





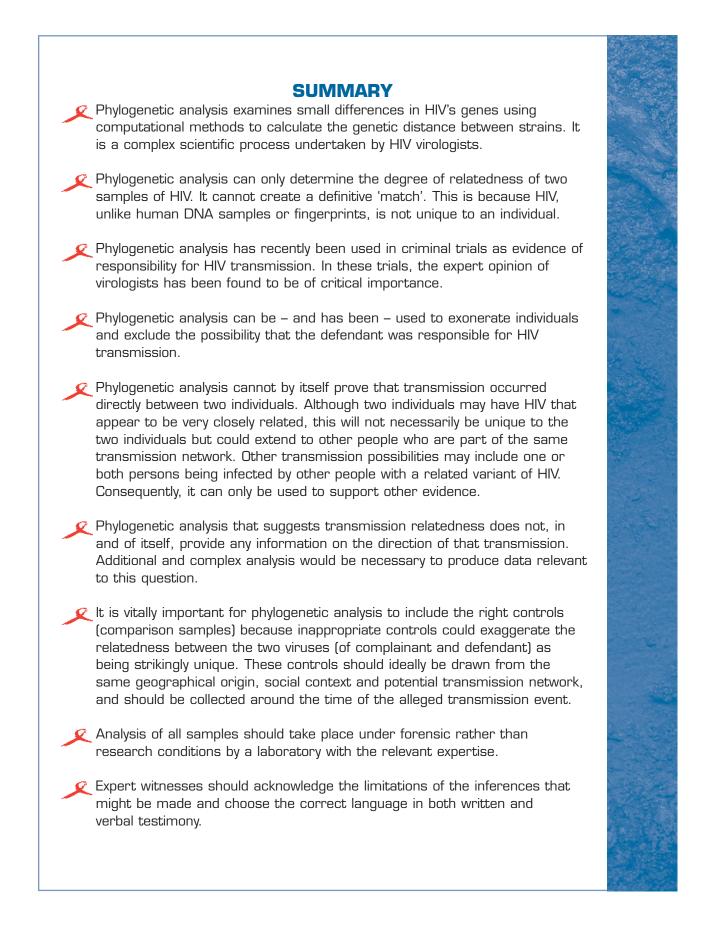
The use of phylogenetic analysis as evidence in criminal investigation of HIV transmission

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This short briefing paper is aimed at professionals working in the criminal justice system and HIV professionals who may be called as expert witnesses in criminal HIV transmission cases. It may also be useful for people working in HIV support organisations and HIV-positive individuals. It aims to explain how phylogenetic analysis should and should not be used in criminal trials for the reckless transmission of HIV.

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PROSECUTION FOR SEXUAL TRANSMISSION OF HIV: LEGAL BACKGROUND

Since 2001, a number of prosecutions have taken place in the United Kingdom for the sexual transmission of Human Immunodeficiency Virus (HIV), with more cases awaiting trial.

Prosecutions for the reckless transmission of HIV in England and Wales have all taken place since 2003, under section 20 of the Offences Against the Person Act 1861 (OAPA 1861); most have pleaded guilty, two were convicted following trials, and one was acquitted.1

In a number of the cases to date the prosecution used scientific evidence – specifically, phylogenetic analysis of the virus samples of complainant and defendant – to 'prove' that the defendant infected the complainant.

Expert evidence in the one acquittal, however, has demonstrated serious flaws in the way this scientific evidence has been used by prosecutors. There has been an incorrect assumption that phylogenetic analysis can provide definitive evidence of the route, direction, and timing of HIV transmission. There are, in fact, many limitations regarding what this scientific evidence can 'prove', and these will be discussed in detail in this paper.

It should be noted that the offence that people have been convicted of under section 20 of the OAPA 1861 is one of reckless trans-mission – there is no offence simply of risk taking behaviour, exposing others to the risk of transmission, or 'endangerment'.

