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Prevalence of drug resistance and newly recognised treatment-related substitutions in the HIV-1 reverse transcriptase and protease genes from HIV-positive patients naïve for anti-retrovirals

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ABSTRACT

The aim of this study was to assess the prevalence of genetic changes in either the HIV reverse transcriptase (RT) or protease (Pro) genes in a cohort of patients naïve for anti-retroviral therapy. Of 61 patients, 43 (70.5%) were infected with HIV strains harbouring at least one resistance-related mutation, with 41 (67.2%) harbouring newly recognised treatment-related mutations. Among the 61 patients, the prevalence of specific mutations in the RT gene was as follows: 39A, 1.6%; 43E, 1.6%; and 228H, 1.6%. The prevalence of specific mutations in the Pro gene was as follows: 11I, 1.6%; 13V, 26.2%; 35D, 19.6%; 45R, 1.6%; 58E, 1.6%; 62V, 31%; 72V, 11.4%; 72M, 6.5%; 72T, 3.2%; 75I, 1.6%; and 89M, 13%. A higher prevalence of newly recognised mutations was found in strains from patients infected through sexual practices (30/36 = 83.4% vs. 11/25 = 44%; p 0.0023; OR 10.91; 95% CI 3.14–40.39). These findings support the use of resistance testing in patients naïve for anti-retroviral therapy, and suggest that the possible impact of newly recognised treatment-related mutations on clinical outcome requires further investigation.

Keywords AIDS, anti-retroviral therapy, HIV, resistance mutations

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INTRODUCTION

The advent of highly active anti-retroviral therapy has resulted in significant reductions in opportunistic infections and deaths among HIV-positive patients. These benefits continue, but the incidence of treatment failures is increasing, mainly because of the emergence of HIV drug resistance mutations [1]. These mutations may also be present in therapy-naïve patients, either from a stochastic process or following transmission from index patients. The clinical impact of these mutations has been demonstrated by evidence from patients harbouring resistant strains of the prolonged time required to reach an undetectable viral load and the higher risk of virological relapse [2].

The prevalence of resistance mutations in therapy-naïve patients may vary depending on the country in which a survey is conducted, epidemiological features (e.g., drug exposure and treatment response at a population level, risk factors for HIV transmission), technical features (type of test used and mutations considered to predict resistance) and the duration of HIV infection [3]. Secondary mutations or natural polymorphisms at numerous protease codons have been identified in therapy-naïve HIV patients, with possible implications for viral fitness evolution and response to anti-retroviral therapy [4–6]. The possibility of novel mutations in either the reverse transcriptase (RT) [7] or the protease (Pro) [8] genes has been suggested following treatment exposure, but few data have been collected regarding their prevalence in therapy-naïve patients.

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The aim of this study was to assess the prevalence of any amino-acid changes resulting from mutations in either the RT or in the Pro gene in a cohort of patients naïve for anti-retroviral therapy. A second aim was to assess whether these changes were related to epidemiological and clinical characteristics among the patients in the study cohort.

MATERIALS AND METHODS

Patients were identified from the clinical and genotypic database at the Institute of Infectious and Tropical Diseases (Brescia, northern Italy). Each patient was interviewed to confirm that no anti-retroviral agents had been prescribed previously. Patients were stratified into two groups by time of infection: acute or recently infected patients, and patients whose duration of HIV infection was either unknown or >1 year. Patients were also stratified by gender, risk factors for HIV acquisition (intravenous drug use), HIV viral load (≤ 10 000 or >10 000 copies/mL plasma), $CD4^+$ T-cell count (≤ 200 or >200 cells/mL) at the time of resistance testing, and the time when resistance testing was performed (last calendar year or before; May 2002 to May 2003; or April 1998 to April 2002).

HIV genotyping was performed with a TRUGENE HIV-1 Genotyping kit (Bayer Diagnostics, Milan, Italy). Amino-acid substitutions were categorised into five different groups: (1) mutations known to confer resistance to anti-retroviral drugs, as listed on the International AIDS Society (IAS) mutation list [9], plus the mutation V179D in the RT gene, which is not listed by the IAS, but which is responsible for low-level resistance to each of the non-nucleoside RT inhibitors (NNRTIs) [10], but since mutation L63P in the Pro gene does not cause any appreciable increase in the phenotypic resistance for any protease inhibitor (PI) [11], this mutation was considered to be a polymorphism; (2) mutations that have been identified recently as treatment-related (i.e., their prevalence increases according to the number of drugs prescribed) [7,8]; (3) any amino-acid variations, compared to the wild-type, that occurred at codon positions listed by the IAS, but which differed from the specific amino-acid change listed by the IAS (i.e., atypical IAS mutations) [9]; (4) silent nucleotide changes at codon positions listed by the IAS (i.e., a nucleotide change that did not result in an amino-acid change with respect to the HIV wild-type reference sequence); and (5) amino-acid substitutions at positions not listed by the IAS or recognised as resistance-related to date (i.e., polymorphisms).

