The Use of Genetic Evidence to Defend Against Toxic Tort Claims—Part I

By Susan E. Brice and Dr. Whitney V. Christian

"Most common complex diseases are believed to be the result of the combined effect of genes, environmental factors and their interactions."[1]

Toxic tort cases generally involve claims that an individual was harmed as a consequence of exposure to a chemical(s) (including a medication). These cases can be particularly difficult to litigate because of the challenges presented by demonstrating or disproving causation. Because we do not fully understand the extent to which a chemical exposure can affect a particular individual, experts typically offer opinions based on the general risk posed to the plaintiff by the exposure in question. Judge and juries find this lack of plaintiff-specific evidence unsatisfying.

This multi-part article explores how genetic and epigenetic biomarkers of cause and effect can be used to fill this gap for defendants. By permitting the demonstration of alternative causation or the absence of a known cause and effect genetic pathway, genetics and epigenetics are quickly becoming a powerful tool in toxic tort litigation. For the attorney, this article provides the necessary scientific background to understand how genes operate and interact with the environment. It also discusses tools that can be used to employ genetic/epigenetic testing in the courtroom, including an examination of case law where genetic testing has defeated toxic tort claims. For the scientist, the article explains how genetic and epigenetic studies fit into the current and future legal framework. Finally, the article discusses how CRISPR genomic editing techniques can be used to refine causation analysis. In fact, the genetic tools furnished by the CRISPR system can provide scientists with the tools necessary to study how an individual plaintiff 's genome reacts to a given chemical, revolutionizing the science of cause and effect. In this first part of the article, the authors introduce the topic, discuss the human genome, genes, the environment, susceptibility, and disease. The final parts of the article, which will appear in upcoming issues of Intellectual Property & Technology Law Journal, will explore genetic data and toxic tort law, genetic biomarkers, genomics and toxicogenomics, epigenetics, and tools for understanding causation at the genomic level.

Susan E. Brice (susan.brice@bryancave.com) is a partner at Bryan Cave LLP defending toxic tort and environmental cases brought against corporate clients in cases involving complex scientific issues. **Dr. Whitney V. Christian** formerly was a health scientist with Cardno ChemRisk, and is now a senior toxicologist at Medtronic, focusing on genetics, toxicology, and gene-environment interactions and their contribution to the development of human disease.

The Toxic Tort Case

"The term 'toxic tort' refers to circumstances under which plaintiffs attempt to prove that they suffered harm as a result of exposure to a substance."[2] The term applies in a "variety of cases, ranging from exposure to harmful external substances, such as asbestos or nuclear material, to the adverse affects [sic] of substances deliberately ingested into the body, including prescribed medicines."[3] Accordingly, toxic tort claims can be related to environmental exposures or to consumer products.

To succeed in a toxic tort case, a plaintiff must satisfy a number of elements, including that he or she was sufficiently exposed to the toxic substance and that the substance caused their disease.[4] This second prong of "causation" must be shown in two ways: (1) general causation and (2) specific causation.[5]

Plaintiffs prove general causation by showing that it is more probable than not that the substance is *capable* of causing the disease. Specific causation is demonstrated by proving that the substance *did*, in fact, cause the disease in the plaintiff. In most states, in order to demonstrate specific causation in a toxic tort case, a plaintiff must demonstrate through expert testimony that the exposure in question was the cause, or a "substantial factor" in the cause, of the disease.[6] In Illinois, for example, an exposure has met the "substantial factor" test when "it was a material element and a substantial factor in bringing the event [the disease] about."[7] General causation typically is proven through experts who rely on scientific studies, including epidemiological and toxic¬ological studies, to support the proposition that the substance could cause the disease.[8] Specific causation is more complex. Parties rely on medical doctors and other scientists to assess and opine on the risk posed to the plaintiff by the specific exposure in question.[9]

This article was printed in the September issue of the IPT Journal.

Based on this risk and other lines of evidence, the experts then render an opinion on specific causation.

Expert testimony is critical in a toxic tort case. The U.S. Court of Appeals for the Seventh Circuit stated: "when there is no obvious origin to an injury and it has multiple potential etiologies, expert testimony is necessary to establish causation."[10] Because of this, parties in toxic tort cases often target the opposing side's expert and file motions to limit or exclude the expert from testifying at trial. The standards that govern the admissibility and relevancy of expert testimony are set forth in the applicable court's procedural rules as well as case law.[11]

This strategy often is successful. In *Higgins v. Koch Development*, for example, after the plaintiff 's causation expert was disqualified, the defendants moved for summary judgment, arguing that the plaintiff could not prove that the exposure to chlorine gas caused his respiratory disease.[12] After deciding that expert causation testimony was necessary and that the plaintiff 's treating doctor could not offer the requisite causation opinion, the U.S. District Court for the Southern District of Indiana granted, and the Seventh Circuit affirmed, summary judgment for the defendants.[13] The same strategy was employed by defendants in multi-district litigation pending in the U.S. District Court for the Eastern District of Pennsylvania involving the antidepressant Zoloft. The multidistrict litigation (MDL) judge granted the moving defendants summary judgment in all pending cases after ruling that the plaintiffs could not establish general causation through expert testimony.[14] The case is currently on appeal.

Given that many disease processes are multifactorial and that diseases often manifest themselves years after exposure, it often is challenging for plaintiffs to prove that a specific chemical exposure caused or served as a substantial factor in bringing about their disease. But there are exceptions to this norm. For example, there are "signature diseases," ones that "are uniquely related to exposure to a certain substance and are rarely observed in individuals that are not exposed."[15] One of the most well-known signature diseases is mesothelioma, a type of lung cancer that is linked to asbestos. Because of the known causal relationship between a sufficient dose of certain types of asbestos and mesothelioma, asbestos cases can be an uphill battle for defendants.

Signature diseases, however, are few and far between.[16] Thus, the science of causation has been and continues to be inexact. Disease can result from a variety of sources, including infections, viruses, inherited genetic defects, lifestyle choices and environmental factors. Some diseases are idiopathic, meaning their cause is unknown. While there is still much to learn about how environmental factors impact disease, scientific research is beginning to untie this knot. Advances in genetic research, in particular, are providing experts with new tools to bolster specific causation testimony.

In fact, the recent developments in genomic editing could revolutionize the science of cause and effect and furnish the precision needed to eliminate much of the guesswork that currently exists in toxic tort cases. As one legal commenter recently put it, "[g]enetic research towers over its rival scientific methodologies with its ability to provide answers to many of the questions that have, so far, stumped the courts."[17] While genetic evidence certainly can be used equally by the plaintiffs and the defense, this article focuses on how defendants can employ genetic evidence to combat toxic tort claims.[18]

The Human Genome

As we know from basic biology, a human offspring's mother and father each contribute one chromosome to each of the 23 pairs to make up the offspring's genome. Every chromosome therefore contains DNA inherited from one parent, and its complement contains DNA inherited from the other parent.

DNA is constructed from a combination of the four "nucleotide" building blocks, guanine (G), cytosine (C), thymine (T), and adenine (A). The DNA is tightly wrapped around proteins (histones) that hold the chromosome together. A gene is a small segment of the wrapped DNA and is found at a specific location on one of the 23 pairs of chromosomes (see Exhibit 1).

Humans have two copies of each gene (a paternal and a maternal copy) and one copy is often slightly different from the other copy. Each copy is referred to as an allele.[19] The two alleles of each gene encode instructions, called messenger RNA (mRNA), for building the proteins that carry out the functions of every cell (see Exhibit 2).

The Human Genome Project was an international collaborative research project aimed at understanding the genes of humans. In 2003, the Project successfully completed the sequencing of the human genome. In other words, the researchers developed a map showing how all the genetic material (gene coding and non-coding DNA) contained in a human's 23 chromosome pairs is arranged.

Exhibit 1

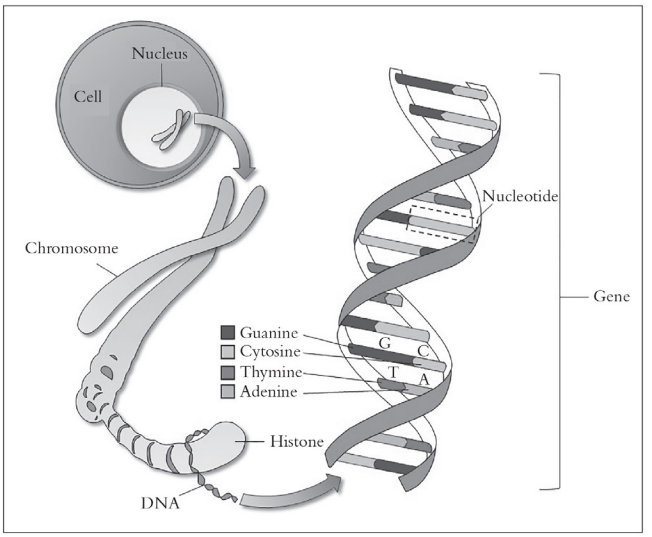
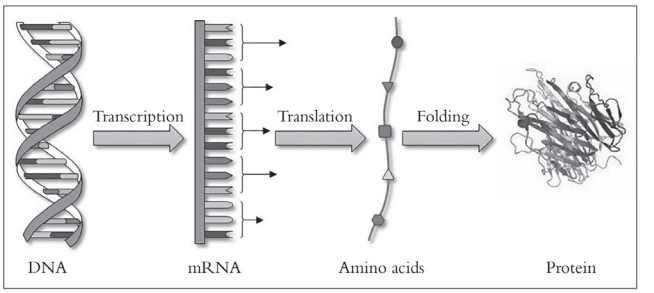


Exhibit 2



The Project's results surprised scientists. At that time, it was thought that out of the approximately two meters of DNA in each nucleated cell of the human body, only two percent of this DNA comprised actual genes with around 20,000 to 25,000 genes in the human genome. Since then, it has been estimated that humans actually have 19,000 to 20,000 genes and that nearly 99.5 percent of these genes are identical among the general human population.[20]

Conversely, our differences are determined by only about 0.5 percent of variable human genetic material (i.e., 1.6 Å~ 107 nucleotide base pairs out of the 3.2 Å~ 109 nucleotide base pairs in the entire genome), appearing in different parts of the genome within the population. One small change in the nucleotide sequence of a gene, which is one out of 6.4 billion nucleotides that make up the entire genome, can result in meaningful genetic variation.

The Human Genome Project spawned a number of research projects seeking to identify genes that impact health and disease. Over the past few decades, researchers have been studying this variable 0.5 percent to identify how specific genes function and whether they are associated with disease. According to GeneCards, a database developed at the Weizmann Institute of Science, to date over 10,464 "disease genes" have been identified.[21] The identification of these disease genes—including their location, function and how they interact within gene networks—can help unwrap the proverbial black box of what causes humans to develop diseases, including whether or not a particular exposure to a chemical or substance can cause or contribute to the manifestation of cancer or other diseases in an individual.

