Molecular Epidemiology of HIV in Public Health Surveillance: a Position Paper

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Summary

In the National Programme HIV and Sexually Transmissible Diseases (NPHS) 2011-2017 molecular epidemiology is explicitly mentioned as a supportive means to direct and optimize intervention strategies. Despite this specification, molecular epidemiology has not been implemented yet. Work Group 2 Laboratory and Diagnostics was mandated to prepare a position paper on molecular epidemiology of HIV, and to make recommendations whether or not molecular epidemiology should be implemented and in which way it may be best applied to complement and improve the existing basis for decision making.

In Switzerland all technical and organizational requirements to generate and collect viral sequence information are met and in place for many years. Two data bases exist in Switzerland with a national coverage of HIV viral sequences, one supported by the Swiss HIV Cohorts Study for patients enrolled in this study, and one supported by the Federal Office of Public Health (SFOPH), which stores viral sequences from the newly diagnosed HIV patients notified to the SFOPH since 2006. Although there is a substantial overlap between these two data bases, both of them contain unique sequences as well as specific gaps. To answer specific question, either one data base alone or a combination of the two can be used.

WG2 recommends to implement molecular epidemiology for HIV surveillance. It proposes to maintain and improve the existing infrastructure for collecting and analyzing viral sequence data, to introduce the surveillance of HIV subtype, antiviral resistance, and transmission-chain length, and to support the further development of molecular epidemiology for public health interventions.

1. Introduction

The NPHS 2011-2017 specifies several support measures which shall help to achieve the main goals of the programme, most notably the reduction of the HIV transmission risk. Among these molecular epidemiology of HIV is specifically named as means to identify HIV transmission chains within the Swiss HIV epidemic(s), which should allow to devise appropriate prevention stategies:

> *c) Molekulare Epidemiologie*
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> *Untersuchungen auf molekularer Ebene (mittels phylogenetischer Analysen) geben Aufschluss, über welche Übertragungsketten sich das HI-Virus in der Schweiz verbreitet. Diese Untersuchungen werden regelmässig durchgeführt – sie entstehen mit dem Einverständnis der Betroffenen. Datenschutz und Persönlichkeitsrechte werden dabei*

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respektiert. Die Untersuchungen haben zum Ziel, besonders geeignete oder besonders notwendige Präventionsmassnahmen zu eruieren.

c) Epidémiologie moléculaire

Des études au niveau moléculaire (au moyen d'analyses phylogénétiques) renseignent sur les chaînes de transmission par lesquelles le virus IH se propage en Suisse. Ces études sont régulièrement réalisées avec le consentement des personnes concernées. La protection des données et les droits de la personnalité sont respectés. Les études ont pour objectif de déterminer les mesures de prevention appropriées ou particulièrement importantes.

In the past years molecular epidemiology of HIV-1 in Switzerland has been pursued along two related but independent initiatives. HIV sequence data collected in the context of the Swiss HIV Cohort Study (SHCS) were analyzed for scientific projects by members and collaborators of the SHCS. Although epidemiological questions were adressed in these studies, they were neither driven nor financed by the public health authorities. On the other hand the Swiss Federal Office of Public Health (SFOPH) is actively supporting the acquisition of HIV sequence data from all HIV infections newly diagnosed within Switzerland. Attempts were made to link the surveillance data collected at the SFOPH for HIV with the viral sequence. While some progress was made, legal issues obstructed further development of this.

The goal of this position paper of work group 2 is severalfold. It shall outline the key principle on which molecular epidemiology is built, describe the infrastructure that is available in Switzerland, illustrate some of the epidemiological questions which have been adressed for HIV by molecular epidemiology, and to make recommendations to the EKSG/CFSS how molecular epidemiology of HIV could be used to support The NPHS.

2. Molecular epidemiology: Requirements and status quo

Molecular epidemiology is based on the insight that pathogen evolution and epidemiology occur often at similar scales (GRENFELL *et al.* 2004), such that the spread of pathogens can be derived from the signatures evolution has left in the pathogens' genomes (DRUMMOND *et al.* 2003; PYBUS and RAMBAUT 2009). Hence, the underlying idea of molecular epidemiology is that, since pathogens evolve and diverge along their transmission chains, similarity in pathogen sequences indicates closeness in the transmission network.

Molecular epidemiology requires the collection of viral sequence information in addition to the epidemiological information from infected people. Viral sequences need to be deposited in a centralized data base. that can be linked to the epidemiological information by way of a unique identifier. If the sequences do not represent the entire viral genome, the sequenced region of the viral genome must always be homologous in order to allow a direct comparison of the viral evolution and the evolutionary relationship. Furthermore, meaningful interpretations can only be achieved, if the

sequence quality is high, i.e. if the frequency of errors in the nucleotide calls is smaller than the frequency of mutations that is due to viral sequence evolution.

In Switzerland all these technical and organizational requirements to generate and collect viral sequence information are met and in place for many years: Four laboratories (in Basel, Geneva, Lausanne, and Zürich) exclusively carry out all genotypic HIV resistance tests that are ordered for diagnostic purposes for newly diagnosed HIV infection, in accordance with the national HIV test concept. Focusing on the target of antiretroviral drug resistance ensures the necessary homology of the sequences. The laboratories have access to a central database (SmartGene GmbH, Zug), in which all these sequences are entered via a web browser-based interface. Quality is assured by participation in an annual external quality control program (ANRS).

