we can use this scientific approach to help us track down and convict criminals, to determine whether we carry a gene for a particular disease, and to obtain personalized medical care for diagnosis, treatment, and disease management, based on our own genetic makeup. As predicted more than a decade ago, because we have a better understanding of the human genome and an improved ability to understand our own individual risks of disease and biological responses to drugs and other chemicals, these techniques, along with related technologies, are also gaining traction in civil litigation as ways to support or refute toxic tort exposure claims.

One recent technology helping make all of this possible, RNA sequencing, has become so sophisticated that scientists can use it to help prove or disprove specific disease causation *and* to determine the innate presence of certain genes that can lead to an individual's particular disease. Consequently, in toxic tort lawsuits where these sciences and technologies could be applied, causation may rise and fall upon the presence, or absence, of a toxicogenomic response. If that toxicogenomic response is not found—meaning that the gene expression levels in a sample of the individual's diseased tissue are inconsistent with the known expressions associated with the particular toxicant to which the individual was allegedly exposed, or going even one step further, are not only inconsistent with the toxic exposure but also *consistent* with an inherited genetic predisposition for the disease—causation can be strongly refuted. The reverse, however, also is true.

Using a recent case-specific example, this article gives a brief overview of genomics and toxicogenomics and then explains how these techniques and technologies can be applied in toxic tort cases to help prove or disprove specific causation.

The Science

An individual's genetic information is encoded in chromosomal DNA within a cell's nucleus. DNA consists of four building-block nucleotides, represented by the letters "A," "T," "C," and "G." "Genes" are the molecular unit of heredity of a living organism, and specific segments of nucleotides are like a sentence. The sequence of the "letters," or nucleotides, on each gene determines the meaning of its genetic message and carries instructions on how to make proteins. To do this, the genetic message is transcribed, or "copied," from that DNA into an intermediary molecule called the messenger ribonucleic acid or mRNA. The mRNA carries "instructions" from the DNA to the cell machinery that makes proteins, each of which has a specific function in the body. Thus, the DNA acts as an instruction manual for our bodies.

Some differences in our DNA are "expressed" as our individual genetic traits, such as the color of our eyes and hair, while other variations may be "silent" and have no recognizable consequence. Genetic variations may be hereditary, caused by environmental factors, or both. Potentially gene expression-altering environmental factors include diet, lifestyle choices, geography, and exposure to pollutants and chemicals. Unfortunately, sometimes these factors combine to affect our health negatively. Indeed, as Francis Collins, Nobel-prize winner and Director of the NIH Human Genome Project, explained, "[c]ancer is a disease of the genome. It arises when genes involved in promoting or suppressing cell growth sustain mutations that disturb the normal stop and go signals." *See* NIH Director's Blog, Oct. 22, 2013.

The word "genome" is used to identify an organism's complete set of DNA, including all of its genes. Thus, while genetics is the study of genes, their functions, and resulting effects—and focuses principally on the mutations in individual genes—genomics is the study of *all* the genes in a person, as well as the interactions of those genes with each other and a person's environment. *See* Public Health Genomics, Centers for Disease Control and Prevention, http://www.cdc.gov/genomics (last visited Feb. 12, 2015). In April 2003, the Human Genome Project sequenced and mapped all of the genes of a human being to reveal the human genome, thereby revolutionizing genomic research. *See* All About the Human Genome Project, Nat'l Human Genome Project Research Inst., http://www.genome.gov/10001772 (last visited Feb. 12, 2015). As a result, we have at our disposal the complete genetic blueprint for a human being, which can be studied to help science gain insight into how the individual parts work together and help establish a genetic basis for health and the pathology of human disease.

Toxicogenomics uses genomic technologies to study how the genome responds to environmental toxicants and other stressors.