Given the significant role genetics plays in explaining disease causation, genetic evidence is finding its way into the courtroom in toxic tort litigation.

Genes, the Environment, Susceptibility, and Disease

While the intersection of genetics and disease is complex, the fundamental tenet—that environmental exposures can damage genetic material and lead to genetic defects that can then cause disease—is well established. The conundrums lie in the details. Certain flawed genes and genetic mutations are inherited while others develop over the course of a lifetime. Science has a good grasp on the former category; however, the latter group, involving what appear to be spontaneous mutations and aberrations, remains somewhat elusive.

There exists a good reason for this divergence. Inherited diseases, including those that result from a flaw in a single gene (*i.e.*, monogenic diseases, such as Huntington's disease or Cystic Fibrosis) are easy to spot. By contrast, complex diseases, such as cancer, can evolve from an accumulation of aberrations in multiple genes over time and may be potentiated by certain environmental factors or may develop spontaneously. In other words, diseases that are not inherited are often multifactorial and polygenic.

Environmental factors or exposures can originate from many sources. These include, but are not limited to, dietary intake, chemicals, ambient conditions, viruses, and pharmaceuticals. While much work remains to be done, scientists have been able to identify causal connections between specific environmental exposures and certain diseases. In fact, in some instances, they have been able to elucidate how the exposure in question initiates or causes the disease.

With respect to environmental impacts on genes, we know that environmental exposures can operate both directly and indirectly on DNA and genes to lead to disease. For instance, environmental exposures can damage DNA by causing chromosomal aberrations or specific gene mutations. A chromosomal aberration can occur when DNA is damaged to such an extent that the structure or number of chromosomes changes. A genetic mutation can happen when exposure produces a change in the nucleotide sequence of a specific gene.

The relationship between ultraviolet radiation and skin cancer provides a well-established illustration of exposure-related DNA damage. Ultraviolet B (UVB) radiation can directly break the bonds between certain nucleotides within a gene. It also can indirectly damage DNA through the production of free radicals and reactive oxygen species (ROS),[22] which can harm the nucleotides within a gene. If not repaired, this damage can result in a change in the nucleotide sequence that modifies or impairs the protein produced by that gene. If such mutations accumulate and impact important skin cell genes such as those that prevent cancer, those that repair genes or those that promote cell growth, skin cancer can develop. More specifically, if the UVB radiation were to modify the nucleotide arrangement of the highly significant p53 tumor suppressor gene[23] in an individual's skin cells, that person may be more likely to develop skin cancer.

In other situations, an interplay occurs between the environment and one's genetic make-up, known as a gene-environment interaction. For instance, genetic variations themselves influence susceptibility to environmental factors. The susceptibility then, in turn, determines whether specific environmental factors increase the risk of acquiring certain diseases. This explains why the human population responds differently to the same environmental factors.

Researchers have discovered that for each gene there is a more common or expected nucleotide sequence found within a population. The most commonly occurring sequence is called the "wild type," but here we will refer to it as the "expected" sequence. Any variation from the expected sequence is known as a polymorphism. Polymorphisms provide genetic variation and diversity.[24] As a result, there are many common inherited polymorphisms.

Mutations represent a subset of polymorphisms. They differ from inherited polymorphisms in many ways. These departures include that mutations are more sporadic; mutations typically result from an external stimuli, such as UVB radiation; and mutations are confined to specific tissue, as opposed to being present in every cell. Unlike common polymorphisms, mutations often detrimentally impact affected cells.[25]

Individuals with a specific genetic polymorphism may respond differently to their environment than those without the sequence change or with a different sequence change in the same gene, exemplifying a gene-environment interaction. For instance, humans differ in how their bodies metabolize alcohol. Researchers have determined that this, in part, derives from the existence of genetic polymorphisms within a specific gene, known as the aldehyde dehydrogenase gene (ALDH2). The ALDH2 gene creates a protein enzyme that regulates the metabolism of alcohol. The DNA sequence of this gene for some individuals results in the production of an enzyme that metabolizes alcohol more slowly than normal. In turn, individuals with this genetic alteration experience a flushing response after alcohol consumption.[26]

The type of polymorphism responsible for the flushing is known as a single nucleotide polymorphism (SNP). These polymorphisms occur when the allele found in an individual differs from the expected allele due to just one nucleotide change. For instance, the expected sequence of a segment of the ALDH2 gene is GAA (i.e., the ALDH21 allele), but sometimes a sequence of AAA is found (an adenine replacing a guanine at nucleotide position 1510).

DNA sequence alignment:

```
Expected ALDH2[1] …1501     GCATACACT***G***AAGTGAAAACTGTCACAGTCAAAGTGCCTCAGAAGAACTCATAA 1554
                            |||||||||*|||||||||||||||||||||||||||||||||||||||||||||||||
Polymorphism ALDH2[2] …1501 GCATACACT***A***AAGTGAAAACTGTCACAGTCAAAGTGCCTCAGAAGAACTCATAA 1554
```

This small variation in the expected DNA sequence changes one amino acid (a glutamate (E) is exchanged for a lysine (K) at position 487 (E487K)) in the protein produced by the ALDH2 gene, which results in the functional deficit seen with the ALDH22 allele.

Protein sequence alignment:

```
Expected
ALDH2[1] …484        AYT***E***VKTVTVKVPQKNS 500
                          ||| * ||||||||||||||||
Polymorphism         AYT***K***VKTVTVKVPQKNS 500
ALDH2[2] …484
```

The example above underscores how just one small variation, whether inherited or caused by an external stimulus, can make a significant difference.

In 1997, the NIEHS Environmental Genome Project set out to identify human SNPs indicative of susceptibility to specific environmental factors. The goal was to better understand the relationships between environmental exposures, DNA sequence variation and the risk of disease in the general human population.[27] The Project therefore looked for SNPs known to influence whether a person is more prone to injury from environmental factors.

For example, a SNP known to influence susceptibility is found in the gene for N-acetyltransferase 2 (NAT2), an enzyme that participates in the metabolism of xenobiotics, which are chemicals or substances that are foreign to an organism's biological system. Arylamines, a class of chemicals historically used in the production of industrial dyes, are xenobiotics, and occupational exposure to arylamines has been associated with bladder cancer in humans.[28]

Research has found that certain SNPs within the NAT2 gene may influence susceptibility to bladder cancer from arylamine exposure because they are associated with slower than expected arylamine detoxification.[29] In other words, a person with the version of the NAT2 gene that metabolizes arylamines more slowly may have an increased risk for bladder cancer, if exposed to a sufficient dose of arylamines.

Of course, not all SNPs related to susceptibility result in a negative effect. In fact, some SNPs, and even some mutations, can provide benefits. For instance, it is well established that the faulty allele that leads to sickle cell anemia also confers resistance to malaria.[30] Accordingly, while some SNPs may increase a person's risk of disease relative to the general population, other SNPs may decrease that same risk.

Understanding how the genetic component varies biological responses and disease outcomes has been the focus of several projects, including the Environmental Genome Project and the International HapMap Project. These projects employed DNA sequencing to identify patterns of SNP groups (or SNP haplotypes) that influence human susceptibility to environmental chemicals. For example, the inheritance of certain CYP2E1-NQ01 haplotypes[31] influences benzene metabolism and can confer susceptibility or protection to benzene exposure-related diseases (*e.g.*, hematological malignancies), depending on the specific SNPs present in the inherited haplotype.[32]

* * *

The final parts of this article, which will appear in upcoming issues of *Intellectual Property & Technology Law Journal*, will explore genetic data and toxic tort law, genetic biomarkers, genomics and toxicogenomics, epigenetics, and tools for understanding causation at the genomic level.

Notes

1. *H. Aschard, et al.*, "Challenges and opportunities in genome-wide environmental interaction (GWEI) studies," 131 *Hum Genet*. (2012).
2. *Rimbert v. Eli Lilly and Co.*, No. CIV 06-0874, 2009 WL 2208570 (D. N.M. July 21, 2009).
3. *Id.*
4. As stated by Paracelsus, "[a]ll things are poison and nothing is without poison; only the dose makes a thing not a poison." This ancient adage, which is often shortened to "the dose makes the poison," has been engrafted into toxic tort litigation. According to the Federal Judicial Center's Reference Manual on Scientific Evidence, "a plaintiff with a medical condition known from epidemiological studies to be caused by a specific chemical may not be able to substantiate his or her claims without evidence of exposure to that chemical of a sufficient magnitude. Exposure experts are needed to quantify the exposures incurred …." Federal Judicial Center's Reference Manual on Scientific Evidence (3rd Ed.) at 512.
5. *See, e.g.*, *Higgins v. Koch Development Corp.*, 794 F.3d 697, 701-02 (7th Cir. 2015); *Acosta v. Shell Western Exploration and Production, Inc.*, 370 P.2d 761, 767 (N.M. 2016); *Raynor v. Merrell Pharm., Inc.*, 104 F.3d 1371, 1376 (D.C. Cir. 1997).
6. *E.g.*, *Jones v. Ortho Pharm. Corp.*, 163 Cal.App.3d 396, 402-03 (Cal. Ct. App. 1985); *see generally*, 2 Owens & Davis On Prod. Liab. Åò 11:19 (4th Ed.). Asbestos cases often involve a variant of the substantial factor test. *Nolan v. Weil-McLain*, 910 N.E.2d 549 (Ill. 2009) (stating that Illinois uses the "frequency, regularity and proximity" test "as a means by which an asbestos plaintiff can prove more than minimum contact to establish that a specific defendant's product was a substantial factor in being a cause in fact of a plaintiff 's injury.").
7. *Nolan*, 910 N.E.2d at 557.
8. It is generally accepted that "epidemiology is the best evidence of general causation in a toxic tort case." *In re Zoloft Products Liability Litigation*, 2016 WL 1320799, E.D. Pa (April 5, 2016), *appeal filed* (May 4, 2016). In fact, some courts have held that "where epidemiology is available, it cannot be ignored." *Id.*
9. For an epidemiological study to be used to prove specific causation, some courts require that the exposure double the risk of harm. *See, e.g.*, *Merrell Dow Pharm. Inc. v. Havner*, 953 S.W.2d 706, 716 (Tex. 1997).

