Treatment-Naive Individuals Are the Major Source of Transmitted HIV-1 Drug Resistance in Men Who Have Sex With Men in the Swiss HIV Cohort Study

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Background. Human immunodeficiency virus type 1 (HIV-1) transmitted drug resistance (TDR) can compromise antiretroviral therapy (ART) and thus represents an important public health concern. Typically, sources of TDR remain unknown, but they can be characterized with molecular epidemiologic approaches. We used the highly representative Swiss HIV Cohort Study (SHCS) and linked drug resistance database (SHCS-DRDB) to analyze sources of TDR.

Methods. ART-naive men who have sex with men with infection date estimates between 1996 and 2009 were chosen for surveillance of TDR in HIV-1 subtype B (N = 1674), as the SHCS-DRDB contains pre-ART genotypic resistance tests for >69% of this surveillance population. A phylogeny was inferred using *pol* sequences from surveillance patients and all subtype B sequences from the SHCS-DRDB (6934 additional patients). Potential sources of TDR were identified based on phylogenetic clustering, shared resistance mutations, genetic distance, and estimated infection dates.

Results. One hundred forty of 1674 (8.4%) surveillance patients carried virus with TDR; 86 of 140 (61.4%) were assigned to clusters. Potential sources of TDR were found for 50 of 86 (58.1%) of these patients. ART-naive patients constitute 56 of 66 (84.8%) potential sources and were significantly overrepresented among sources (odds ratio, 6.43 [95% confidence interval, 3.22–12.82]; $P < .001$). Particularly large transmission clusters were observed for the L90M mutation, and the spread of L90M continued even after the near cessation of antiretroviral use selecting for that mutation. Three clusters showed evidence of reversion of K103N or T215Y/F.

Conclusions. Many individuals harboring viral TDR belonged to transmission clusters with other Swiss patients, indicating substantial domestic transmission of TDR in Switzerland. Most TDR in clusters could be linked to sources, indicating good surveillance of TDR in the SHCS-DRDB. Most TDR sources were ART naive. This, and the presence of long TDR transmission chains, suggests that resistance mutations are frequently transmitted among untreated individuals, highlighting the importance of early diagnosis and treatment.

Keywords. molecular epidemiology; transmitted antiretroviral drug resistance; genotypic resistance testing.

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Antiretroviral treatment (ART) has drastically reduced human immunodeficiency virus (HIV)–related morbidity and mortality since the late 1980s [1–3]. However, ART has been shown to be less effective against drug-resistant HIV type 1 (HIV-1) [4], which may take longer to be suppressed and may cause virological failure earlier [5]. Resistance is seen in individuals undergoing ART (acquired drug resistance), and in those who have never undergone ART (transmitted drug resistance [TDR]) [6, 7]. Estimates of the prevalence of drug resistance among new infections (TDR) vary worldwide between 4% and 22% [8–12]. Thus, reducing TDR is an important public health goal.

Several studies have documented stable or decreasing TDR rates in Europe and North America in recent years [10, 12–15]. In addition to such direct quantification of TDR cases, molecular epidemiology offers a unique tool for characterizing the sources of transmitted drug resistance in HIV-1 [14, 16]. Phylogenetics reveals evolutionary relationships between genetic sequences, and is an established method for characterizing transmission of rapidly evolving pathogens [17–21]. Usually, sources of TDR remain unknown, but if 2 patients cluster on a phylogeny, one is a potential source of the other's infection and TDR. Many previous phylogenetic analyses of HIV-1 TDR were limited by the number of patients included, the representativeness of the study population, and/or limited information on infection dates or patient ART histories. Thus, a data set with high coverage of a (sub-)epidemic provides a unique opportunity to investigate the sources of TDR.

The Swiss HIV Cohort Study (SHCS) is a clinic-based prospective cohort study continuously enrolling patients from all of Switzerland. The linked drug resistance database (SHCS-DRDB) contains all genotypic resistance tests (GRTs) conducted in Switzerland, providing a uniquely representative source of genetic, epidemiologic, demographic, and clinical data, which we use here to characterize the sources of TDR with a molecular epidemiologic approach.