Put simply, two facts therefore need to be proved: i. that the defendant infected the complainant, and ii. 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'², the Crown Prosecution Service (CPS) has rightly required scientific evidence to support a prosecution case, even where the defendant wishes to plead guilty. The defendant might 'feel guilty' at having had unprotected sex without disclosure of HIV-positive status but this is not the same as knowing with an appropriate degree of certainty that they are actually responsible for the fact of HIV infection.

Much will depend on evidence as to the complainant's sexual history. If, for example, the complainant has had unprotected sex with other people, it could quite plausibly be the case that another of those sexual partners was the person who transmitted HIV to the complainant. It could also be the case that it was the complainant who transmitted HIV to the defendant – again, it all depends on the facts of the case and the quality of the evidence provided.

In addition, 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. The timing of HIV infection can therefore be relevant to proving a case of reckless HIV transmission.

It is, however, worth emphasising that even where phylogenetic analysis is properly used in accordance with the standards set out in this paper, matters of timing will remain to be proved where there is the possibility that transmission took place either before the diagnosis of the defendant or after the complainant was made aware of the defendant's HIVpositive status (because such awareness will be relevant to the question of whether the defendant can raise the defence of consent). There are, therefore, real complexities in proving the fact, timing and direction of HIV transmission between two people.

Proving the fact of HIV transmission (including both direction and timing) beyond reasonable doubt will, as the remainder of this briefing paper makes clear, ordinarily require a combination of scientific and other evidence, such as the documented sexual health histories of both defendant and complainant. Consequently, it is extremely important to understand the degree of weight and certainty that can be attached to phylogenetic analysis alone.

There is also an offence of intentional HIV transmission under section 18 of the OAPA 1861. No prosecutions for this offence have as yet taken place. Being a distinct offence, different facts need to be proved in court from those in reckless transmission cases. But to the extent there is a need to provide scientific evidence of virus relatedness between two parties, the content of this briefing paper applies. There is also a separate common law offence in Scotland of 'reckless injury' under which prosecutions have occurred. Again, the contents of this briefing paper apply in any attempt to use phylogenetic analysis to provide evidence of virus relatedness and direction of transmission.
http://www.cps.gov.uk/news/consultations/sti_policy.html

PHYLOGENETIC ANALYSIS IN HIV FORENSICS: A BRIEF HISTORY

Phylogenetic analysis has taken on increasing importance as legally admissible evidence in the tracking and investigating of events leading to HIV infections, also known as HIV forensics.

Phylogenetic analysis, as used for HIV forensics, first entered mainstream public awareness in 1990, when the United States Centres for Disease Control (CDC) began investigating the alleged transmission of HIV between a Florida dentist and his patients during the course of routine invasive dental surgery³. The investigation lasted two years, during which time the dentist and some of his patients subsequently died. Although the CDC's reports⁴,⁵ and an independent review concluded that up to six patients may have been infected by the dentist, questions persist regarding the methodology used⁶, and there were no criminal charges 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 due to ill health. Phylogenetic analysis by CDC investigators concluded 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 male had already been convicted of rape and 'deliberate' transmission of HIV in the Stockholm district court, without any 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, Stockholm, to determine the degree of relatedness between the strain of HIV in the suspect and the alleged victim. On the basis of their 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

Phylogenetics (from the Greek *phylon*, meaning tribe or race and *genetikos*, meaning relative to birth) is the field of biology that studies and identifies the evolutionary relationship among the many different kinds of life on earth.

Phylogenetic analysis examines small differences in HIV's genes using computational methods to calculate the genetic distance between strains. Unlike human DNA, which remains stable for a lifetime, HIV's RNA changes very rapidly, leading to a huge amount of genetic diversity. This diversity means that scientists, using phylogenetic analysis, have been able to ascertain where HIV comes from, as well as track the various strains of HIV that exist worldwide.

