# What role does HIV-1 subtype play in transmission and pathogenesis? An epidemiological perspective

**Dale J. Hu, Anne Buvé[a], James Baggs[b], Guido van der Groen[a] and Timothy J. Dondero**

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## Introduction

HIV exhibits an extremely high genetic variation with rapid turnover of virions [1,2]. To date, two major viral types have been characterized: HIV-1, the predominant HIV type throughout the world; and HIV-2, first reported and still found primarily in persons from west Africa [2–4]. A system that is currently widely used for classifying HIV-1 and HIV-2 into a number of subtypes is based primarily on genetic sequences coding for the envelope (Env) and structural (Gag) proteins and methods to infer the phylogenetic relationships between them [5–10].

The implications of this variability have been reviewed previously [2,11,12]. In particular, the potential role of HIV genetic variation in influencing transmissibility and pathogenesis has been at the center of debate and the focus of research interest in recent years. In this paper, we describe, primarily from an epidemiological perspective, a framework with which to assess the potential role of virological factors in influencing HIV-1 transmissibility and pathogenesis. The background of this framework is based on a number of well-developed principles of infectious disease epidemiology, which is the study of the distribution and determinants of infectious disease in different populations [13,14]. Within this framework, we will focus on HIV-1 subtype as the main 'risk' factor of interest. As we will assume that most, if not all, variants of HIV-1 are transmissible and pathogenic, our outcomes of interest are the relative variability observed in the transmissibility and pathogenicity of HIV-1. From this perspective, we will review the current epidemiological and biological evidence related primarily to HIV-1 genetic variation and potential subtype differences in transmissibility and pathogenesis. In addition, we would like to show that not only is it extremely difficult to find consistent associations between HIV-1 subtype and correlates of transmission and pathogenesis, but that it is highly unlikely that a single characteristic such as subtype can account for significant differences in transmission and disease progression.

## Epidemiological framework

An important initial step is to establish if there are consistent and strong associations between HIV-1 subtype and observed differences in transmissibility and pathogenesis. The second and more difficult step is to evaluate critically, within the context of other contributing factors, whether any association between an exposure or factor and an outcome, such as increased transmissibility and/or pathogenesis, is a cause and effect relationship. Because an association can be produced by mechanisms other than causation such as bias, confounding, and chance, we must consider these possibilities in our evaluation of different factors [14].

From the Division of HIV/AIDS Prevention, National Center for HIV, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, USA, the [a]Institute of Tropical Medicine, Antwerp, Belgium, and [b]EDS, Plano, Texas, USA.

Requests for reprints to: Dale J. Hu, International Activities Branch, Division of HIV/AIDS Prevention, NCHSTP, Centers for Disease and Prevention, E-50, Atlanta, GA 30333, USA.

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In assessing the potential causative role of a factor in affecting transmissibility or pathogenesis, we need to define a causal or risk factor as one which increases the frequency or probability of an event or outcome occurring. For example, it has been clearly established that HIV-1 is the etiologic agent of the long-term infection that leads to progressive immunodeficiency and eventual AIDS. However, it is not clear what specific virological properties contribute to the outcomes of increased transmissibility or faster disease progression. When the outcome is not an 'absolute' dichotomous outcome such as infected versus not infected, but is a relative increase or decrease in transmissibility or disease progression, assessment becomes more difficult.

In any epidemiological study, the first important consideration is the selection of an appropriate comparison group. Secondly, the sample size must be large enough to permit study to detect a statistically significant difference between groups; if the magnitude or strength of an association is large, then a smaller sample is sufficient. Comparisons between groups which differ on one or more important factor(s) may lead to misleading findings due to confounding caused by unmeasured factors. Confounding is defined as a distortion of an exposure–outcome association brought about by another factor which is independently associated with both the outcome and the exposure [13,14]. Finally, even a true causal factor may have a very small effect because the factor may be one of many factors contributing to a particular outcome such as more rapid disease progression.

