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BRIEF 2 of 3

Practical uses of HIV phylogenetics in public health

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Introduction

Phylogenetic approaches have been used to study HIV biology and epidemiology since the early 1980s. In the last 15 years, advances in sequencing have led to an increase in the information obtained and a sharp drop in costs, making it feasible to use phylogenetics in larger studies and public health HIV surveillance programmes.

This is the second brief in a series of three on HIV phylogenetics. The first brief, *The Application of Phylogenetics to HIV—Insights into Biology and Epidemiology of HIV*, explains the methods and approaches used to generate genetic and phylogenetic data and analyses. This brief summarises the ways in which phylogenetic (and other sequence-based) analyses are currently being used and reflects on how they could be used in the future in the field of HIV research and public health. The third brief discusses the ethical challenges of using HIV phylogenetics in research and public health surveillance.

The ability to study and compare the genetic make-up of pathogens such as HIV has revolutionised infectious disease biology. Phylogenetic analyses have made it possible to determine if pathogens found in two individuals are closely related or not, thus allowing us to learn more about how pathogens travel between humans and animal hosts and how they change in the process. In the case of HIV, some information can be obtained directly from a single sequence, for example the drug resistance profile. However, most public health-relevant information derived from sequences is relational, i.e., it is obtained by comparing a set of sequences. Phylogenetic techniques aim to understand how sequences are related and which process of evolution could have given rise to the sequences that we see in a data set. Genetic clustering approaches typically compare only the differences between the sequences and do not make any assumptions about how these changes might have arisen. They can often be run on a personal computer whereas phylogenetic analyses on large datasets usually require a high-performance computer. Genetic clustering provides less detailed results, but also generates less data that is potentially sensitive. Both phylogenetic and clustering approaches have been used for HIV public-health related research and surveillance programmes, often in combination. Table 1 provides an overview of use cases and the insights they have provided. These will be discussed in more detail in the following sections.

Table 1.

Insights from and use cases for phylogenetic and other sequence-based analyses

Technique	Area of work	Insights / use cases	Ref.
Analysis of individual sequences	Drug resistance profiling	Starting patients on second-line therapy if their virus is resistant to current treatment; monitoring evolving drug resistance at the population level.	Many
	bnAb* resistance profiling	Active area of research—predicting if bnAbs would benefit an individual patient, designing optimal bnAb combinations based on circulating variants.	[1–3]
Phylogenetic approaches	Origins and subtypes of HIV	Helped people understand where HIV was coming from, reduced stigma for individuals and groups associated with HIV transmission at the beginning of the epidemic. Monitoring the global spread of HIV and the spread of subtypes with different clinical properties in a population.	[4–14]
	Estimating recency of infection and incidence	Active area of research—phylogenetic estimates for recency of infection can help to better estimate incidence.	[15–19]
	Quantification of risk factors for individual transmission	Established that $U=U^{**}$ for both heterosexual couples and men who have sex with men. Demonstrated that the risk of HIV acquisition per sex act is low, that acquisition risk increases with increasing viral load and that circumcision decreases the risk of acquiring HIV but not of passing on the virus.	[20–34]
	Identifying correlates of transmission at the population level	Estimating from which age and sex group most transmissions originate from to focus prevention efforts, assessment of which fraction of transmissions comes from neighbouring regions / countries to assess local / in-country testing and treatment programmes, assessment of which fraction of transmission arises from recent infections to support decisions about testing strategies for different populations.	[35–57]
	Assessment of population clinical trial outcomes	Estimating the fraction of transmission in clinical trial communities that originated from control communities or from outside the trial area to assess how successful the trial could have been if conducted in a larger area.	[56–58]
	Understanding HIV outbreaks	Assessing how HIV spread during the outbreak and which changes to services and messaging could have prevented the outbreak.	[59–65]
Clustering approaches	Public health molecular surveillance programmes	Identifying outbreaks quickly, increasing messaging in affected communities, deploying additional resources to linking people who acquired HIV during the outbreak to care quickly.	[36,47,50, 63,66–74]

* broadly neutralising antibodies
 ** Undetectable = untransmissible

Analyses based on individual sequences

Some analyses can be performed on individual sequences in a dataset, without the need to consider their relationship to other sequences in the dataset. These include drug resistance analyses and estimation of susceptibility to broadly neutralising antibodies.