³ CDC. *Possible transmission of human immunodeficiency virus to a patient during an invasive dental procedure.* MMWR 39: 489-93, 1990. Available at http://www.cdc.gov/MMWR/preview/mmwrhtml/00001679.htm

⁴ Ciesielski C et al. Transmission of human immunodeficiency virus in a dental practice. Ann Intern Med 116: 798-805, 1992.

⁵ Ou CY et al. Molecular epidemiology of HIV transmission in a dental practice. Science 256: 1165-1171, 1992.

⁶ 5. Altman LK. AIDS *mystery that won't go away: did a dentist infect 6 patients?* New York Times, 5 July 1994. Available at http://query.nytimes.com/gst/fullpage.html?sec=health&res=9C02E0DB1E3CF936A35754C0A962958260

⁷ Jaffe HW et al. Lack of HIV transmission in the practice of a dentist with AIDS. Ann Int Med 121 (11): 855-859, 1994.

⁸ Myers G. Molecular investigation of HIV transmission. Ann Int Med 121 (11): 889-890, 1994.

⁹ Albert J et al. *Analysis of a rape case by direct sequencing of the HIV-1 pol and gag genes*. J Virol 68: 5918-5924, 1994. Available at http://jvi.asm.org/cgi/reprint/68/9/5918.pdf

In the 1997 case of State of Louisiana vs. Richard J Schmidt, a doctor was alleged to have tried to kill his former mistress by injecting her with HIV (and hepatitis C) -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 admissibility¹⁰. Dr Schmidt was found guilty of attempted second-degree murder and the verdict was appealed to the Louisiana State Supreme Court, where it was upheld in 2000. In March 2002, the United States Supreme Court also rejected an appeal. 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

Since then, several other jurisdictions have allowed phylogenetic analysis to be utilised 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 man¹²; a man sentenced to six years imprisonment in Denmark for sexually abusing a 12 year-old boy and also transmitting HIV¹³; and a man prosecuted for raping and transmitting HIV to six women in Belgium¹⁴.

PHYLOGENETIC TESTING: WEIGHT OF EVIDENCE IN AN ENGLISH AND WELSH COURT OF LAW

The evidence of virologists who may be called upon to present the results of phylogenetic testing is expert evidence, which is considered a form of opinion evidence. Experts may give evidence within their area of competence, which may include explaining technical information, and to express an opinion about the significance of that information; but they not permitted to give their opinion on matters that are within the ordinary competence of the jury (the *Turner* rule)¹⁵.

Traditionally the common law prevented an expert witness from giving an opinion on the ultimate fact in issue (which in an HIV transmission case could, for example, be whether the defendant was the source of the complainant's infection). This appears to have been abandoned now, to all intents and purposes.

When expert evidence 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 answer that question¹⁶.

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 or not.

10 State of Louisiana vs. Richard J. Schmidt, 15th Judicial District Court, Lafayette Parish, LA, Criminal Docket No. 73313, *Reasons For Ruling* of Louisiana State 15th Judicial District Court Judge Durwood Conque (1997); State of Louisiana vs. Richard J. Schmidt, 699 So. 2d 488, K97–249 LA Court of Appeal, 3rd Circuit (1997); writ denied 706 So. 2d 451, 97–2220 LA (1997).

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¹³ Machuca R et al. *Molecular investigation of transmission of human immunodeficiency virus type 1 in a criminal case.* Clin Diagn Lab Immunol. 8(5):884-90, 2001. Available at http://cvi.asm.org/cgi/reprint/8/5/884.pdf

¹⁴ Lemey P et al. *Molecular testing of multiple HIV-1 transmissions in a criminal case.* AIDS19(15): 1649-1658, 2005. Available at http://www.aidsonline.com/pt/re/aids/pdfhandler.00002030-200510140-

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¹⁵ *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 judgment by the application of these criteria to the facts proved in evidence" (R v Gilfoyle [2001] 2 Cr App R 5)

PITFALLS IN THE USE OF HIV PHYLOGENETIC ANALYSIS FOR FORENSIC PURPOSES

The reliability of phylogenetic analysis to 'prove' HIV transmission between two individuals must be addressed in some detail. It is important that everyone involved in the criminal justice system is made fully aware of the limitations of phylogenetic analysis before using such evidence as conclusive or even suggestive of HIV transmission between two individuals. Phylogenetic analysis can and does include a certain degree of approximation and error.

