Genomics and Genetics in Toxic Tort Litigation

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Our society has an increasing rate of cancer and other diseases. This increasing rate can be attributed to a number of factors when looking across large populations. They include increased lifespan, inherited risks, environmental or occupational exposure to toxicants and lifestyle choices. When someone develops a disease, such as cancer, they want to know why and they often want to assign blame. They may believe that there has been an exposure to a toxicant and want to seek remedy from others. Historically, the primary way to determine if the alleged exposure was causal was for the court to draw inferences from population-based epidemiological studies. If experts determine that an increased risk exists, the courts are disposed to find a causal connection. With new technologies, such as massively parallel sequencing, often called Next Generation Sequencing or NGS, the courts can now include less speculative, more quantitative information from genetic signatures to establish causation based on the individual instead of a population. With today's NGS technologies, the blueprint of the genomes can tell the story.

Advancements in NGS technologies have pushed forward the fields of genetics and genomics, enhancing our understanding of the onset and progression of diseases and providing society and the medical community with better tools for the diagnosis, monitoring and guiding treatment of many diseases. These tools are now routinely used in the scientific and medical arena and have also recently been adopted by the legal community. Such advances have resulted in the ability of NGS data to be used as highly probing and discerning additional pieces of scientific evidence to support or refute toxic tort claims. Since the first use of gene expression signatures in January 2003 by the Netherlands Cancer Institute in Amsterdam to make treatment decisions regarding women with breast cancer, significant new research (more than 9,000 academic publications) used genetic signatures to elucidate possible causes of tumors and predisposition to cancer and other diseases. Right now, juries are deciding cases with genetic signature data presented, as to the source of tumorigenesis and familial predisposition. This evidence is part of a collection of scientific information used to depict an overall likelihood of disease causation picture to the jury for establishing causation.

ArrayXpress (AX) is a genomics and genetics service provider specializing in NGS that has experience in toxic tort cases. We help the genes tell you the story. In this white paper we will seek to explain, at a high-level, how and when genomics and genetics testing can and cannot be used in toxic tort cases, as well as explain the strengths and limitations of the technology. We are also producing white papers on specific applications of genomics to exposure cases such as low and high dose ionizing radiation, various aromatic hydrocarbons, in particular benzene, toluene,

ethylbenzene, and xylene isomers (also collectively referred to as BTEX), asbestos, and heavy metals. These are the so-called "invisible agents of harm" that have traditionally been very difficult to adjudicate using traditional population-based epidemiological study information.

What is Genetics and Genomics Testing?

Genetic information is encoded in a chemical molecule called deoxyribonucleic acid (DNA) that is tightly packed inside our cells in structures called chromosomes. In simple terms, DNA consists of a string of four building blocks called bases (also known as nucleotides). Each is represented by a letter: A, T, C, G. Genes are specific segments of nucleotides or "letters", like a sentence. The sequence of the "letters" on each gene determines the meaning of its genetic message and carries instructions on how to make proteins. In order to do this, the genetic message is transcribed, or "copied", from the DNA into an intermediary molecule called the messenger ribonucleic acid or mRNA. The messenger RNA carries the "instructions" from the DNA to the cell machinery that makes proteins. Each protein has a specific function, like the enzymes that digest our food. The DNA thus acts as an instruction manual for our body where each chromosome is a "chapter" with lots of "sentences" (the genes), carrying detailed directions on how our cells must function. The word "genome" is used to refer to the entire set of genetic information across all chromosomes. The human genome was first sequenced in April of 2003 and since then the field of genomics has continued to grow.

One of the goals in human genomics research is to find variations in the DNA sequence and to determine its biological significance across various populations. At the whole organism level, these differences in the DNA are expressed as our genetic traits, such as the color of our eyes and hair, which make each person unique. Some variations may be "silent" and have no consequence altogether. Conversely, other variations are associated with a genetic predisposition to a disease or a response to a particular medication or toxicant. However, not all of our traits are governed by genetics. As a matter of fact, the majority of our characteristics are determined by varying degrees by both genetics and the environment in which we live and grow. Environmental factors determining phenotypic outcomes include diet, lifestyle choices, and pollutants. Through the course of our lifetime we are exposed to a countless number of such environmental factors, some of which can alter the sequence of our DNA ever so slightly. An example is too much exposure to a specific toxicant such as UV light that may cause alterations in our skin cells to produce aberrant proteins that alter normally non-growing cells to begin to grow into melanomas. How we respond to these various environmental factors can also be influenced by our inherited genetic variations that are passed on from our parents to us.