METHODS

Subject/Sequence Selection

The SHCS has >17 900 patients followed semiannually (www.shcs.ch) [22]. Informed consent was obtained from all patients and the study was approved by the ethics committees of the participating institutions. The SHCS-DRDB [23] contains all GRTs from the 4 laboratories in Switzerland that conduct HIV drug resistance testing. Sequences include the full protease and at least codons 28–225 of the reverse transcriptase. Sequences were obtained from routine clinical testing (60%) and systematic retrospective sequencing of stored plasma samples (40%). The SHCS-DRDB contains >19 000 partial *pol* gene sequences from >10 000 patients. It is unique in achieving good coverage of the population of HIV-infected patients for an entire country: Approximately 54% of all HIV cases ever diagnosed in Switzerland and 75% of antiretroviral-treated patients are in the SHCS [22]. Viral nucleotide sequences are available for 62.5% of patients in the SHCS [22, 24]. Infection dates were imputed from CD4 cell counts by the back-calculation method published in [25].

To analyze the incidence and phylogenetic linkage of TDR in a highly representative population, we use ART-naive men who have sex with men (MSM) infected between 1996 and 2009 as a surveillance population (see Results and Figure 1). We focused on this group for the following reasons: First, subtype B is the main subtype transmitted in Switzerland [26]. Second, there is substantial ongoing transmission in Switzerland among MSM [27]. Third, stored plasma samples for systematic retrospective sequencing were available starting from 1996. Finally, there was typically a delay of several years (median, 4.17 years [interquartile range, 2.98–5.82 years]) between infection and enrollment in the cohort [25]. Along with 1674 surveillance patients, all 6934 remaining SHCS-DRDB patients with subtype B HIV were included as a background for the phylogenetic tree and as additional potential sources of TDR.

To assess representativeness of the surveillance population, we used data from the Swiss Federal Office of Public Health (SFOPH) on the yearly HIV-1 diagnoses in Switzerland, by transmission group. As the SFOPH data do not contain subtype information or infection dates, we used diagnosis date as a proxy for infection date and pooled all subtypes for our representativeness calculations. We calculated the representativeness of the SHCS-DRDB for given ranges of diagnosis dates by comparing the number of patients with available pre-ART GRTs in the SHCS-DRDB to the number of newly diagnosed HIV cases in Switzerland, according to the SFOPH (http://www.bag.admin.ch/hiv_aids/05464/12908/12909/12913/index.html?lang=de).

Patients carrying TDR ("recipients" of TDR) were identified from the surveillance population. However, potential transmitters of TDR are not restricted to this group; all other patients (drug naive, treated, or treatment failing) with available HIV-1 subtype B *pol* gene sequences were included as a background for tree building ("background population"), and both populations were considered possible transmitters of drug-resistant HIV (potential "sources" of TDR). In total, 15 681 HIV-1 *pol* gene sequences from 8608 subtype B patients from the SHCS were analyzed (Figure 1). Because sequences for several time points could be included for the same patient, only mutations found while a patient was ART naive were considered TDR. However, mutations from any time were considered when determining if a patient was a source of TDR. For definitions of all terms used for groups of patients, see Table 1.

Drug resistance mutations were defined as amino acid substitutions on the World Health Organization HIV Drug Resistance Surveillance list [28].