Mutation patterns were interpreted with the TRUGENE HIV-1 Genotyping Test Resistance Report (Guidelines 5.0) rules-based algorithm (Bayer Diagnostics). This system uses three categories to predict HIV drug susceptibility: resistance, possible resistance, and sensitivity. In this study, resistance and possible resistance results were grouped together for analysis. Viral subtypes were determined by comparing HIV *pol* sequences with the HIV reference sequence using a BLAST database search (http://www.hiv.lanl.gov/content/hiv-b/basic_blast/basic_blast.htm). Viraemia was determined with the VERSANT HIV-1 RNA 3.0 bDNA Assay with a System 340 bDNA Analyzer (Bayer Diagnostics). Chi-squared tests were used to compare the prevalence of resistance between stratification groups (see above), with $p < 0.05$ considered to indicate statistical significance.

RESULTS

Sixty-one patients were included in the study between April 1998 and May 2003. Patient characteristics at the time of resistance testing are listed in Table 1.

All of the patients harboured viruses with at least one amino-acid change, with respect to the wild-type, in either the RT gene or the Pro gene. Among the 61 patients, 43 (70.5%) were infected with HIV carrying at least one mutation either listed by the IAS or resistance-related (six (9.8%) to nucleoside or nucleotide reverse transcriptase inhibitors (N(t)RTIs), five (8.2%) to NNRTIs, and 40 (65.5%) to PIs; Table 2). Ten (16.4%) HIV isolates had at least one IAS or resistance-related mutation in the RT gene. No major mutations listed by the IAS were found in the Pro gene.

The TRUGENE HIV-1 rules-based interpretation system did not predict resistance to any of the PIs. However, in the RT gene, the prevalence of resistance to N(t)RTIs, as interpreted by TRUGENE, correlated with the prevalence of IAS or resistance-related mutations (Table 2). Ten (16.4%) HIV resistance patterns were interpreted as resistance to at least one RT inhibitor. With respect to particular N(t)RTIs, the prevalence of resistance indicated by TRUGENE interpretation

Table 1. Characteristics of the 61 patients included in the study

Characteristic	Patients
Age (years)	
Median (IQR)	37 (23–64)
Gender; *n* (%)	
Male	48 (78.7%)
Female	13 (21.3%)
HIV transmission category; *n* (%)	
IVDU	25 (41.0%)
Not IVDU	36 (59.0%)
$CD4^+$ cell count (cells/mm^3)	
Median (IQR)	289 (4–992)
Patients with $CD4^+$ count ≤200 cells/mm^3	14 (23.0%)
HIV RNA (copies/mL)	
Median (IQR)	21 000 (500–750 000)
Patients with HIV RNA ≤10 000 copies/mL	21 (34.4%)
Time of infection; *n* (%)	
Acute or recent (≤ 1 year)	10 (16.4%)
Chronic or unknown	51 (83.6%)
Time of testing; *n* (%)	
Last year	31 (50.8%)
Previous years	30 (49.2%)
Viral subtype; *n* (%)	
B	56 (91.8%)
Others	5 (8.2%)

IQR, interquartile range; IVDU, intravenous drug use.

Table 2. Genotypic changes and related rules-based interpretation in HIV strains

Resistance	N(t)RTI	NNRTI	PI
TRUGENE prediction[a]	6/61 (9.8%)	5/61 (8.2%)	0/61 (0%)
IAS[b] or resistance-related mutations	6/61 (9.8%)	5/61 (8.2%)	40/61 (65.5%)
Recently identified treatment-related mutations[c]	3/61 (4.9%)	0/61 (0%)	40/61 (65.5%)
Atypical mutations at IAS positions[d]	4/61 (6.5%)	2/61 (3.2%)	8/61 (13.1%)
Silent nucleotide substitutions[e]	43/61 (70.5%)	32/61 (52.4%)	37/61 (60.6%)
Polymorphisms[f]	60/61 (98.4%)	34/61 (55.7%)	60/61 (98.4%)
Total (any of the above)	60/61 (98.4%)	48/61 (78.6%)	61/61 (100%)

