ABSTRACT

Phylogenetics of HIV Used as Forensic Evidence in Court

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Forensic Phylogenetics is a scientific technique used to compare different disease strands in order to discern which of the strands is the parental strand. This technique is used in forensic science when a malicious transmission of diseases has occurred between two individuals. This type of evidence has increasingly been used within the last decade in court to help convict people for the transmission of HIV. This technique is still considered very new in the field of forensic science, and many question whether or not it should be allowed as evidence. The goal of this thesis is to determine if this piece of forensic evidence should be admissible in court. This thesis will specifically look at the positives and the negatives of forensic phylogenetics in relation to cases involving HIV transmission.

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PHYLOGENETICS OF HIV USED AS FORNESIC EVIDENCE IN COURT

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CHAPTER ONE

Introduction to Forensic Phylogenetics

As technology and research begin to rapidly develop in society, so too do forensic science techniques. The world of forensic science is always looking to advance and improve different methods to collect and analyze evidence effectively and efficiently. Throughout the years forensics has developed many different techniques such as DNA fingerprinting, tire track marks, ballistics, and many more that have all contributed to the conviction of criminals in court. These techniques are aimed at helping to definitively prove who committed a crime. As society continues to advance and grow, people have started to become creative in the ways that they commit crimes hoping that they can get away with them. Because of this, the discipline has had to come up with new techniques that can prove conclusively whether a person or persons committed a crime. One of the newest concepts in forensic science is forensic phylogenetics. For the purpose of this thesis, forensic phylogenetics is the study of genetic relatedness that is found between different disease strands (Bernard et al. 2007). This technique combines evolutionary-biology with modern genetic sequencing technology in order to compare side by side the structure of two disease strands. By comparing the two different disease strands, it is possible to discern how closely related they are to one another. This comparison helps to prove or disprove if a person was infected with a certain disease by another person (Bhattacharya 2014). This may seem like an odd thing to test for in the field of forensic science, but over the last 30 years it

has been suggested that there are individuals in the world who are purposefully trying to infect others with diseases for the intended purpose of hurting or killing them. This has especially become prevalent in the malicious transmission of HIV from person to person.

Human Immunodeficiency Virus is a retrovirus that was first detected back in the 1980's. This virus leads to the development of AIDS which is considered an uncurable and chronic disease. Through scientific study, it has been determined that the HIV virus can only be transmitted through bodily fluids such as blood, semen, or breast milk. The primary routes of transmission are through sexual contact and contaminated needles ("HUMAN IMMUNODEFICIENCY VIRUS | Encyclopedia of Bioterrorism Defense - Credo Reference" n.d.). The virus works by inserting itself into the host's DNA. It then uses the host's cells replication mechanism to grow and spread. HIV is considered such a deadly disease because the infection cannot be cured with our current technology and medications. The virus attacks a person's immune systems. With a weakened or nonexistent immune system, the person's body is no longer able to fight off any foreign pathogens or disease. With no way to fight off diseases, the body becomes susceptible to foreign pathogens and eventually will lead to death. There are different drugs that can target the life cycle of the virus to try and disrupt the cycle to help manage some of the symptoms of HIV, however none of these drugs are able to entirely eradicate the disease from a person's body.

People do not always know that they have HIV right away sine the virus has an incubation period that is commonly measured in months or years. It is possible to have the virus for a long time before symptoms present, making it very difficult to eradicate

the virus and treat the symptoms in time. Thanks to modern medicine it is possible to mitigate the symptoms of HIV and prolong life, but it is still considered a deadly disease to contract ("HUMAN IMMUNODEFICIENCY VIRUS | Encyclopedia of Bioterrorism Defense - Credo Reference" n.d.).

The idea that people were maliciously infecting others with the HIV virus was brought to the public's attention in the early 1990's (Bernard et al. 2007). One of the first documented cases in the United States was that of Graham Farlow. In July of 1990, a prison guard was attacked by a prisoner. The inmate injected the guard with HIV positive blood. The guard, Geoffrey Pearce, tested positive for HIV four weeks after the incident. Farlow died before he could ever stand trial, and as a result not much came of the incident in the legal system. Unfortunately, even with treatment, Pearce Contracted AIDS and died seven years later. While this case was never brought to court, it served to demonstrate to the public that the transmission of harmful diseases was an issue that needed to be addressed in a legal format.