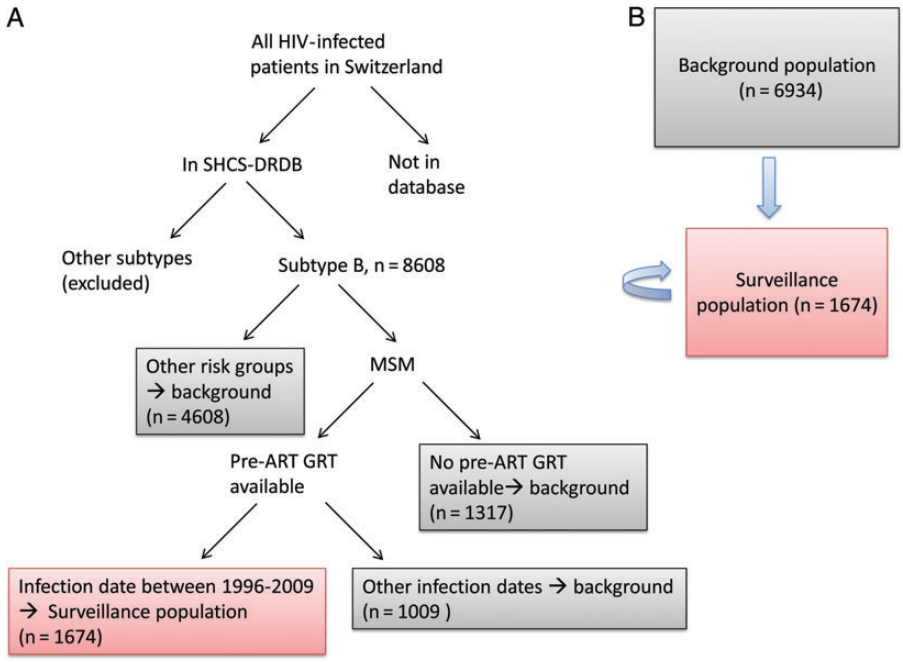

Figure 1. *A*, Sequence selection for inclusion in the analysis, and definition of the surveillance (red box) and background populations (gray boxes). *B*, Possible transmission of drug resistance mutations. Transmitted drug resistance can be transmitted to the surveillance patients (red box) from either the background population (gray box) or another member of the surveillance population. Abbreviations: ART, antiretroviral therapy; GRT, genotypic resistance test; HIV, human immunodeficiency virus; MSM, men who have sex with men; SHCS-DRDB, Swiss HIV Cohort Study drug resistance database.

Phylogenetic Analysis

A phylogeny was inferred from 15 681 *pol* gene nucleotide sequences. Nucleotide positions where resistance mutations are documented [28] were excluded for tree inference. The tree was created using the GTR model in the software FastTree2 [29, 30]. Support values for the branching pattern were calculated from 100 bootstrap replicates.

Monophyletic groups with bootstrap support ≥70% ("transmission clusters") were investigated for TDR recipients and sources. Two very large clusters containing sequences from 210 and 43 patients were excluded and smaller clusters nested within them analyzed instead. In all other cases, clusters nested within larger clusters were excluded. Additionally, a sensitivity analysis was conducted with a bootstrap threshold of ≥98%.

Potential sources of TDR were identified from those patients who clustered with and shared ≥1 drug resistance mutations with a recipient of TDR and satisfied 2 criteria: The estimated infection dates with 95% confidence intervals (CIs) did not exclude that the source was infected before the recipient, and the genetic distance of the source viral sequence from that of the corresponding recipient was <1.5% (Table 1).

Primary/Recent Infections

We calculated the fraction of primary/recent infections among sources of TDR. Primary/recent infections were defined by diagnosis during clinically defined acute HIV infection (http://www.shcs.ch/56-definitions#2.1) or during recent infection defined by seroconversion (<1 year between last negative and first positive HIV test), or by an ambiguous nucleotide count of <0.5% in a baseline, ART-naive GRT [31] (Table 1).

Statistical analyses were done using R version 2.15.1 and Stata/SE12.1. Comparisons between groups were performed with Fisher test for count data, or Welch *t* test for means. For surveillance patients with and without TDR, we assessed the role of infection date, clustering status, age at time of infection, and whether a patient was diagnosed in primary infection, using univariable and multivariable logistic regression. For potential sources vs nonsources, we additionally analyzed the role of sex, transmission group, and TDR. The multivariable logistic regressions controlled for the potential confounders listed above (see also Tables 2 and 3).

RESULTS

Representativeness

We considered the phylogenetic linkage of transmitted drug resistance mutations in subtype B HIV-1 in a surveillance population of therapy-naive MSM with estimated infection dates between 1996 and 2009 (see Methods) and calculated the representativeness for this group using data from the SFOPH.

Table 1. Definitions of Terms Used

Term	Definition
Surveillance population	MSM, subtype B patients with at least 1 genetic resistance test prior to beginning ART, and who were infected between 1996 and 2009 (based on a back-calculation method from Taffé et al [25])
Background population	All subtype B patients in the analysis who were not in the surveillance population
Recipient (of TDR)	A surveillance patient with a TDR mutation
Potential source (of TDR)	Any patient (from surveillance or background population) who shares a resistance mutation with a patient with TDR in a well-supported cluster, and meets the following criteria: • Genetic distance to the corresponding TDR patient is <1.5% • Estimated infection dates with 95% confidence intervals do not exclude that the potential source was infected before the corresponding recipient of TDR (estimated date for source is earlier, or confidence intervals overlap)
Treatment-naive potential source	A potential source of TDR for whom a genetic test indicates that the relevant mutation was present before the patient had begun ART
Treatment-experienced potential source	A potential source of TDR for whom the first available genetic test with the relevant mutation present was conducted after the patient had begun ART
Non–source patient with the potential to transmit resistance	A patient with a resistance mutation, from the background or surveillance population, who clusters with a surveillance patient, but is not a potential source
Patient with primary or recent infection	A patient with a primary/recent infection by 1 of 3 criteria: • Clinical identification of acute HIV infection • <1 year between the last documented negative and first documented positive HIV test • <0.5% ambiguous nucleotides in a baseline, ART-naive genetic sample

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; MSM, men who have sex with men; TDR, transmitted drug resistance.