N(t)RTI, nucleoside (nucleotide) reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
[a]Resistance patterns interpreted by the TRUGENE HIV-1 Genotyping Test Resistance Report (Guidelines 5.0). 'Resistant' and 'possible resistant' results are ranked together for the prevalence count.
[b]Mutations known to confer resistance to anti-retroviral drugs as listed by the International Aids Society (IAS) [9]. Prevalence includes V179D in the RT gene [10], but excludes L63P in the Pro gene, which is currently considered to be a natural polymorphism [11].
[c]Recently identified treatment-related mutations [7, 8].
[d]Amino-acid changes at positions indicated by the IAS, but different from the specific changes indicated [9].
[e]Mutations at the codon positions listed by the IAS, but which do not correspond to amino-acid substitutions.
[f]Amino-acid changes at positions neither indicated by the IAS nor recognised as resistance-related, including those at position 63 in the Pro gene, found in 45 (73.8%) patient isolates. L63P was found in 36 (59%) cases. At this position, amino-acids AFIQST have also been found. R57K has been associated with a high rate of virological failure in patients prescribed regimens containing a PI (especially nelfinavir) [6]; this mutation was found in eight (13.1%) of 61 patients.

was highest for zidovudine (4/6), and lowest for stavudine and tenofovir (1/6 for both drugs). For NNRTIs, the TRUGENE system successfully predicted all five patients with viruses resistant to nevirapine, delavirdine and efavirenz. Only one strain, from a patient with acute HIV infection, had multidrug resistance to both NNRTI and N(t)RTI classes.

The prevalence of newly recognised treatment-related mutations in the Pro gene was high, with three (4.9%) patients having at least one such mutation conferring resistance to N(t)RTIs, and 40 patients (65.5%) having at least one such mutation conferring resistance to PIs (Table 2). Specific mutations in the Pro gene were as follows: 11I, 1.6%; 13V, 26.2%; 35D, 19.6%; 45R, 1.6%; 58E, 1.6%; 62V, 31%; 72V, 11.4%; 72M, 6.5%; 72T, 3.2%; 75I, 1.6%; and 89M, 13%. In the RT gene, newly identified treatment-related mutations were as follows: 39A, 1.6%; 43E, 1.6%; and 228H, 1.6%.

Atypical mutations at codon positions listed by the IAS were found either in mixed populations together with a major mutation (i.e., 36I/L) or singly (i.e., 67G, 75L and 215D/C in the RT gene, and 20I and 82I in the Pro gene). Only mutations listed by the IAS, or resistance-related mutations, were found at other positions (i.e., 41, 69, 103, 108, 179, 181, 184, 210 and 219 in the RT gene, and 10, 71 and 77 in the Pro gene; Fig. 1).

Following stratification by relevant epidemiological and clinical features (including HIV subtype), the only statistically significant difference was a higher prevalence of the newly recognised treatment-related mutations in strains from patients infected through sexual practices, as opposed to intravenous drug use (30/36 = 83.4% vs. 11/25 = 44%; p 0.0023; OR 10.91; 95% CI 3.14–40.39).

DISCUSSION

Mutations conferring resistance to anti-retroviral drugs exert a negative impact on the virological response to highly active anti-retroviral therapy, both in patients who have received anti-retroviral therapy previously (secondary resistance) and in therapy-naïve patients (primary resistance) [2]. Since the end of 1990, the prevalence of primary resistance in the USA and Europe has been increasing. Thus, in the USA, the prevalence increased from 3.4% in 1995 to 12.4% in 2000 [2]. However, an opposite trend has been demonstrated in certain countries, e.g., Switzerland, where the prevalence of primary resistance was 8.6%, 14.6%, 8.8% and 5.5% in 1996, 1997, 1998 and 1999, respectively [12], while a large collaborative multinational study found a primary resistance prevalence of *c.* 10% across Europe [13]. In the present study involving 61 patients, the overall prevalence of primary resistance mutations considered in the IAS resistance tables to be resistance-related [9] was 16.4% after the exclusion of minor mutations found in the Pro gene. The rules-based TRUGENE algorithm suggested a 16.4% prevalence of resistance, support-