Drug resistance profiling

If HIV replication is not fully suppressed in an individual taking antiretroviral therapy (ART), variants of the virus can evolve that are drug resistant. The variants carry changes in the genetic code that appear as drug resistance mutations in the HIV sequences isolated from this individual. The number of virus particles (viral load) can increase sufficiently for this person to become infectious again and at risk of developing AIDS. A person who is living with a drug-resistant strain of HIV may not become virally suppressed if the resistance is for the same drugs used for their treatment. In many countries, the relevant parts of the HIV genome are sequenced routinely to identify a treatment regimen that will suppress the replication of each patient's virus. In limited resource settings, this knowledge is often only available through research studies. Monitoring drug resistant mutations in a population is important to inform standard treatment guidelines. These analyses can be conducted without using phylogenetics. However, phylogenetic methods can be used to distinguish between people who developed drug resistance during treatment and those who contracted a drug-resistant virus. This knowledge can inform public health decisions on how often viral load tests should be used in a clinic.

Predicting resistance to bnAbs

The genetic sequence of a given virus can also be analysed to show if it is likely to be resistant to broadly neutralising antibodies (bnAbs). BnAbs are antibodies generated by certain individuals living with HIV which, when given in combination, can suppress viral load in other individuals [1,2]. In addition to their therapeutic potential, bnAbs will be the kind of antibodies that a successful preventive HIV vaccine will need to elicit. Thus, using sequences from all areas of the world for these analyses is important for developing vaccines that work everywhere. Determining whether a virus with a given sequence is likely to be resistant or susceptible to a given bnAb or a panel of bnAbs is much more complicated than determining if a virus is resistant to antiretroviral drugs, and is an active area of research using machine learning [3].

Phylogenetic analyses

Phylogenetic analyses aim to determine the evolutionary relationship between sequences. They are therefore always performed on a group of sequences. In the context of HIV, public-health related uses include determining the origins of HIV, subtype analyses, estimating recency of infection, studying individual factors for HIV transmission, identifying unrelated samples in randomised control trials to assess the effectiveness of antiretroviral therapy (ART), and learning more about where in population transmissions are occurring. Phylogenetic techniques are mostly used in research that aims to inform public health but have on occasions also been used by public health agencies. HIV phylogenetics has also been used in court cases to disprove genetic linkage between individuals or to support genetic linkage where other evidence existed. Ethical challenges in the use of HIV phylogenetics are discussed in the third brief of this series.

Origins and subtypes of HIV

One of the first applications of phylogenetics to HIV was the reconstruction of its origins. Phylogenetic analyses revealed that there were two different human immunodeficiency viruses—HIV-1 and HIV-2 [4–6]—and showed conclusively that HIV had originated from equivalent viruses found in non-human primates, called Simian Immunodeficiency Viruses (SIVs) [7–10]. When worries surfaced that HIV had been transmitted via chimpanzee cells harvested locally to produce oral polio vaccine and threatened to slow the polio eradication campaign, phylogenetics could show that the SIV circulating in local chimpanzees was very different from circulating HIV strains and ruled out that the vaccine was a source of HIV transmission [11]. Phylogeography, which combines phylogenetics with geographical information, showed that the pandemic strain of HIV-1, group M, originated in Kinshasa in the 1920s [12]. Until the 1960s, this HIV-1 lineage mostly spread in sub-Saharan Africa. From Africa, HIV spread to Haiti, most likely due to the return of Haitian professionals who had been working in the newly independent Congo in the 1960s [13], and eventually reached the USA, Europe and then the rest of the world. At a time when very little was known about the new virus, the first phylogenetic analyses provided an understanding of where HIV had come from and reassured people that introductions into the human population were rare. The analyses showed that HIV had been introduced to different countries on different occasions and that it wasn't confined to the groups it had first been identified in, like Haitians and men who have sex with men, thus dispelling myths that had increased stigmatisation of these groups.

