Infrequent transmission of HIV-1 drug-resistant variants

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Transmission of drug-resistant variants is influenced by several factors, including the prevalence of drug resistance in the population of HIV-1-infected patients, HIV-1 RNA levels and transmission by recently infected patients. In order to evaluate the impact of these factors on the transmission of drug-resistant variants, we have defined the population of potential transmitters and compared their resistance profiles to those of newly infected patients. Sequencing of *pol* gene was performed in 220 recently infected patients and in 373 chronically infected patients with HIV-1 RNA >1000 copies/ml. Minimal and maximal drug-resistance profiles of potential transmitters were estimated by weighting resistance profiles of chronically infected patients with estimates of the Swiss HIV-1-infected population, the prevalence of exposure to antiviral drugs and the proportion of infections attributed to primary HIV infections. The drug-resistance prevalence in recently infected patients was 10.5% (one class drug resistance: 9.1%; two classes: 1.4%; three classes: 0%). Phylogenetic analysis revealed significant clustering for 30% of recent infections. The drug-resistance prevalence in chronically infected patients was 72.4% (one class: 29%; two classes: 27.6%; three classes: 15.8%). After adjustment, the risk of transmission relative to wild-type was reduced both for one class drug resistance (minimal and maximal estimates: odds ratio: 0.39, $P<0.001$; and odds ratio: 0.55, $P=0.011$, respectively), and for two to three class drug resistance (odds ratios: 0.05 and 0.07, respectively, $P<0.001$). Neither sexual behaviour nor HIV-1 RNA levels explained the low transmission of drug-resistant variants.

These data suggest that drug-resistant variants and in particular multidrug-resistant variants have a substantially reduced transmission capacity.

Introduction

Transmission of human immunodeficiency type 1 virus (HIV-1) resistant to antiretroviral drugs may reduce treatment efficacy and future treatment options [1,2]. Moreover, as antiretroviral drugs are increasingly used in countries with limited medical infrastructure, there is a risk for rapid spread of resistance both in the HIV-1-infected population and in newly infected patients [3,4].

The transmission of HIV-1 drug-resistant variants in Western Europe and North America where antiretroviral drugs have been available since the early 1990s ranges between 5 and 27% [1,2,5–11]. There have been alarming reports suggesting a steep increase, while others show or predict a relatively stable transmission rate of drug-resistant HIV-1 [1,2,8,9,12,13]. We have shown previously that the relatively low prevalence of drug resistance in recently infected patients in Switzerland is related to treatment efficacy in chronically infected patients, and partially related to the impact of recently infected patients on the spread of new infections [8]. Another factor that might limit

the spread of drug resistance in recently infected patients is an impaired replication of multidrug-resistant HIV-1 [14,15].

The main limitation of previous studies of drug resistance in both recently infected and chronically infected populations is that study samples were not representative of these populations [1,2,5–11,16,17]. Indeed, most studies on recent infection include small numbers and/or subgroups, whereas investigations on the prevalence of drug resistance in chronically infected patients have focused on patients enrolled in clinical trials.

To address the issue of transmission of drug-resistant variants, we compared drug resistance profiles in recently infected patients and in potential transmitters in Switzerland. Resistance profiles among potential transmitters were calculated on the basis of the resistance profiles determined among chronically infected patients from the Geneva Swiss HIV Cohort Study (1999–2001) weighed for the Swiss population and for the impact of recently infected patients on transmission.

Methods

Selection of patient populations

Recent infection was defined by either a documented acute HIV-1 infection based on symptomatology and dynamics of HIV-1 antibodies and HIV-1 RNA, or a documented negative HIV-1 antibody test in the year preceding the first HIV-1-positive antibody test. All recently infected patients identified between 1 January 1999 and 31 December 2001 in both the SHCS AIDS Centers and large laboratories were included [8]. The first plasma sample collected before treatment initiation was analysed.

Chronic infection was defined by at least a 24-month delay after a documented positive HIV-1 antibody test. All patients enrolled in the SHCS in Geneva with at least one clinical visit and a laboratory follow-up between 1 January 1999 and 31 December 2001 were included. We identified 977 chronically infected patients. Eighty-nine percent were drug exposed (either treated in the past or on antiretroviral drugs) and HIV-1 RNA was >1000 copies/ml in 373 (43%) of them. This cut-off for HIV-1 RNA was selected because large studies have shown that HIV-1 transmission rarely or never occurs through sexual contact for patients with <1000 RNA copies/ml [18].

