A Primer Presented by ArrayXpress, Inc.

ArrayXpress (AX) has produced a series of white papers on specific applications of NGS technologies to tort cases with claims based on exposure to agents such as ionizing radiation, asbestos, heavy metals, and carcinogenic aromatic hydrocarbons, in particular benzene, toluene, ethylbenzene, and xylene isomers (collectively referred to as BTEX). This paper is intended to serve as a primer on the technology and its application to toxic tort litigation.

AX is a genomics and genetics service provider specializing in the application of Next Generation Sequencing (NGS) technologies in toxic tort cases. We use NGS technologies to reveal the evidence of toxicant exposure and damage that is locked within the genetic blueprint of an individual(s) and in appropriate cases to assess alternative causation.

Introduction

Our bodies are complex biological systems that are influenced by our inherited genetic makeup, the foods we eat, the places we grew up, and the environment we are exposed to. Diseases arise when the body’s normal physiological balance among its gene networks is broken by some perturbation, such as exposure to a toxicant. A lot of financial resources go into understanding causes and prevention of diseases. Every year the body of knowledge regarding the genetics of diseases grows larger. Traditionally, research has been based on population wide studies, but more and more medical research is focusing on the individual. When looking across large populations, the incidence of cancer or other diseases may be attributed to any number of factors. It includes increased human lifespan, inherited risks (genetic predisposition), lifestyle choices, and exposure to environmental or occupational toxicants. Among these, it is well known that environmental risk factors play a role in more than 80% of the diseases regularly reported to the WHO.

Genetics 101: What is a Gene?

Genes are the molecular units of heredity of all living organisms. They are encoded in a chemical molecule called deoxyribonucleic acid (DNA). The building blocks of DNA are called nucleotides. There are four possible nucleotides in DNA, which are represented by the letters: A, T, C or G. The sequence of the nucleotides in each gene determines the genetic message. The genetic message is transcribed, or “copied”, from the DNA to a transient molecule called the messenger ribonucleic acid (mRNA), a process referred to as “gene expression”. The mRNA then carries some form of restitution from an alleged guilty party. Historically, courts ruling on causation relied primarily on the exposure claims or inferences from population-based epidemiological studies. If epidemiology experts determined that an increased risk existed, the courts were disposed to accept the epidemiology association as causation evidence. With the application of new genomics technologies the courts can now consider less speculative, more quantitative information from an individual’s own genetic blueprint. In a growing number of cases, exposure and/or causation can be refuted or verified based on the individual instead of only population based studies.
the genetic message from the DNA to the cell's protein manufacturing machinery (the ribosomes) where the proteins are constructed following the information provided in the genetic message. A genome is the collective name for all the genetic information (DNA and RNA) of an organism.

Genes, Diseases, and the Environment

The majority of our characteristics are determined in varying degrees by both our genes and the environment working together in a biological system. Environmental factors that affect our health include diet, lifestyle choices, and exposure to pollutants. Through the course of our lifetime we are countless exposed to factors that can alter our DNA sequence ever so slightly. For example in skin cancer, these alterations ultimately translate to the production of aberrant proteins that alter normal skin cells to grow out of control resulting in the formation of skin cancers. From the genetics standpoint, some people are more prone to develop skin cancer upon exposure to UV light (genetic susceptibility) while others are more prone to develop skin cancer even without excessive UV light exposure (genetic predisposition). Everyone is different and population studies can not take into account the individual.

Because of its potential medical implications and benefits, some genomic research specifically concentrates on developing strategies for the early detection, diagnosis, and treatment of diseases. Testing for genetic predisposition can help to identify individuals with increased risk of developing a disease. It is based on whether an individual has one or more variations in particular genes that could either increase or decrease their risk of developing an inherited disorder or disease. Such variations are called mutations. Simply put, a mutation is a permanent change in the DNA sequence that makes up a gene. Examples of mutations are nucleotide changes, deletions and insertions that alter the meaning of the genetic message. Some DNA differences may be “silent” and have no consequence, i.e. it does not influence the phenotype (trait). Conversely, other differences may influence the phenotype unfavorably, for example a genetic predisposition to a disease or an adverse response to a particular medication or toxicant.