Two HIV sequence databases exist, which can be used for molecular epidemiology. The so-called BSV data base (Bundesamt für Sozialversicherungen) contains exclusively the prospectively collected viral sequence information from newly diagnosed patients. It started in 2006 and continues to this date. This data-base is financed by the SFOPH. Origjnally it was intended that the sequence information is linked with the epidemiological information collected by the FOPH. For this reason the sequence entries are accompanied by only a very limited set of additional information. However, linking surveillance data from the FOPH with the viral sequence from these newly diagnosed patients is a labor-intense task, and it suffers from non-negligible uncertainties regarding the correctness of matching, because there is no unique common identifier in the sequence and epidemiological data sets.

The same four laboratories also contribute the genotypic resistance tests for patients enrolled in the SHCS. These sequences are deposited in the SHCS sequence database, which is also stored at SmartGene GmbH, Zug, but in a separate environment. The SHCS sequences database is financed by core money from the study and by additional funds to individual members of the SHCS. The SHCS sequence database is more comprehensive (due to retrospective sequencing of stored plasma samples) and contains the sequences from the earliest virus isolates date to the 90s of the last century. These sequences can be linked with all the data, which are stored in the SHCS data base, and they are regularly used for scientific projects of the SHCS.

Although many of the sequences in the BSV database are also in the SHCS data base, both data sets contain unique sequences. In a recent study by Shilaih et al (submitted), it was shown that the SHCS database alone may be sufficient to answer many questions. This study also suggested that in combination with the SHCS data set the unique sequences from the BSV data base may be used anonymously, i.e. without linkage to FOPH data, and that epidemiologically relevant information the anonymous sequences can be obtained by inference).

3. Molecular epidemiology: Applications in the field of HIV

3.1. A brief scientific literature survey

Molecular epidemiology analyses have made important contributions to understanding transmission. The key additional insight gained by molecular epidemiology (compared to traditional epidemiological surveillance) is a characterization not only of newly infected individuals (recipients) but also that of their transmitters (see (LEMEY *et al.* 2006; PYBUS and RAMBAUT 2009; BRENNER and WAINBERG 2013; DENNIS *et al.* 2014) for reviews of molecular epidemiology in HIV).

In HIV, molecular epidemiology approaches have been particularly important, given that contact tracing has not been used widely for this infection, mainly because of practical or ethical/privacy concerns. Accordingly, molecular and phylogenetic methods have led to several key insights about the spread of HIV that would not have been possible with other methods. These insights range over a broad range of scales: From the uncovering of individual transmission events in forensics (OU *et al.* 1992; DE OLIVEIRA *et al.* 2006; BERNARD *et al.* 2007) up to the large evolutionary patterns such as the origin of HIV-1 and HIV-2 (GAO *et al.* 1999; SHARP *et al.* 2001; WOROBEY *et al.* 2004), molecular signatures were the main tool used to track the spread of HIV. At an intermediate scale, such approaches were also instrumental in characterizing the structure of the HIV transmission networks at the local, national and global level:

Several studies have shown that in HIV transmission networks the number of links or size of clusters is distributed according to a power law(LEWIS *et al.* 2008; HUGHES *et al.* 2009; LEIGH BROWN *et al.* 2011), suggesting that the epidemic is driven by a small number of individuals (super spreaders). It has also been argued that the frequent clustering of acutely infected patients indicates that this stage is particularly important for the spread of HIV(BRENNER *et al.* 2007). Phylo-geographical analyses have shown the high interconnectedness of epidemics in different places(PARASKEVIS *et al.* 2009; WERTHEIM *et al.* 2013); but have also highlighted that the spread of HIV is often structured by geography even at a local scale(KOUYOS *et al.* 2010). Interestingly it has been recently argued that the HIV-1 spread in endemic settings (sub-Saharan Africa) exhibits almost no local geographical structure beyond the household levels(GRABOWSKI *et al.* 2014). Phylo-geographical analyses can become particularly valuable with time-trees (phylogenies in which branch length can be interpreted as calendar time), because those allow estimating the date when a HIV strain has been introduced into a particular setting: for example it has been shown with this method that HIV-1 subtype B has been introduced into the USA via Haiti “in or around 1969” (GILBERT *et al.*). Similar to geographical structure, preferential transmission within risk groups leaves traces in the viral sequences and can hence be recovered with phylogenetic analysis(KOUYOS *et al.* 2010). The power of molecular approaches can be boosted by combining them with mathematical models: This has allowed the quantification of key parameters of the spread of HIV such as the transmissibility of the virus (PYBUS *et al.* 2001; STADLER *et al.* 2012), or the fraction of transmission events attributable to the acute infection (VOLZ *et al.* 2013).