Because of its potential health and medical implications and benefits, some genomics research concentrates on developing strategies for the early detection, diagnosis and treatment of diseases, based on whether an individual has one or

more of these variations in particular genes that could either increase or decrease their risk of developing an inherited disorder or disease. Breast cancer is an example of a disease where genetic testing can identify gene mutations inherited from a parent that increase the risk of developing breast cancer. In 2013, actress Angelina Jolie brought considerable attention to the application of the science when she elected for a double mastectomy, based on risk factors largely driven by the presence of specific variations found in her BRCA1 and BRCA2 genes, inherited from her parents.

In a clinical or research setting, "genetic" testing refers to the examination of specific stretches of DNA that have a known function, the genes. Genetic testing requires that an investigator know which gene or genes to look at based on some prior understanding of the biological contribution to a phenotypic trait or disease. "Genomic" testing on the other hand, looks for variations across the entirety of genetic material. Genomic testing produces large amounts of data that must be interrogated in order to identify genetic variants and/or mutations that are associated with a particular trait or disease (e.g., cancer).

Why is Genomic and Genetic Testing Relevant to Toxic Tort?

Back to the question then of what caused the disease? Mutations in DNA sequences that result in a disease occur through one of three processes:

1. *Acquired DNA Mutations*: DNA damage from environmental agents such as ionizing radiation, heavy metals, gases, organic solvents or certain chemicals can change the DNA. These are the mutations that a plaintiff wants to prove and a defendant wants to disprove with regard to a claimed exposure.
2. *Familial Inherited Mutations*: These are passed down from generation to generation. They are primarily of use to a defendant in showing that they are not to blame, it was just due to the inheritance of "faulty genetics".
3. *Random Mutations:* Errors that occur when a cell copies its DNA in preparation for cell division. Our bodies are a precision machine, but once in every billion or so copies of the "letters" (A, T, C, and G) in the DNA an error is made. It just happens. No one is to blame.

According to the National Academy of Sciences National Research Council Committee on Applications of Toxicogenomic Technologies to Predictive Toxicology, the definition of *toxicogenomics* is the application of genomic technologies to study the adverse effects of environmental and pharmaceutical chemicals on human health and the environment. Toxicogenomics combines toxicology with information-dense genomic technologies to integrate toxicant-specific alterations in gene, protein, and metabolite expression patterns with phenotypic responses of cells, tissues, and organisms. Toxicogenomics can provide insight into gene-environment interactions and the response of biologic pathways and networks to perturbations. As a result, it may lead to information that is more discriminating, predictive, and sensitive than that currently used to evaluate toxic exposure or to predict effects on human health.

NGS is the most powerful technology currently available in the toxicogenomics toolkit. Genomic technologies can provide a nearly binary answer to many legal questions, e.g. paternity, DNA presence at a crime scene, etc. Genetic signatures as evidence in response to toxic tort claims provide analytical and often quantitative data that is less population based and more individually focused than prior epidemiological evidence. Depending on the claims made regarding mode of action (MOA) and the implicated toxicant, as well as the impacted organ(s), NGS often provides very strong guidance to a source of tumorigenesis, or conversely, indicates the presence of biomarkers for familial inherited genetic mutations. This genomic evidence can be used as part of a suite of tools including epidemiological analyses, environmental assessment, medical and toxicological information, etc.

In some limited situations, genetic signatures and robust assays to unveil these signatures that are relevant to a legal case may already exist, particularly for genetic predisposition. However, in most cases the literature contains many individual pieces of the puzzle that need to be validated against each other to form a testable scientific hypothesis suitable to the claims being made. AX specializes in conducting such multi-disciplinary research. Working with counsel, AX will design an appropriate experiment to query the specific modes of action and disease states presented in the initial claims or those claims presented by experts during discovery. Utilizing a suite of genomics tools and specialized software, AX then analyzes the DNA, mRNA expression, epigenetic markers, and gene mutations in blood and/or clinical formalin fixed paraffin embedded (FFPE) tissue samples as applicable to each specific case and according to the availability of the corresponding samples. The results are then carefully analyzed and interpreted in light of the latest scientific and medical literature to give guidance as to whether a patient suffered DNA damage from toxicants such as ionizing radiation, heavy metals or organic chemical solvents, and/or whether there is any indication of a familial genetic predisposition to the disease being linked to a claimed exposure.

The Scientific Admissibility

One area of concern for all new technology is application of the Frye and Daubert standards for the admissibility of new science. It is not in the scope of this paper to elucidate on these two standards or their application: however, we believe that this issue has been settled. Genetic and genomic testing are no longer new science. In the United States, 100% of the Top 100 Cancer Hospitals currently utilize genetic or genomic testing to classify the source of tumors and determining the appropriate treatment protocol. Most insurance companies, often the last to accept new technology until significant efficacy and clinical utility has been demonstrated, now offer coverage for many types of genomic and genetic testing. As of the writing of this white paper, Aetna covers 96 different genetic tests and Blue Cross & Blue Shield covers 27 genetic tests. Numerous legal cases have previously been conducted where genetic signature evidence was admitted and the jury considered the evidence as part of their ruling. Numerous other cases are now in progress

where judges at both the State and the Federal level have ordered the tissues be provided and the genomic and genetic testing to proceed.