A number of widely used criteria are used to evaluate the likelihood that an observed association between exposure and outcome is causal [14,15]. The applicable criteria include the consistency of an association such that the likelihood of causality is increased when different studies give similar relationships. Similarly, the stronger an association is, the less likely it is to be due to any of a number of error sources that may distort the results [15]. In general, findings and associations should be biologically plausible and make sense in the context of the current biological knowledge of risk factors and transmission and disease outcomes. Although stringent criteria are required to establish a causal relationship between a risk factor such as HIV-1 subtype and variations in HIV transmissibility and pathogenicity, there are, of course, many important relationships which are not necessarily cause and effect. Establishing the existence of such associations and ascertaining the meaning of such relationships can still be very useful.

Transmissibility is particularly difficult to evaluate because the most direct measure – per sex act transmission probability – can only be estimated indirectly by prospective follow-up of uninfected contacts of known infected persons. This approach is, in turn, biased because those persons for whom transmission already (and perhaps more readily) occurred are excluded from the study cohort. Prospective couples studies may be used to determine the probability of transmission assuming that a susceptible person's risk is limited to their sexual relationship with the known HIV-infected partner [16]. However, it is still difficult to control for potential confounding factors which may affect transmission such as the frequency and nature of sexual activity, condom use, presence of other sexually transmitted infections, and duration of infection in the index partner. Cross-sectional and case–control couples studies may be used to evaluate risk factors for transmission. Whereas absolute rates or probabilities of transmission cannot be determined, relative rates (more or less transmissible) may be determined after appropriate adjustment for confounding factors. The probabilities and risk factors for other modes of HIV-1 transmission such as mother-to-child or intravenous routes can also be evaluated through prospective and cross-sectional designs.

In evaluating pathogenesis and disease progression, differences in the probability of a particular event, such as death or stage of immunodeficiency, would be compared over time. Ideally, to minimize potential confounding, these studies require prospective follow-up of comparable groups of individuals which differ mainly in the particular risk factors of interest. Cross-sectional studies may compare the proportions of persons at certain stages of immunodeficiency in similar groups to infer potential risk factors related to disease progression. The studies make the general assumption that persons are infected for similar average lengths of time although this assumption is difficult to verify. Thus, whereas cross-sectional studies are easier to perform than prospective studies, they are also subject to more bias [14]. In any type of study, internal validity concerns the validity of the inferences to the target or study population and external validity refers to generalized inferences beyond the study population. In view of the epidemiological framework just outlined, we will examine examples from the relevant literature concerning HIV-1 subtype and possible differences in transmissibility and pathogenicity.

## Molecular epidemiology of HIV

The global molecular epidemiology of HIV has been reviewed in detail elsewhere based on extensive international efforts to collect and characterize HIV isolates from around the world [2,17,18]. However, as the thousands of isolates which have been characterized were usually obtained from non-systematic samples of convenience, often from persons at later stages of infection, and are orders of magnitude less than the millions

of HIV-infected persons in the world, they may not be representative of the actual distribution of HIV strains.

It would be expected that as information on HIV variation increases as more viral isolates are characterized (especially through full-length genome sequencing), taxonomic classifications will be continually modified. Furthermore, as the amount of variation between strains may differ in certain parts of the genome, classification schemes may vary depending on what particular portion of the genome is chosen for comparison. For example, possible recombination events were first detected when phylogenetic analyses of isolates differed by which gene or region within the same gene was used for analysis. Because of the potential for coinfection and genetic recombination between distinct viral strains, both a wider distribution of recognized strains and the existence of yet more divergent variants seem likely [19–22].

Despite these caveats and limitations, a broad picture of the distribution of HIV strains around the world emerges. One important observation is the nonuniform distribution of the various subtypes of HIV-1 and HIV-2. The greatest diversity of HIV strains, so far, has been found in sub-Saharan Africa, especially in central Africa, with almost all known HIV-1 strains found in this region [2,12,17,23–28]. Although the diversity of HIV strains appears to be increasing all over the world, the degree of heterogeneity in most regions of the world is much less than that in central Africa. Let us examine two examples.