Estimates for the Swiss HIV-1-infected population

In order to evaluate the prevalence of drug resistance in the whole Swiss HIV-1-infected population, we needed estimates of the total number of HIV-1-infected persons in Switzerland and the proportion of drug-exposed patients. The estimates of the number of HIV-1-infected persons in Switzerland were based on the mandatory declaration of all HIV-1-infected patients to the Swiss Federal Office of Public Health (SFOPH) [19]. After correction for double reporting of HIV-1-infected cases in Switzerland in the 1980s, for deaths and for reports concerning persons with short stay in Switzerland, the minimal estimate of HIV-1 infected persons living in Switzerland in 2001 was 14000 [19 and M Gebhardt, personal communication]. After correction for persons not diagnosed (estimation 10%), the maximal estimate of HIV-1-infected persons living in Switzerland in 2001 was 15400.

Estimation of the number of drug-exposed patients was based on sales of antiretroviral drugs in Switzerland in 1998 [20]. For the estimation of year 2000, we included a documented sale increase of 7% per year (T Waegli, GlaxoSmithKline, Switzerland, personal communication), and a 15% increase taking into account treatment interruptions and antiretroviral drugs provided free by the pharmacological industry within controlled clinical trials or for compassionate use. This leads to a number of drug-exposed patients in 2000 of 7930 patients. Based on these numbers, the proportion of drug-exposed patients in the Swiss HIV-1-infected population varied from 51 to 57% for the minimal and maximal estimates, respectively.

Plasma HIV-1 RNA levels

Quantitation of HIV-1 RNA was performed using Amplicor HIV Monitor version 1.5 (Roche Diagnostics, Basel, Switzerland) according to manufacturer's instructions.

Sequencing of *pol* protease, reverse transcriptase and env C2V3 regions

The procedure of viral isolation, reverse transcription, amplification and sequencing were described earlier [8]. Briefly, viral RNA was isolated from 100 µl of plasma using Amplicor HCV Cobas reagents (Roche). Viral RNA was transcribed into cDNA, which was subjected to nested PCR for the reverse transcriptase (RT) (574 bp), protease (PR) (291 bp) and env C2V3 (383 bp) regions. Direct double-stranded sequencing was performed on an automatic sequencer with Big Dye terminator kit (Applied Biosystems, Foster City, Calif., USA).

Analysis of drug-resistance mutations

Genotypic resistance was defined as the presence of mutations in the RT and PR genes associated with impaired drug susceptibility or virological response according to the consensus USA Panel [21]. Detection of one or more of the following mutations was retained to define genotypic resistance to nucleotide reverse transcriptase inhibitors (NRTIs) (RT: 41L, 44D, 65R,

67N, any insertion 69, 70R, 74V, 75T, 118I, 151M, 184V/I, 210W, 215Y/F/C/D/S) and non-nucleotide reverse transcriptase inhibitors (NNRTIs) (RT: 100I, 103N, 106A, 108I, 181C/I, 188C/L/H, 190A). The presence of at least one primary mutation (PR: 30N, 46I/L, 48V, 82A/F/T/V, 84V, 90M) was required for genotypic protease inhibitors (PIs) resistance. Resistance profiles were defined as resistance to one, two or three classes of drugs (NRTI, NNRTI or PI).

Phylogenetic analysis

Alignment of sequences was performed using ClustalW version 1.7 [22]. Phylogenetic analyses were carried out using the PHYLIP package [23]. Neighbour-joining trees with 100 replicates were used in the bootstrap analysis, and values above 85 were considered as supporting the grouping. These clusters were confirmed using Fitch-Margoliash and least-squares analysis with 100 bootstrap replicates [24].

Analysis of sexual behaviour

At their regular clinical visits, during planned follow-ups of the SHCS, patients are requested to answer a short questionnaire about sexual activities during the preceding 6 months. Results were exploited to assess whether sexual behaviour differs among chronically infected patients with different drug-resistance profiles.