10. *Higgins*, 794 F.3d at 703.
11. *See, e.g.*, Federal Rule of Evidence 702. The federal courts follow the principles set forth in *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 U.S. 579 (1994) (*Daubert*) and the state courts generally follow either *Daubert*, a standard set forth in *Frye v. United States*, 293 F. 1013 (D.C. Cir. 1923) (*Frye*) or a different standard. Based on a review done by The Expert Institute in 2016, 76 percent of states follow *Daubert*, 16 percent follow *Frye* and eight percent follow a different standard. Michael Morgenstern, "*Daubert v. Frye*—A State-by-State Comparison" (February 15, 2016) at *https://www.theexpertinstitute.com/daubert-v-frye-a-state-by-state-comparison/*.
12. *Higgins v. Koch Development Corp*., 997 F.Supp.2d 924, 928 (S.D. Ind. 2014); aff 'd 794 F.3d 697.
13. *Id*. at 932; 794 F.3d 705.
14. In re Zoloft Products Liability Litigation, 2016 WL 1320799 at *10.
15. *Hurtado v. Purdue Pharma Co*., No. 12648/03, 2005 WL 192351 (N.Y. Sup. Ct. January 24, 2005).
16. Rigorous research into chemicals mandated by regulatory initiatives such as the European Union's Registration, Evaluation, Authorisation and Restriction of Chemicals regulation (REACH) might reveal more signature diseases.
17. Allison Hite, "Who's To Blame?: How Genetic Information Will Lead to More accurate Decisions in Toxic Tort Litigation," 63 *S.C.L.Rev*. 1031 (Summer 2012).
18. The issue of whether a court can compel genetic testing, and the scope of such testing, is brewing in the courts. In general, courts will require a plaintiff to submit to blood testing. *See, e.g.*, *Harris v. Mercy Hospital*, 231 Ill. App. 3d 105 (1st Dist. 1992) (child could be compelled to submit to blood test to determine whether she suffered from Angelman Syndrome). But there are limitations to what courts will mandate. Two recent trial court orders delineate some of these boundaries. *See Meyers v. Intel Corp*. 2015 WL 3643470 (Super. Del. June 11, 2015) (court would not compel father to undergo "state of the art" "Trio Whole Exome Sequencing" when father was not a party to the case and the court rules governing physical or mental examinations only applied when the condition of a party or of a person in the custody or under the legal control of the party was in controversy; *Sheley v. Shiffman*, 2014 WL 8060034, *3-4 (N.Y. Sup. Court Feb. 14, 2014) (discussing prior ruling allowing genetic blood testing of infant, but not allowing skin biopsy, which was allegedly a more reliable test yet more painful, until blood results were obtained and good cause was shown).
19. In fact, the lack of allelic variation, such as from inbreeding, can result in the accumulation of deleterious alleles that decreases survival and reproduction. This occurred with the Hapsburgs, a royal family in Medieval and Renaissance Europe that was known for inbreeding and resultant high infant mortality rates.
20. *I. Ezkurdia, et al.*, "Multiple evidence strands suggest that there may be as few as 19 000 human proteincoding genes," 22 *Hum Mol Genet*. (2014); *S. Levy, et al.*, "The Diploid Genome Sequence of an Individual Human," e254 PLoS Biol. (2007).
21. Weizmann Institute of Science, "GeneCards Human Gene Database," *http://www.genecards.org/cgi-bin/listdiseasecards. pl?type=full*.
22. Free radicals and ROS are produced by ionizing radiation and are also natural byproducts of biochemical reactions within cells. They are short-lived but highly reactive molecules that can damage DNA, mRNA, and proteins when present at sufficient levels.
23. Tumor suppressor gene p53 functions by sensing DNA damage and either arresting the cell cycle, such as by inhibiting cell growth, or by inducing apoptosis through initiating programmed cell death. P53 functions as a transcription factor. If the DNA damage is minor, it activates the expression of proteins that halt the cell cycle and repair the DNA. If the DNA damage is extensive and unrepairable, it causes the expression of apoptotic proteins that lead to cell death. Both of these functions prevent the accumulation of mutations and, in turn, the development of cancer. Approximately 50 percent of cancers exhibit a loss of p53 tumor suppressor function.
24. Genetic variation, including SNPs, can occur naturally when modifications are incorporated into the genome as a result of DNA replication errors. They can also be chemically induced, such as when chemical exposures cause DNA damage, which upon repair results in a non-lethal mutation being incorporated into the genome.
25. Mutations can fall into three categories: (1) silent mutations (non-functional changing genetic alterations); (2) mutations that lead to a loss of function; and (3) mutations that lead to a gain of function. In the context of cancer, if a tumor suppressor gene acquires a loss of function mutation over time, the ability to suppress tumors is lost and cancer risk is increased. If a tumor promoter gene, such as an oncogene, *infra*, acquires a gain of function mutation, the ability to promote cancer is acquired and cancer risk is increased. On the other hand, gain of function mutations exist that decrease cancer risk such as a gain of function in a gene that improves the body's ability to metabolize man-made carcinogens.
26. *T. Takeshita, et al.*, "The contribution of polymorphism in the alcohol dehydrogenase beta subunit to alcohol sensitivity in a Japanese population," 97 *Human Genet*. (1996).
27. National Institute of Environmental Health Sciences Environmental Genome Project, Home Page, *http://egp.gs.washington.edu*.
28. M.C. Yu, et al., "Arylamine exposures and bladder cancer risk," 506-507 *Mutat Res*. (2002).
29. A. Hirvonen, "Polymorphic NATs and cancer predisposition," *IARC Sci Publ* 1999. (1999); *J. Green, et al.*, "N-acetyltransferase 2 and bladder cancer: an overview and consideration of the evidence for gene–environment interaction," 83 *Br J Cancer*. (2000); Le Johns & R.S. Houlston, "N - acetyl transferase - 2 and bladder cancer risk: A meta - analysis," 36 *Environ Mol Mutagen*. (2000).
30. H.F. Bunn, "The triumph of good over evil: protection by the sickle gene against malaria," 121 *Blood* (2013).
31. CYP2E1 is a cytochrome P450 enzyme largely expressed in the liver that is responsible for the metabolism of xenobiotics, including drugs such as acetaminophen (Tylenol), ethanol, and benzene. CYP2E1 can convert or activate benzene into toxic metabolites that are detoxified by NAD(P) H:Quinone Oxidoreductase 1 (NQ01), which is an antioxidant enzyme.
32. *N. Rothman, et al.*, "Benzene poisoning, a risk factor for hematological malignancy, is associated with the NQ0l 609C→T mutation and rapid fractional excretion of chlorzoxazone," 57 *Cancer Res*. (1997).

This article was printed in the September issue of the IPT Journal.

The Use of Genetic Evidence to Defend Against Toxic Tort Claims—Part II

By Susan E. Brice and Dr. Whitney V. Christian

This second part of a multi-part article explores genetic data, toxic tort law, and genetic biomarkers. The first part appeared in the September 2017 issue of *Intellectual Property & Technology Law Journal*. The final part of the article will appear in upcoming issues of the *Intellectual Property & Technology Law Journal* with a discussion of genomics, toxicogenomics, epigenetics, and tools for understanding causation at the genomic level.

Genetic Data and Toxic Tort Law

There are two types of genetic data that likely will shape toxic tort litigation in the near future: (1) data on individual genetic susceptibility[33] and (2) genetic evidence, including biomarkers of cause and effect.[34] This article addresses both, but focuses more on the latter.

The generally accepted process by which testifying experts determine specific causation is known as differential diagnosis. "Differential diagnosis, or differential etiology, is a standard scientific technique of identifying the cause of a medical problem by eliminating the likely causes until the most probable one is isolated."[35] In the toxic tort context, an expert performs a differential diagnoses by examining which of two or more etiologies, or causes, might have led to the disease. As recently stated by the U.S. Court of Appeals for the Seventh Circuit, for differential etiology to be "validly conducted, an expert must systematically 'rule in' and 'rule out' potential causes in arriving at her ultimate conclusion."[36] Consequently, alternative causation can be a powerful tool in defending toxic tort cases.

While the published opinions are scant, litigants for years have employed genetic evidence to demonstrate or suggest alternative causation, such as showing that an inherited genetic defect caused a disease as opposed to a toxic exposure. In *Jones v. NL Industries*, the court allowed expert testimony about the plaintiff 's family history to defeat a claim that lead paint caused mental disabilities in children.[37] The defense argued that, based on medical evaluations of the plaintiffs and their families, the plaintiffs' learning disabilities were inherited from their parents as opposed to being related to lead poisoning.[38] The court allowed the testimony, even though the defendants did not offer genetic evidence to support their opinions.

Recent advances in genetic testing make this type of argument even more compelling. In *Deribeaux v. Secretary of Health and Human Services*, the Secretary of Health and Human Services relied on genetic testing to demonstrate that the child's seizures were caused by mutations in her SCN1A gene and not by a vaccine given to the child. The Federal Circuit held that the Secretary "carried her burden and that the SCN1A gene mutation was the sole substantial cause of Deribeaux's seizure disorder and developmental delays."[39]

In *Bowen v. E.I. Dupont de Nemours*, the defendant was permitted to conduct newly available genetic testing on the plaintiff to show that the birth defect at issue was inherited instead of being caused by prenatal exposure to Benlate, a fungicide.[40] The testing showed that the plaintiff had a specific genetic mutation associated with an inherited syndrome, CHARGE, that was known to cause the birth defects at issue. The defense therefore argued that the mutation, as opposed to Benlate, caused the defects. According to the opinion, the genetic testing results were so compelling that they caused one of the plaintiff 's experts to switch sides and agree with the defense that the birth defects were related to CHARGE and its associated mutation. The court granted the defendant's motion to exclude the plaintiff 's expert testimony that Benlate caused the defects, finding that the expert could not rule out CHARGE and the mutation as the cause; "there is no evidence of any cause other than the CHD7 mutation."[41] As a result, the court also granted defendant's motion for summary judgment because "without the testimony of those witnesses [the experts] the plaintiffs could not establish that Benlate was a human teratogen or that it was a specific cause of the injuries being complained of by either plaintiff."[42]

Likewise, in *Wintz v. Northrop*, parents brought an action against the manufacturer of photographic developing materials containing bromide and the mother's employer, claim-

Susan E. Brice (susan.brice@bryancave.com) is a partner at Bryan Cave LLP defending toxic tort and environmental cases brought against corporate clients in cases involving complex scientific issues. **Dr. Whitney V. Christian** formerly was a health scientist with Cardno ChemRisk, and is now a senior toxicologist at Medtronic, focusing on genetics, toxicology, and gene-environment interactions and their contribution to the development of human disease.

This article was printed in the October issue of the IPT Journal.

ing their infant suffered developmental problems from in utero exposure.[43] The infant's bromide levels were elevated and her symptoms were similar to another case where an infant was injured from bromide. The infant underwent genetic testing and it was found that the infant possessed a genetic disorder known as Prader-Willi Syndrome, which is caused by a deletion in paternal genetic material and is not environmentally related.[44] The lower court excluded the testimony of the plaintiff 's causation expert, and the Seventh Circuit affirmed. The courts took issue with the expert's qualifications as well as his methodology. The courts were particularly troubled by the fact that the expert, a toxicologist and not a medical doctor, lacked any specific experience with bromide, Prader-Willi Syndrome, or birth defects in general.45 The exclusion of the expert testimony led to summary judgment for the defendants.