Another one of the early cases brought to attention in media was that of Brian Stewart. In 1992, Brian Stewart worked in a hospital as a lab technician, as a technician,he came into contact with many different bodily fluid samples. Some of the fluid samples were blood that had been contaminated with HIV. He took one of the HIV blood samples and injected his 11-month old son with it. Stewart did not want to have to pay child support for his son. He believed that if he injected his son with HIV that his son would die a quick and painless death and he would no longer be required to pay the mother child support. This case went to court and Stewart was charged with first-degree assault. The jury found him guilty and he was sentenced to life in prison.

Forensic phylogenetics was not a technique that had made its way to the United States at this time. As a result, forensic phylogenetics was not considered as a type of evidence for this case. The case was purely built on circumstantial evidence in the court, but the evidence was strong enough to warrant a guilty conviction ("HUMAN IMMUNODEFICIENCY VIRUS | Encyclopedia of Bioterrorism Defense - Credo Reference" n.d.). While these cases did not incorporate forensic phylogenetics themselves, they helped to establish the need to introduce a type of evidence that could help to prove or disprove the transmission of diseases between individuals.

Viruses such as HIV mutate very quickly, so in order to see similarities between the viruses it is necessary to sequence virus samples from all the infected individuals and compare small differences in their genomes as fast as possible. Through comparisons, it is possible for scientists to trace the evolution of the viruses and put them on a "family tree". This tree is constructed in order to more accurately compare the disease strains and see if or how they are related. The phylogenetic tree works by placing each of the disease strands on their own branch. The closer together two branches are, the more closely related the disease strands are to one another. As Oliver Pybus said, "What we are doing is a virus genealogy" (Bhattacharya 2014). By mapping these viruses out and placing them on a tree, it is possible to predict how closely related two or more virus strains are.

The process of creating a forensic phylogenetic tree begins by first collecting samples from all the individuals involved. The chain of custody must be closely followed from the time the samples are collected to the time that they are tested in the lab. All specimen movements and people who encounteres the specimens must be

closely recorded so that any chance of contamination is reduced as much as possible. In order to reduce the risk of any errors occurring in the laboratory, several samples should be tested from each individual under investigation. Control samples also need to be included in each investigation. These control samples should be taken from other individuals infected with the HIV virus in the same geographical area. Control samples are used so that the scientists analyzing the disease strains are not biased when looking for comparisons between strains. Risk of bias can also be reduced by ensuring that the scientists performing the analysis are not aware of the proposed direction of transmission in relation to the HIV strains being tested. This ensures that the scientists are not looking for a specific transmission direction, but are objectively interpreting the data they are receiving (Abecasis et al. 2011).

When scientists start the analysis, they begin by first extracting the HIV RNA from the plasma of the sample. They then reverse transcription about ten microliters of the RNA at 42 degrees Celsius for about 30 minutes. Reverse transcription is used to turn the strand of RNA into a strand of DNA that can then continue the process of polymerase chain reaction. About four microliters of the product is used to perform the polymerase chain reaction for around 30 cycles. A polymerase chain reaction is used in order to amplify the number of strands of DNA for testing. Once that process is complete, the product can then go through DNA sequencing (Kaye, Chibo, and Birch 2009). Sequencing the DNA is what yields the results that can then be compared against each other. The results themselves are then represented as a phylogenetic tree. Each branch of the tree represents a different individuals HIV sequence (Volz and Frost 2013).

When analyzing the tree, two strains are compared against one another to determine their relationship. In 2010, a biologist at the University of Texas at Austin described a method that gave support to the direction of viral transmission. Because the HIV gene mutates very quickly, one million times faster than human DNA, a person harbors many different variants and only a small subset of those variants is transferred when an infection occurs (Vogel 1997). This means that some viruses in the host may be more closely related to the viruses in a recipient than to some of their own (Bhattacharya 2014). Comparison of how alike strains are is what determines the percent that a transmission event occurred between two individuals. The more similar the strains are between two people, the more likely it is that one of them was contracted from the other.