DNA sequence mutations can occur through one of two ways. When it is inherited from a parent, it is called a familial mutation. For example, families with inherited cancer syndromes have incidences of the same type of cancer in two or more generations. Breast cancer is an example of a disease where genetic testing can identify individuals carrying familial inherited gene mutations that increase their risk of developing breast cancer like other members in the families affected by this disease. Actress Angelina Jolie brought considerable attention to the matter of genetic testing when she elected for a preventive double mastectomy in 2013. Her decision was based on the presence of specific DNA mutations found in her BRCA1 gene and the fact that her mother died from ovarian cancer at the age of 56.

Mutations can be acquired during a person's lifetime. These are known as somatic mutations. Although our bodies are a precise machine, once in every billion or so nucleotides, an error is made. These are considered spontaneous somatic mutations and occur during cell division when a cell copies its DNA. Sometimes however it is induced by environmental agents, such as ionizing radiation, heavy metals, gases, organic solvents or certain chemicals. We refer to these as acquired somatic mutations. These mutations cannot be passed on to the next generations but can negatively impact the person acquiring them.

What is Next Generation Sequencing (NGS)?

Technology advancements in genomics have led to major scientific and medical breakthroughs. NGS is a high-throughput sequencing technology proven to be a powerful, cost effective tool for studying a person's genes (DNA-seq) and its expression (RNA-seq) at the whole genome level. With whole genome DNA sequence information researchers are able to look for DNA sequence variations (mutations) among and within populations and determine if there is a significant biological outcome connected to these mutations. NGS allows for this whole genome perspective in searching for possible DNA mutations that might be the cause of the disease in question. In a Toxic Tort scenario, familial (inherited) mutations are primarily of use by a defendant (or defendants) that want to show that they are not the primary reason for a particular disease to have developed. It can also help to determine if they
are spontaneous somatic mutations, where it literally happens by chance or reasons we do not yet understand.

In the same way as DNA research, NGS has revolutionized RNA research in that gene expression profiling (or mRNA expression) is now performed at the whole genome level using RNA-seq. Researchers are able to characterize all the genes expressed in a certain tissue and under specific conditions. With mRNA expression information, it is possible to precisely characterize an individual’s response to environmental changes. Exposure to substances (medication, ionizing radiation, benzene, heavy metals, etc.) affects the expression of genes and leaves behind a measurable gene “signature”. Over the past decade, many research groups have worked on developing and validating gene signatures that indicate such exposures. Using these published and validated gene signatures, we can differentiate exposed individuals from non-exposed ones.

**NGS in Toxicogenomics**

The National Academy of Sciences’ National Research Council Committee on Applications of Toxicogenomic Technologies to Predictive Toxicology defines toxicogenomics as “the application of genomic technologies (for example, genetics, genome sequence analysis, gene expression profiling, proteomics, metabolomics and related approaches) to study the adverse effects of environmental and pharmaceutical chemicals on human health and the environment. It combines toxicology with information-dense genomic technologies to integrate toxicant-specific expression pattern alterations of genes, proteins, and metabolites with phenotypic responses of cells, tissues, and organisms. Toxicogenomics provide insight into gene-environment interactions and the response of biological pathways and networks to perturbations. The resulting information is more discriminating, predictive, and sensitive than what are currently used to evaluate toxic exposure or to predict effects on human health”.

NGS is without doubt the most powerful technology currently available in the toxicogenomics toolkit. Genomic technologies have provided a nearly binary answer to many legal questions including paternity or the owner of DNA at a crime scene. Genetic signatures as evidence in response to toxic tort claims provide analytical and often quantitative data. Unlike the inferences from epidemiological data that are based on associations found in the general population, validated genetic signatures go one step further. They use well characterized associations to provide an answer specific to the individual. Based on well-established scientific evidence linking a specific toxicant to a specific disease, genetic signatures can provide evidence that: (i) the individual was or was not actually exposed to the toxicant, and (ii) that the individual’s disease was or was not caused by the claimed exposure. Depending on the claims made regarding the mode of action (MOA) of a specific toxicant in a specific organ(s), and if that topical area has been the subject of peer reviewed scientific literature, NGS may provide very strong guidance as to a source of tumorigenesis, or conversely, indicate the presence of biomarkers for familial, inherited genetic mutations. This genomic evidence can be used as part of a suite of tools that include, but are not limited to epidemiological analyses, environmental assessment, medical, and toxicological information.