While not a published opinion, a recently resolved case from the Superior Court of Delaware serves as a good example of how expert testimony and genetics are employed in the courtroom today. In that case, *Pallano v. AES Corporation*, residents of Dominican Republic sued coal-fired power plant operators alleging that coal ash waste deposited on beaches caused them to suffer a myriad of personal injuries, including birth defects.[46] According to the court, there were 19 *Daubert* motions filed by the parties in "this hotly and heavily litigated dispute."[47] Some of the *Daubert* motions focused on testimony involving genetic evidence. For example, the plaintiffs moved to exclude a defense expert who opined that a plaintiff 's gastrointestinal neurological disease (Hirschsprung's disease) was most likely caused by genetic variation and not environmental factors. The doctor opined that Hirschsprung's disease "follows a multigenic model" (associated with the interaction of variants of several genes) and noted that genetic testing showed that the child plaintiff possessed at least three genetic variations associated with the disease. The court ruled that the defense expert's opinion "passes muster under D.R.E. 702 and *Daubert*."[48]

The defense attacked the plaintiff 's medical geneticist. The court "was more than satisfied" that the plaintiff 's medical geneticist met the requirements under the rules and *Daubert* as he analyzed each child's "genetic testing results, relevant medical literature, and how each child Plaintiff 's individual gene variants relate (or do not relate) to their individual congenital anomalies" and he also analyzed literature relating to "gene-environment interactions in the etiology of birth defects, embryology and organ formation, and human epidemiological and animal studies concerning Coal Ash Waste and its toxic constituents, … and discusses how each of these studies factor into his causation analysis for plaintiffs."[49] After these key *Daubert* motions failed, the matter settled one month later, in April 2016.[50]

In some cases, courts have excluded expert testimony simply for neglecting to consider genetics as an alternative cause. These courts view the expert's failure to "rule in" genetics as a potential cause as a misapplication of the differential diagnosis methodology and therefore fatal to the expert's opinion.[51] In *Palmer v. Asarco*, the northern district of Oklahoma excluded expert testimony that exposure to lead caused the plaintiff 's learning disabilities and IQ loss. The court reasoned that the doctor failed to "consider factors such as genetics, parental intelligence and psychosocial settings" and thus did not perform a proper differential diagnosis.[52] Likewise, in *Lofgren v. Motorola*, for example, the court excluded an expert's attempt to tie trichloroethylene exposure to brain cancer.[53] The court held: "There are a number of inherited or genetic syndromes that may contribute to the development of brain tumors. Dr. Kilburn apparently did not consider any genetic components or attempt to discuss or evaluate how they may have caused or contributed to the plaintiff 's concern. Dr. Kilburn's elimination from consideration alternative risk or confounding factors does not appear to be in step with mainstream scientific thought on proper methodology for arriving at causation opinion. Dr. Kilburn's testimony is, therefore, for the above reasons inadmissible."[54]

Genetic Biomarkers

In more recent years, plaintiffs and defendants alike have begun to explore the next level of genetic evidence—biomarkers of cause and effect.[55] Biomarkers in general are defined as: (1) a chemical, (2) its metabolite, or (3) the product of an interaction between a chemical and some target molecule or cell that is measured in the human body.[56]

A genetic biomarker of effect represents an interaction between a chemical and a target molecule in the human body. In other words, the biomarker comes in the form of a genetic fingerprint, or to use a more simple analogy, a trail of breadcrumbs. A geneticist looking in the right place can see the breadcrumbs and know that a specific chemical exposure could have caused a mutation associated with the initiation of a disease process. The "could" caveat is explained in more detail hereinafter.

The courts are becoming accustomed to the introduction of biomarkers into evidence. In *Cord v. City of Los Angeles*, plaintiffs in California claimed that benzene and other chemicals emanating from a landfill near a high school caused lymphatic cancer.[57] To rebut the plaintiffs' claim that Mr. Cord experienced "chronic exposure to benzene and other volatile compounds during his years of employment at the high school," the court allowed the testimony of the City's expert, who argued that

> [b]iomarker [testing] can be performed utilizing blood, urine or fat samples … Such biomarkers can test for 180,000 diff erent chemicals, including the chemicals to which plaintiff s claim Mr. Cord was exposed resulting in his cancer … because no such test were performed on Mr. Cord, "it is impossible to determine to a medical certainty" whether Mr. Cord's exposure, absorption or toxicity to benzene or other chemicals exceeded normal and expected levels. In other words, existing tests were available to measure whether Mr. Cord in fact had excessive exposure to benzene and other chemicals but plaintiff s' experts did not use them.[58]

The appellate court found that this rebuttal opinion was properly considered in the granting of summary judgment for the defense.[59]

Chromosomal aberrations and specific gene mutations exemplify two different types of genetic biomarkers of effect.[60] A good example of a biomarker of effect associated with chemical exposure can be found in how benzo(a) pyrene impacts the p53 tumor suppressor gene. If this gene is not functioning properly, tumor suppression is hindered and a person is more likely to develop tumors. Researchers have discovered that exposure to benzo(a)pyrene (a chemical contained in cigarette smoke) can produce DNA adducts. DNA adducts can occur when carcinogens chemically bind to the nucleotides (the G, C, T or A) in the DNA sequence of our cells. These abnormal adducts then mechanically interfere with the DNA replication process and lead to mutations that can cause cancer.

It has been determined that DNA adducts created by benzo(a)pyrene (benzo(a)pyrene bonded to DNA) can result in specific mutations within the p53 tumor suppressor gene that are linked to smoke-induced lung cancer.61 Consequently, the presence of benzo(a)pyrene-DNA adducts at certain locations within the p53 tumor suppressor gene in a lung cancer patient suggests that the patient's lung cancer could have been caused by benzo(a)pyrene. In other words, the adducts allow an expert performing a differential diagnosis to "rule in" benzo(a)pyrene as a possible cause. The absence of adducts, however, can largely rule out benzo(a)pyrene as the cause.

The reason for this disparity in proof lies in the intricacy of the human biological process. As was noted at the beginning, "[m]ost common complex diseases are believed to be the result of the combined effect of genes, environmental factors and their interactions."[62] Therefore, a geneticist's investigation into cause and effect must take into account the reality that humans are exposed to thousands of chemicals and other substances each day in what they eat, what they drink, which medicines they take, the composition of the air they breathe and the surroundings they encounter.[63] While the science of biomarkers is rapidly developing, in most cases, there still remains a lack of specificity to prove that one cause, such as a single chemical exposure as opposed to a number of factors, created the genetic biomarker and was a substantial factor in bringing about the disease.

It is easier, though, to prove the negative. If it is well known that benzo(a)pyrene causes lung cancer through one pathway and evidence of that pathway is absent in the plaintiff, then it is unlikely that the benzo(a)pyrene caused the plaintiff 's disease. If multiple known pathways exist, however, the ability to rule out benzo(a)pyrene as the cause becomes more difficult. As a result, the efficacy of the studies supporting the pathogenesis of the disease and of the chemical-to-disease connection is of paramount importance.

While published decisions are scant, courts have entertained the absence of a known pathway to find for the defense. In *Tompkin v. Philip Morris USA*, the defendant argued that asbestos, as opposed to smoking, caused the plaintiff 's lung cancer.[64] The defense expert opined that the decedent lacked mutations in the p53 and Ki-ras genes known to be caused by smoking, and thus his cancer likely stemmed from asbestos.[65] The jury ultimately found that the cancer was caused by asbestos, a win for the defense. The decision was affirmed on appeal in which the U.S. Court of Appeals for the Sixth Circuit noted that the trial court had stated that the genetic testimony was particularly "devastating" to plaintiff 's case.[66]

This article was printed in the October issue of the IPT Journal.

In *Milward v. Acuity Specialty Products Group, Inc*., the initial case turned on general causation, whether benzene could cause the unique type of acute myelogenous leukemia (AML) contracted by the plaintiff.[67] The parties agreed that benzene could cause chromosomal aberrations that lead to certain forms of AML, but benzene had not been linked to the characteristic genetic alteration (t(15;17)translocation) almost always found in this plaintiff 's type of AML.[68] The expert attempted to opine that because benzene is known to cause some chromosomal damage, it probably also causes the (t(15;17)translocation) linked to the AML contracted by the plaintiff. The court held that the expert's "general extrapolation" was unjustified and not a "reliable scientific conclusion," therefore excluding the opinion.[69] On appeal, the U.S. Court of Appeals for the First Circuit reversed. The court did not disagree with the merits of the court's criticism of the opinion, but rather opined that the lower court went too far and that the "alleged flaws identified by the court go to the weight of Dr. Smith's opinion, not its admissibility. There is an important difference between what is unreliable support and what a trier of fact may conclude is insufficient support for an expert's conclusion."[70]

In *Henricksen v. ConocoPhillips Co*., the court excluded the plaintiff 's causation experts, in part, because they failed to consider adequately the possibility of an alternative cause, de novo AML (AML that is unrelated to chemical exposure).[71] Science distinguishes between de novo AML and secondary AML (AML caused by an external stimulus, including benzene exposure).[72] According to the court, the majority of adult AML cases (80-90 percent) were de novo. In secondary AML cases, 90 percent showed chromosomal aberrations and typically were preceded by myelodysplatic syndrome. Mr. Hendricksen had neither.[73] Because Mr. Hendricksen's presentation was much more closely aligned with de novo AML, it was improper methodology for plaintiff 's causation experts not to rule in de novo AML as a potential cause then to rule out de novo AML as the cause.[74] Accordingly, the court excluded the experts and granted summary judgment to the defendants.

* * *

This article will continue in the *Intellectual Property & Technology Law Journal* with a discussion of genomics, toxicogenomics, epigenetics, and tools for understanding causation at the genomic level.