• Phylogenetic analyses are generally carried out in research settings rather than forensic laboratories and there are only a few laboratories with forensic 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 and dual blind testing (see 'Acceptable standards', p7).

• For forensic purposes, at least two independent samples need to be blindly tested at two different time points, and the results between the time points should be consistent (see 'Acceptable standards').

• There are many different ways of constructing a phylogenetic tree (See appendix for explanation and examples.) 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. Several types of methods have been tested for HIV contract tracing for forensic purposes (see 'Acceptable standards').

• When constructing a phylogenetic tree for HIV forensic analysis, it is vital that the tree is as unbiased as possible. This includes choosing sufficient and appropriate epidemiological controls. This means analysing approximately 30 other strains of HIV from individuals who are from the same geographical origin, social context and potential transmission network as the defendant and complainant(s). These should then be compared with the strains under investigation. Using inappropriate controls may wrongly emphasise any relatedness detected between two viruses as being strikingly unique.

• In addition, the controls should be collected around the time of the alleged transmission event. This is crucial 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 (MSM). In most cases, it will be difficult and often impossible to obtain samples from the appropriate controls. As a result, interpretation of the findings will need to be particularly cautious.

• Current techniques are not reliable enough to estimate the direction of transmission. Research is being done in this area, but multiple samples would need to be obtained very soon after the presumed transmission event from both the defendant and the complainant, and full-length sequencing would need to be performed (see 1.7 in 'Acceptable standards').

• It is important to remember that similar strains may be found in many more than two individuals if they are both part of a wider transmission network. The majority of individuals with HIV are part of such a network. Consequently, even with the appropriate controls, phylogenetic analysis cannot 'prove' transmission. Yet, when there is statistical support to link the individual under investigation closer to one of the controls rather than to the complainant, the technique is reliable enough to exclude the possibility of transmission. Investigations can therefore exonerate suspected individuals.

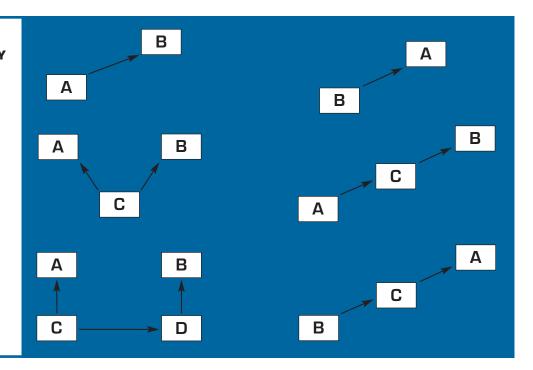
• When phylogenetic analysis appears to show a probable relationship between two parties, it is important to remember that this does not exclude other possibilities. All of the following circumstances can yield similar results in phylogenetic analysis (Fig 1):

• the defendant was infected by the complainant

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

FIG 1. HOW TWO INDIVIDUALS WITH PROBABLY RELATED HIV STRAINS MAY BE CONNECTED.

If we take two individuals (named A and B) that are infected with HIV that are probably 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 unknown third parties.



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. The process must meet the judicial standards for evidence admissibility.

1. Methodology¹⁷

1.1 HIV sequencing and the analysis of sequences to build phylogenetic trees is commonly done in research settings rather than in 'forensic' facilities used to handling samples under vigorous sample 'tracking' systems. Thus, it becomes paramount that precautions are taken to minimise the possibility of sample error (for example, through contamination or mislabelling). Maintenance of the chain-of-custody must, therefore, receive the highest priority and specimen movements must be closely recorded and rigorous protocols applied.