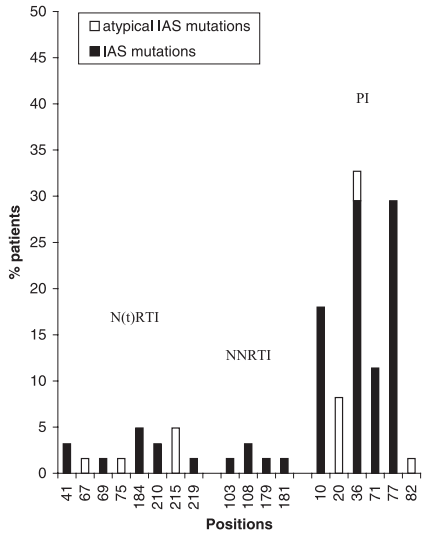

Fig. 1. Prevalence of mutations (both canonical and atypical) at positions indicated by the International AIDS Society (IAS) in HIV strains from the study patients. N(t)RTI, nucleoside (nucleotide) reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

ing the possible relevance of resistance testing in anti-retroviral-naïve patients.

Even though protease resistance patterns were not interpreted as conferring resistance by the rules-based algorithm used, some minor mutations found at high prevalence, such as those at positions 10 or 36, have been associated with increased risk of virological failure [5]. Moreover, one study [6] has associated the presence of polymorphism R57K in the protease gene at baseline with early virological failure in anti-retroviral-naïve patients commencing first-line therapy containing PIs (mainly nelfinavir), and this polymorphism was found in eight (13.1%) isolates from plasma in the present cohort. In some resistance positions listed by the IAS (positions 67, 75 and 215 in the RT gene, and positions 20, 36, 63 and 82 in the Pro gene), substitutions were found that are not considered by the IAS to be resistance-related. Some of these mutations (e.g., so-called 'revertant' mutations at position 215) have been associated with a risk of virological failure in previously therapy-naïve patients [14]. Overall, these findings suggest that the broad spectrum of mutations that may occur at high prevalence in therapy-naïve patients should be investigated further to assess their possible impact on the virological treatment response.

Interestingly, a high prevalence of newly recognised treatment-related mutations was found in the present cohort, either in the RT gene [7] or in the Pro [8] gene. In the RT gene, positions 43 and 228 were invariably conserved in the HIV genome in therapy-naïve patients in the reference study [7], while 43E and 228H mutations were found, albeit at low frequencies, in the present study. More striking was the observation that mutations at positions 11, 35 and 45 in the Pro gene were found at a prevalence comparable to that in patients treated with at least two PIs, while mutations at positions 72 and 89 were found at a prevalence comparable to that in patients treated with at least four PIs [8]. In addition, substitutions at positions 45 and 58 in the Pro gene were never found in a large population of naïve patients [15], while they were found at a low frequency in the present study. The possibility that these mutations involving fitness or phenotypic resistance traits may then promote persistence or accumulation of further mutations, in turn increasing the risk of virological failure, remains to be elucidated in further studies.

The prevalence of newly identified treatment-related mutations was significantly higher in patients who acquired HIV infection through sexual practices, as opposed to intravenous drug use. Several factors may have an impact on the transmissibility of resistant strains. It is generally thought [16,17] that resistant HIV strains are less transmissible because of a negative impact of major resistance mutations, either on viral fitness (which contributes to an explanation of the lower viral load found in patients under drug selective pressure) or on the molecular mechanisms involved in transmission. It has been demonstrated that some minor mutations can restore viral fitness that has been compromised by other mutations [4]. To complicate this matter further, compartmentalisation of resistance strains in the genital system may be favoured by weak penetration of active drugs and a poor immune response at these sites. The complexity of this issue means that it is difficult to identify the factors that explain the high prevalence of newly identified treatment-related mutations, especially in patients infected through sexual practices. However, it can be hypothesised that these new mutations could also have a role in improving transmission fitness via the sexual route.

The present study had several limitations. First, the sample size was small and, since all the patients were recruited from a limited area, the results cannot necessarily be applied to other geographical areas. Second, the study was retrospective and no results on the treatment outcome of the patients studied were available. Thus, although a high prevalence of known mutations or newly recognised treatment-related mutations was found in therapy-naïve patients, further prospective studies that address the possible clinical impact of these mutations are required to provide information on the clinical utility of resistance testing in these circumstances.

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