Many different things can affect the similarity between transmission pairs. One of these is time of transmission. Because the HIV gene mutates so rapidly within the cell, the longer a person is infected the more diverse the individuals strain of HIV will become. This means that the longer it takes to analyze the sequence the more different two related strains will become from one another. The mutation rate makes it difficult to be able to discern the relatedness with the more time that passes. The substitution rate of the retrovirus also changes over time to make it more difficult to compare strains over time. This means that the retrovirus in the host cell is constantly incorporating new strands of the host's DNA into itself. The rate at which this substitution occurs also affects how related the strands of virus are and can affect how similar two strains appear to be (Volz and Frost 2013). The interpretation of the results are critical. It is important that the scientists remember to ask the appropriate questions when trying to

make comparisons, such as have the proper controls been included in the samples, are there two viruses that are more closely related than others and are there other epidemiological evidence of linkage between the individuals (Bernard et al. 2007). If these questions can be answered properly, a determination of relatedness can be reached.

The first time forensic phylogenetics was attempted to be used in a United States court was during a 1990 case involving a dentist (Kaye, Chibo, and Birch 2009). A Florida dentist, who was HIV positive, was accused of purposefully infecting five of his patients. He had supposedly contracted HIV while working on one of his patients early on in his career. He was upset over his diagnosis and began trying to purposefully infect his patients so that they too would be affected by the disease that had caused him distress. Phylogenetic analysis was conducted to see if the dentist was the probable source of the HIV infection for all five of the patients. Results revealed that the dentist was most likely the source of all five of this patient's HIV strands. Unfortunately, the case never had the chance to make it to trial. The civil case was settled outside of court and never required the phylogenetic evidence to be presented in court (Smith and Waterman 1992). Because the phylogenetic forensic evidence never made it to court, the technique was not introduced to the general public. This case did however help to start the precedent for how the analysis between disease strands should be conducted when the time came. The case illustrated that the comparison the disease strands was a feasible option that could now help to provide answers for direction of transmission.

In 1994, the time did come for forensic phylogenetics to make its first introduction in court. Forensic phylogenetic evidence was presented in a Swedish

court. Sweden beat the United States to the application and used the phylogenetic analysis to help convict a rapist who was accused of deliberately transmitting HIV to his victim (Vogel 1997). This court case set the precedent for the use of forensic phylogenetic analysis as evidence in court. Following this conviction, the use of forensic phylogenetic evidence slowly started to be introduced into court systems all around the world.

Today, society is aware that malicious transmission of diseases is a problem that needs to be resolved. To help better address the problem, countries and states have started to put laws into effect that assist in controlling the transmission problem. In Australia, criminal law provisions include intentionally causing a very serious disease such as HIV in the category of endangering a person (Kaye, Chibo, and Birch 2009). In England, included under section 20 of the Offences Against the Pearson ACT is conviction of reckless transmission of HIV. Under this law two factors need to be proved before a conviction can occur. The first factor that needs to be verified is that the defendant was the one that infected the complainant, and the second factor is that the defendant conducted the transmission "recklessly" (Bernard et al. 2007). Both factors need to be proved in order to warrant a conviction under British law. As of 2008, 32 states in the United States of America have statues that criminalize the deliberate or reckless transmission of HIV. The severities of the crime vary from being classified as either a misdemeanor or a felony depending on the state (Abecasis et al. 2011). Even though this problem has just been presented within the last couple of decades, it has become increasingly evident that malicious transmission is a problem that requires laws to effectively address. As technology begins to develop so too will

the technique of forensic phylogenetics. In the future we could see the possibilities of this type of forensic evidence increase tenfold.

CHAPTER TWO

Positives of Allowing Forensic Phylogenetics in Court

As forensic phylogenetics is being introduced into society, people are beginning to question whether or not it should be allowed in court. Because this type of evidence is relatively new, not many standards have been set regarding how this type of evidence should be conducted and handled in court. The admissibility of forensic evidence in court revolves around either the Frye or Daubert standard depending on the state. The Frye standard states that a scientific principle presented in court "must be sufficiently established to have gained general acceptance.", while the Daubert standard states the judge is the "gatekeeper" and has full discretion on what evidence should be admissible in their court. When looking at what evidence to allow in court, the judge should consider factors such as: the general acceptance of the theory, the scientific principles used, and the expert's knowledge on the subject. While most states have adopted the Daubert standard to decide what evidence is permissible, there are still states which continue to use the Frye standard ("Forensic Evidence Admissibility and Expert Witnesses" n.d.).