In some situations, genetic signatures and robust genetic assays relevant to a legal case may already exist. However, in most cases the literature contains many additional individual pieces of the puzzle. These pieces need to be critically evaluated against each other to form a working hypothesis and dataset suitable to the claims being made. Working with counsel, AX determines if there is an appropriate study that can be designed to query the specific MOA and disease states presented in each claim. Utilizing a suite of genomics tools and specialized software, AX analyzes the relevant tissue samples available and generates the appropriate information (DNA sequence, mRNA expression, Epigenetic gene regulation, and spontaneous or inherited gene mutations). This information is carefully analyzed and interpreted in light of the most current scientific and medical literature and, in the case of a toxicant claim, will guide whether
or not there is scientific evidence to support or refute the exposure and disease causation claims. Depending on the experimental design, the results can also indicate if there is scientific evidence to support or refute familial genetic predisposition to the disease.

Scientific Admissibility

One area of concern for all new technology is the application of the Frye and Daubert standards for the admissibility of new science. Genetics and genomic testing are no longer new science. In the United States, 100% of the Top 100 Cancer Treatment Hospitals currently utilize some kind of genetic or genomic testing to classify the source of tumors and determine an 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 tests. ArrayXpress has provided genomics analyses and scientific support in legal cases where genetic evidence was admitted and the jury considered the evidence in their decision. There are a number of other cases in progress where court orders, at both the State and the Federal level, were issued to provide tissue samples to be used in a genomic and/or genetic test. It is important to understand that this testing does not produce a medical diagnosis. It is scientific evidence of causality that can be provided to medical professionals for consideration.

Types of Causation Claims

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

The second claim 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 MOA of toxicant damage. Given the number of toxicants, genetic variation and incalculable number of other variables, the MOA of toxicant damage is not always clearly defined and presents an opportunity for creative interpretation. In some cases a theory is presented by a plaintiff on how a toxicant exposure caused a disease that is not clearly supported in literature. In these instances, a study can be designed with knowledge from the literature along with the plaintiff’s own genetic data to support or refute the claim(s).

The third claim is when contributory damage (environmental harm and genetic susceptibility) is asserted. 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 lifestyle choices or exposures. Depending on the jurisdiction of the case, the type of contributory choices may significantly impact the design of study.

In all three types of claims, AX can investigate the feasibility of the application of genomics to conduct investigational studies to support or refute the claims.

The ArrayXpress Strategy: Matching the Claims

AX develops a unique strategy and experimental design to match the claim or claims in a specific toxic tort case. Many times a very specific MOA is claimed. It is therefore critical that the correct questions are formulated to address the claim(s). Pathology reports, the claims in each case, and the information available in the scientific literature will ultimately determine a unique strategy and tailored scientific study. Following 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 and predisposition 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). The defense might employ an investigational study that analyzes mutations or deletions in mitochondrial DNA, or gene specific mutations found in the plaintiff’s DNA indicating genetic predisposition to AML.

The abovementioned cases require very different study designs as different MOAs are involved. 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 before any study design is selected and conclusions are drawn. There are also many, many cases where NGS is not applicable, or the development of an appropriate study would be cost prohibitive.

How does ArrayXpress support 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 and genetic signatures of exposure or limitations in tissue availability for testing. 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 and other diseases. The results AX provides 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 provides an end-to-end service. We work with the client and their medical and toxicology experts to review the pathology reports, and if for the defense, the claims, and then design the most appropriate and scientifically valid approach. If for the defense, we collaborate in the development of affidavits to secure tissue(s) for genomic testing and subsequently conduct all the laboratory 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 interpretations 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.

Conclusion
The applications of NGS technologies have brought significant benefits to society in many ways. In the toxic tort arena it can be equally helpful to plaintiffs and attorneys for shedding light into disease causation claims. 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 technology is proven and the number of cases are expanding. NGS is emerging as a powerful tool for justice.