Can it be Abused and How Does ArrayXpress Make Sure it is NOT?

As with all technology, there will always be some "snake-oil salesman" that will abuse its application. We have only been able to find one such situation. A test called msds1™, that presumably determined whether an individual's leukemia, cancer or other disease was caused by exposure to benzene, according to published commentary, may have been abused. This application was not in the courts but in an insurance context. We stand by our position that overall the science is quite irrefutable, having been studied by government, academic and corporate researchers the world over. With our systems biology, multidisciplinary approach and techniques, scientists looking at the use of genomics for classifying tumorigenesis and genetics for determining predisposition have found the technology to be sound. We focus on solid, supportable, repeatable systems-wide biology science.

What CAN AX do for Counsel?

Genomic sciences and genetics can have very strong evidentiary value and depending on the case can be very conclusive. In some cases it simply may not be possible to provide scientifically valid conclusions to the exposure and causation questions, mainly because of the lack of supporting peer reviewed research data. In many cases, however, they are very powerful tools that, depending on the disease, the proposed mode of action, the tissues available, the toxicants and the availability of published literature, can provide excellent information on sources of tumorigenesis. The results AX provides on sources of tumorigenesis do not constitute a stand-alone challenge. The results of our investigational studies strengthen the case along with the epidemiology, toxicology and medical and environmental data. AX investigational studies can be used for claims including cancer, cardiac disease, asbestos related disorders, disorders associated with exposure to heavy metals (e.g. chromium, cadmium, lead), as well as organic chemical solvents (such as BTEX and its toxic metabolites) and ionizing radiation exposure (e.g. x-rays and naturally occurring radioactive materials (NORM)). Although genomics does not usually indicate exposure levels, it can often indicate whether exposure and resulting DNA damage did or did not occur. Separate from issues of exposure and causation, the individual's genetic susceptibility through familial inheritance to a particular disease or disorder can also be determined.

AX provides an end-to-end service. We work with the client and their medical and toxicology experts to review the pathology reports and the claims, and to ultimately design the most appropriate and scientifically valid approach to evaluation. We then use our internal databases we have developed from prior research, or we develop new databases according to peer-reviewed published literature to address the disease and the MOA. We actively collaborate with the legal team in the

development of affidavits to secure the tissue(s) (discriminated by an external pathologist) required for genomic testing, then conduct the laboratory and sequencing work. Finally, we analyze the data using state-of-the-art bioinformatics and statistical tools and provide written reports to confirm or reject the scientific hypothesis. These data are then submitted to the medical and toxicological experts for their subsequent diagnoses and conclusions. Upon the client's request we can also provide expert or fact witness testimony, attorney assistance and supporting research during the conduct of a case.

The AX Strategy: Matching the Claims

For each case, AX develops a unique strategy and experimental design to match the claims in the specific toxic tort case. Many times a very specific MOA is claimed. For example, that the toxicant is still circulating in the blood, and therefore chronically exposing a particular organ. Conversely, other cases may claim past exposure and damage. These studies will require different strategies and experimental approaches to answer the questions on disease causation. The pathology reports, the claims in the case, and the information available in the literature will ultimately determine the strategy and structure of the scientific study. Answering the correct questions is extremely important. For example, here are two similar cases with very different study designs:

Case 1: A plaintiff claims exposure to a toxicant that was absorbed into the bloodstream via the lungs, where it currently remains, chronically exposing the blood, resulting in tumorigenesis in another organ. In this case the simplest and most straightforward approach for the defense is to look for genetic signatures of ongoing exposure and damage in the blood. This is less costly than studying the actual tumor. Alternatively, if cancerous tissue is available, the defense could also look for additional corroborating data, such as the presence of genetic markers indicative of biological pathways for cancer development other than those claimed by the plaintiff. In addition, the defense would also look at biomarkers for genetic susceptibility to the disease affecting that particular organ.

Case 2: A plaintiff claims exposure to a toxicant such as benzene, which was absorbed through the lungs, gastrointestinal tract, and across skin, entering the blood stream and ultimately causing Acute Myeloid Leukemia (AML). In this case, studying the blood may be a fruitless strategy. The defense might instead employ an investigational study that analyzes mutations or deletions in mitochondrial DNA, or gene specific mutations found in the plaintiff's DNA of the affected organ or extracted from epithelial cells from the oral mucosa to determine if any of the markers are associated with previous benzene exposure. Similar to the hypothetical Case 1, the defense would also determine if the plaintiff has any gene mutations associated with an inherited risk of developing the alleged type of cancer.