The debate in the forensic community revolves around whether this type of evidence should be allowed in court according to the Daubert standard. Since the technology behind forensic phylogenetics is still so new, people are still discovering the positives and negatives of this kind of evidence and what it can offer in both criminal and civil court proceedings. Since the evidence was first allowed in a Swedish court in 1994,

it has been setting precedents for the positives that phylogenetic data can provide (Abecasis et al. 2011). Dr. Jan Albert and his colleagues were responsible for presenting the phylogenetic evidence in Sweden to prove a man had deliberately transmitted HIV while raping a woman. Based on Dr. Albert's phylogenetic evidence, the court made the decision to uphold the validity of the evidence and determined that the man and his victim shared similar HIV strains (Bernard et al. 2007). The similar strains were able to show that the disease were related to one another. Since the direction of transmission was proposed by the rape, it was concluded that the man did in fact recklessly transmit HIV to the victim. Analysis of phylogenetic disease strains have increasingly been determining criminal court convictions ever since its introduction in 1994 (Abecasis et al. 2011).

While the idea of forensic phylogenetics was brought to the United States in the early 90's in a case involving a Florida dentist who infected his patients with HIV, it wasn't until 1997 that phylogenetics was first admitted as evidence in the United States (Smith and Waterman 1992). The phylogenetic evidence was presented in a Louisiana court. The case began in 1995 when a nurse, Janet Trajan Allen, accused a gastroenterologist, Richard J. Schmidt, of deliberately infecting her with HIV (Vogel 1998). Janet and Richard worked together at the same hospital and were having an affair with each other. One day, Janet confronted Richard and told him she wanted to end the affair. Richard became very angry and begged for her not to leave. When Janet refused to take him back he became resentful. Dr. Schmidt was responsible for helping to give Janet Allen her weekly vitamin B-12 shot. Dr. Schmidt visited Janet's house the week she broke up with him and gave her what she thought was her typical "Vitamin B-12

shot" ("HUMAN IMMUNODEFICIENCY VIRUS | Encyclopedia of Bioterrorism Defense - Credo Reference" n.d.). Instead of injecting her with her regular vitamin injection, he injected her with tainted HIV blood that he got from one of his patients (Vogel 1998). Six months after the shot, Janet tested positive for HIV. Janet pressed charges against Dr. Schmidt for the deliberate infection of HIV and the case went to court ("HUMAN IMMUNODEFICIENCY VIRUS | Encyclopedia of Bioterrorism Defense - Credo Reference" n.d.).

For the first time in the United States, the district attorney wanted to introduce a type of genetic analysis, forensic phylogenetics, that had never been used in a criminal trial in the US before (Vogel 1997). The district attorney wished to present an analysis of the two strains of HIV, Janet's strain and a strain taken from one of Dr. Schmidt's patients, to show that they were from the same strain of the disease. The analysis was conducted by Michael Metzger. At the time, Metzger was a graduate student at Baylor College of Medicine in Houston and was a student in the molecular biology lab of Richard Gibbs (Vogel 1998). The analysis included appropriate controls that were collected from the local Louisiana population. The analysis revealed there was evidence of relatability between the two strains that could have been from a transmission event. The results showed that the strains were more closely related to each other than they were to any of the controls that were tested with the strains (Bernard et al. 2007).

The phylogenetic analysis was ruled admissible in a preliminary hearing of the court. Louisiana District Judge, Durwood Conque, ruled that the technique was considered a reliable and valid science in the eyes of the law (Vogel 1997). Richard's defense attorney tried to argue that the evidence did not hold up in a court of law

("HUMAN IMMUNODEFICIENCY VIRUS | Encyclopedia of Bioterrorism Defense - Credo Reference" n.d.). The defense lawyers claimed that the laboratory work had been sloppily conducted and the risk that contamination had occurred was too high. Metzger had admitted that two of the control samples that he had been given were contaminated with a laboratory strain of HIV at some point during the analysis. The defense stressed that this should render the evidence inadmissible due to the fact there was no way of knowing what else could have been contaminated. The risk of contamination could alter the results making them unreliable. The defense also argued that the analysis was considered meaningless without a proper epidemiological study of other routs of possible infection (Vogel 1998). With the contamination of two of the control samples being brought to attention, the court agreed to let the phylogenetics be repeated at a different laboratory to see what they found. Two Separate laboratories in Austin repeated the analysis and both found similar results to those that Metzger had found.