Statistical analysis

Comparison of HIV-1 RNA levels across drug-resistance profiles was performed using the Mann-Whitney test. A χ^2 test with multinomial probability [25] was used to compare drug-resistance profiles between the 220 recently infected patients and a theoretically infinite population of potential transmitters. Two versions of the population of transmitters were used (maximal and minimal estimate, described above). The relative risk for transmission according to drug-resistance profiles was calculated using logistic regression, where the 220 recently infected patients were compared with the theoretical populations of transmitters (a population of size 100000 was simulated). Since the number of recently infected patients in each resistance category was small, we used exact estimation algorithms (StatXact and LogXact, version 4.0, Cytel Software, Cambridge, Mass., USA).

Results

Population characteristics

From 1 January 1999 to 31 December 2001, 225 consecutive recently infected patients were analysed for drug resistance. Five patients were excluded because genotype was not available. During the same period, all patients (n=373) from the SHCS in the Geneva Center with chronic infection and HIV-1 RNA >1000 copies/ml were analysed for drug resistance. Table 1 reports patient characteristics.

There were no significant differences between patients with recent and chronic infection for gender (P>0.1) and risk groups (P>0.5), whereas HIV-1 RNA and the proportion of non-B subtype infections were significantly (P>0.001) higher in recently infected patients as compared to chronically infected patients (Table 1). The demographic characteristics were similar for chronically infected patients included in the genotypic study and for the whole Geneva SHCS population.

Genotypic analysis

Table 2 reports the profiles of drug resistance in recently and chronically infected patients. Genotypic evidence of resistance was detected in 23 (10.5%) recently infected patients. Among the three classes of antiretroviral drugs, the highest prevalence was observed for mutations associated with NRTI resistance (8.6%), followed by mutations associated with PI resistance (2.3%) and NNRTI resistance (0.9%). The prevalence of genotypic resistance was 9.1% for one class of antiretroviral drug and 1.4% for two classes, whereas none of the recently infected patients in 1999–2001 had genotypic resistance to three classes.

Genotypic evidence of resistance was detected in 270 (72%) chronically infected drug-exposed patients. The highest prevalence was observed for mutations associated with NRTI resistance (67%) followed by mutations associated with PI resistance (37%) and NNRTI resistance (28%). The prevalence of genotypic resistance was 29% for one class of drug, 28% for two classes and 16% for three classes.

Phylogenetic analysis

A phylogenetic tree was constructed using neighbour-joining method for the 220 available RT sequences of recently infected patients. In order to avoid missing potential clusters in patients infected in 1999, all RT sequences (n=57) of recently infected patients from 1998 [8] were included in the tree. Using neighbour-joining method, significant clustering (bootstrap value ≥85/100) was found for 94 patients infected in 1999–2001. A phylogenetic tree was constructed using Fitch-Margoliash method with RT sequences of patients included into clusters. Significant clustering (>90/100) was confirmed for all patients (Figure 1). The proportion of recently infected patients who were infected by patients with recent infection was estimated at 30% (number of patients in clusters minus the number of clusters). The proportion of recently infected patients included in clusters was 24% for patients infected with B subtype and 41% for patients

Table 1. Baseline characteristics of study populations with drug resistance testing

	Recent HIV-1 infection	Drug-exposed chronic HIV-1 infection
n	220	373
Female, *n* (%)	56 (25)	118 (32)
Age, median (range), years	32 (18–69)	39 (18–68)
Risk factors, *n* (%)		
MSM	74 (34)	131 (35)
Heterosexual	73 (33)	133 (36)
IVDU	62 (28)	98 (26)
Others	0	6 (1.6)
Unknown	11 (5)	5 (1.4)
HIV-1 RNA, median (range), $\log_{10}$ copies/ml	5.00 (3.00–7.45)	4.08 (3.00–6.26)
CD4, median (range), cells/mm^3	525 (32–1384)	355 (2–1733)
Non-B subtypes, *n* (%)	76 (35)	58 (16)

MSM, men who have sex with men; IVDU, intravenous drug user.

Table 2. Drug-resistance profiles in recently and chronically HIV-1-infected populations

Drug-resistance profiles, *n* (%)*	Recent HIV-1 infection (*n*=220)	Drug-exposed chronic HIV-1 infection (*n*=373)
No mutation	197 (89.5)	103 (27.6)
Any mutations	23 (10.5)	270 (72.4)
Any NRTI mutations	19 (8.6)	248 (66.5)
M184V/I mutation only	0 (0.0)	39 (10.5)
Any NNRTI mutations	2 (0.9)	106 (28.4)
Any primary PI mutations	5 (2.3)	137 (36.7)
Mutations affecting one class	20 (9.1)	108 (29.0)
Mutations affecting two classes	3 (1.4)	103 (27.6)
Mutations affecting three classes	0 (0.0)	59 (15.8)

*See Methods section.