Building phylogenetic trees of viruses from different locations led to the description of different HIV subtypes: groups of viruses that are more closely related to each other than to other HIV samples. Using this established framework, each patient's virus can be assigned to a subtype. This is of interest when different subtypes circulating in a population are known to have different disease progression, like subtype A and D viruses circulating in East Africa [75,76]. Within each subtype, many different variants

exist. These do not differ in the disease they cause as much as variants of SARS-CoV-2 do, although recently a variant which leads to faster disease progression has been identified in the Netherlands [14].

Estimating recency of infection

Recency of infection is usually determined by diagnostic tests. These tests are adversely affected by several factors like variability in immune responses and biomarker progression as well as use of ART and pre-exposure prophylaxis (PrEP), which can lead to a substantial overestimation of recent infections [77]. In the last few years, methods have been developed to use sequence-derived estimates of recency of infection alone or in combination with other tests. The estimates are based on how genetically diverse the virus population isolated from an individual is [15–19]. The greater the diversity of HIV within an individual, the longer since the individual likely acquired HIV. Currently, these sequence-based methods are not accurate enough to be used on an individual level, but when used in conjunction with other methods, they can help to establish how many people recently acquired HIV in a given population. This is an active area of research and further advances could lead to more accurate and less costly incidence estimates.

Quantifying risks for individual transmission

Once it was established that HIV could be transmitted through sexual intercourse, a stream of research embarked on identifying the risk factors associated with transmission, mostly in established cohorts in Africa. Many of these studies enrolled serodiscordant couples—i.e., couples in stable partnerships in which one partner tested positive for HIV and the other tested negative—at a time when ART was only given in the later stages of disease. When sequencing became available, the studies started to use phylogenetics to determine if, in the case of a transmission event, the HIV sequences of both partners were dissimilar enough to assume that transmission had occurred outside the partnership, which made the analysis more accurate.

Insights from studies using phylogenetics, among other techniques, included: the per-contact risk of acquiring HIV is low [20]; viral load is an important predictor of transmission risk [21], confirming previous studies; and viral loads are similar between transmission pairs [22]. Also, the couples studies showed that even though male circumcision was previously shown to reduce HIV infection in males, it did not reduce the incidence in their female partners [23], i.e., circumcision reduced the probability of acquisition but not the probability of onward transmission. The studies also broadened general understanding of the virus, comparing the transmissibility of variants [24,25], confirming that usually only a single genetic variant is transmitted and goes on to establish a productive infection [26] and that the risk of acquiring HIV again after already being infected is relatively higher than had previously been recognised [27].

Later, when ART became available, couples studies crucially demonstrated that ART was effective in stopping transmission [29–34]. These were randomised control trials that used phylogenetics to assess the outcome of the study. One was the HPTN 052 study on the effect of ART on transmission [32,33], which took place in nine countries across three continents between 2005 and 2011, monitoring the impact of ART on HIV transmission between couples in which one partner was HIV positive. Out of 39 acquisitions that occurred during the study, only four occurred when the individual's HIV positive partner was on ART, and phylogenetic analysis revealed that in three out of the four cases HIV had not been transmitted from the partner on ART. The study concluded that regular use of ART reduced the chances of transmission to a partner

by approximately 96%. This showed that ART, in addition to providing individual clinical benefits, is a highly effective tool to prevent new HIV infections. This insight is the basis for the “undetectable means untransmittable” (U=U) campaign. HPTN 052 was followed by the PARTNERS [31] study, the Opposite Attract study [29] and the PARTNERS2 [30] study, which together established that ART is also highly effective at preventing transmission between men who have sex with men, recording no transmission from individuals taking ART as prescribed. Without phylogenetic analysis, these findings would not have been so categorical, and advice would have to be more nuanced.

Identifying correlates of transmission at the population level

Population-level phylogenetic analysis offers a way to understand how HIV spreads in a population and where services need to be improved to reduce ongoing transmission. Most studies have taken place in Europe and North America. In these regions HIV epidemics are concentrated rather than generalised (prevalence is less than 1% of the overall population) and most transmission occurs between members of key populations—mostly men who have sex with men and people who inject drugs, but also transgender women and sex workers and their clients [35,36]. These networks can be highly connected [37,38] and can experience a growing epidemic even when prevalence in the rest of the population is extremely low.