Notes

33. Research has shown that some individuals are more genetically susceptible to disease, including disease caused by environmental exposures. As a result, plaintiffs in toxic tort litigation have attempted to use evidence of genetic susceptibility to prove they are more at risk than the average person to contracting a particular ailment due to chemical exposure. So far, these efforts have not been fruitful in large measure because the plaintiffs who have tried have been unable to show that they possess and/or are expressing the genetic variant that makes them susceptible. *See, e.g., Hall v. Baxter Healthcare Corp*., 947 F. Supp. 1387, 1456 (D. Or. 1996) (rejecting introduction of evidence of genetic susceptibility to silicone because the breast implant plaintiffs had failed to show that they carried the specific genes allegedly conferring susceptibility); *Pohl v. NGK Metals Corp*., 2003 WL 25871522 (Pa. Com. Pl. July 9, 2003 (Philadelphia)) (order denying class action when evidence demonstrated that in order to develop Chronic Berryllium Disease, plaintiffs must have a genetic predisposition to beryllium sensitization and that none of the named plaintiffs tested positive for beryllium sensitization). However, even if a plaintiff demonstrates susceptibility, there is a question whether the law would recognize the injury. In some states, such as Illinois, courts protect manufacturers from liability when a plaintiff suffers from an "idiosyncratic" reaction to a product. *See Presbrey v. Gillette Co*., 105 Ill. App. 3d 1082, 1091, 435 N.E.2d 513, 520 (2d Dist. 1982) ("[t]he unusual susceptibility of the consumer is generally recognized as a complete defense where the manufacturer did not know and had no reason to know that a very few users of his product might be injured."); *Bear v. Power Air, Inc*., 230 Ill. App. 3d 403, 595 N.E.2d 77 (1st Dist. 1992) (building owner not liable for eye injury resulting from dust generated during installation of new air conditioning equipment because conduct was not in and of itself inherently dangerous, but became hazardous only to person with employee's sensitive eye condition.) This concept, which is somewhat at odds with the "eggshell plaintiff " doctrine, is exemplified in the Food Allergen Labeling and Consumer Protection Act of 2004 (Public Law 108-282), which requires only that food labels warn about eight major allergens.
34. Gary E. Marchant, "Genetic Data in Toxic Tort Litigation," J. of L. & Policy, (2006).
35. *Clausen v. M/V New Carissa*, 339 F.3d 1049, 1057 (9th Cir. 2003) (quoting Stedman's Medical Dictionary 474 (26th Ed. 1995)); *Higgins v Koch Development Corp.*, 794 F.3d at 705 (explaining that "differential etiology" is the proper term for "causation-determination methodology").
36. *Higgins*, 794 F.3d at 705; *Clausen*, 339 F.3d at 1058 ("[a]fter the expert rules in all of the potential hypotheses … he or she must then engage in a process of elimination, eliminating hypotheses on the basis of a continuing examination of the evidence so as to reach a conclusion as to the most likely cause of the findings in that particular case"); *see also Reference Manual on Scientific Evidence* (3rd Ed.) at 617 ("[e]liminating other known and competing causes increases the probability that a given individual's disease was caused by exposure to the agent.")
37. Staff Report, *Federal jury accepts paint manufacturer's gene defense*, (Aug. 6, 2006) *http://www.picayuneitem. com/2006/08/federal-jury-accepts-paint-manufacturersgene- defense* (discussing *Jones v. NL Industries*).
38. Sheila Byrd, *Gene Defense in Lead Paint Case Rankles* (July 13, 2006), *http://www.washingtonpost.com/wp-dyn/content/article/2006/07/13/AR2006071301205_pf.html*.
39. *Deribeaux v. Secretary of Health and Human Services*, 717 F.3d 1363, 1368 (Fed. Cir. 2013); *see also Myers v. United States*, 2014 WL 611398, *47-49 (S.D. Ca. Nov. 20, 2014) (bench trial order finding that plaintiff 's alopecia was more likely than not the result of genetics as opposed to thallium exposure).
40. *Bowen v. E.I. Dupont de Nemours*, No. Civ.A. 97C-06- 194 CH, 2005 WL 1952859 (Del. Super. June 23, 2005).
41. *Id.* at *11.
42. *Id.* at *6.

This article was printed in the October issue of the IPT Journal.

43. *Wintz v. Northrop Corp*., 110 F.3d 508 (7th Cir. 1997).
44. *Id*. at 511.
45. *Id*. at 513-14.
46. *Pallano v. AES Corp*., 2016 WL 930545, *1 (Del. Super. March 10, 2016) (trial court order noting that Defendants challenged seven of Plaintiff 's causation experts and plaintiffs have challenged six of Defendants' causation experts).
47. *Pallano v. AES Corp*., 2015 WL 7776612, at *1 (Del. Super. Nov. 24, 2015) (trial court order).
48. *Pallano v. AES Corp*, 2015 WL 9008641, *2-3 (December 11, 2015).
49. *Pallano v. AES Corp*, 2016 WL 930545, *2 (March 10, 2016).
50. *http://www.bloomberg.com/news/articles/2016-04-04/aes-settles-suit-over-coal-ash-dumping-in-dominican-republic*.
51. *C.f., Tumlinson v. Advanced Micro Devices*, No. 08C- 07-106 FSS, 2013 WL 7084888 (Del. Super. October 15, 2013) (in a case alleging that exposure to multiple chemicals at semiconductor plant caused birth defects, court struck epidemiologist's opinion for failure to apply adequately differential diagnosis to support her opinion; epidemiologist did not show that there was only one possible cause of the birth defects and epidemiologist did not explain her rejection of other possible causes, such as mother's obesity and the fact that, though rare, the birth defects did occur in the population at large without evidence of causation.").
52. *Palmer v. Asarco Inc*., 2007 WL 2298422,*9 (N.D. Okla. Aug. 6, 2007); see Brown v. Burlington N. Santa Fe Ry. Co., 2013 WL 1729046, at *10 (C.D. Ill. Apr. 22, 2013), aff'd, 765 F.3d 765 (7th Cir. 2014) (excluding doctor that admitted that "there is a genetic predisposition to CTD [carpal tunnel syndrome], yet the doctor failed to 'rule in' Brown's [family] history [of CTD].")
53. *Lofgren v. Motorola*, No. CV 93-05521, 1998 WL 299925 (Ariz. Super. Ct. June 1, 1998).
54. *Id*. at 33; *Nat'l Bank of Commerce v. Dow Chemical Co*., 965 F.Supp. 1490 (E.D. Ark. 1996) (in a case alleging that in utero exposure to pesticide caused birth defects, the court excluded the plaintiff 's expert who lacked specialty in genetics or teratology and attempted to rule out genetics as a cause based solely on anecdotal family history).
55. Biomarkers largely fall into one of three classification groups: (1) biomarkers of exposure, (2) biomarkers of effects, and (3) biomarkers of susceptibility. F Gil and A Pla, "Biomarkers as biological indicators of xenobiotic exposure," 21 J *Appl Toxicol*. (2001). For example, a biomarker of exposure to lead can be determined by looking at the amount of lead in a person's blood or urine; a biomarker of effect can be depressed Δ-aminolevulinic acid dehydratase (ALAD) activity, the presence of coproporphyrinogen III in urine, or the accumulation of protoporphyrin XI and zinc protoporphyrin in red blood cells; and a biomarker of susceptibility, can be specific ALAD polymorphisms or a lack of allelic variation in expression (e.g., ALAD1 homozygotes). T SAKAI, "Biomarkers of lead exposure," 38 Ind Health. (2000).
56. World Health Organization, Environmental Health Criteria 237, "Principles for Evaluating Health Risks in Children Associated with Exposure to Chemicals," published under UNEP-ILO-WHO, Geneva. (2006).
57. *Cord v. City of Los Angeles*, No. EC032513, 2004 WL 2189182 (Sept. 30, 2004).
58. *Id*. at *9.
59. *Id*. at *1.
60. Testing exists to examine both types. Examples of testing for chromosomal aberrations include karyotyping, extended banding, fluorescence in situ hybridization or chromosomal microarray analysis to determine whether whole chromosome or chromosome fragments have been deleted, duplicated, inverted, translocated or otherwise rearranged. Genetic mutations can be identified by analyzing the DNA sequence of specific genes. This can be done through DNA microarray analysis, Sanger sequencing, shotgun sequencing, as well as next-generation sequencing, which includes highly accurate techniques such as polony sequencing, SOLiD sequencing, and SMRT sequencing. These tests look at the order of the nucleotides within cells' DNA in comparison to control, or "normal", DNA.
61. *MF Denissenko, et al*., "Preferential formation of benzo [a] pyrene adducts at lung cancer mutational hotspots in P53," 274 Science (1996).
62. *H. Aschard, et al*., "Challenges and opportunities in genome-wide environmental interaction (GWEI) studies," 131 *Hum Genet*. (2012).
63. It is estimated that humans are exposed to 1–3 million discrete chemicals in a lifetime. J.R. Idle, et al., "Metabolomics," 6 Cell Metab. (2007).
64. *Tompkin v. Philip Morris USA, Inc*., 362 F.3d 882 (6th Cir. 2004).
65. *Id*. at 890 n.5.
66. *Id*. at 894.
67. *Milward v. Acuity Specialty Products Group, Inc*., 664 F.Supp.2d 137, 146-147 (D. Mass. 2009), rev'd 639 F.3d 11 (1st Dist. 2011).
68. *Id*. at 146-147.
69. *Id*. at 147.
70. *Milward v. Acuity Specialty Products Group, Inc*., 639 F.3d 11, 22 (1st Cir. 2011).
71. *Henricksen v. ConocoPhillips Co*., 605 F.Supp.2d 1142 (E.D. Wash. 2009).
72. *Id*. at 1149-50.
73. *Id*.
74. *Id*. at 1163.

The Use of Genetic Evidence to Defend Against Toxic Tort Claims—Part III

By Susan E. Brice and Dr. Whitney V. Christian

This final part of a three-part article discusses genomics, toxicogenomics, epigenetics, and tools for understanding causation at the genomic level. The first part, which appeared in the September 2017 issue of *Intellectual Property & Technology Law Journal*, introduced the topic, discussed the human genome, genes, the environment, susceptibility, and disease. The second part, which appeared in the October 2017 issue of *Intellectual Property & Technology Law Journal*, explored genetic data, toxic tort law, and genetic biomarkers.

Genomics and Toxicogenomics

Genomics is the study of the structure and function of the complete set of DNA within an organism. Human genomics therefore encompasses more than just the sequencing and mapping of human genes. It also looks at how these genes collaborate to perform cellular functions. Every nucleated cell in the human body contains the same DNA. Then how does a skin cell know to be a skin cell and a liver cell to be a liver cell? The answer is gene expression. In order for a skin cell to differentiate itself from a liver cell, certain genes encoded by the DNA (skin genes) must be expressed while others must be repressed, or silenced. In other words, a gene is expressed (on) and active or it is repressed (off) and inactive. A useful way to think of this is a circuit breaker panel. When switched on or off, a circuit breaker modulates the electricity that operates a light.

Similar to genetic mutations (*i.e.*, changes in the nucleotide sequence), gene expression impacts disease. For example, an oncogene (a gene that possesses the potential to cause cancer and generally is involved in controlling the rate of cell growth or cellular differentiation) can be turned on and expressed at a high level, contributing to the development of cancer. However, if this high expression is turned off, the cancer risk diminishes. When it comes to disease, therefore, geneticists not only can look at the sequence of the plaintiff 's nucleotides and for mutations, but also can analyze which genes are being expressed and which are not. One way to study this is through "gene expression profiling," which is the measurement of the expression of all the genes at once to develop a global picture of the cellular functional state.

Toxicogenomics is the study of how an organism's genome responds to toxic substances. Toxicogenomics looks at both whether a chemical causes a genomic change as well as whether it elicits a change to gene expression. Researchers have discovered that environmental exposures can induce specific "gene expression profiles," or a characteristic pattern of genes expressed in response to the exposure, which is distinguishable from the gene expression profile exhibited absent the exposure.

Recently, researchers realized that radiationinduced cancer in certain tissue induces a gene expression profile distinct from idiopathic cancer in that same tissue type.[75] Accordingly, specific gene expression profiles can be used to infer causal events. For instance, one can analyze the gene expression profile and make a reasonable determination as to whether a carcinogenic event was caused by radiation or something else.