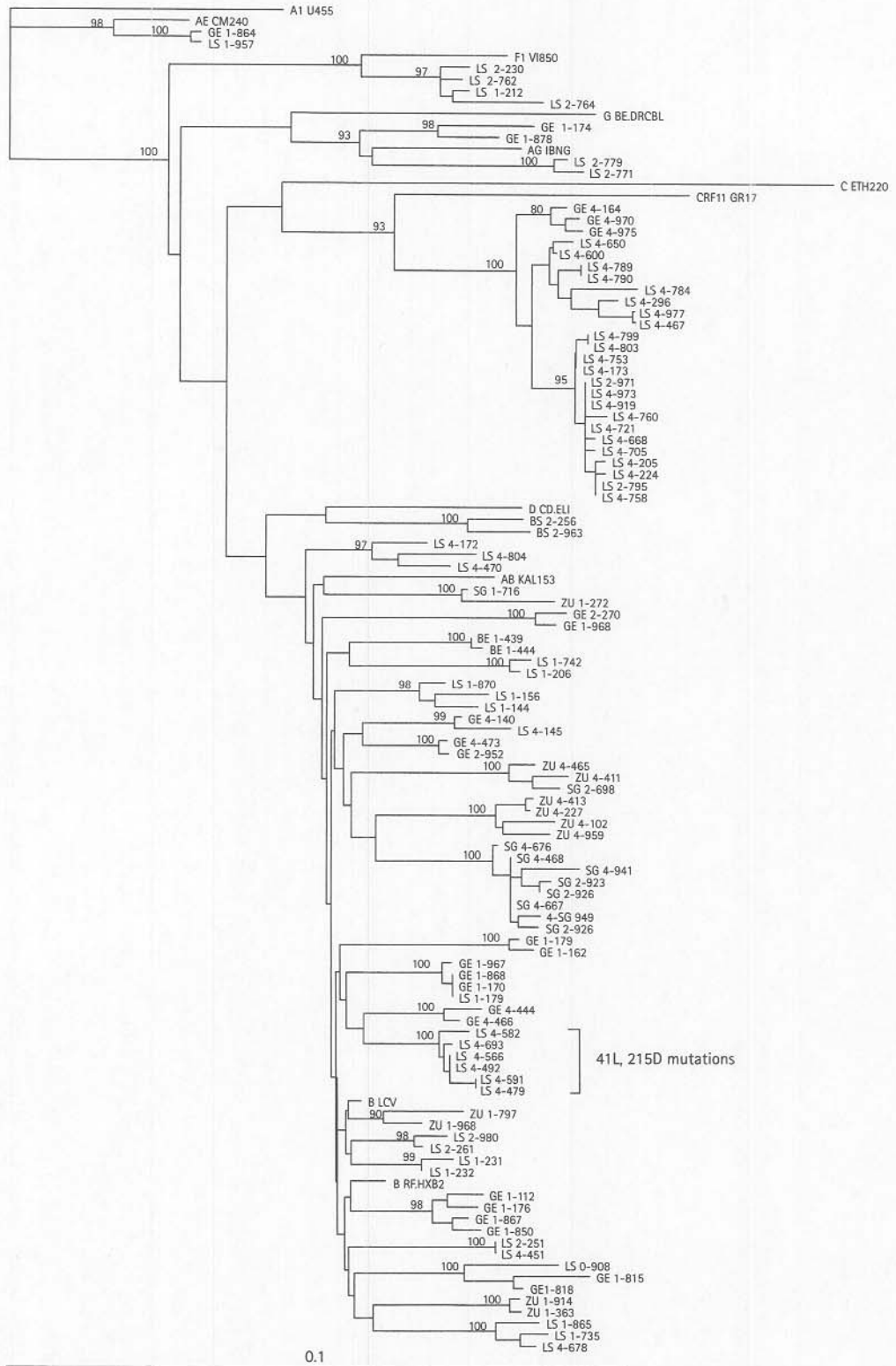
infected with non-B subtypes. The latter reflected the identification of a large cluster of Caucasian intravenous drug users living in the western part of Switzerland [26] infected with a recombinant circulating form, CRF-11. Removal of this large cluster resulted in a similar proportion of patients infected by recently infected patients among B and non-B subtypes (20%). All sequences included in clusters had a wild-type genotype except for a cluster of six patients with the 41L and 215D mutations. These results were confirmed using phylogenetic analyses of PR and env C2V3 regions (data not shown).

