

ORIGINAL RESEARCH

HIV forensics: pitfalls and acceptable standards in the use of phylogenetic analysis as evidence in criminal investigations of HIV transmission*

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Background

Phylogenetic analysis – the study of the genetic relatedness between HIV strains – has recently been used in criminal prosecutions as evidence of responsibility for HIV transmission. In these trials, the expert opinion of virologists has been of critical importance.

Pitfalls

Phylogenetic analysis of HIV gene sequences is complex and its findings do not achieve the levels of certainty obtained with the forensic analysis of human DNA. Although two individuals may carry HIV strains that are closely related, these will not necessarily be unique to the two parties and could extend to other persons within the same transmission network.

Acceptable standards

For forensic purposes, phylogenetic analysis should be conducted under strictly controlled conditions by laboratories with relevant expertise applying rigorous methods. It is vitally important to include the right controls, which should be epidemiologically and temporally relevant to the parties under investigation. Use of inappropriate controls can exaggerate any relatedness between the virus strains of the complainant and defendant as being strikingly unique. It will be often difficult to obtain the relevant controls. If convenient but less appropriate controls are used, interpretation of the findings should be tempered accordingly.

Conclusions

Phylogenetic analysis cannot prove that HIV transmission occurred directly between two individuals. However, it can exonerate individuals by demonstrating that the defendant carries a virus strain unrelated to that of the complainant. Expert witnesses should acknowledge the limitations of the inferences that might be made and choose the correct language in both written and verbal testimony.

Keywords: phylogenetic analysis, criminal investigation, HIV transmission, forensic investigation, molecular epidemiology

Received: 19 February 2007, accepted 20 April 2007

Introduction

Since 2001, several prosecutions have taken place in the UK for the sexual transmission of HIV, with more cases awaiting trial. In England and Wales, people have been

convicted of 'reckless transmission of HIV' under section 20 of the Offences Against the Person Act (OAPA) 1861. There is no offence of risk-taking behaviour, exposing others to the risk of transmission, or 'endangerment'.¹ Put simply, two facts need to be proved:

*A longer version of this paper was originally published by NAM and the National AIDS Trust in March 2007.

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¹There is a separate common law offence in Scotland of 'reckless injury' under which prosecutions have taken place. In Scotland, it is also possible that prosecutions may take place for HIV exposure, as 'reckless endangerment', although none has taken place to date.

- (1) that the defendant infected the complainant, and
- (2) that the defendant was 'reckless' (i.e. that at the relevant time he or she was aware of the risk of infecting the complainant).

In its draft policy on 'Prosecuting cases involving the sexual transmission of infections which cause grievous bodily harm' [1], the Crown Prosecution Service (CPS) requires scientific evidence to support a prosecution case, even where the defendant wishes to plead guilty. A defendant might 'feel guilty' at having had unprotected sex without disclosure of HIV-positive status, but this is not the same as knowing that he or she is actually the source of the complainant's infection.

Phylogenetic analysis – the study of the degree of relatedness between HIV genetic sequences – has been used as scientific evidence to determine whether the defendant and the complainant share similar HIV strains. However, expert evidence in the only trial that resulted in acquittal [2] demonstrated serious flaws in the way in which this scientific evidence has been used. Many of the issues pertinent to the correct use and interpretation of HIV forensics have been raised before in the USA and in Europe. The aim of this article is to address the still widely held but incorrect assumption that phylogenetic analysis can provide definitive evidence of the source, route, direction and timing of HIV transmission. There are, in fact, many limitations regarding what this scientific evidence can prove beyond doubt.

One of the requirements for recklessness to be proven is that the infection took place *after* the defendant was made aware of his or her HIV-positive status. Establishing the timing of HIV infection is therefore highly relevant to the case. Where there is the possibility that transmission took place either before the defendant's HIV diagnosis or after the complainant became aware of the defendant's HIV-positive status, such awareness will be relevant to the question of whether the defendant can raise the defence of consent.

Thus, there are real complexities in proving that HIV transmission occurred between two people. The building of a prosecution case and, crucially, drawing the right conclusions will typically require a combination of scientific and other clinical and epidemiological evidence, including a detailed and documented sexual health history of both defendant and complainant.

Cases and legal precedents

Forensic use of phylogenetic analysis of HIV sequences first entered public awareness in 1990, when the United States (US) Centres for Disease Control and Prevention

(CDC) began investigating the alleged transmission of HIV from a Florida dentist to his patients during the course of dental surgery [3]. The investigation lasted 2 years, during which time the dentist died. Although the CDC's reports [4,5] concluded that up to six patients may have been infected by the dentist, questions persist regarding the methodology used [6] and no criminal charges were ever brought against the dentist.