1.2 In addition, given that there are many ways of constructing and analysing phylogenetic trees, sequence analysis should be 'blinded': in other words

the person 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.

1.3 To minimise errors in sampling, and to confirm the results, two different samples need to be taken from the individuals in the investigation, at two different time points. However all time points need to be close enough to the time point of taking the control samples, e.g. within a few years.

1.4 The composition of the control population should be clearly stated. Controls should be derived from a relevant setting and should be temporally and geographically relevant to the cases under investigation. In addition, the samples should be taken from the same risk group and the appropriate social context. Thus, if a certain social network is apparent (i.e. club, cruising park, sauna, etc.) the controls should reflect this. Obtaining the correct controls, however, raises further issues related to consent for use of sequencing data and protection of sequence databases¹⁸.

¹⁷ For detailed methodological reviews of phylogenetic methods for HIV forensics, see: Leitner T and Albert J. *Reconstruction of HIV-1 transmission chains for forensic purposes.* AIDS Rev 2: 241-251, 2000. Available at www.aidsreviews.com/files/2000_2_4_241_252.pdf; Learn GH and Mullins JI. *The microbial forensic use of HIV sequences.* HIV Sequence Compendium 2003, Los Alamos National Laboratory: 22-37, 2004. Available at http://hiv-web.lanl.gov/content/hiv-db/COMPENDIUM/2003/partl/Learn.pdf; and Lemey P, Vandamme AM et al. *Molecular testing of multiple HIV-1 transmissions in a criminal case.* AIDS 19(15): 1649-1658, 2005. Available at http://www.aidsonline.com/pt/re/aids/pdfhandler.00002030-200510140-00012.pdf

¹⁸ Further discussions regarding the difficulties around consent can be found in Anderson J et al. *HIV transmission, the law and the work of the clinical team: a briefing paper,* available at www.bhiva.org.

1.5 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.

1.6 At least two genetic regions should be sequenced of reasonable length (\geq 500 nucleotides, depending on the gene under investigation). Selection should target genes with different biological functions, different rates of evolution, different selective pressures. In particular, care should be taken when using the *pol* region for patients under therapy, since similar treatment regimens can drive the virus to accumulate similar mutations, causing an apparent relatedness in the absence of direct epidemiological link. This problem can be addressed by excluding drug resistance positions from the analysis as described in Lemey et al.¹⁹.

1.7 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. In addition, rigorous statistical analyses should be performed.

2. Interpretation

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.

2.1 Phylogenetic trees cannot be 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. They 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 other 'partners' involvement in the transmission chain?"

2.2 The appropriate selection of controls will increase confidence that the relationship observed reflects a true direct transmission. However even with the best controls, 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, which would be

'beyond reasonable doubt'. Even if statistical support for a closer link between the investigated individuals, compared with the controls, is 100%, this does not imply that the evidence for a direct transmission is 100%. As stated before, it all depends on the controls, and an unknown third party can never be excluded.

2.3 The first use of phylogenetic analyses of HIV sequence as admitted evidence in a US criminal court showed the following key aspects²⁰:

• Clear evidence of possible transmission between two individuals was available prior to phylogenetic testing

• Appropriate controls were obtained from the local population

• Independent testing was carried out by different laboratories

• The evidence provided by phylogenetic analysis was only part of the prosecution's case.

Thus, the appropriate interpretation would include the following questions:

• Have the appropriate controls been included?

• Are the two viruses more related to each other than to the controls?

• Is there anybody else who is, or could be, also related?

• Is there any other epidemiological evidence of linkage between individuals?

2.4 Even in cases in which patterns are consistent with a direction of transmission from the defendant to the complainant, it may be impossible to know with certainty that transmission occurred directly from one to the other without an intervening individual.

2.5 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" and statements should include the possibility that an unknown third person might be involved, and that the direction of transmission cannot be proven.