Estimation of resistance in potential transmitters and comparison with recently infected patients

We next estimated the prevalence of drug resistance in potential transmitters considering that recently infected patients are responsible for 30% of HIV-1 transmission. Drug resistance in potential transmitters is thus based on the following equation: (resistance profiles in the viraemic Swiss chronically infected population × 0.70) + (resistance profiles in the recently infected × 0.30). The drug-resistance profiles in the Swiss chronically infected population were estimated by weighing the observed drug-resistance profiles in the Geneva SHCS chronically infected population against the proportion of drug-exposed patients in the Swiss chronically infected population. The minimal estimate of drug resistance was calculated by considering 51% of the Swiss chronically infected population as drug-exposed and a prevalence of resistance for drug-naive patients of 0%. The maximal estimate of drug resistance was calculated by considering 57% of the Swiss chronically infected population as drug-exposed and a prevalence of resistance in drug-naive patients corresponding to values measured in recently infected patients. The prevalence of drug resistance varied from 13.1 to 17.0% for one class drug resistance, 10.3 to 11.9% for two classes, and 5.6 to 6.3% for three classes (Table 3).

Overall, the distribution of drug-resistance profiles estimated in potential transmitters was different from that observed in recently infected patients (χ^2: 43.7 and 63.3; P<0.001 for minimal and maximal estimates,

Figure 1. Phylogenetic analysis of reverse transcriptase region from recently infected patients included into clusters



Patient codes represent location (BS, Basel; LS, Lausanne; GE, Geneva; ZU, Zurich; SG, St-Gallen), followed by risk factors (1, men who have sex with men; 2, heterosexual; 4, intravenous drug user) and patient identification number with three digits. Numbers at branch nodes represent bootstrap values for 100 replicates. Values <80 are not reported. HIV-1 reference sequences according to GenBank.

Table 3. Estimates of drug-resistance profiles in recently infected patients and in potential transmitters

Resistance profiles	Recently infected patients		Estimates of potential transmitters* (%)	
	Percentage	95% CI	Min †	Max §
Wild-type	89.5	83.5–94.1	71.0	64.8
One class resistance	9.1	5.1–14.9	13.1	17.0
Two classes resistance	1.4	0.2–4.6	10.3	11.9
Three classes resistance	0.0	0.0–2.1	5.7	6.3

*Corrected for transmission among recently infected (30% of related sequences). Potential transmitters = (resistance profiles Swiss chronically infected × 0.70) + (resistance profiles recently infected × 0.30).
† The minimal estimate of drug resistance was calculated by considering 51% of the Swiss chronically infected population as drug exposed and a prevalence of resistance for drug-naive patients of 0%.
§ The maximal estimate of resistance was calculated by considering 57% of the Swiss chronically infected population as drug exposed and a prevalence of resistance in drug-naive patients corresponding to values measured in recently infected patients.

respectively, see Table 3 for minimal and maximal estimates). A decreased relative risk of transmission was found for both one class and two to three classes of drug resistance as compared to the wild-type; with odds ratios of 0.39 and 0.55 for 1 class (P=0.011 and P<0.001) for maximal and minimal estimates of resistance prevalence among potential transmitters, and of 0.05 and 0.07 (P<0.001) for two to three classes (Figure 2). We next explored the impact of the removal from the analysis of patients with isolated 184V/I mutations, which is known to decrease viral replicative capacity. This resulted in a relative risk of transmission for drug resistance to one class that differed significantly from the wild-type only for the maximal resistance estimates (odds ratio: 0.48; P=0.0009), but not for minimal resistance estimates (odds ratio: 0.71; P=0.17).