One of the first and most completely documented epidemics was the extensive spread of HIV-1 in Thailand, where studies clearly documented the independent introductions and spread of two different HIV-1 subtypes, E and B, refuting earlier assumptions of a single expanding epidemic [29–33]. Although the two subtypes were introduced during approximately the same period, the proportion of new infections due to subtype E has increased in almost all population groups in Thailand [34]. These and other ecological observations showing variations and changes in the proportion and distribution of subtypes over time have led to the hypothesis that certain non-B subtypes, such as subtype E, may be transmitted more easily through heterosexual intercourse than subtype B and that the rapid spread of HIV in heterosexual populations in Africa and Asia may be explained by biologic properties of circulating HIV strains [35].

In contrast, in the Americas and in Europe, HIV-1 infection initially spread rapidly in the early 1980s, primarily by male-to-male sexual contact and by injecting drug use, and subsequently by heterosexual transmission. Whereas most HIV-1 isolates reported have been subtype B, other HIV-1 subtypes, as well as HIV-2, have been reported in recent years with increasing frequency [36–40]. Although some reports show evidence of gradual increases in strain heterogeneity [41–43], non-B strains in Europe and in the Americas have still been found predominantly in persons with links to regions of the world where these strains are prevalent [44–46] and the wider spread of these strains, including subtypes that are associated with explosive epidemics in Africa and Asia, has not been recognized beyond the imported cases themselves or their direct contacts.

Observations on differences and changes in subtype distribution in different populations are ecological and, by themselves, do not constitute definitive evidence for differences in transmissibility between subtypes. Although it is tempting to speculate that virological factors, as represented by HIV-1 subtype, play a major role in determining patterns of transmission and disease progression around the world, one must be cautious of the 'ecological fallacy', which is the danger of making conclusions about individual risk based on apparent associations at the population level [47]. Associations based on the heterogeneous distribution of different HIV-1 subtypes may not be due to any inherent properties of the viral strains themselves but rather the chance results of a founder effect where one or more subtypes are introduced and consequently established in a population before other subtypes.

## Evaluating possible subtype associations from epidemiological studies of transmission

Many factors that can influence HIV-1 transmission have been well described from epidemiological studies. These include the prevalence and distribution of different risk behaviors in the population, such as sexual behavior, sexual network patterns, and the degree of exposure through commercial sex, the prevalence of co-factors for the sexual transmission of HIV, such as other sexually transmitted diseases and condom use as well as non-immune host factors, such as male circumcision, and immune host factors [16,48–55]. Due to all of these various factors, in addition to the great challenge in measuring incidence, differences in transmission rates are very difficult to document unless the magnitude of the difference is sufficiently large.

The difference between HIV-1 and HIV-2 is a useful illustration. Although HIV-2 shares the same modes of transmission as HIV-1 [4,56,57], the distribution of HIV-2 infection remains restricted primarily to west Africa, where prevalence rates have been stable over time, in contrast to the global distribution and rising rates of HIV-1 [3,4]. To follow up on these ecological observations, prospective epidemiological studies clearly demonstrated large differences in transmissibility

between the two HIV types, with much lower rates of sexual and mother-to-infant transmission for HIV-2 than for HIV-1 [58–61]. For example, whereas one-quarter to one-third of infants born to HIV-1-infected women around the world become infected in the absence of preventive interventions [62–64], only about 1% of HIV-2-infected mothers infect their infants [59–61]. Had the relative differences in transmissibility been small, even well-designed studies might not have been able to demonstrate significant differences.

Reported HIV-1 transmission rates from studies are variable, and it has been especially difficult to delineate what factors contribute to differences in the rates of transmission because of the variations in study methodologies and potential confounding factors. For example, the per sex act transmission probabilities estimated from studies of populations in which the predominant subtypes were not subtype B have in certain cases been higher than estimates of transmission rates found in countries where subtype B is common [65,66]. However, there has not been a consistent difference, as other studies have reported similar transmission frequencies, irrespective of subtype, for both sexual [16] and vertical transmission [67].