Overall, the SHCS contains at least 53.6% of all patients ever diagnosed in Switzerland, according to data from the SFOPH. The SHCS-DRDB contains pre-ART GRTs for 22.1% of patients (see Methods). Overall, a higher fraction of patients diagnosed from 1996 to 2009 had pre-ART sequences in the DRDB than did all patients (50.8% vs 22.1%). Combining this with the restriction of transmission group to MSM, we found that 69.3% of MSM diagnosed from 1996 to 2009 had pre-ART sequences in the SHCS-DRDB. Although we selected surveillance patients using estimated infection dates and not diagnosis dates, the above percentages support our choice to limit the transmission group and infection dates of the surveillance population.

Clustering

Of the analyzed patients, 3225 of 8608 (38.0%) belonged to transmission clusters with bootstrap support ≥70%, including 1119 of 1674 (66.8%) surveillance patients. Thus, surveillance patients had a much higher rate of clustering than nonsurveillance patients (odds ratio [OR], 3.36 [95% CI, 3.0–3.77]; $P < .001$). Of surveillance patients with TDR, 86 of 140 (61.4%) belonged to transmission clusters. The presence of TDR did not impact clustering (OR, 0.77 [95% CI, .53–1.12]; $P = .16$).

Characteristics of Transmitted Resistance

Of 1674 surveillance patients, 140 (8.4%) carried virus with TDR [28]. Notably, patients with TDR were more often diagnosed during primary infection (OR, 1.51 [95% CI, 1.06–2.14]; $P = .02$). See Table 2 for further characteristics of surveillance patients with and without TDR.

Of the 140 surveillance patients carrying TDR, 114 (81.4%) carried virus with mutations against 1 drug class, 16 (11.4%) against 2 classes, and 10 (7.1%) against 3 classes. Ninety-three (66.4%) of surveillance TDR sequences carried resistance against nonnucleoside reverse transcriptase inhibitors, 45 (32.1%) against protease inhibitors, and 38 (27.1%) against nonnucleoside reverse transcriptase inhibitors. Moreover, 61 carried >1 mutation (range, 1–9). The most commonly mutated position in surveillance-patient viruses was codon 215 in the RT, similar to [14]. A total of 53 RT mutations at codon 215 were found (T215Y/S/C/D/E/L/F). In the surveillance population, T215F/Y constitute only 10 of 53 (18.9%) resistance mutations at position 215, whereas they constitute 1124 of 1234 (91.1%) resistance mutations for ART-experienced patients. See the Supplementary Data for complete lists of transmitted/acquired resistance mutations.

Evidence of Resistance Transmission

We could link most surveillance population TDR in clusters to a potential source of the drug resistance mutation(s). Specifically, we found that of the surveillance patients who had TDR and belonged to transmission clusters, 50 of 86 (58.1%) clustered with at least 1 potential source. For 35 of 50 (70%), >1 possible source was identified (range, 1–7). Sources were often associated with multiple surveillance patients.

We found that surveillance patients were more likely to carry resistant virus if they belonged to a cluster containing resistant virus in other patients. For surveillance sequences with TDR in clusters, there was at least 1 other patient carrying resistant

Table 2. Characteristics of Surveillance Patients With and Without Transmitted Drug Resistance

Characteristic	With TDR, No. (%)	Without TDR, No. (%)	Total, No.	Univariable		Multivariable	
				OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Age at time of infection					.09		.07
≤29	30 (21)	287 (19)	317	1 (Reference)		1 (Reference)	
30–39	64 (46)	614 (40)	678	1.00 (.63–1.57)		1.01 (.64–1.61)	
40–49	39 (28)	439 (29)	478	0.85 (.52–1.4)		0.87 (.53–1.44)	
≥50	7 (5)	194 (13)	201	0.35 (.15–.8)		0.35 (.15–.83)	
Patient is in a cluster					.16		.08
No	54 (39)	501 (33)	555	1 (Reference)		1 (Reference)	
Yes	86 (61)	1033 (67)	1119	0.77 (.54–1.1)		0.73 (.51–1.04)	
Year of infection					.62		.65
1996–2000	39 (28)	463 (30)	502	1 (Reference)		1 (Reference)	
2001–2003	35 (25)	352 (23)	387	1.18 (.73–1.9)		1.17 (.72–1.89)	
2004–2006	37 (26)	455 (30)	492	0.97 (.6–1.54)		0.97 (.60–1.55)	
2007–2009	29 (21)	264 (17)	293	1.3 (.79–2.16)		1.24 (.74–2.08)	
Primary infection at registration					.02		.029
No	59 (42)	803 (52)	862	1 (Reference)		1 (Reference)	
Yes	81 (58)	731 (48)	812	1.51 (1.06–2.14)		1.46 (1.02–2.09)	

The odds ratios in the multivariable model are adjusted for all variables listed in the table.