HIV-1 RNA in chronically infected patients

As viraemia level affects transmission efficiency [18], HIV-1 RNA levels were analysed in the Geneva SHCS chronically infected, drug-exposed patients with HIV-1 RNA >1000 copies/ml according to resistance profile (Figure 3). Similar HIV-1 RNA levels were found for patients with wild-type and three classes drug-resistant variants (Mann-Whitney test, P=0.97), whereas HIV-1 RNA levels of patients resistant to one or two classes of drugs were significantly lower than HIV-1 RNA of patients resistant to the three classes (P=0.007 and P=0.05, respectively).

Sexual activity of patients

The data of SHCS were analysed for the clinical visit closest to the time of sample collection for resistance typing, covering the 6-month period prior to the clinical visit. There were no significant differences between patients with resistance to one, two and three classes, regarding the proportion who reported anal or vaginal intercourse during the recall period, the irregular use of condoms during anal/vaginal sex, or having occasional non-stable partners. Data regarding the number of partners and the frequency of sexual intercourse were not available.

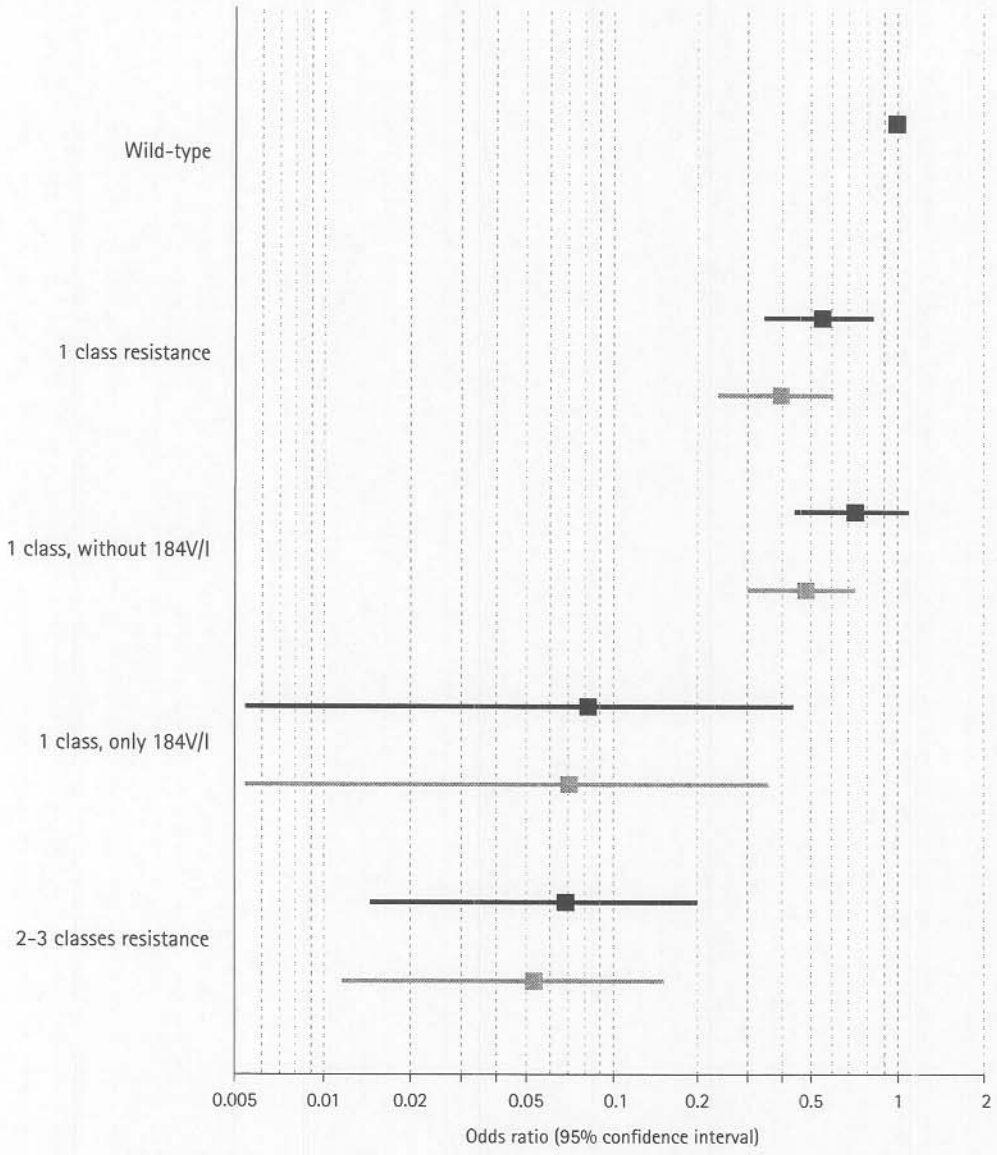
Discussion

In this investigation, we compared sequences of the *pol* gene in 220 patients with recent infection and in 373 drug-exposed, viraemic patients with chronic infection. We next evaluated maximal and minimal drug-resistance profiles among potential transmitters by combining results obtained in chronically infected patients with estimates of antiretroviral drug consumption in Switzerland. These results were further adjusted to take into account the impact of recently HIV-1-infected patients on transmission. We found that: 1) the prevalence of drug-resistant HIV-1 in potential transmitters was between 29 and 35%; and 2) the relative risk for transmission of drug resistance was decreased by about twofold for one class drug resistance and from 14- to 20-fold for two to three classes.

Several contributions have previously reported the prevalence of drug resistance in recently infected patients [1,2,5–11] and in drug-exposed patients [2,16,17]. However, there is no systematic study relating the viral genotypic characteristics in potential transmitters to those of newly HIV-1-infected patients. This issue has only been addressed recently in theoretical models based on sets of partially representative data requiring large ranges of estimates [12,13].

Our first step was to define the resistance profiles in two groups of patients, recently infected and chronically infected viraemic patients, respectively. Before adjustment, there was a much lower prevalence of resistance in recently infected than in chronically infected patients, particularly for multidrug-resistant variants. Our prevalence rate of drug resistance in chronically infected drug-exposed patients is in the same range of that reported in HIV-1 drug-exposed patients in Western Europe and North America [2,16,17]. A meaningful comparison between the two populations in the context of HIV-1 transmission