In July 1991, various US media published the name of a second Florida dentist who had been diagnosed with AIDS, and who had subsequently closed his practice because of ill health. Phylogenetic analysis by CDC investigators exonerated the dentist by concluding that he had not infected any of his 28 HIV-positive patients [7,8].

Phylogenetic analysis was first used as evidence in a court of law in Sweden in 1992. An HIV-positive man had already been convicted of rape and deliberate transmission of HIV in the Stockholm district court, without the use of forensic evidence. In preparation for his appeal, the prosecution asked virologist Dr Jan Albert and his colleagues from the Karolinska Institute and the Royal Institute of Technology in Stockholm to determine whether the suspect and his alleged victim shared similar HIV strains. On the basis of their phylogenetic analysis and other evidence in the case, the verdict from the district court was upheld in the court of appeal. 'It is important to stress', wrote Albert and colleagues, 'that even though our investigation showed that the strains carried by the male and the female were epidemiologically linked, we could not determine the direction of transmission, nor could we formally rule out the possibility that both the male and the female were infected by a third party. Thus, it was essential that the results from our sequence investigation be used in conjunction with other epidemiological information in the case.' [9]

In the 1997 case of *State of Louisiana v. Richard J. Schmidt*, a doctor was alleged to have tried to kill his former partner by injecting her with HIV- and hepatitis C virus-infected blood obtained from his patients. Phylogenetic analysis was ruled admissible in a preliminary hearing, and then challenged by the defence. The Louisiana Court of Appeal found that phylogenetic analysis met the judicial standards of evidence of admissibility [10]. Dr Schmidt was found guilty of attempted second-degree murder and the verdict was upheld by the Louisiana State Supreme Court and the US Supreme Court. Virologist Dr Michael Metzger and his colleagues – who had performed the phylogenetic analysis on behalf of the State of Louisiana – wrote in a 2002 article detailing their methods: '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.' They stressed

that 'the increasing role of scientific methods and hypothesis testing within the legal system challenges scientists to uphold the highest possible levels of rigor and objectivity' [11].

The first use of phylogenetic analyses of HIV sequence as admitted evidence in a US criminal court showed the following key aspects.

- Clear evidence of possible transmission between two individuals was available prior to phylogenetic testing.
- The evidence provided by phylogenetic analysis was only part of the prosecution's case.
- Independent testing was carried out by different laboratories.
- Appropriate controls were obtained from the local population.

Several other jurisdictions, other than those in Scotland (2001) and England and Wales (2003), have allowed phylogenetic analysis to be utilized as forensic evidence in criminal HIV transmission prosecutions. These include a man prosecuted in Australia for 'knowingly and recklessly' transmitting HIV during the rape of an intellectually disabled man [12]; a man sentenced to 6 years imprisonment in Denmark for sexually abusing a 12-year-old boy and also transmitting HIV [13]; and a man prosecuted for raping and transmitting HIV to six women in Belgium [14].

Weight of expert testimony in a court of law²

The evidence of virologists called upon to present the results of phylogenetic analysis is regarded as expert evidence, which is a form of opinion evidence. Experts may give evidence within their area of competence, which includes explaining technical information and expressing an opinion about the significance of that information; they are not permitted to express opinions on matters that are within the ordinary competence of the jury (the Turner rule).³

Traditionally the common law prevented an expert witness from giving an opinion on the ultimate fact in issue, which includes whether a defendant was the source of a complainant's HIV infection. This appears to have been abandoned. When expert opinion is given on an ultimate issue, it is important that the jury is told that they are not bound by the expert's opinion and that it is for them to

decide what weight they give to it. However, it is wrong to direct a jury that they may disregard scientific evidence when the only such evidence adduced on a particular question dictates one answer and only a scientist is qualified to provide that answer [15].

In HIV transmission cases the expert opinion of virologists is of critical importance. They may be allowed to express an opinion on whether the phylogenetic evidence is sufficiently persuasive to indicate that the defendant was the only possible source of the complainant's infection.

Potential pitfalls in the use of HIV phylogenetic analysis for forensic purposes

The reliability of phylogenetic analysis to investigate HIV transmission between two individuals must be addressed in some detail. The following considerations should be kept in mind.

Phylogenetic analysis is most often used in the context of scientific research on general populations rather than specific individuals. In the research setting, phylogenetic analysis can and does tolerate a certain degree of approximation and error.