Abbreviations: CI, confidence interval; OR, odds ratio; TDR, transmitted drug resistance.

virus in the same cluster in 74 of 86 (86%) cases. For the surveillance population patients without TDR mutations, only 246 of 1033 (23.8%) clustered with another patient carrying resistant virus. The presence of a viral resistance mutation is thus a very strong predictor of other patients with resistance mutations in the same transmission cluster (OR, 19.7 [95% CI, 10.4–40.4]; $P < .001$).

Sources of Transmitted Resistance

Fifty-six of 66 (84.8%) potential source patients were ART naive when the relevant mutation was first observed. We compared this with the fraction of ART-naive patients among nonsource patients with the potential to transmit resistance. These nonsource patients were defined as patients with HIV-1 resistance mutations who belonged to a cluster with a surveillance patient but were not sources of TDR (Table 1). Treatment-naive patients were significantly overrepresented among potential sources of TDR compared to nonsources (OR, 6.43 [95% CI, 3.22–12.82]; $P < .001$).This significance remains when controlling for potential confounders (Table 3). Of 50 surveillance patients with viral TDR and a potential source, 43 (86%) had at least 1 ART-naive patient among their potential sources. By contrast, only 17 of 50 (34%) were in clusters with treated potential sources. Thus, most resistance mutations were transmitted to >1 naive individual, and a single initial transmission event from a treated patient may lead to several new infections with drug-resistant HIV. Sources were infected later than nonsources, likely because the surveillance population was characterized by more recent infection dates than the background population (see Methods). As the sources of TDR will be more similar to recipients (which stem by definition from the surveillance population), this will result in sources having more recent infection dates than nonsources. Further comparisons are shown in Table 3.

Potential Role of Recent Infections

Given the possibility of reversion of drug resistance in untreated individuals, transmission of drug-resistant virus may be more likely if the source has been recently infected. We thus considered the fraction of primary and recent infections among the sources of TDR (see Methods). Thirty-four of 66 (51.5%) potential sources of TDR were individuals diagnosed in primary/recent infection, compared with only 57 of 187 (30.5%) of nonsource patients who carried resistance mutations and clustered with surveillance patients. The difference was significant (OR, 2.42 [95% CI, 1.36–4.3]; $P \leq .001$), but when controlling for estimated date of infection, significance was lost ($P = .98$).

Notable Individual Clusters

Two striking examples of long TDR transmission chains are shown in Figure 2. In Figure 2*A*, 2 treated patients and 7 drug-naive patients carry virus with L90M in the protease gene. In Figure 2*B*, 11 drug-naive patients carry virus with L90M, but no treated patients are present. These transmission chains indicate that either a treated patient transmitted resistant virus to several uninfected people, or that patients infected with

Table 3. Description of Sources and Nonsources Among Patients With Resistance Mutations Who Cluster With Surveillance Patients

	Sources, No. (%)	Nonsources, No. (%)	Total No.	Univariable		Multivariable	
				OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Transmission group					.69		.34
MSM	56 (85)	155 (83)	211	1.00 (Reference)		1 (Reference)	
Heterosexual	6 (9)	19 (10)	25	0.87 (.33–2.30)		1.15 (.33–4.03)	
IDU	1 (2)	7 (4)	8	0.40 (.05–3.29)		0.75 (.07–8.40)	
Other/unknown	3 (5)	5 (3)	8	1.66 (.38–7.18)		6.61 (.94–46.30)	
Sex					.19		.14
Male	65 (98)	177 (95)	242	1 (Reference)		1 (Reference)	
Female	1 (2)	9 (5)	10	0.30 (.04–2.44)		0.18 (.02–2.12)	
Age at time of infection					.98		.73
≤29	28 (42)	79 (42)	107	1 (Reference)		1 (Reference)	
30–39	26 (39)	73 (39)	99	1.00 (.54–1.87)		0.87 (.42–1.8)	
40–49	8 (12)	25 (13)	33	0.90 (.37–2.23)		0.56 (.2–1.6)	
≥50	4 (6)	9 (5)	13	1.25 (.36–4.40)		1.09 (.22–5.49)	
Year of infection					<.001		<.001
1985–1994	3 (5)	24 (13)	27	1 (Reference)		1 (Reference)	
1995–1999	7 (11)	90 (48)	97	0.62 (.15–2.59)		0.37 (.08–1.76)	
2000–2004	27 (41)	40 (22)	67	5.40 (1.48–19.73)		2.36 (.53–10.54)	
2005–2009	22 (33)	28 (15)	50	6.29 (1.67–23.62)		2.81 (.59–13.37)	
2010–2013	7 (11)	4 (2)	11	14.00 (2.51–77.99)		4.89 (.64–37.22)	
Primary infection at registration					.003		.98
No	32 (48)	130 (70)	162	1 (Reference)		1 (Reference)	
Yes	34 (52)	57 (30)	91	2.42 (1.36–4.30)		1.01 (.49–2.10)	
ART-naive at when mutation was observed					<.001		.011
No	12 (18)	110 (59)	122	1 (Reference)		1 (Reference)	
Yes	54 (82)	77 (41)	131	6.43 (3.22–12.82)		3.20 (1.27–8.05)	