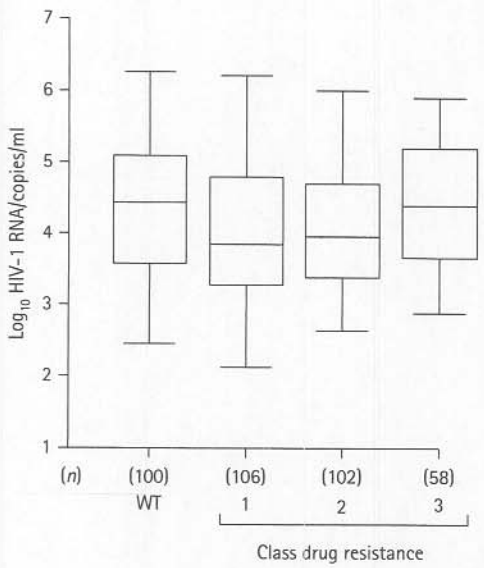
Figure 2. Relative risk for transmission according to drug-resistance profiles



The odds ratios are represented in reference to wild-type virus, with maximal (black line) and minimal (grey line) estimates, for one class drug-resistant variants with and without inclusion of patients with the 184V/I, for 184V/I only and for two to three class resistance (combined data).

required an adjustment of the chronically infected population in order to define the population of potential transmitters. Taking advantage of two large databases and of data on antiretroviral drug consumption, we first estimated the size of the Swiss HIV-1-infected population and the proportion of drug-exposed patients in order to evaluate the resistance profiles in the chronically infected Swiss population with HIV-1 RNA >1000 copies/ml. This cut-off was selected due to the infrequent transmission by patients with lower HIV-1 RNA [18]. In order to estimate the resistance profile in potential transmitters, the resistance profiles in the Swiss chronically infected population were finally weighed according to the impact of recently infected patients on transmission based on the percentage of related sequences in this population [8]. Analyses of HIV-1 *pol* sequences revealed a higher percentage of non-B subtypes in recently infected as compared to chronically infected individuals, reflecting the observed progressive increase of non-B infections in recent years in Western Europe [27]. The excess of non-B infections in recently infected individuals was associated with a local epidemic of a particular circulating recombinant form (CRF-11) in Caucasian intravenous drug users. This suggests that new infections with non-B are actively

Figure 3. HIV-1 RNA levels in the Geneva SHCS chronically infected patients according to drug-resistance profiles



The number of patients included in the analysis is reported in parenthesis for patients with wild-type (WT) and for patients with, respectively, one, two and three class drug-resistant variants.

transmitted in Switzerland. Moreover, in chronically infected patients with non-B subtypes, the percentage of patients with drug resistance (69%) was similar to that measured in chronically infected patients with B subtype (72%), suggesting equal access to antiretroviral treatment. As the resistance profile in potential transmitters has been weighed for the impact of higher percentages of related sequences and of non-B infections in recently infected individuals, the overall two- to fourfold higher prevalence of drug resistance in recently infected patients derives from other factor(s).