One of the goals of human genomics is to identify variations in the DNA sequence and to determine the significance of the variation across populations. By studying the relationship between a person's genes and his or her environment, scientists may be able to discern why some people develop diseases while others do not. One of the ways this is accomplished is through the study of toxicogenomics. Toxicogenomics uses genomic technologies to study how the genome responds to environmental toxicants and other stressors. *See* J. C. Rockett, *The Future Of Toxicogenomics*. Chapter 15, Michael E. Burczynski (ed.), An Introduction to Toxicogenomics (CRC Press LLC, Boca Raton, FL), 299–317, (2003), http://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=65899&CFID=156832895&CFTOKEN=53949242&jsessionid=8630ee3c1e716bb97cd4541317711c545f15 (last visited Feb. 12, 2015). Toxicogenomics can identify toxicant-specific alterations in gene, protein, and metabolite "expression" patterns. These expressions show how exposures to certain chemicals cause some genes to "turn on" or "turn off," which affects the proteins produced by the cell. *See Applications of Toxicogenomic Technologies to*

Predictive Toxicology and Risk Assessment, The National Academy of Sciences (2007). The resulting "on/off" pattern of genes creates a "signature" for the particular toxicant. *Id.* Studying the gene signatures helps explain how the chemicals act on our bodies to cause disease.

In September 2000, the National Institute of Environmental Health Sciences (NIEHS) created the National Center for Toxicogenomics (NCT) for the primary purpose of collecting gene expression data to build a knowledge base of chemical effects on biological systems. *See* Raymond W. Tennant, *The National Center for Toxicogenomics: Using New Technologies to Inform Mechanistic Technology*, Environmental Health Perspectives, Vo. 110, No. 1 Jan. 2002.

In certain cases, genomics, genetics, and toxicogenomics can both eliminate the alleged cause of a disease and reveal its true cause.

A new technology available to detect the gene expressions for a large number of genes simultaneously is "massively parallel" or "high throughput" gene sequencing, which is known as "next-generation sequencing" or "NGS." NGS is considered one of the most advanced and most reliable and quantitative forms of genetic testing. Significantly, NGS can be used to establish specific causation based on an individual, rather than on a generalized population. RNA-sequencing, a type of NGS, is the first sequencing-based method that allows the entire transcriptome—the RNA expressed from the genome—to be surveyed in a very high throughput and quantitative manner. This allows an in-depth, sometimes quantitative analysis of gene expression with great accuracy compared to the other microarray platforms. The resulting data can reflect whether gene expression levels in an individual's diseased tissue are consistent, or inconsistent, with the toxic exposure alleged, otherwise known as a "toxicogenomic response."

The results of the RNA sequencing are subject to principal component analysis, which means that the results produced are a pure mathematical and statistical finding. Any "opinion" in interpreting the data is essentially removed, as the data speaks for itself. Thus, the NGS data can provide strong guidance and scientific reliability to a source of tumorigenesis, including the presence of biomarkers for inherited genetic mutations. This makes NGS particularly well suited for litigation, where scientific evidence must satisfy an admissibility threshold.

The data is analyzed by principal competent analysis (PCA). This is a proven statistical method that separates variance and covariance to defer the meaning of large complex data. Using PCA allows this complex data to be viewed and compared to published gene signatures in peer-reviewed publications.

Applying the Science and Technology to Toxic Tort Litigation

In a toxic tort lawsuit, it is the plaintiff's burden to prove both general and specific causation. That means that the plaintiff must prove both that the substance that allegedly caused the disease is capable of causing the type of injury that the plaintiff suffered *and* that the particular substance to which the plaintiff allegedly was exposed actually caused the plaintiff's injury. Proving specific causation—that the exposure actually caused the plaintiff's injury—typically is the greater challenge. A defendant's position often is that a plaintiff's disease was not caused by the exposure, or even that the plaintiff had genetic traits that predisposed him or her to the disease and that inherited genetic mutation, not the defendant's product, was the legal cause of the disease.