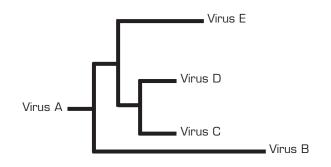
¹⁹ Lemey P, Vandamme AM et al. *Molecular footprint of drug-selective pressure in a human immunodeficiency virus transmission chain.* J Virol. 79(18):11981-9, 2005.

²⁰ Further detailed discussion regarding the limitations of phylogenetic analysis can be found in Budowle B and Harmon R. *HIV legal precedent useful for microbial forensics.* Croat Med J 46 (4): 514-521, 2005. Available at http://www.cmj.hr/2005/46/4/16100753.pdf

APPENDIX

Phylogenetic trees

Phylogenetic trees are scientific illustrations that represent the results of phylogenetic analysis. They show pictorially the relationship between different strains of HIV. The concept of a tree – with roots and branches – comes from early ideas of life as a progression from lower (older) to higher (more recent) forms (hence the term 'family tree'). HIV virologists use phylogenetic trees to depict HIV's relatedness because they effectively capture the idea that changes occur through the splitting of common ancestors. In this simple phylogenetic tree, you can see how different viruses are related to each other. All of the viruses are descendents of Virus A, but Viruses C and D are more closely related to each other than to Viruses B and E.

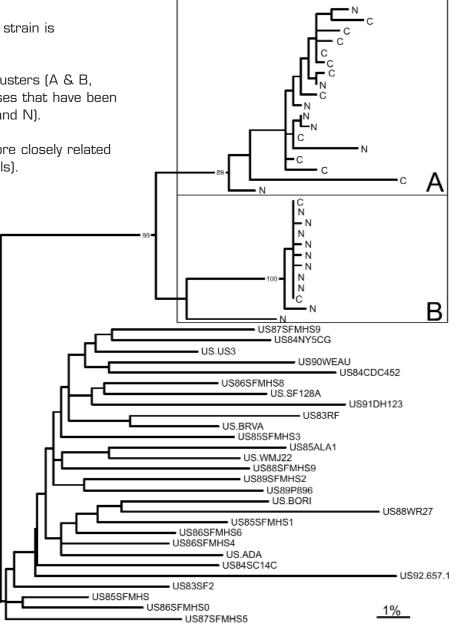


Example of a phylogenetic tree.

In this phylogenetic tree each HIV strain is represented by a branch.

You can see that there are two clusters (A & B, highlighted) of closely related viruses that have been found in two individuals (called C and N).

The two clusters appear to be more closely related than the other strains (the controls).



HIV'S GENETIC DIVERSITY

Types, groups, subtypes, recombinants, and *quasispecies* are scientific terms used to classify different strains of HIV, from the global, regional and country level (types, groups, subtypes/recombinants) to the individual level (quasispecies).

There are two *types* of HIV that infect humans – **HIV-1** and **HIV-2**. Both HIV-1 and HIV-2 are descendants of SIV (simian immunodeficiency virus) found in wild chimpanzees in Cameroon, in western Africa. HIV-1 is the type of HIV that is seen globally, whereas HIV-2 is limited predominantly to the areas around Cameroon.

HIV-1 is further classified into three main *groups* called M, N and O. Again, it is group M that is seen globally, whereas groups O and N are limited predominantly to the areas around Cameroon.

Group M viruses are again further classified into *subtypes* (represented by letters of the alphabet, e.g. A, B, C) and *recombinants*, which are a combination of two subtypes. Recombinants are officially known as circulating recombinant forms (CRF) and represented by a number followed by the two combined subtypes (e,g. CRFO1_AE, is a combination of subtypes A and E).

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NAM is an award-winning, community organisation, based in London. We deliver reliable and accurate HIV information across the world to HIV-positive people and to the professionals who treat, support and care for them.

NAM exists to support the fight against AIDS with independent, accurate, accessible and comprehensive information. We aim to create and disseminate information resources rooted in the experience of those most affected, enabling individuals and communities to take action and control in responding to HIV and AIDS.

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