For forensic purposes, HIV sequencing and the phylogenetic analysis of sequences are commonly performed in research settings rather than in forensic facilities used to handling samples under vigorous sample tracking systems. Thus, the setting is not generally comparable to that of forensic laboratories performing human DNA analysis and the experimental conditions are likely to be less stringent.

The strength of any apparent link between two HIV strains cannot approach the levels of certainty generally expected of human DNA analysis commonly used in the criminal courts.

The results of phylogenetic analysis are represented as phylogenetic trees, with each branch of the tree representing the HIV sequence of one individual sample. In the analysis, the HIV sequences of the two parties under investigation are compared with sequences derived from other HIV-infected persons. The latter represent the 'controls'. When constructing a phylogenetic tree for HIV forensic analysis, it is vital that the tree is as unbiased as possible and this implies using well-chosen controls. The use of inappropriate controls may wrongly emphasize any relatedness detected between two HIV strains under investigation as being strikingly unique. Obtaining the correct controls raises further issues related to consent for use of sequencing data and protection of databases generated in routine clinical practice. (Further discussions regarding the difficulties around consent can be found in reference 19.) In most cases, it will be difficult and often

²This relates only to the law in England and Wales.

³*R v Turner* [1975] 1 All ER 70. '... expert witnesses must furnish the court with the necessary scientific criteria for testing the accuracy of their conclusions, so as to enable the judge or jury to form their own independent judgement by the application of these criteria to the facts proved in evidence.' (*R v Gilfoyle* [2001] 2 Cr App R 5).

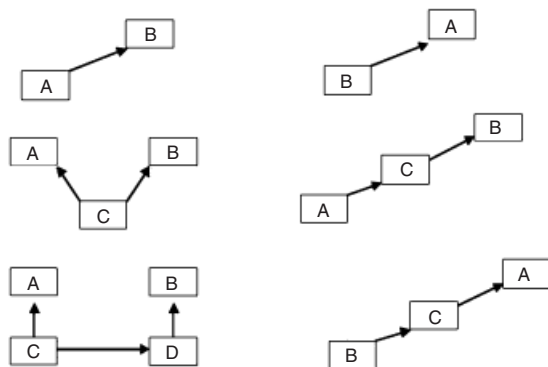


Fig. 1 How two individuals with related HIV strains may be connected. If we take two individuals (A and B) who are infected with HIV strains that are related by phylogenetic analysis, several scenarios can be proposed that may yield similar results in the phylogenetic tree. Arrows indicate direction of transmission. C and D refer to third parties.

impossible to obtain samples from the appropriate controls. In these circumstances, convenient sets of controls can be downloaded from public databases. However, interpretation of the findings will need to be particularly cautious.

When phylogenetic analysis shows that the two HIV strains under investigation are more related to each other than to the control strains, it is nonetheless important to remember that similar HIV strains may be found in many more than two individuals if one or both are part of a wider transmission network. The majority of individuals with HIV infection are part of such a network. Consequently, even with the appropriate controls, phylogenetic analysis cannot prove transmission. All of the following circumstances can yield similar results in phylogenetic analysis (Fig. 1):

- the defendant was infected by the complainant;
- the complainant was infected by a third party with a similar viral strain;
- both the complainant and the defendant were infected by one or more third parties with similar viral strains;
- the complainant was already HIV-positive and was re-infected (also known as super-infected) with another strain of HIV, either by the defendant or by a third party.

Laboratories should apply great care when performing phylogenetic analysis of HIV sequences generated for routine purposes (typically drug resistance testing) as the identification of possible 'matches' may carry serious implications. Clear guidelines should be adopted about when and how the analysis should be conducted and whether consent should be obtained and results communicated to the patients.

Acceptable standards

Given the above considerations, evidence from phylogenetic trees must be seen in the context of the totality of other evidence and never be the starting or central point of an investigation. In addition, certain standards must be met in the analysis [14, 16, 17]. The process must meet the judicial standards for evidence admissibility.

Methodology

It is paramount that precautions are taken to minimize the possibility of sample error (for example, through contamination or mislabelling). Maintenance of the chain of custody must receive the highest priority and specimen movements must be closely recorded and rigorous protocols applied. There are only a few laboratories with adequate experience. If phylogenetic analysis is requested from a research laboratory without forensic experience, it is the task of the requestor to stress the importance of sample tracking.

To minimize the risk of laboratory errors, at least two samples should be tested from each party under investigation from two different time-points, and the results should be consistent.