The Louisiana Court of Appeals found the phylogenetic evidence met judicial standards and upheld the ruling to allow it as evidence in court (Bernard et al. 2007). Phylogenetic evidence was not the only evidence presented in the case, there were many other forms of evidence that were used to help build the case against Richard Schmidt. Forensic phylogenetics helped to provide further support to the case that DR. Schmidt was responsible for intentional transmission of the HIV virus. At the end of the trial, Richard was found guilty of attempted murder and sentenced to 50 years in jail. Virologist Dr. Michael Metzger, who performed the analysis for the trial, stated that a "precedent for the use of phylogenetic analysis to support or reject criminal viral transmission cases has thus been established in United States courts of law." (Bernard et

al. 2007). This case was ground breaking for the field of forensic phylogenetics and helped to demonstrate the power that this evidence could provide in court.

Many people believe that this scientific technique's greatest strength might be in its ability to exonerating and clear individuals of crimes that they were not responsible for committing. In 2004, a Palestinian doctor and five Bulgarian nurses were sentenced to death for the belief that they had infected 426 children with HIV at the hospital that they were working at in Benghazi, Libya. These individuals earned the name the "Benghazi Six". They were all detained in 1999 and were being imprisoned and tortured in prison. They stayed in the Libya prison being treated unfairly until their court hearing was scheduled five years later. A phylogenetic analyst looked at the strains of HIV and discovered that the strain that the kids at the hospital all possessed matched a strain that had been circulating the country for almost a decade. The specific HIV strain had been present long before the foreign health workers ever arrived in the country of Libya. Because the strain of HIV had been circulating in the country for several years, the doctor and the nurses could not have been responsible for infecting the kids with HIV. As a result of the use of forensic phylogenetic evidence, the six people were exonerated for their crimes and finally released (Bhattacharya 2014). The powerful use of phylogenetics to exonerate people of crimes that they did not commit was demonstrated in this court case. With the ability to determine if different disease strains are related it is possible to clear people of crimes in instances when there are no evidence of linkage between the disease strains.

The forensic phylogenetic application has also proved to be beneficial in more than just HIV transmission cases. It is also possible to track transmission of other

transferable diseases through this science. There has been notable success in using forensic phylogenetics to track transmission of Hepatitis C. In 2007, a Spanish anesthesiologist, Juan Maeso, was found guilty of infecting at least 275 people with Hepatitis C. The Hepatitis C infection ultimately resulted in the death of four of the anesthesiologist's patients. Juan Maeso was employed in a hospital in Valencia, Spain for over 20 years. During the time that he was working he had been skimming morphine off his patients before they went into surgery. He would inject himself with the morphine before using the same needle to inject the rest of the morphine into his patients. He did this so the hospital would not know that he was stealing morphine from them. The hospital allotted a certain amount of morphine for each patient about to go into surgery. Maeso would use a little of the morphine each time so that no one could track that he was stealing it. By using the needle on himself first, his blood contaminated the needle and transmitted Hepatitis C to many of his patients. Fernando Gonzalez Candelas, working with the University of Valencia, took almost 4,200 viral samples from the patient and the anesthesiologist and analyzed them all. He was able to determine the path of the infection proving that Maeso was guilty of infecting all of his patients (Bhattacharya 2014).

Forensic phylogenetics doesn't just have to apply to the transmission of diseases through bodily fluids. Forensic phylogenetics was also used to help track the source of anthrax spores that were sent to several different U.S. politicians and media outlets back in 2001 (Bhattacharya 2014). This unique use of the technology helped to catch the criminal who wished to do harm to some of our countries most influential people. As the field is maturing with new analytical tools and advance sequencing methods, people are

discovering many different ways that this technology can be used. With so many different application possibilities, there is no telling what this scientific technology could be used for in the future. One thing is for sure though, forensic phylogenetics has the potential to help a lot of people.

There are many positives to allowing forensic phylogenetics to be admissible in court. While it is still a new technique that is constantly being redefined and developed, it has opened a whole new world for forensic evidence. It has provided evidence that is very important in our current societal age. It is an unfortunate world we live in where others would maliciously intend to infect someone with a disease; however, it is important that we are able to identify and incriminate these people for their acts. This technique is very new and just starting to be developed.