Even in studies conducted in the same country or location, there may be risk group differences. For instance, a cross-sectional study from Thailand of HIV-1 infected men and their sex partners showed that there was a higher seroconcordance among couples infected with subtype E than among those infected with subtype B [68]. As most of the men infected with subtype B were injecting drug users and those infected with subtype E were most probably infected sexually, it would not be possible to control completely for potential confounding factors such as differences in frequency of sexual contact with partners and concomitant sexually transmitted diseases, which may vary between the two risk groups. As mentioned earlier, this type of study cannot measure actual transmission probabilities, but rather identifies potential risk factors. However, it is difficult to determine if there were other factors that may have biased this association between subtype and couple seroconcordance. The lack of strong and consistent associations with transmission rates and certain subtypes would suggest that observed associations may well be due to other factors.

## Evaluating possible subtype associations from epidemiological studies of pathogenesis and disease progression

As with transmission, there are many factors that have been shown to be associated with pathogenesis and disease progression [69]. The rate of disease progression appears to vary greatly from person to person and appears to be influenced by a multitude of virological and host factors which have been reviewed previously [70,71].

Differences in the rate of immunological deterioration and disease progression resulting from HIV-2 compared with HIV-1 or HIV-1/HIV-2 dual infection have been well documented [72–75]. However, the question of whether there are differences in disease progression between different HIV-1 subtypes is far from resolved.

Data on the clinical progression of HIV-1 infection in many developing countries, where the predominant subtypes are not subtype B, are scant compared with those for industrialized countries where subtype B is common. Therefore, it is unclear whether HIV infection progresses more rapidly among persons in developing countries than in industrialized countries. For example, it has been hypothesized that chronic immune activation from many infectious pathogens among HIV-infected persons in some developing countries may contribute to faster disease progression [70]. However, any comparisons of disease progression between developing and industrialized countries must take into account a wide range of factors which may influence the spectrum of disease and clinical progression of HIV infection [76–81].

Some studies from different developing countries have suggested that median AIDS-free survival time was shorter than in industrialized countries, even before the institution of antiretroviral therapy [74,75,82–85]. On the other hand, among patients in a rural population in Uganda, progression to AIDS 5 years after seroconversion was not very different from what has been estimated in cohorts of homosexual men in industrialized countries, prior to the availability of antiretroviral therapy [83].

In addition to the many factors which may affect pathogenesis and disease progression, late diagnosis of HIV and lack of quality medical care, plus higher prevalences of tuberculosis and other opportunistic pathogens, may be important factors explaining the apparently shorter survival and poorer clinical outcomes of AIDS patients in developing countries as compared with those in industrialized countries [81–86]. To reduce the potential for confounding, studies should compare similar groups of individuals infected with different subtypes. Two cross-sectional studies from Thailand had initially suggested the possibility that disease progression may be faster for subtype E than for subtype B [87,88]. However, these studies were not prospective and did not account adequately for possible differences in time from infection. In

addition, subtype was highly correlated with mode of transmission, sociobehavioral factors, and potentially, access to care in Thailand. Further review of the data suggested that overall, the clinical and immune presentation of persons infected with different subtypes were very similar [89]. In another study, no difference was found in disease progression and survival with AIDS between Africans living in London (typically infected with non-B subtypes) and native Europeans, all of whom have more or less similar access to care [90,91].

Thus far, very few prospective studies have been conducted to address specifically the issue of differences in disease progression by HIV-1 subtype. A study among Caucasian French men with known date of seroconversion found some evidence of a more rapid decline of CD4 T-cell counts in patients infected with subtype E than in patients infected with subtypes A, B, C or D, which seems to support the preliminary findings from Thailand [92]. Ugandan patients infected with subtype D and with A/D recombinants appeared to have a more rapid decline in CD4 T-cell counts than patients infected with subtype A, but the difference was not statistically significant and longer follow-up times are needed before any conclusions can be drawn [93]. A study of known seroconvertors suggested that women infected with subtype A were less likely to develop AIDS by 5 years post-infection than women infected with other subtypes [94]. Finally, in a cohort of Israeli men infected with subtype B and Ethiopian immigrants into Israel infected with subtype C, clinical evolution and decline in CD4-T cell counts were similar in the two groups [95]; however, the two populations differed in terms of demographic characteristics, infection route, genetic make up and concomitant infections, and according to the authors it can not be excluded that the lack of difference in disease progression was the result of opposing influences.