It is preferable for those carrying out the phylogenetic testing and analysis to be blinded to the identity of the samples under investigation. Given that there are many ways of constructing and analysing phylogenetic trees, those performing the analysis should not be aware of the proposed direction of transmission and of the other circumstances of the case.

Ideally, therefore, samples from each person should be tested at two independent laboratories under blinded conditions, thus removing the possibility of laboratory error and investigator bias.

At least two genetic regions should be sequenced of reasonable length (≥ 500 nucleotides, depending on the gene under investigation). Selection should target genes with different biological functions, different rates of evolution, and different selective pressures. In particular, care should be taken when using the polymerase (*pol*) region for patients under therapy, as similar treatment regimens can drive the virus to accumulate similar mutations, causing an apparent relatedness in the absence of a direct epidemiological link. This problem can be addressed by excluding drug resistance positions from the analysis [18]. The best strategy, however, would be the analysis of the full genome, also known as full-length sequencing, although in most circumstances this is not economically feasible.

There are many different ways of constructing a phylogenetic tree and the choice is based on the reliability of the methods used for building the tree – including the particular HIV genes analysed – as well as the purpose of the tree. Rigorous statistical analyses should be performed.

The composition of the control population should be clearly stated. Choosing sufficient and appropriate epidemiological controls is critical in the setting of often-complex sexual networks that exist primarily (but not exclusively) among gay men and other men who have sex with men. In practical terms, this means analysing approximately 30 other HIV strains from individuals who are from the same geographical origin, social context and potential transmission network as the defendant and complainant. Thus, if a certain social network is apparent (e.g. a club, cruising park or sauna), the controls should reflect this.

The samples from the parties under investigation and the controls should be collected at around the time of the alleged transmission event or at least within a few years.

When a simple phylogenetic tree is suggestive of genetic relatedness between viruses carried by two individuals, analysis of multiple genetic clones from each person can strengthen the proposed relationship.

Current techniques do not allow a reliable estimation of the direction of transmission. However, research is being carried out on this topic, which indicates that such an estimate becomes more reliable if the samples are obtained very soon after the presumed transmission event; if clonal analysis is performed; and/or if multiple sequences are obtained from the source and the recipient.

Interpretation

Phylogenetic analysis can never provide the sole proof of transmission and should not act as the starting point around which to build ‘a story’ by choosing convenient pieces of evidence that would support the relationship. The analysis must be used in the context of all the evidence available. The important question to be asked when interpreting the information provided by a phylogenetic tree is: ‘How confident can one be in excluding other risk factors for infection and the involvement of other parties in the transmission chain?’

When the phylogenetic analysis indicates that the two HIV strains under investigation are not related, the evidence should be regarded as solid enough to exclude the possibility of transmission. The investigation can therefore exonerate suspected individuals.

When the phylogenetic analysis indicates that the two HIV strains under investigation are related, the appropriate selection of controls will increase confidence that the

relationship observed reflects a true direct transmission. However, even with the best controls, it may be impossible to know with certainty that transmission occurred directly from one to the other without one or more intervening individuals. It should be acknowledged that the relationship shown by the phylogenetic tree cannot be easily translated into a definite statement about the possibility of transmission that would be beyond reasonable doubt. Even if statistical support for a close link between the investigated individuals is 100%, this does not imply that the evidence for a direct transmission is 100%.

Thus, the appropriate interpretation would include the following questions.

- Have the appropriate controls been included?
- Are the two viruses more closely related to each other than to the controls?
- Is there anybody else infected with the virus that is, or could be, also related?
- Is there any other epidemiological evidence of linkage between individuals?

Experts must be ready to acknowledge the limitations of the inferences that might be made and choose the correct language in both written and verbal testimony. For example, the correct language should be: ‘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’. Statements should include the possibility that an unknown third person might be involved, and that the direction of transmission cannot be proven.

Conclusion

It is important that everyone involved in the criminal justice system is made fully aware of the limitations of phylogenetic analysis before using its findings as conclusive evidence of HIV transmission between two individuals. Phylogenetic evidence, in the context of other clinical and epidemiological evidence, can provide support for linkage between cases, but cannot, in itself, be proof of transmission. Expert witnesses should acknowledge the limitations of the inferences that might be made and choose the correct language in both written and verbal testimony. Over-interpreting the results of phylogenetic analyses is unacceptable, regardless of how convinced an expert may be of the guilt or innocence of the accused.

Acknowledgements

The authors would like to thank the following for their comments and insights during the drafting of this

paper: Catherine Dodds, Sigma Research; Lisa Power, Terrence Higgins Trust; Quincy Whitaker, Doughty Street Chambers.

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