In 2012, defendants were able to use this type of genetic evidence to prevail in a toxic tort case in Louisiana involving Naturally Occurring Radioactive Materials (NORM). In the case, *Guzman v. Exxon Mobil*,[76] a plaintiff alleged that her second hand exposure to NORM contained in pipe scale caused her to contract papillary thyroid cancer. The defense filed a motion to conduct genetic testing on the plaintiff 's preserved thyroid tissue, which the court granted. The defense expert opined that previously conducted toxicogenomic research, which analyzed gene expression caused by specific toxic exposures, had identified a "radiation induced gene 'signature' in radiation induced thyroid cancers that was not present in sporadic thyroid cancers."[77]

When the plaintiff 's thyroid tissue was subjected to gene expression analysis, the signature for radiation-induced thyroid cancer was absent. After hearing all the scientific testimony, the jury returned a verdict for the defendants.

Susan E. Brice (susan.brice@bryancave.com) is a partner at Bryan Cave LLP defending toxic tort and environmental cases brought against corporate clients in cases involving complex scientific issues. **Dr. Whitney V. Christian** formerly was a health scientist with Cardno ChemRisk, and is now a senior toxicologist at Medtronic, focusing on genetics, toxicology, and gene-environment interactions and their contribution to the development of human disease.

This article was printed in the November issue of the IPT Journal.

Epigenetics

Gene expression profiling is a useful tool in that it can promote the linking of certain chemicallyinduced diseases to a characteristic overall gene expression profile. But its utility is limited. The critical work, the work that could crack open the causative mechanics between exposure and disease, lies in epigenetics.

The emerging field of epigenetics promises to alter how we understand disease and disease processes. Epigenetics is the "devil in the details" of gene expression profiling. Epigenetics, which literally means "above" or "on top of" genetics, is one biological mechanism that impacts altered gene expression. Epigenetics examines the biochemical modifications placed "above" or "on top of " DNA, which do not alter the actual sequence of the DNA, but can cause a gene to be turned on or off or to be expressed more strongly or weakly.[78]

In order to better understand this concept of epigenetics, it makes sense to return to and add another dimension to the circuit breaker example. We know that genes turn on/off and expression/repression occurs with respect to the circuit breaker being turned on/off, but we have not yet explored how the breaker flips. The answer is biochemistry. Normally, the cell initiates certain biochemical modifications that flip the circuit breaker and turn the gene on/off.[79] In a house, the circuit breaker typically is set in one position, such as the position that maintains electricity running to the lights in the kitchen. Sometimes, however, it becomes necessary to turn all the kitchen lights off at once. This can be done by manually flipping the circuit breaker associated with kitchen lighting. The same thing happens with epigenetics, but through a biochemical process. For instance, in the normal cellular process, if the kitchen light gene needs to be deactivated, biochemical processes are initiated to turn the circuit breaker associated with the kitchen light gene off. In other words, the biochemical processes act as if they are manually and intentionally flipping the circuit breaker. When the kitchen light gene needs to be illuminated, the biochemical processes reverse themselves, as if they were being manually flipped back, facilitating illumination of the kitchen lights.

Like the house, cells function more effectively and efficiently with certain genes turned on and others turned off. In fact, humans are born with a set gene expression profile. Over time, this pattern changes. For example, although twins are born with the same epigenome (pattern of genes turned on and off or modulated), their epigenomes diverge as they age.[80]

Not surprisingly, research has shown that epigenetic modifications and corresponding gene expression changes can be caused by gene-environment interactions, such as genes interacting with chemical exposures, ambient environmental conditions, pharmaceuticals and diet. When this happens, instead of the normal process of manually flipping the circuit breaker, it is unexpectedly tripped. This unforeseen "tripping" can happen in different ways, but seems to involve xenobiotic exposures interfering with epigenetic processes. Stated differently, the "tripping" would involve: (1) xenobiotic exposures promoting epigenetic modifications; (2) those epigenetic modifications causing aberrant changes in gene expression; and (3) then those changes to gene expression resulting in altered gene expression profiles that can lead to disease. It is important to underscore the ripple effect potentially generated by just one epigenetic modification. Genes work in networks. Therefore, when the circuit breaker is tripped, it impacts not only the kitchen light gene, but all related genes. In our example, therefore, the tripping of the light gene might impact all lights in the house.

While scientists understand epigenetic mechanisms on a molecular level, they do not yet fully understand how xenobiotic exposures trigger epigenetic changes. That being said, discoveries are made every day and researchers successfully have connected some chemical substances to specific epigenetic alterations. For example, arsenic exposure has been shown to cause target organ-specific global and gene-specific epigenetic change.[81]

Studies have shown that arsenic can produce a characteristic epigenetic pattern modification that silences specific tumor suppressor genes, including p53.[82] Similar epigenetic alterations have been employed to underlie causation in toxic tort cases. In 2014, the Western District of Louisiana denied a *Daubert* challenge to a Plaintiff expert expected to testify that pioglitazone, an anti-diabetic drug, was an "epigenetic modifier" that rebalanced the tumor suppressive environment toward a tumor conducive environment.[83] The case settled in favor of the Plaintiff, and exemplifies the increasing use and recognition of epigenetic evidence in the courtroom.

Tools for Understanding Causation at the Genomic Level

Some chemical-mediated changes in the human genome may be well documented, while others require investigating the alleged connection between exposure and alteration(s). Several techniques exist for characterizing genetic

and epigenetic modifications that may result from specific chemical exposures and, in turn, serve as biomarkers or footprints of exposure (Table 1). Genetic mutations in the sequence of DNA or at the chromosomal level may be detected via next-generation DNA sequencing (DNA-Seq) or modern karyotyping techniques, respectively. Likewise, techniques have been developed to identify epigenetic changes that result in gene expression profile alterations.[84] But how do we break through the next ceiling? We need to transition from understanding general causation at the genomic level, establishing an association between specific chemical exposures and genetic/epigenetic changes, to establishing specific causation in an individual.

Genomic Editing and CRISPR

The answer may lie in the emerging field of genomic editing. Genome editing is the process by which scientists modify the genes in an organism and then observe how the forced modification impacts cellular biology. In recent years, one specific form of genome editing, the CRISPR system (Clustered Regularly Interspaced Short Palindromic Repeats), has transformed traditional genome editing. Scientifically speaking, the CRISPR system is a RNA-guided DNA endonuclease. In layman's terms, it is a highly specific pair of genetic scissors. With CRISPR, scientists can conduct genome editing that targets the genetic sequence of a specific gene or alters the expression of a specific gene.

Table 1—Methods to Link Genetic/Epigenetic Biomarkers of Exposure and Disease Causation

Biomarker	Type	Effect of Chemical Exposure	Identification	Characterization	Causation Analysis
Genetic	nucleotide sequence	somatic/germline mutation: deletion, insertion, substitution, and frameshift	Sanger sequencing, DNA-Seq: Genome-Seq, Exome-Seq, and Target-Seq	CRISPR/Cas9	Studying effect of specific genetic alteration(s) in whole animal, specific human cell type(s), and/or human organoid
	genomic imbalance	chromosomal abnormaility: structural - deletion, insertion, inversion, duplication, and translocation; numerical - aneuploidy and euploidy	Conventional karyotyping, Fluorescence in situ hybridization (FISH), Comparative genomic hybridization (CGH)		
Epigenetic	gene expression profile	gene upregulation and downregulation, mRNA stabilization and degradation	RT-qPCR, RNA-Seq	CRISPRi and CRISPRa	Study effect of specific epigentic alteration(s) by mimicing the resulting upregulation or downregulation of a specifi c gene(s) in whole animal, specifi c human cell type(s), and/or human organoid
	DNA methylation pattern	CpG hypomethylation and hypermethylation, abnormal DMR methylation	Bisulfite sequencing (BS-Seq), OxBSSeq and TAB-Seq, MeDIP-Seq		
	ncRNAome	expression of non-coding RNA (ncRNA): long non-coding RNA (lncRNA), microRNA (miRNA), and piwiinteracting RNA (piRNA)	Tiling arrays, Serial analysis of gene expression (SAGE), Dumbbell-PCR, miRDeep		
	chromatin remodeling	histone modifi cation: active transcription (H3K4me1, H3K4me3, H3K9me1, H3Ac, H4Ac); repression/ gene silencing (H3K9me3, H3K27me3)	ChIP-Seq, ChIP-on-Chip		

At the sequencing level, scientists can modify the nucleotide sequence of a gene or multiple genes, by cutting out nucleotides in a strand of DNA and replacing them with different nucleotides.[85] For instance, in our example (found in the first part of this article) of the single nucleotide polymorphism (SNP) responsible for the flushing response to alcohol in which the ALDH2 gene is AAA instead of the expected GAA (*i.e.*, the ALDH22 allele), scientists could use CRISPR to replace the A with the G and theoretically eliminate the flushing response.[86]

In order to edit gene expression, scientists may take one of two paths. There are two main layers of gene expression control, transcriptional and epigenetic, and in a way, these are organized like layers of an onion. In the bottom transcriptional layer, biological processes occur that cause the nucleotides that make up the gene to be transcribed into mRNA, and therefore expressed. After the mRNA is generated, it is translated into a protein and becomes functional. In the upper epigenetic layer, epigenetic modifications, such as DNA methylation and histone modification, allow or hinder the bottom layer biological processes responsible for transcription, and therefore also modulate activation or repression of gene expression. At both layers, CRISPR can be exploited to increase or decrease the expression of specific genes.

At the transcription level, CRISPR can be used to create a roadblock or repel the transcription of a gene, a process known as CRISPR interference (CRISPRi). On the other hand, CRISPR can be used to increase gene expression by attracting transcription; this process is referred to as CRISPR activation (CRISPRa).[87] At the epigenetic level, CRISPRi and CRISPRa can be used to alter the epigenetic profile of a gene, including through methylation.[88]

Using CRISPR to Study Toxicants

The ability to alter genes and therefore study how various genotypes react to outside stimuli is an ideal tool for investigating disease states that may result from chemical exposures that induce genetic and epigenetic changes within the human genome. For example, scientists in the laboratory can investigate how cells with different genotypes, the wild type and various polymorphisms, are impacted when exposed to a chemical. They can then take it one step further and test how *a specific plaintiff's genomic profile reacts to a chemical.*

This can be done by designing and growing tissue cells in the lab that have the plaintiff 's genomic profile, including the exact nucleotide sequence of a gene and how that gene is being expressed through transcriptional and epigenetic mechanisms.[89] Once the cells are created, they can then be exposed to various doses of the chemical in question. Thereafter, the cells' dose response can be examined. If disease phenotypes do not develop in the plaintiff-specific cell line, then the defense has a strong argument against specific causation. However, if they do, then it is more likely that the plaintiff 's exposure to the chemical in question caused his or her disease.