Studies of biological differences between HIV subtypes will continue to be difficult to conduct and to interpret. Globally, there are only a few regions in the world where there are sufficiently high prevalences of two or more different subtypes in otherwise similar groups of people to permit the previously described studies to be conducted. For example, in Bangkok, Thailand, where both subtypes B and E are found among the population of injecting drug users, a very recent prospective study of seroconvertors showed that whereas early post-infection viral load may be higher for subtype E, the viral load and CD4 and CD8 cell counts after 1 year are similar in persons infected with either subtype [96]. Only cohort studies of persons with incident infections in which virological, immunological, epidemiological and sociobehavioral parameters are monitored will provide convincing evidence of biological differences in the viral subtypes.

## Biological plausibility of subtype associations and biological correlates of transmissibility and pathogenesis

In addition to examining evidence from epidemiological studies, it is necessary to evaluate potential associations between subtype and our outcome variables within a framework of biological plausibility and coherence. In other words, proposing subtype as a 'risk' factor should make sense within the context of the current scientific knowledge of risk factors affecting transmission and disease outcomes. It should be pointed out, though, that current biological knowledge may not be adequate to explain some observations or apparent inconsistencies.

Entry of most strains of HIV into target cells requires binding to both CD4 and at least one of a number of other coreceptors [97–100]. As viral isolates can also be classified by their use of different coreceptors [99,101], which appear to be associated with infectability [53–55] and disease progression [52,102], to what extent is coreceptor usage associated with genetic subtype? A recent study examined 81 primary isolates representing nine different HIV-1 subtypes and suggested that there may be subtype-specific differences in coreceptor usage [103]. Although this study did support previous work showing associations with coreceptor utilization *in vitro* and other genotypic and phenotypic characteristics, more studies are needed to confirm these findings [99,101,103–106]. In other studies, syncytium-inducing (SI) phenotype has been associated with faster disease progression in certain cases [107] and on the molecular level, with positively charged amino acids in the third variable region of Env, which appear to be conserved across different subtypes [108–109]. It has also been suggested that the frequency of SI variants may differ by subtype [108–110]. There are other examples of varying associations between different biological properties and HIV-1 subtype. Although preliminary reports had suggested that selected isolates of HIV-1 Env subtypes E and C infected Langerhans cells (found in the genital tract) more readily than isolates of subtype B [35,111], two subsequent studies failed to show any subtype-specific differences in the ability of isolates to infect Langerhans cells and infectiousness for Langerhans cells may be a function of strain differences that is independent of subtype [104,105]. A lack of consistency in the biological evidence suggests that these associations may be due simply to chance variation or may be confounded by other unknown factors. Furthermore, it should always be stressed that associations from studies *in vitro* in the laboratory may not translate to associations seen in human populations.

Finally, the ability of the host immune response elicited by HIV does not appear to correlate closely with

HIV-1 subtype. For instance, there have been several studies examining inter- and intraclade antibody neutralization patterns of different HIV-1 subtype isolates by both autologous and heterologous sera from HIV-infected persons [112–114]. Whereas some primary HIV-1 isolates, regardless of the genetic subtype that they belong to, are highly sensitive to neutralization, others are less sensitive or relatively resistant to neutralization [115–117]. Similarly, for cell-mediated immunity, studies suggest that cytotoxic T-lymphocyte epitopes from different HIV-1 genetic subtypes are often conserved rather than segregating specifically by subtype [118,119]. In general, these analyses show that immunogenicity does not appear to correlate directly with HIV-1 subtype and that many epitopes may be conserved between subtypes [12,112,116,117,120].