Phylogenetic analyses of epidemics of men who have sex with men have helped to understand the dynamics of social and sexual networks, for example how individuals interact within and between age groups and ethnicities [39,40]. The studies showed that membership of a cluster (geographically close, or epidemiologically linked HIV diagnoses) was, among other things, correlated with not being on ART, having transmitted drug resistance, having a high viral load and having an acute infection. Other approaches have identified risk factors for transmission and acquisition of HIV in a fast-growing sub-epidemic or cluster [41–45]. Risks identified in several studies included being a man who has unprotected sex with men, being recently diagnosed with HIV, having an acute HIV infection, living with another sexually transmitted infection, and having previously injected drugs. These studies can help to identify where testing and treatment services are failing to reach individuals who have recently acquired HIV or are at risk of acquisition.

Phylogenetics has also been used to estimate the fraction of infections that were acquired locally rather than from abroad or from a different community [46–50]. These data can be used to assess if local or in-country prevention programmes are working and to offer testing to groups who are at risk of acquiring HIV abroad. Another important question in HIV prevention is the best frequency of seeking HIV testing. A study in the Netherlands used phylogenetics to estimate the fraction of transmissions that originated from individuals who had recently acquired HIV themselves and were unaware of their positive status [51]. Another study estimated risk factors for being a source in a transmission pair, and the fraction of these infections that could have been prevented by making changes to testing and treatment policies [50].

Recently, phylogenetic studies became available in settings experiencing a generalised epidemic, where more than 1% of the population is living with HIV. A study in South Africa addressed the origin of infections in young women [52]. Soon after, two studies analysing the transmission dynamics between inland and fishing communities in Uganda showed that contrary to common belief, the marginalised fishing communities were not a source of new cases in the inland communities, but had a net influx of infections [78,79]. These and other studies highlighted that phylogenetics can help to confirm or refute assumptions of where infections originate.

Phylogenetic studies linked to two of the major universal test and treat population trials, HPTN 071 (PopART) [53] and Ya Tsie [54,55], addressed a range of questions that can be answered from a large population sample but not by the main trial. HPTN 071 PopART Phylogenetics took place in Zambia between 2015 and 2018 and phylogenetically determined the fraction of transmission from different age and gender groups, from recent versus non-recent infections, from inside and outside the study communities, and from drug resistant viruses. The analysis showed that men living with HIV infect more women per capita than vice versa, in particular those aged between 25 and 40, and that the majority of infections arose from partnerships in which the source does not belong to a high-risk group [56]. The phylogenetic analysis of the Ya Tsie trial produced similar results, and also emphasised that relationships between partners registered in different trial arms can make the intervention seem less effective than actually is the case [57].

Assessment of population trial outcomes

The phylogenetic studies linked to HPTN 071 (PopART) [56] and Ya Tsie [57] showed that a phylogenetics component can help interpret the results of the main trial and assess how much an intervention would add if rolled out in a larger geographical area. In addition to analysing the residual sources of HIV transmission, phylogenetic analyses can be used to monitor the spread of acquired versus transmitted drug resistance. These analyses can help to assess how well certain drug combinations are working at the population level, and whether a new regimen needs to be introduced before drug resistance to established backbones becomes too widespread to be brought under control [58]. Phylogenetic analyses are likely to become an integral feature of large population trials in the future.

Understanding HIV outbreaks

HIV phylogenetics and clustering approaches have also been used to study outbreaks, often in networks of men who have sex with men and among people who inject drugs. An HIV outbreak is loosely defined as an increase in the rate of transmission in an area or a sexual or social network. Until recently, outbreaks were mostly studied retrospectively. Men who have sex with men are at risk of acquiring HIV in an outbreak as networks are often highly connected. People who inject drugs are at risk both from sharing needles and from engaging in sexual activity within the same network. Several studies have used phylogenetics to study outbreaks among people who inject drugs in Greece, Romania, Scotland and the USA [59–62], and among men who have sex with men in the USA [63,64]. The studies showed that transmission in highly connected networks can be rapid, and that needle-exchange programmes are needed to reduce transmission among people who inject drugs. The outbreak in Scotland led to a reassessment of how HIV care was implemented for these groups. HIV services were established closer to the affected communities and ART was made available via community pharmacies. The new model reduced time to ART initiation from 264 to 23 days and increased viral load suppression rates from 35% to 86% [65]. Increasingly, sequencing and phylogenetic analyses are carried out in real time to support the outbreak response and minimise further infections.