The odds ratios in the multivariable model are adjusted for all variables listed in the table.

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; IDU, injection drug user; MSM, men who have sex with men; OR, odds ratio.

resistant HIV transmitted the resistant virus further. Thus, for the L90M mutation, reversion does not necessarily occur quickly or completely in absence of ART.

The 90M mutation confers resistance to nelfinavir and saquinavir [32], protease inhibitors whose use has decreased dramatically over time. Specifically, saquinavir and nelfinavir were used in ART for 30% and 10% of patients in the SHCS in the late 1990s, but for <5% of patients by 2005 and 2000, respectively. However, no similar decrease was seen in the incidence of transmitted 90M mutations (Figure 3). This strongly suggests continuing transmission among naive patients, independent of ART usage, as does our finding of large 90M transmission chains dominated by ART-naive transmitters.

One cluster contained signatures of reversion of drug resistance mutations. Figure 2*C* shows a cluster with 5 drug-naive sequences sharing at least 1 mutation. In addition, 1 viral sequence (labeled with **) harbors the 215F mutation. Interestingly, the 215F mutation is not present in 2 sequences from earlier infections in the cluster (green triangles). The infection dates and close phylogenetic relationships imply that this patient likely acquired his HIV infection from one of the earlier infected patients in this cluster; however, we did not observe the 215F mutation in virus from either of the earlier infected patients. Notably, the earlier infected patients (green triangles) had GRTs from a later date than the patient marked by **, so reversion of the 215F mutation could have occurred between transmission and sampling of the source patient's virus. Viruses from the other 4 patients in this cluster have the rare, but not resistance conferring, 215L mutation. 215L occurs only in 62 of 15 681 (0.4%) subtype B sequences in the SHCS-DRDB.

Sensitivity Analysis

A sensitivity analysis explored how the results changed depending on the criteria defining potential TDR sources (Figure 4).