Because a number of functional properties and responses can be found across the different genetic subtypes of HIV-1, it appears that the subtype classification of HIV-1 isolates does not appear to be closely correlated with a number of biological or phenotypic characteristics examined. Hence, if associations are weak or inconsistent, it may suggest that the exposure or risk factor we have chosen, in this case HIV-1 subtype, may be a very inaccurate, inconsistent, or indirect measure of the true biological factors affecting transmissibility and disease progression. While it may be plausible that subtype differences in transmissibility or pathogenicity exist, it is probably more likely that differences in transmissibility or pathogenesis are influenced by virological strain differences other than represented by HIV-1 subtype or the multitude of host and sociobehavioral factors mentioned previously.

## Discussion

In this overview, we have shown that not only is it extremely difficult to find consistent associations between HIV-1 subtype and correlates of transmission and pathogenesis, but that it seems unlikely that a single characteristic such as subtype can account for significant differences in transmission and disease progression. In summary, the evaluation of associations between HIV subtype and potential differences in transmissibility and disease progression is difficult and depends on the systematic consideration of many factors which affect both transmission and pathogenesis. It is therefore extremely difficult to establish whether an observed association is causal or is due to other mechanisms such as bias, confounding, or chance. The outcomes of interest are highly variable and multifactorial, and are associated with host, societal, and possible viral factors.

Information in the literature range from ecological observations to results of epidemiological and laboratory studies of viral and host factors. Observations on differences and changes in subtype distribution in different populations are ecological and by themselves they do not constitute definitive evidence for differences in transmissibility between subtypes. Associations based on the heterogeneous distribution of HIV-1 subtypes may not be due to inherent properties of the virus itself but rather to the chance results of a founder effect where one or more subtypes are introduced and consequently established into a population before other subtypes are introduced.

Whereas convincing evidence from epidemiological studies would provide stronger support, the lack of strong and consistent associations between transmission rates and certain subtypes would suggest that the associations observed in independent studies are probably due to other factors. In addition, although there are a few studies suggesting differences in disease progression by HIV-1 subtype, more prospective studies are needed to confirm the consistency of these associations. As a number of functional properties and responses can be found across the different genetic subtypes of HIV-1, it appears that the subtype classification of HIV-1 isolates is not closely correlated with a number of the biological or phenotypic characteristics that have been examined. Hence, if associations are weak or inconsistent, it may suggest that the exposure or risk factor chosen for examination, in this case HIV-1 subtype, may be an inaccurate or indirect measure of the true biological factors affecting transmissibility and disease progression.

Although classification of the wide genetic variation of HIV by Env or Gag subtypes has played an important role in elucidating the epidemiological and historic aspects of transmission in the global epidemic, it is unlikely that a single classification can comprise all aspects of the genetic variability of HIV, the dynamics of transmission, and the relationship of genetic variability to phenotypic properties. Rather, as knowledge of the virus increases, separate classification systems may emerge which are dependent on the question of interest. For example, the classification of viral strains into SI and non-SI (NSI) phenotypes makes biological sense as it is classifiable (SI viruses are different from NSI) and this classification has been associated with the biology of the virus and disease progression studies. However, SI/NSI does not correlate well with subtype and is not useful in tracking epidemics. HIV-1 subtype genetic variation will remain an important and essential factor in the continued surveillance of the HIV-1 epidemic worldwide.

Studies of biological differences between HIV subtypes will continue to be difficult to conduct and interpret. Studies describing differences in transmissibility and pathogenesis of different HIV subtypes are complex due to the many confounding variables. Only cohort studies of persons with incident infections in which

virological, immunological, epidemiological and sociobehavioral parameters are monitored will provide convincing evidence of biological differences in the viral subtypes.

Molecular epidemiology is a hybrid discipline, only one of whose ultimate aims is to identify and to characterize viral and host factors that contribute to transmission and disease. We hope that this review using HIV-1 subtype as an example will be a useful framework with which to evaluate other putative factors. By using approaches which combine methods from epidemiology, virology, immunology, genetics, molecular biology and statistics, researchers can maximize the potential that these disciplines have to work together.

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