This issue was explored by analysing the relative risk of transmission for one and two to three classes of drug resistance as compared to the wild-type. There was about twofold decrease risk of transmission for one class drug resistance. In contrast, the risk of transmission was decreased by more than 10-fold for two to three classes. This suggests that multidrug-resistant variants are transmitted with a lower efficiency than wild-type variants.

Others have reported higher frequencies of transmission of drug-resistant variants [1,2]. However, the high resistance prevalence in recently infected patients observed in California, was, as in our investigation, associated with a gradient in the percentage of transmission of one, two and three drug class resistance (16.4, 6.2 and 0.4%, respectively) [1]. Moreover, a recent contribution based on comparison of resistance in acutely infected patients in southern California and in chronically infected patients but without adjustment for transmission by acutely infected patients also concluded that transmission of resistant variants was lower than expected [28]. Another factor that might explain the relatively low prevalence of resistance in our recently infected patients is a reversion of mutant variants to a wild-type but this has been shown to be rare in recently infected patients infected for less than 1 year [29–31]. It has been reported that resistant variants have lower fitness or replicative capacity, and lower infectivity than wild-type virus [14,15,31–35]; in particular, the 184V/I mutation has been consistently associated with reduced fitness [31–36]. Since we cannot completely exclude the reversion to wild-type of 184V/I mutation in some of the recently infected patients, we repeated the analysis after removal of patients with isolated 184V/I mutation. This results in a risk of transmission of drug resistance to one class that did not differ significantly from the wild-type for the minimal estimate. The detection of a cluster of six patients with the 41L and 215D mutations also suggests a low impact on transmission efficiency of one class drug-resistant variants.

The first limitation of this investigation is due to the fact that we analysed blood virus, which is only a surrogate compartment for the transmitting virus in patients infected through sexual contacts. HIV-1 transmission is quantitatively linked to viral load in transmitters, with each log increase in HIV-1 resulting in a 2.45-fold increase in the risk of transmission [18]. This factor can be ruled out for decreased transmission of two to three class drug-resistant variants since the HIV-1 RNA levels of drug-exposed wild-type patients are similar to those of two to three class drug-resistant patients. Moreover, our results were adjusted for a 30% transmission by patients with recent infection who had 1 log higher HIV-1 RNA than chronically infected patients. Other potential drawbacks are the underestimation of the percentage of patients unaware of their HIV status and a higher proportion of transmission by recently infected patients. When we increase in the model of transmission the percentage of patients unaware of their infection status to 30%, and consider that 50% of transmissions are due to recently infected patients, the relative risk of transmission for minimal estimate of resistance was similar for wild-type and for one class drug-resistant variants (odds ratio: 0.74; P=0.23), but was still significantly decreased for the two to three class of drug resistance (odds ratio: 0.12; P<0.001). This confirms, under extreme constraints, the validity of our main finding, that is, the reduced transmission of the two to three class drug-resistant variants.

Another potential confounding factor is the risk behaviour of transmitters carrying wild-type or drug-

resistant variants. The patients with resistance to several classes of drugs may be more ill, more likely to have a reduced sexual activity and/or to respect more universal precautions to prevent transmission of HIV-1 infection. However, the data on sexual behaviour available in the SHCS do not support this hypothesis.

In conclusion, this investigation indicates that multidrug-resistant and likely the 184V/I variants are transmitted at a lower rate than expected, based on resistance profiles in potential transmitters. This is good news for countries with widespread drug availability but also lessens concern about the rapid spread of resistance in newly infected patients in the developing world. However, these findings also underline that, owing to the relatively high transmission of one class drug-resistant variants, antiretroviral treatment in resource-limited countries should be based on optimal combination of drugs and not be limited to the availability of one or two classes of antiretroviral drugs.

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