Clustering approaches

Clustering approaches aim to group objects such that objects in the same group are more similar to each other, and objects in different groups are less similar, according to some measure of similarity. For genetic clustering, similarity between sequences can be based on their closeness in a phylogenetic tree, but also on how many mutations distinguish them. While phylogenetic approaches always aim to describe how sequences are related to each other, clustering approaches often group sequences without making any assumption on how this similarity has arisen. In a transmission analysis, a phylogenetic approach would make assumptions about who infected whom while a clustering approach might only state that within a cluster, all sequences are closely linked to each other. Compared to phylogenetic approaches, clustering approaches therefore generate less rich information, but are faster, require less computational power and return less sensitive results as they make no assumptions about the direction of transmission. All of these features are useful for applications in public health surveillance.

Public health molecular surveillance programmes

In many countries, HIV sequencing is routinely carried out to determine the drug resistance profile of the virus at diagnosis and during care. In the last few years, sequencing has become fast enough to use clustering and phylogenetic approaches to study outbreaks in real time and thus support the public health response. Some of the programmes have met with opposition, criticising, among other things, the lack of informed consent, the inability to opt out, and the risk of stigmatising marginalised groups further [66,74]. Recommendations for best practice in the use of HIV phylogenetics in public health are discussed in the third brief of this series.

The United States Centers for Disease Control and Prevention, for example, uses cluster detection and response (CDR) as part of the US Government's Ending the HIV Epidemic in the US initiative [67,68,80,81]. CDR relies on genetic analysis of routinely collected HIV sequence data to identify clusters or groups of genetically similar HIV sequences that point to rapid HIV transmission within a closely related time frame or geographical area [67]. This is done using computational tools that approximate transmission chains based on the genetic relatedness between HIV genetic sequences. Health officials monitor these cluster data to quickly detect and disrupt emerging outbreaks, and to prioritise HIV service delivery to people who acquired HIV during the outbreak [67]. Phylogenetic approaches have also been used by public health bodies and/or affiliated research teams to gain an insight into the nature of local transmission networks as they evolve over time [36,47,50,69–72]. Public health cluster detection response and phylogenetic molecular surveillance are currently used in several countries, including Canada, and China and the USA [63,73,82].

Conclusion and Outlook

HIV phylogenetic analyses have been used in public-health related research since the beginning of the epidemic. Phylogenetic analyses have contributed to understanding the biology of HIV and individual risks for transmission among other things. They have helped to show that U=U, to monitor the emergence of drug resistance, to find and address gaps in the care system, and to design more effective prevention interventions.

The recent widespread use of phylogenetic analyses of SARS-CoV-2 for public health purposes during the COVID pandemic has firmly established phylogenetics in the public health tool kit. Many countries have expanded their sequencing capacities and will likely also consider using them for the surveillance of endemic diseases. Currently, systematic evaluations of national HIV molecular surveillance programmes are still pending. Once undertaken, these will show if molecular surveillance is a safe and effective tool to stop outbreaks and link people living with HIV to care in different populations. These programmes are likely to work best if patient privacy is preserved as much as possible, aims of the programme are clearly communicated, and non-health related uses of the data, e.g., by law enforcement agencies, are ruled out. It will be key to engage with all groups of affected communities, explain the technology and the associated risks and benefits, and address any concerns.

As incidence is declining in many countries, new strategies are needed to reach those that are not currently benefiting from HIV programmes. Used in the right way, HIV phylogenetics can help to identify gaps in prevention and treatment. For more background on phylogenetics, see the first brief in this series “The application of phylogenetics to HIV—insights into biology and epidemiology of HIV.”

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UNAIDS
Joint United Nations
Programme on HIV/AIDS

20 Avenue Appia
1211 Geneva 27
Switzerland

+41 22 791 3666

unaids.org