Before now society has not needed a reason to track the transmission of disease. We used to live in a society where older forensic techniques, such as fingerprinting, were enough to catch a criminal and bring them to justice, but this is no longer where we stand as a society. The crimes of our society have continued to advance and similarly the technology used to prosecute and hopefully convict criminals has advanced in response to those changes. Due to the changes in our criminal society, our justice system must follow in suit to advance their techniques in order to catch other criminals like Juan Maeso. Because forensic phylogenetics is seen as such a new technique in the world of forensics, people continue to question the usefulness of the advancing technique. However, the more we use this technique and continue to introduce compelling evidence to the courts, its usefulness will become more apparent to the justice system and our society. By using forensic phylogenetics, we will be able to further develop scientific

processes and work to convict and exonerate people who continue to commit, or are innocent of, these heinous crimes.

CHAPTER THREE

Negatives of Allowing Forensic Phylogenetics in Court

While many people feel like forensic phylogenetics provides many positives as a form of evidence, there are still some who believe that its pitfalls are too great a risk to allow this evidence into a court. Ever since phylogenetics was introduced as a form of evidence, its intersection with the legal system has made many uneasy (Bhattacharya 2014). People in the legal system are afraid that this evidence could cause more problems than it would solve. Since this piece of evidence is new it is still very underdeveloped. The underdevelopment of this piece of forensic science means that there have been no set requirements that help to standardize this type of evidence. This has led to some uneasiness with presenting this evidence in court for fear of getting the association wrong and wrongly convicting people of a crime. These critics feel that even though forensic phylogenetics had the potential for good, its risk of getting the information wrong is too high to risk.

While the scientists working with this type of evidence understand its limitations, the public is under the misconception that phylogenetic analysis works a lot like DNA evidence does. This is not true. Unlike DNA evidence, forensic phylogenetics is more complex. Forensic phylogenetics does not have the same degree of certainty that DNA analysis provides (Bernard et al. 2007). By people equating forensic phylogenetics to DNA, they are creating a flawed view of the science that can lead to problems when the evidence is being testified in court. DNA fingerprinting can be used to calculate a

likelihood of a match between the DNA sample taken from the victim and the DNA sample taken from the crime scene. DNA does not mutate or change under normal circumstance within an individual. The lack of mutation that occurs in the genes means that a percentage match can be calculated to determine if the two DNA strands being tested against each other are a match. Phylogenetics of diseases and especially HIV is different because there can never be a complete match between the RNA or DNA of the HIV strands between two samples. There can never be a complete match of the two strains due to the rapidly evolving nature of the HIV virus. HIV is constantly being mutated and changed with individuals so it can never be an exact match with another strand of HIV. Even a strain taken from the exact person 5 minute later would not exactly match the first sample that was taken. The strength of association between HIV strains cannot approach the level of certainty that is found in DNA comparison (Bernard et al. 2007). This flawed view can lead to a risk for wrongful conviction based on the misunderstanding of the science. Many people in the field have started referring to phylogenetics as HIV fingerprinting. This further creates an association between phylogenetics and DNA analysis raising unrealistic expectations (Abecasis et al. 2011). It is important that people are educated on the differences between DNA and phylogenetics if this form of evidence is going to be used in the court.

Phylogenetic evidence has a degree of uncertainty and error that make professionals in the field very uneasy (Bernard et al. 2007). When conducting the analysis, the two strains that are suspected of being related are compared against multiple control samples. Since these control samples are so important, it is critical that care is taken to choose suitable controls for the analysis (Brooks and Sandstrom 2013). These

controls are the basis for helping to determine which strains are potentially related epidemiologically. Because of this, if these controls are not properly selected the data has the potential to be skewed or biased. If the controls are not selected from the same demographic, then the HIV strains will be wildly different from the two samples that are trying to be compared. With such different control samples, it would become obvious to the scientists which strains are trying to be examined against each other. This could once again cause scientists to exhibit bias by saying the tested strains are similar just because they are more closely related to each other than they are to the other samples that are being tested.