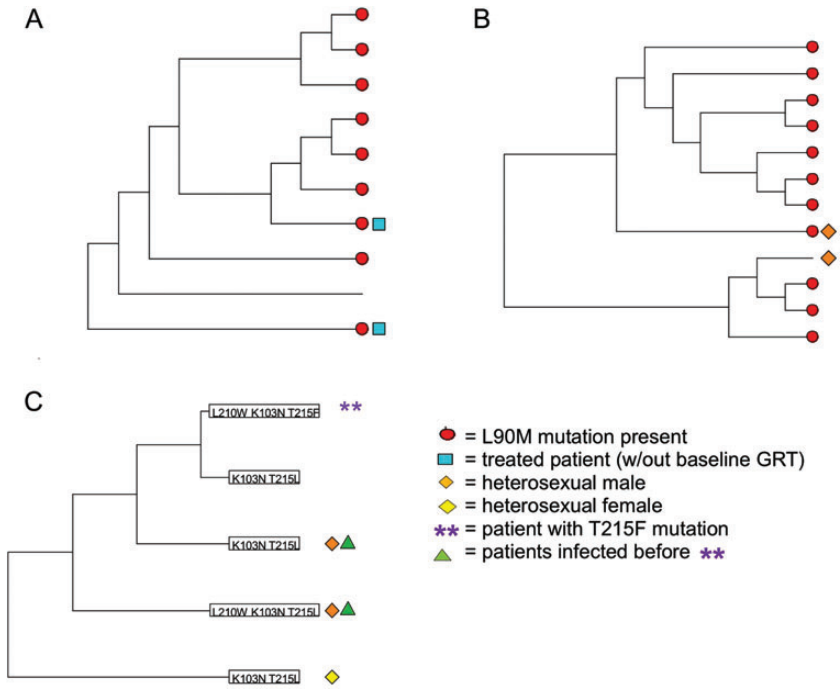

Figure 2. Patient clusters. For clarity, only the earliest sequence from each patient is shown. *A* and *B*, Clusters with 9 and 11 patients, respectively, with the L90M mutation in the protease gene (red circle), of which 2 and 0 were treatment experienced (blue square), respectively. *B*, Two heterosexual males (orange diamonds) are present. *C*, Transmission cluster including the T215F mutation in a drug-naive sequence (**), which is not present in 2 earlier infected sequences in the cluster (green triangles). Drug resistance mutations are shown at the tips. The cluster also contains 3 heterosexuals, 2 men (orange diamonds), and 1 woman (yellow diamond). Abbreviation: GRT, genotypic resistance test.

The number of source-recipient pairs in clusters decreases with stricter bootstrap or distance criteria. The fraction of naive sources, however, did not change significantly with the bootstrap or distance criteria (results not shown).

DISCUSSION

Here we investigated HIV-1 TDR in the SHCS-DRDB, which is representative for the HIV-infected population in Switzerland and contains exact treatment histories and estimates of infection dates. The high proportion (86/140 [61.4%]) of TDR-carrying individuals who belonged to Swiss transmission clusters indicates a major role for domestic transmission in the acquisition of TDR in our study population. Of TDR-carrying patients in domestic clusters, we linked many (50/86 [58.1%]) to sources, underlining the high-quality surveillance of domestic TDR in the SHCS-DRDB. We also found that drug-resistant HIV is transmitted between MSM and heterosexuals or injection drug users (Supplementary Data) [27].

We tested whether primary/recent infections were particularly important for the transmission of drug resistance. The frequency of primary infections in transmission clusters has been used to assess the importance of early infections in HIV transmission [14, 21, 33]. As diagnosis and treatment can lead to decreased risk behavior and lowered viral loads, transmission is less likely to occur after diagnosis [34]. Therefore, the proportion of infections diagnosed during early infection is a proxy for the amount of HIV transmission that occurred during early infection. We found a significant increase in the fraction of individuals diagnosed in primary infection among potential sources of TDR. However, when controlling for year of infection, the effect became nonsignificant. More generally, the increasing fraction of patients diagnosed during primary infection in recent years may confound assessments of the importance of primary infection.

We found some evidence of reversion. Figure 3*C* showed possible reversion of the 215F mutation, as potential sources in this cluster carried mutation 215L, an atypical amino acid that has been shown to emerge after 215F [35]. For all mutations found at codon 215, a much higher percentage were 215F or Y among treated patients than among patients with TDR. T215Y and T215F mutations have significant fitness costs in the absence of ART, but these costs are reportedly lower for T215C, D, and S [36]. Seeing higher-cost mutations in treated patients implies that these mutations may revert in the absence of drug pressure. Additionally, we found that diagnosis during primary

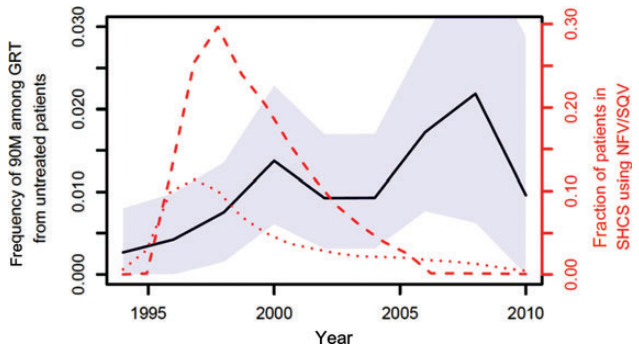

Figure 3. There has been a strong decrease in use of saquinavir (red dotted line) and nelfinavir (red dashed line), but not in the incidence of 90M mutations in newly diagnosed patients (black line). The confidence interval for the 90M mutation incidence (derived by bootstrapping) is shown in blue shading. Due to small numbers, incidence data were pooled every 2 years. The smaller confidence intervals for the use of saquinavir and nelfinavir are omitted for visual clarity. Abbreviations: GRT, genotypic resistance test; NFV, nelfinavir; SHCS, Swiss HIV Cohort Study; SQV, saquinavir.