Proving, or disproving, specific causation traditionally has involved an application of differential diagnosis to produce an ultimate opinion on medical causation, followed by a battle of the experts. In certain cases, genomics, genetics, and toxicogenomics can both eliminate the alleged cause of a disease and reveal its true cause.

Two of the authors were involved in a recent case in which both genetic and genomic testing was used to refute a plaintiff's claim that she developed thyroid cancer resulting from exposure to "NORM," or "naturally occurring radioactive material." *Naomi Guzman vs. Exxon Mobile Corporation, et al*; 24th Judicial District Court for the Parish of Jefferson, Civil Action No. 693-606, Division "I." The plaintiff was the daughter of a former pipe yard worker who cleaned drilling pipes allegedly contaminated with NORM. The plaintiff's uncle also worked at the pipe yard. The plaintiff claimed that she was exposed through four pathways:

1. She was exposed to gamma radiation while in utero, when her mother brought lunch and dinner to her father at work;
2. She ingested and inhaled radioactive dust particles when she was an infant/toddler while accompanying her mother to bring meals to her father;
3. She inhaled and ingested radioactive dust from her father's clothes when he returned home after work; and
4. As a young girl, while staying with her uncle after school, she was exposed to radioactive dust on his clothes.

Tissue specimens had been obtained by the plaintiff's physicians when she underwent a thyroidectomy as part of her cancer treatment. The court granted Exxon's motion to compel production of the plaintiff's tissue specimens for genetic and genomic testing. The purpose of the genetic tests was to detect inherited traits that would have predisposed the plaintiff to papillary thyroid cancer. And the objective of the genomic testing was to establish whether the plaintiff, who was diagnosed specifically with papillary thyroid cancer (PTC) and Hashimoto's thyroiditis, had been exposed to external or internal radiation, which caused the diseases.

The tissue specimens were delivered to scientists at ArrayXpress in Raleigh, N.C. Array Xpress established a quantitative gene expression profile using NGS and in particular, RNA sequencing on mRNA extracted from cancerous formalin-fixed paraffin embedded tissue sections collected from the plaintiff. Then the scientists attempted to correlate the resulting signature with the expression signatures

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for thyroid tumors previously published in peer-reviewed scientific journals. In this case, the scientists were able to rely on a recently published paper that provided a comprehensive overview of gene expression signatures related to radiation-induced thyroid tumors. This published gene list served as the reference point for radiation-induced cancer. The scientists then established the genetic signature for the plaintiff's cancerous tissue. That produced the plaintiff's personal gene expression profile, which was compared to the published gene expression profiles for radiation-induced thyroid tumors to determine if any similarities existed.

When the results came in, the plaintiff's gene expressions demonstrated a "gene signature" for sporadic thyroid cancer as opposed to radiation-induced thyroid cancer resulting from exposure to NORM. And genetic test results added another layer to Exxon's causation defense. Those tests revealed that the plaintiff's predisposition to developing thyroid cancer was five times greater than that of the general population. Based on the genetic test results alone, Exxon presented an expert in medical pathology who testified that the plaintiff most likely would have developed PTC without exposure to any other risk factors.

Ultimately, the jury never reached the medical causation questions because it found Exxon not liable on the strict liability and negligence claims. It appeared, however, that the genetics and genomic test results firmly established an alternative causation and may have aided the jury in reaching their verdict.

Conclusion

The "Brave New World" is here. Current and forthcoming developments in genetics and genomics will have profound effects on several fronts. Already we see personalized medicine, and widespread use of tailor-made drugs is on the horizon. Similarly, genetics and genomics will play an increasingly important role in courtrooms. While lawyers historically have relied on epidemiology and scientific studies to prove or to disprove causation, especially in toxic torts and pharmaceutical product liability cases, new genomic technology has the potential to be a game changer. As more and more gene signatures are identified and published, gene expression tests and mRNA sequencing will bring specific causation to a new level. We literally will have the ability to present competent evidence to prove or to disprove that a party's injury or condition was caused by exposure to a specific substance, chemical, or drug. FD