As you can see, the cases appear similar, but require very different study designs. AX works closely with counsel and their medical and toxicological experts to determine

if there is an appropriate study, and if so, what the design should be, all while focusing on cost efficiency. It is important to note that each case is unique, with very specific confounding factors that have to be taken into consideration on a case-by-case situation before any experimental design is selected and conclusions are drawn. Applying genomics and genetics testing in toxic tort is not a cookie-cutter approach; it is unique to each particular case.

Types of Claims

There are three common types of causation tumorigenesis claims that genomics and genetics are used to address. The first is when a *normal mode of action* is assumed. A normal mode of action means that a claim is made that a disease was caused through a known, thoroughly studied and well-published genetic progression. This could be familial inheritance or by naturally acquired mutation or by acquiring a mutation induced via exposure to a toxicant with a documented mode of action.

The second is when a *hypothetical mode of action* is presented. The World Health Organization International Agency for Research on Cancer along with the US National Institutes of Health, have tried to define the modes of action of toxicant damage. In most cases a theory is presented by a plaintiff on how a toxicant exposure caused a disease. Sometimes that theory is unsupported in the literature from these and other agencies. In the Case 1 example above, a claim was presented indicating a toxicant was inhaled, lodged in lung tissue and from the lungs the toxicant made it into the blood and that in turn led to the plaintiff's cancer in an organ other than the lungs. The specific study was designed given this hypothetical mode of action.

The third is when *contributory damage* (environmental harm & genetic susceptibility) is claimed. The toxicant may have caused the disease but the plaintiff has a genetic predisposition to developing the disease or may have knowingly contributed to it by smoking, drinking or other actions. Depending on the jurisdiction of the case, this may significantly impact the design of studies.

In all three types of cases, AX can investigate the feasibility of the application of genomics to conduct investigational studies in response to the claims.

Future Applications

The future holds the ability to use genetic and genomic signatures for even greater causal understanding, but also using the technology to assess risk or future disease. As the field of genomics and genetics research continues to grow, AX will continue to grow its database to be used for assessments. We are continuously growing our proprietary database by aggregating the latest literature in the fields of toxicogenomics and molecular epidemiology, by collaborating on scientific projects with key academic and strategic partners and by conducting our own tissue and blood sequencing studies. As the database grows, more well-characterized genetic

signatures that are associated with exposure to specific toxicants or with genetic susceptibility or increased risk to developing a disease or disorder will be identified. As the database grows, the genomic evidence becomes even stronger.

In addition AX is working to develop ways to further quantify *Increased Genetic Risk/Susceptibility*. This is when someone feels that because of an alleged exposure or genetic predisposition they are at a higher risk in the future for developing a disease. We can test for the signatures indicating exposure but also for predisposition. The presence of a mutation for predisposition is often binary, it is either there or it is not. The technology is very accurate. Each mutation carries with it a quantifiable increase of risk for the individual possessing the mutation. If there is a presence of specific allele or marker, there is often a known percentage increase in susceptibility shown as an odds ratio. If a patient has more than one mutation associated with predisposition we know it translates to an even greater increased risk, but it is hard to say how much of an increase given the other mutations. AX is working to quantify the multi-genetic risk for common toxic tort related SNPs associated with exposure to various environmental, medical and industrial toxicants. This is a long term project and strategic partners are being sought.

Conclusion

The applications of NGS technologies has brought significant benefits to society in many ways. In the toxic tort arena it can be equally helpful to plaintiffs and attorneys for discovering the truth. In the short run, because of the current costs, it is more likely to be used by the defense than plaintiffs. However, as NGS is applied to more cases the costs will drop rapidly. The only ones that should have concern are the occasional plaintiff attorneys that bring frivolous cases and the rare defense counsel that uses the deep pockets of a corporate client to bludgeon a legitimate plaintiff out of the legal system. Those with true harm will be able to prove it and those who accused of having caused it will sleep better at night knowing they only paid damages when they have actually done harm.

In toxic tort cases that can benefit from the application of genomics sciences it is a very powerful tool for establishing causation. Precedent has been set, the number of cases are expanding and the technology is proven. NGS is emerging as a powerful tool for justice.

AX is a Next Generation Genomics and Bioinformatics laboratory services company specializing in biomarker development, bioprocess optimization, gene expression studies and diagnostic solutions. AX is able to provide extremely powerful and unparalleled bioinformatics and statistical analytical capabilities to investigational studies. AX designs and conducts investigational studies to examine the validity of toxic tort claims. We can be reached at toxictort@arrayxpress.com or on the web at www.ArrayXpress.com