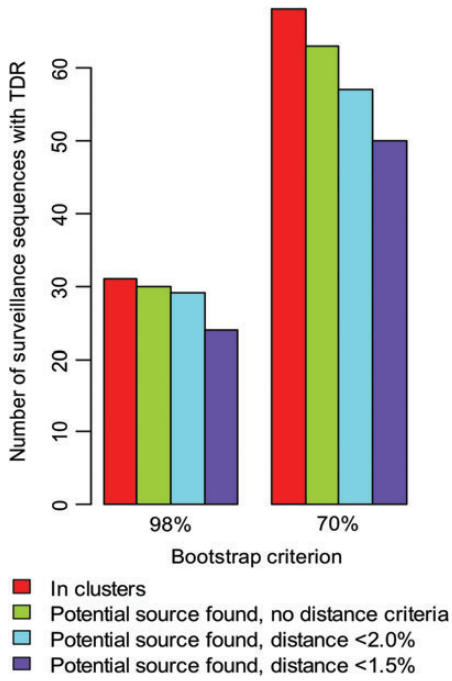

Figure 4. Sensitivity analysis. In the main analysis we used a bootstrap criterion of 70% with a genetic distance of <1.5% to determine source–recipient relationships, but here we considered the impact that varying these criteria had on the number of recipients for whom a source could be identified. Abbreviation: TDR, transmitted drug resistance.

infection was more common for surveillance patients with ART-resistant virus. This could reflect reversion occurring between transmission and viral sequencing; if more time passes before a GRT is conducted, less TDR may be detected.

Despite this evidence for TDR reversion, our results indicate that, overall, therapy-naive patients are the predominant sources of TDR. Two patterns suggested that long transmission chains of TDR occur in Switzerland. We found that a majority (56/66 [84.8%]) of potential TDR sources are ART-naive patients. We also identified several large clusters of TDR containing mainly ART-naive patients. These results highlight that although reversion occurs in patients not taking antiretrovirals, it often does not occur quickly or completely enough to prevent resistance mutations from being transmitted further. Particularly, the example of L90M in the protease demonstrates that resistance mutations can exhibit an apparently undiminished spread even after the drugs selecting for them dramatically decreased in use. A similar cluster was found in [37]. This implies, more generally, that even if the evolution of drug resistance mutations during treatment were completely preventable, the spread of TDR might continue. Several previous studies have also found that ART-naive patients contribute to the transmission of drug-resistant HIV-1 [11, 14, 38]. Thus, screening should be intensified to detect HIV infections earlier, to allow for early treatment as recommended in some recent treatment guidelines [39, 40]. Early treatment of patients with drug-resistant HIV would rapidly reduce the circulation of resistant viruses in the ART-naive population and interrupt this vicious cycle.

Although our study has a high representativeness of the surveillance population, we do not have GRTs for the entire epidemiologic network included in the study (patients not included in the SHCS-DRDB, not diagnosed, or infected outside the country), and can only assess patients in clusters in our sample population. We also focused on subtype B HIV in MSM in a resource-rich country. Finally, our methods also could not always resolve the direction of infection. However, this potential limitation has no major effect on our results because we found only 1 cluster in which infection dates of sources and surveillance patients were ambiguous and directionality thus was not clearly defined. Although it is thus unclear to what extent our results are generalizable to other risk groups, subtypes, or countries, they indicate a high potential of HIV drug resistance to circulate among treatment-naive patients. This highlights the importance of limiting the acquisition of drug resistance before it becomes established in untreated patients, and of early test-and-treat strategies to prevent resistance transmission from untreated patients.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org/). Supplementary materials consist of data

provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Members of the Swiss HIV Cohort Study. Aubert V, Barth J, Battegay M, Bernasconi E, Böni J, Bucher HC, Burton-Jeangros C, Calmy A, Cavassini M, Egger M, Elzi L, Fehr J, Fellay J, Furrer H (Chairman of the Clinical and Laboratory Committee), Fux CA, Gorgievski M, Günthard H (President of the SHCS), Haerry D (deputy of "Positive Council"), Hasse B, Hirsch HH, Hösli I, Kahlert C, Kaiser L, Keiser O, Klimkait T, Kovari H, Kouyos R, Ledergerber B, Martinetti G, Martinez de Tejada B, Metzner K, Müller N, Nadal D, Pantaleo G, Rauch A (Chairman of the Scientific Board), Regenass S, Rickenbach M (Head of Data Center), Rudin C (Chairman of the Mother & Child Substudy), Schmid P, Schultze D, Schöni-Affolter F, Schüpbach J, Speck R, Staehelin C, Tarr P, Telenti A, Trkola A, Vernazza P, Weber R, Yerly S.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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