Because of its precision, the CRISPR system can be employed to study carcinogenesis in a whole new way. It is well known that DNA mutations that result in loss-of-function in a tumor suppressor gene or gain-of-function in an oncogene can promote the development of cancer. However, the acquisition of one mutation typically is insufficient and the disease itself occurs in the context of gene-environment interactions. Therefore, characterizing an exposure to a foreign chemical(s) in the background of a specific combination of genetic alterations, such as the genetic profile of a plaintiff, is an effective approach to determining the validity of a plaintiff 's allegation that exposure to a specific foreign chemical(s) caused their cancer. For example, if someone alleged they were suffering from kidney cancer due to exposure to chloroform (a non-genotoxic carcinogen),[90] a model could be created using CRISPR that mimicked the genetic profile of the plaintiff in human kidney cells.[91] Those kidney cells could then be exposed to the alleged amount of chloroform to see if it produced the disease phenotype associated with the plaintiff 's kidney cancer.

Similarly, exposures with alleged gene expression profiles can be studied. Human cells can be treated with a specific chemical(s) (*e.g.*, recreating a plaintiff 's acute exposure) and the resulting expression profile, or epigenetic footprint, can be identified (Table 1). After characterizing the gene expression profile, it can be replicated in the laboratory. Scientists can create cells, or even animals,[92] that exhibit this gene expression profile through CRISPRi and/or CRISPRa. These models can then be observed over a period of time to see if they develop the phenotype of the disease in question, thereby permitting long-term observation after acute exposure. In other words, CRISPRi and/or CRISPRa can be used to modulate the expression of targeted genes. That is, the expression of specific genes can be turned up or down, much like the volume of music or an electrical rheostat, and whether the gene expression alterations lead to a disease state can be studied. By doing

this, scientists might determine that an adverse effect does not occur until a certain gene is expressed, expressed at a certain level or expressed for a certain amount of time. This type of information would be invaluable to determining causation.

The ability of CRISPR to sharpen our understanding of how chemicals cause disease cannot be understated. As we know, chemical exposures may result in both genetic and epigenetic alterations. CRISPR can be employed to study both simultaneously. That is, multiple mutations can be introduced into a genome, and at the same time, the expression level of multiple genes (genes with or without introduced mutations) can be finely controlled. The ability to modulate both genetic and epigenetic change together adds a new dimension to toxicogenomics. However, CRISPR has not been without its limitations. In particular, some instances of off-target effects resulting in unwanted genetic alterations have been observed.[93] Fortunately, the reasons for this are well understood and advances have been made in CRISPR technology that address, and for the most part, overcome concerns with off-target genomic effects, ensuring the high-fidelity of this technique.[94]

It is important to emphasize that while CRISPR technology is in its infancy, it is currently being used to create models for studying human disease and/or the adverse effects of chemical exposures. While some of the examples discussed above are more theoretical at this point, as CRISPR technology advances, so will the ability to use CRISPR as a means to study cause and effect in the human body. Such advancements are exemplified by recent clinical trials utilizing CRISPR-based cancer treatments.[95] Like its developing role in modern medicine, CRISPR could become a critical tool for understanding the significance of chemical exposures in toxic tort cases.

* * *

Notes

75. C. Ory, *et al*., "Comparison of transcriptomic signature of post-chernobyl and postradiotherapy thyroid tumors," 23 *Thyroid* (2013); C. Ory, *et al*., "Gene expression signature discriminates sporadic from postradiotherapy-induced thyroid tumors," 18 *Endocrine-Relat Cancer* (2011); C. Ory, *et al*., "Discriminating gene expression signature of radiation-induced thyroid tumors after either external exposure or internal contamination," 3 *Genes* (Basel) (2012).
76. *Guzman v. Exxon Mobil*, Jefferson Parish, Louisiana Case No. 693-606.
77. Expert Report of Dr. John B. Sullivan Jr. dated June 21, 2012 (on hand with author).
78. Epigenetics can control expression in a gradient fashion from low to high (*e.g*., hyper-to hypo-methylation of a gene promoter, respectively). In this case, the gene is not silenced but on, and its expression can range from low to high levels. There are instances where genes are completely off, such as during cellular differentiation, although gradients of expression are also prominent.
79. Two ways genes are turned on or off or modulated in tone are through DNA methylation and histone modification. DNA methylation occurs when methyl groups are attached to cytosine nucleotides in special places (CpG islands) within the sequence of DNA (often in gene promoters), which silences genes. Histone modification impacts DNA more indirectly. Histone modification involves attaching chemical tags to the tails of histones (the proteins the DNA is spooled around). These chemical tags include acetyl groups as well as methyl groups. On a very basic level, the addition of acetyl groups (histone acetylation) relaxes how tightly the DNA is wrapped around the histones. If the spooling is loose, transcription is allowed and the associated genes are expressed. Alternatively, removal of acetyl groups (histone deacetylation) tightens the winding of the DNA around the histones. If the DNA is coiled too tightly, the genes associated with the histones cannot be expressed.
80. For instance, twins teach us that a slight change in environment can provoke profound differences. This could be as simple as one twin living in a warm climate and one living in a cold climate. Their respective epigenome will be different due to their body adapting to its surroundings (*e.g*., differential expression of cold and heat-shock proteins). The epigenetic changes might not be one gene off and one on, but rather one turned on low and the other high.
81. C.Q. Zhao, *et al*., "Association of arsenic-induced malignant transformation with DNA hypomethylation and aberrant gene expression, 94 *Proc Natl Acad Sci U S A* (1997); H. Chen, *et al*., "Chronic inorganic arsenic exposure induces hepatic global and individual gene hypomethylation: implications for arsenic hepatocarcinogenesis," 25 *Carcinogenesis* (2004); TJ Jensen, *et al*., "Epigenetic remodeling during arsenical-induced malignant transformation," 29 *Carcinogenesis* (2008); X Ren, *et al*., "An emerging role for epigenetic dysregulation in arsenic toxicity and carcinogenesis," 119 *Environ Health Perspective* (2010).
82. Arsenic also has been shown to impact p16INK4A, RASSF1A, and DAPK and even alters histone modifications and miRNA expression [MJ Mass & L Wang, "Arsenic alters cytosine methylation patterns of the promoter of the tumor suppressor gene p53 in human lung cells: a model for a mechanism of carcinogenesis," 386 *Mutat Res*. (1997); X Cui, *et al*., "Chronic oral exposure to inorganic arsenate interferes with methylation status of p16INK4a and RASSF1A and induces lung cancer in A/J mice," 91 *Toxicol Sci*. (2006); YC Huang, *et al*., "Sodium arsenite-induced DAPK promoter hypermethylation and autophagy via ERK1/2 phosphorylation in human uroepithelial cells," 181 *Chem Biol Interact*. (2009); X Zhou, *et al*., "Arsenite alters global histone H3 methylation," 29 *Carcinogenesis* (2008); K Beezhold, *et al*., "miR-190-mediated downregulation of PHLPP contributes to arsenic-induced Akt activation and carcinogenesis," 123 *Toxicol Sci*. (2011)]. miRNAs are RNA sequences of about 22 nucleotides that do not produce proteins (*i.e*., they are non-coding), but rather silence the expression of proteins by binding in a complimentary fashion to mRNAs encoding proteins and promoting mRNA degradation within the cell. miRNAs are part of the epigenetic repertoire that controls gene expression. Therefore, alterations in the expression of miRNAs result in changes in the expression of the genes that the miRNAs silence.
83. In re Actos Product Liability Litigation, 2014 WL 46818, *1 (W.D. La. Jan. 6, 2014).
84. *Id*.
85. Within a cell, co-expression of Cas9, a DNA endonuclease enzyme, and a single-guide RNA (sgRNA), a targeting RNA, results in the specific and efficient cleavage of sequences of genomic DNA that match the sequence of the sgRNA. M Jinek, *et al*., "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity," 337 *Science* (2012). Cas9 has two catalytic sites that each function as a pair of scissors. With these two pairs of scissors, Cas9 makes doublestranded DNA breaks (DSBs) in the DNA of the target gene it is targeted to via the sgRNA. After cutting and creating DSBs in the target gene, DNA repair pathways are activated within the cell. DSBs often are repaired through a process called Non-Homologous End Joining (NHEJ) [T Lukacsovich, *et al*., "Repair of a specific double-strand break generated within a mammalian chromosome by yeast endonuclease I-SceI," 22 *Nucleic Acids Res*. (1994)]. NHEJ joins the cut DNA ends but

a few nucleotides are frequently deleted in the process, which results in a small deletion or frameshift that inactivates the gene creating a knockout, or removal of a specific gene from the genome. Cas9 also can be employed to create targeted chromosomal rearrangements via NHEJ, showing its utility for altering the structure of DNA on a much larger scale than just a few nucleotides [PS Choi & M Meyerson, "Targeted genomic rearrangements using CRISPR/Cas technology," 5 *Nat Commun*. (2014); D Maddalo, *et al.*, "In vivo engineering of oncogenic chromosomal rearrangements with the CRISPR/Cas9 system," *516 Nature* (2014); R Torres, *et al.*, "Engineering human tumourassociated chromosomal translocations with the RNAguided CRISPR-Cas9 system," 5 *Nat Commun*. (2014); J Jiang, *et al.*, "Induction of site-specific chromosomal translocations in embryonic stem cells by CRISPR/ Cas9," 6 *Sci Rep.* (2016)].

If one of the two catalytic sites in Cas9 is intentionally inactivated through mutagenesis, it creates a version of the endonuclease that can only make one cut or a nick in the target gene, creating Cas9 nickase. Nicked DNA is preferentially repaired by a different process called Homologous Recombination (HR), which utilizes another piece of endogenous undamaged DNA that corresponds to the DNA containing the nick to repair it [P Rouet, *et al.*, "Expression of a site-specific endonuclease stimulates homologous recombination in mammalian cells," 91 *Proc Natl Acad Sci U S A* (1994)]. Taking advantage of this repair process, if Cas9 nickase, a targeting sgRNA, and a piece of exogenous DNA corresponding to the region that will be nicked are introduced into a cell, a knock-in can be created [L Cong, *et al.*, "Multiplex genome engineering using CRISPR/Cas systems," 339 *Science* (2013); P Mali, *et al.*, "RNA-guided human genome engineering via Cas9," 339 *Science* (2013); H Wang, *et al.*, "One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering," 153 *Cell*. (2013)]. That is, the piece of exogenous DNA will be incorporated into the genome. If that exogenous piece of DNA contains a mutation, such as an insertion, deletion, or a change in the nucleotide sequence that results in a loss-of-function or gain-of-function for a gene, the HR repair process will introduce that mutation into the gene where Cas9 makes a targeted nick. This Cas9-mediated knockout/knock-in genetic editing approach can be employed in embryonic stem cells to create knockout animals, or in human pluripotent stems cells, which can then be changed into nearly any human cell type of interest. Also, multiple sgRNAs can be used at once, resulting in multiple genes being specifically and efficiently mutated simultaneously. Therefore, the analysis of how chemically-induced mutations or inadequacies in multiple genes (gene networks) can additively or synergistically produce polygenic diseases is possible in animal models or in human cell types where the disease state originates.