3.2. Applications of molecular HIV epidemiology to public health issues

Several of the abovementioned applications are potentially relevant for (routine) public health surveillance:

- One of the key benefits of molecular epidemiology is distinguishing domestic transmission from imported cases and characterizing the latter one. Traditional surveillance can for example show a decrease in new HIV diagnoses (or infections) --- but in such a case it is not clear whether the time-trend is due to a decrease in domestic infections or a decrease in the number of imported cases. Molecular Epidemiology allows distinguishing those alternative scenarios by considering the clustering patterns of domestic and foreign sequences(KOUYOS *et al.* 2010; WYL *et al.* 2011a). Note that such analyses depend on the availability and representativeness of international sequences. In addition these analyses allow to determine
    - Which foreign countries most matter as sources of imported cases?
    - How often do new imports lead to onward transmission and in which constellations is such onward transmission most likely (WYL *et al.* 2011a)?
- Surveillance of antiretroviral resistance and in particular of transmitted antiretroviral resistance requires viral sequences (phenotypic resistance testing is possible but more expensive and hence not routinely done) and is thus necessarily "molecular" epidemiology. Analyses of transmitted drug resistance mutations have shown that after an early increase, levels of transmitted resistance have stabilized around 10%-15%(WYL *et al.* 2011b). Currently transmitted resistance is associated with subtype B, but this may rapidly change with the roll-out of antiretroviral therapy in Africa. In addition, molecular epidemiology can be used to characterize the sources of TDR, i.e. the patients transmitting the resistant virus. For example, (DRESCHER *et al.* 2014) could show that transmitted drug resistant HIV is mostly transmitted from patients that are themselves treatment naive; implying that further prevention of transmitted drug resistance requires early identification and treatment of HIV-infected individuals.
- Molecular epidemiology can determine the extent to which HIV can spread in specific risk or demographic groups. The weak degree with which HIV sequences from heterosexuals cluster among each other in Switzerland indicates that this mode of transmission is not able to sustain large outbreaks of HIV under the current situation in Switzerland(KOUYOS *et al.* 2010). This is also the case for the migration-related non-B subtypes (WYL *et al.* 2011a), indicating that even though there are frequent introductions of those subtypes into Switzerland, such introductions are followed only by a very limited onward transmission. A further surveillance of these patterns is important, since increasing risk behavior may also boost heterosexual transmission to an extent that it becomes self-sustaining in Switzerland. A quantitative estimation of the ability of HIV to spread is provided by phylogenetic estimators of the basic reproductive number R0 (STADLER *et al.* 2012). However these can currently only be applied to large transmission clusters (corresponding to self sustaining epidemics).
- Molecular epidemiology can be help assessing past prevention measures. For example the results from (KOUYOS *et al.* 2010) indicated that prevention measures addressed to IDUs also lead to a decrease of IDU-related HIV infections among heterosexuals. Inferring, such interactions between different risk or demographic groups relies heavily on molecular epidemiology (because traditional methods allow only the characterization of the recipient but not of the transmitter).

- Often forensic applications of molecular epidemiology can have important implications for public health. This is especially the case for identifying and targeting transmission sources, for example in cases of nosocomial transmission of HIV in a hospital in Benghazi(DE OLIVEIRA *et al.* 2006), iatrogenic transmission of HCV (SHEMER-AVNI *et al.* 2007) or the so-called "Heiler von Bern". Even if such events may only occur sporadically, the quality of insight relies on good background sequences from the same population (BERNARD *et al.* 2007).
- Sequence data can provide estimates of infection dates based on ambiguous nucleotides (KOUYOS *et al.* 2011) or other diversity measures (XIA *et al.* 2014).These estimates can complement serological methods (SCHUEPBACH *et al.* 2012) to identify recent HIV infection and thereby estimate HIV incidence.
- Molecular methods can be used to characterize and identify highly-connected groups in transmission networks (LITTLE *et al.* 2014). Such groups might be the optimal targets for prevention measures.

4. Recommendations by work group 2

There is a consent among the members of Work group 2, that molecular epidemiology is a means that has provided a deeper insight into the epidemiology of HIV-1 in Switzerland, and that its usefulness has been demonstrated in several studies, using data from the SHCS. The group therefore confirms the goal expressed in the NPHS. However, although the application of molecular epidemiology is well established in public health for tracking the origins of viral strains or newly introduced viruses, the work group likes to point out that the field of molecular epidemiology is also explorative and a field of active research, and that hence possibilities to further develop the field should be supported.

Work group 2 makes the following specific recommendations:

- Ensure the activities which are in place to collect and store the HIV sequence informations. This does not imply that the infrastructures need to stay as they are, but they have to be suitable and cost-efficent in order to reach the goal.
- Additional parameters which are based on molecular epidemiological information should be included in the annual statistics of the HIV surveillance. In particular, the following extensions are proposed:

  - a statistical measure of transmission-chain length for the various transmission groups, intervention axes, and socio-economic groups

  - surveillance of antiviral resistance (transmitted and acquired)

  - HIV subtype information

- Evaluate and validate molecular epidemiology to better assess the recency of HIV infection, in order to complement or to reduce the dependency on the current method that is based on the INNOLIA test.

- Provide a general support of the further development of the molecular epidemiological methodology

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