The control samples are not the only known source of error that confounds this type of scientific evidence. The HIV strains themselves are full of variables that make them everchanging and unreliable. Due to the constant and everchanging mutation rate of HIV strains, the time since transmission can cause errors in the comparison. When the transmission between two individuals occurs, the strains start out very similar. As time passes both the HIV strains in the infector and in the infected will mutate. This mutation causes the strains to look more dissimilar from each other with each passing minute. If the strains are compared very soon after the transmission takes place, then there is a chance they will still closely resemble each other enough to be able to conclude that it is possible the strains could be related. However; if the samples are not examined till months or years after the transmission event, then the relatedness between the strands will become harder to conclude (Volz and Frost 2013). This mutation rate makes it difficult to be able to compare HIV strains when the time between transmission has been awhile. The sooner the HIV strands can be compared the less chance of error. Unfortunately, a

comparison usually cannot be made quickly enough resulting in too much mutation to ever be confident about a transmission event. The lack of similarities between strains as time passes is what makes critics of forensic phylogenetics so timid to rely on this evidence with any degree of certainty.

The substitution rate of HIV also creates some errors. The rate of change for the pathogen in each host cell is going to be different for every single person. Different cellular conditions and immunological pressures will cause the HIV strain to mutate and change at different rates. The substitution rate can also change within a single person. depending on certain stressors, the strain can be heavily altered one day and then not changed at all the next. Because of the unpredictability of the mutation rate, it becomes harder and harder to track patterns of suspected changes in the virus. Without the ability to track what the virus was suspected of starting out as, it becomes very difficult to conclude with any certainty that strains are or once were related (Volz and Frost 2013). The uncertainty of forensic phylogenetic evidence can be noted in a study done in the United Kingdom. In this study, the scientists took a stimulated data set in which the order of transmission for HIV was known. Scientists took 159 cases of recent HIV infection in which they knew ahead of time who infected who. They then gave the set to another group of scientists who did not know the source of the transmission and asked them to determine who they believe infected who. The single most likely transmission source was only noted in 41 cases. That turned out to be only 26% of the cases. Even when it was guaranteed that both the infector and infected strain were in the group, scientists could only prove ¼ of the cases (Volz and Frost 2013).

Human error rate also plays a role in the uneasiness of using phylogenetics in court. When forensic phylogenetic evidence is used in court it is usually processed in a research lab and not in forensic facilities. Research labs have the equipment necessary to compare and sequence HIV that forensic labs lack. Research labs are not used to the rigorous sample tracking system that forensic facilities use and this can cause problems. Forensic labs are trained in how not to tamper evidence and how to keep a log of everyone who encountered the evidence to make sure it has not been contaminated in any way. This is not a system research facility are used to dealing with, and because of this there are room for significant errors. The conditions of the lab are likely to be less stringent and allow for accidental contamination (Bernard et al. 2007). Methodology errors such as those in chain of custody, work flow, and lab design can all contribute to the possibility for the HIV sequences not being handled properly (Brooks and Sandstrom 2013). The sources of error present in this process make many question if phylogenetic analysis should ever be used as the sole proof that a transmission occurred between two individuals. It has many people questioning how confident can we really be that two strains are showing a relationship due to similar transmission when there are other risk factors that could have led to an apparent association between the specimen.

The science is not the only piece of this evidence under fire, many see presenting this evidence in court as a challenge in and of itself. When Dr. Richard Schmidt went to court in Louisiana for infecting his girlfriend with HIV, the expert witness on the case had to teach both the judge and the jury about forensic phylogenetics. The witness spent two days lecturing the court about evolutionary terms and concepts. This was background information that was necessary to understanding how phylogenetics works.

Once the court understood the evolutionary terms, the witness proceeded to launch into three weeks of testimonies explaining how forensic phylogenetics works. The most challenging part of teaching the court about the new evidence, was distinguishing it from conventional DNA testing. It took the full three weeks before the judges and lawyers could fully understand the differences of DNA and HIV phylogenetics. Teaching the science was a tedious and long process. Some wonder is the effort that it takes to explain this evidence in court is worth the hassle for some evidence that might not have been done correctly in the first place (Bhattacharya 2014).