86. In an adult organism, it is possible to deliver CRISPR via a virus and edit the genome of a mature organ, such as the liver. The liver exhibits a high capacity for uptake of most viral delivery systems. Because of its ability to regenerate, the organ can be repopulated with "corrected" hepatocytes (*e.g.*, hepatocytes expressing the ALDH2[1] allele). In experiments with mice, viral delivery of CRISPR constructs resulted in 30 to 40 percent of hepatocytes in the liver being edited in one to four weeks [H Yin, *et al.*, "Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo," 34 *Nat Biotechnol*. (2016); FA Ran, *et al.*, "In vivo genome editing using Staphylococcus aureus Cas9," *520 Nature* (2015)]. Therefore, although this type of CRISPRmediated gene therapy has not been conducted in humans to date, it is theoretically possible and presents a promising avenue for treatment of human liver diseases.
87. If both of the catalytic sites (both scissors) in Cas9 are inactivated by mutagenesis, a version of Cas9 is generated that is capable of localizing to the specific DNA sequence corresponding to any sgRNA, but incapable of cutting the DNA. This nuclease-deactivated Cas9 (dCas9) finds the DNA sequence matching the sgRNA and just sits on the DNA. This is useful because if dCas9 is targeted to the promoter of a gene, it will sit on the promoter and cause CRISPR interference (CRISPRi) [LS Qi, *et al.*, "Repurposing CRISPR as an RNAguided platform for sequence-specific control of gene expression," 152 *Cell*. (2013)]. These roadblocks can be specifically placed on regulatory elements of a gene (promoters and/or enhancers) as well as within a gene to decrease the expression of that specific gene [MH Larson, *et al.*, "CRISPR interference (CRISPRi) for sequence-specific control of gene expression," 8 *Nat Protoc*. (2013); IE Lawhorn, *et al.*, "Evaluation of sgRNA target sites for CRISPR-mediated repression of TP53," 9 *PLoS One* (2014)].

    Taking this one step further, transcription activators or repressors can be fused to dCas9, and dCas9 can chaperone them to specific DNA sequences to attract or repel transcription factors and, in turn, obtain more robust gene expression modulation. For example, fusion of four copies of the VP16 transcription activator (VP64) or a single copy of the p65 activation domain (p65AD) to dCas9 led to 25-fold and 12-fold gene activation, respectively, in the presence of a specific sgRNA, a process deemed CRISPR activation (CRISPRa) [LA Gilbert, *et al.*, "CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes," *154 Cell*. (2013); ML MAEDER, *et al.*, "CRISPR RNA-guided activation of endogenous human genes," 10 Nat Methods (2013)]. Conversely, fusion of repressor domains, such as the Krüppel associated box (KRAB), resulted in sgRNA-targeted dCas9 decreasing gene expression by 15-fold, a technique that also is part of the CRISPRi toolbox [LA Gilbert, *et al.* (2013)]. Other activator (SunTag) and repressor domains (Chromo Shadow, WRPW) have been fused to dCas9, and each exhibits a level of effectiveness in up- or down-regulating gene expression. By using different repressor/activator domains and different sgRNAs for a specific stretch of DNA, CRISPRi/CRISPRa can modulate gene expression over a 1000-fold range [LA Gilbert, *et al.* (2013); LA Gilbert, *et al.*, "Genome-scale CRISPR-mediated control of gene repression and activation," 159 *Cell*. (2014); ME Tanenbaum, *et al.*, "A protein-tagging system for signal amplification in gene expression and fluorescence imaging," 159 *Cell*. (2014)].
88. Epigenetic modifiers can be fused to dCas9, and alter the epigenetic profile of a sgRNA-targeted gene. dCas9 fused with the Lys-specific histone demethylase 1 (LSD1) or the catalytic core of the acetyltransferase p300 ($p300^{Core}$) permitted targeted chromatin modification and, in turn, gene expression modulation [NA Kearns, *et al.*, "Functional annotation of native enhancers with a Cas9-histone demethylase fusion," 12 *Nat Methods* (2015); IB Hilton, *et al.*, "Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers," 33 *Nat Biotechnol*. (2015)]. Researchers also have shown that targeting dCas9 fused to the catalytic domain of the DNA methyltransferase DNMT3A can increase methylation of CpG islands within promoters of specific genes [A Vojta, *et al.*, "Repurposing the CRISPR-Cas9 system for targeted DNA methylation," 44 *Nucleic Acids Res*. (2016)]. This methodology can be used to simultaneous activate and repress multiple genes, allowing the replication of specific gene expression profiles within animals or specific human cell types. Furthermore, spatial and temporal control over gene expression can be achieved by using drug or light inducible versions of CRISPRi/ CRISPRa, and all gene regulation changes are reversible [LR Polstein & CA Gersbach, "A light-inducible CRISPR-Cas9 system for control of endogenous gene activation," 11 *Nat Chem Biol*. (2015); Y Nihongaki, *et al.*, "CRISPR-Cas9-based photoactivatable transcription system," 22 *Chem Biol*. (2015); B Zetsche, *et al.*, "A split-Cas9 architecture for inducible genome editing and transcription modulation," 33 *Nat Biotechnol*. (2015); X Sim, *et al.*, "A doxycycline-inducible system for genetic correction of iPSC disease models," 1353 *Methods Mol Biol*. (2016)]. Such fine control over gene dosage permits the examination of how different expression levels of multiple genes may affect the development of specific diseases, and therefore is a valuable tool for establishing or discrediting causation.
89. Human induced pluripotent stem cells (hiPSCs) can be made from somatic cells of plaintiffs, and these stem cells can be differentiated into nearly any cell type (*e.g.*, kidney cells) for further study. Starting material (somatic cells) can be obtained by non-invasive means including buccal cells from a mouth swab, keratinocytes from hair follicles, renal epithelial cells from urine, and hematopoietic cells from blood. However to date, the seminal examples of reprogramming began with fibroblasts, often of dermal origin obtained from skin punch biopsies. The starting cells can then be converted into hiPSCs through "reprogramming", which entails the ectopic expression of four transcription factors (OCT3/4, SOX2, KLF4, and c-MYC known as OSKM or OCT4, SOX2, NANOG, and LIN28 known as OSNL) [K Takahashi, *et al.*,

"Induction of pluripotent stem cells from adult human fibroblasts by defined factors," 131 *Cell*. (2007); J Yu, *et al.*, "Induced pluripotent stem cell lines derived from human somatic cells," 318 *Science* (2007)]. The resultant hiPSCs are in an embryonic stem cell-like state and can be differentiated into the cell type(s) of interest through treatment with specific growth factors. After terminal differentiation, the cells can be further manipulated by CRISPR, if needed, to introduce mutations and/or expression profiles that may only be present in the plaintiffs' organ/tissue of interest (*e.g.*, pre-cancerous mutations or high expression of growth signaling proteins). In addition, generic hPSCs are available that can be purchased, differentiated and engineered to study a specific SNP or SNP haplotype or a specific gene expression profile in the absence of the plaintiff's genetic background and thus used as control.

90. A genotoxic carcinogen alters the DNA sequence to create carcinogenic mutations. A non-genotoxic carcinogen does not directly interact with the DNA sequence but promotes the growth and division of cells that have acquired carcinogenic mutations.
91. hiPSCs can be differentiated, CRISPR-engineered and grown into plai tiff-specific organoids, or mini organs, which display structural and functional resemblance to their physiological counterparts *in vivo*. Human kidney [BS Freedman, *et al.*, "Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids," 6 *Nat Commun*. (2015); BS Freedman, "Modeling kidney disease with iPS cells," 10 Biomark Insights (2015); M Takasato, *et al.*, "Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis," 526 *Nature* (2015)], intestine [J Drost, *et al.*, "Sequential cancer mutations in cultured human intestinal stem cells," 521 *Nature* (2015); M Matano, et al., "Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids," 21 *Nat Med*. (2015)] and brain [MA Lancaster, et al., "Cerebral organoids model human brain development and microcephaly," 501 *Nature* (2013); J Mariani, *et al.*, "FOXG1- dependent dysregulation of GABA/glutamate neuron differentiation in autism spectrum disorders," 162 *Cell* (2015)] organoids have been developed and used as disease models as well as pancreas and liver organoids of mouse origin [M Huch, *et al.*, "Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis," 32 EMBO J. (2013); M Huch, *et al.*, "In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration," 494 *Nature* (2013)]. These can serve as more robust models than cell monolayers that are routinely cultured in vitro.
92. CRISPR-engineered animal models can be created to study the effect of chemical exposures in the context of plaintiff-specific mutations and expression profiles present in an entire, intact organism. These models can be initiated at the embryonic stage of development or in adult animals depending on the extent (*e.g.*, single organ or whole organism) and type (*e.g.*, entire or partial cellular coverage) of genetic editing desired. When implemented in the embryo, entire coverage of a single organ or the whole organism is possible. When implemented in an adult organism, partial coverage of certain organs, such as the spleen, brain, liver, heart, lung, kidneys, muscle and circulating lymphocytes, can be efficiently accomplished. For the latter, viral and non-viral systems can be used to deliver CRISPR constructs. After a single administration, these delivery systems often result in editing ~five –30 percent of cells in the desired organ, which could be used to represent a mixture of normal and abnormal cells [H Yin, *et al*. (2014)].
93. Y Fu, *et al.*, "High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells," 31 *Nat Biotechnol*. (2013); PD Hsu, *et al.*, "DNA targeting specificity of RNA-guided Cas9 nucleases," 31 *Nat Biotechnol*. (2013); V Pattanayak, *et al.*, "Highthroughput profiling of off-target DNA cleavage reveals RNA-programmed Cas9 nuclease specificity," 31 *Nat Biotechnol.* (2013); TJ Cradick, *et al.*, "CRISPR/Cas9 systems targeting ß-globin and CCR5 genes have substantial off-target activity," 41 *Nucleic Acids Res*. (2013).
94. Y Fu, *et al.*, "Improving CRISPR-Cas nuclease specificity using truncated guide RNAs," 32 *Nat Biotechnol*. (2014); BP Kleinstiver, *et al.*, "Engineered CRISPRCas9 nucleases with altered PAM specificities," 523 *Nature* (2015); BP Kleinstiver, *et al.*, "High-fidelity CRISPR-Cas9 nucleases with no detectable genomewide off-target effects," 529 *Nature* (2016); AC Komor, *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage," 533 *Nature* (2016).
95. S Reardon, "First CRISPR clinical trial gets green light from US panel," 534 *Nature* (2016); D Cyranoski, "Chinese scientists to pioneer first human CRISPR trial," 535 *Nature* (2016).