CHAPTER FOUR

Conclusion

There are many positive and negatives to consider when looking at the use of phylogenetic forensic evidence in court. The forensic science application of phylogenetics is still very new. While many people feel that this new application is helpful, others feel that it might be too harmful. This science has the potential to do great work in helping to convict those who wish to maliciously transmit diseases such as HIV. On the other hand, this science is still so new that it does not have any standardizations in how to conduct the genetic comparison or how to testify on the science in court. This lack of standardization makes many scientists uneasy about using this forensic science technique. They are afraid of the error rate and wrongly convicting someone based on the error. When looking at whether this science should be allowed in court it is important to take into accounts all the positives and negatives of forensic phylogenetics. Through the course of this paper I have looked at both sides of the debate and have concluded that I personally believe that forensic phylogenetic evidence should be allowed in court under strict guidelines.

As discussed previously, flawed views in the science behind phylogenetics and the fast mutation rate of HIV increase the risk of falsely convicting a person. Due to this, it is extremely important to emphasize proper scientific validation of the phylogenetic methods before they are used in court (Abecasis et al. 2011). As the use of forensic

phylogenetics increases around the world, the issues surrounding how to correctly use and interpret forensic phylogenetics has been brought up both in the USA and Europe (Bernard et al. 2007). They all agree that more cases are bound to arise that will use this technology to try to prove someone's guilt or innocence in transmitting a disease. Scientists around the world are discussing developing similar guidelines to those used for DNA fingerprinting. DNA fingerprinting guidelines were established by the National Academy of Sciences in 1996 (Vogel 1998). These guidelines helped to standardize the science so that every forensic scientist conducting DNA analysis undergoes the exact same procedures. The standardizing of the science made DNA fingerprint more reliable and more widely accepted in the forensic science community.

Many scientists are concerned about the lack of guidance that surrounds forensic phylogenetic evidence. Scientists and lawmakers alike are currently working on drafting guidelines to help avoid any misinterpretations of the science in the future. The goal is to reach a consensus on issues such as which genetic regions should be assessed when comparing virus strands, as well as developing procedures for how to establish a control population for the samples taken (Bhattacharya 2014). Other future guidelines would require things such as a clear chain of custody for the samples, blind testing samples, and a full epidemiological investigation of the risk factors and inaccuracy's behind the science (Vogel 1998). The hope is that with these guidelines in place the risk of wrongful conviction would be reduced and the science of forensic phylogenetics would be more widely accepted around the world.

To help lower the risk of wrongful convictions, it is also important to know how to present the evidence in court properly. This complicated science is hard for many to

grasp. If the jury does not understand the science they will be unable to comprehend what the results are trying to convey. Because of this, it is important for any future expert witnesses testifying on the validity of forensic phylogenetics to acknowledge the limitations of the science as well as fully express the differences between this science and the science of DNA fingerprinting (Bernard et al. 2007). People on the jury need to understand what forensic phylogenetics is, so that when they are deliberating on a verdict they are doing so based on the proper information.

With the lack of guidelines in place, it is also important to stress that at this point in time phylogenetic evidence should not provide the sole proof of evidence for the conviction (Bernard et al. 2007). There are too many risks associated with forensic phylogenetics that have not been accounted for yet to confidently be able to convict someone of HIV or other disease transmission based on the forensic phylogenetic science alone. Presenting in court, it is imperative to use the proper language when talking about the results. Using proper language can help to avoid any miscommunication or confusion about what the results truly mean. When two viruses appear to be related, they should not be labeled a conclusive match but instead it should be said that, "the viral sequences from the two subjects display a high level of similarity and are more closely related to each other than to other strains circulating in a population with the same epidemiological profile." (Bernard et al. 2007). This language ensures that there is no confusion about the relatedness of the viruses.

The greatest strength this forensic has at this moment is its ability to clear individuals of their suspected crimes (Bhattacharya 2014). Forensic phylogenetics is capable of exonerating people by demonstrating that the viruses of the defendant and the

complainant are unrelated (Bernard et al. 2007). This can provide very helpful in clearing people of suspected crimes that they did not commit. While this is the greatest use of the technology now, the possibilities of where this technology could go are endless. New advances in sequencing and technology are allowing the field to grow and mature. The power of phylogenetic analysis is increasing as new sequence technologies are being developed (Bhattacharya 2014). These new developments will increase what forensic phylogenetics is capable of and what it can prove. While right now this evidence should not be used alone in court, one day it might be able to. As long as proper guidelines and rules are set in place to regulate the use of forensic phylogenetics, it has the possibility to do great things for the future of forensic science and the conviction of those who maliciously infect others with HIV.

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