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Genotypic and Phenotypic Resistance Patterns of Human Immunodeficiency Virus Type 1 Variants with Insertions or Deletions in the Reverse Transcriptase (RT): Multicenter Study of Patients Treated with RT Inhibitors

BERNARD MASQUELIER,[1]* ESTHER RACE,[2] CATHERINE TAMALET,[3] DIANE DESCAMPS,[4] JACQUES IZOPET,[5] CLAUDINE BUFFET-JANVRESSE,[6] ANNICK RUFFAULT,[7] ALI SI MOHAMMED,[8] JACQUELINE COTTALORDA,[9] ANNE SCHMUCK,[10] VINCENT CALVEZ,[11] ELISABETH DAM,[2] HERVÉ FLEURY,[1] FRANÇOISE BRUN-VÉZINET,[4] AND THE ANRS AC11 RESISTANCE STUDY GROUP

The Virology Laboratories of the University Hospitals of Bordeaux,[1] Marseille,[3] Paris-Bichat Claude Bernard,[4] Toulouse,[5] Rouen,[6] Rennes,[7] Paris-Broussais,[8] Nice,[9] Grenoble,[10] and Paris-Pitié Salpêtrière,[11] and IMEA-INSERM, Hôpital Bichat Claude Bernard, Paris,[2] France

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Genomic rearrangements in the 5′ part of the human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) have been involved in multidrug resistance to nucleoside RT inhibitors (NRTI). We carried out a retrospective, multicenter study to investigate the prevalence, variability, and phenotypic consequences of such rearrangements. Data concerning the HIV-1 RT genotype and the biological and clinical characteristics of NRTI-treated patients were collected from 10 virology laboratories. Sensitivities of the different HIV-1 variants to RT inhibitors were analyzed in a single-cycle recombinant virus assay. Fifty-two of 2,152 (2.4%) RT sequences had a rearrangement in the 5′ part of the RT, with an extensive molecular variation. The number of codons inserted between positions 68 and 69 ranged from 1 (3 samples) or 2 (41 samples) to 5 and 11 in one case each. In four cases, codon 67 was deleted. High levels of phenotypic resistance to zidovudine (AZT), lamivudine (3TC), stavudine (d4T), abacavir (ABC), and didanosine (ddI) were found in 95, 92, 72, 62, and 15% of the 40 samples analyzed, respectively. Resistance to AZT, d4T, and ABC could be found in the absence of the T215Y/F mutations. Resistance to 3TC could develop in the absence of specific mutations. Low-level resistance to ddI was noticed in 40% of the patients. The deletions of codon 67 seemed to have little effect on NRTI sensitivity. Most of the rearrangements were shown to contribute to cross-resistance to NRTI. The results regarding susceptibility to ddI raise the question of the interpretation of the phenotypic data concerning this drug.

The efficacy of antiretroviral treatment can be impaired by several factors, including poor compliance with treatment regimens, suboptimal antiviral potency and drug concentrations, and selection of antiretroviral drug-resistant human immunodeficiency virus (HIV) quasispecies (11). The nucleoside analogue reverse transcriptase inhibitors (NRTI) were historically the first group of compounds used in anti-HIV therapy. They selected HIV type 1 (HIV-1) variants with mutations in the RT encoding region of the *pol* gene, which affected resistance to individual drugs (23). Moreover, two different pathways for the selection of HIV-1 showing multidrug resistance (MDR) to NRTI were identified. The first involves the Q151M mutation and four additional mutations (A62V, F77L, V75I, and F116Y) (12, 24). More recently, nucleotide rearrangements coding for different 1-amino-acid (aa) or 2-aa insertions, following position 68 or 69 of HIV-1 RT, have been reported to confer NRTI MDR (7, 8, 17, 19, 21, 22, 26, 29). Deletions at codon 67 associated with a T69G substitution have also been recently described (30; T. Imamichi, H. Imamichi, J. C. Lopez, J. Metcalf, J. Falloon, and H. C. Lane, Abstr. 7th Conf. Retrovir. Opportunistic Infect., abstr. 738, 2000). All these rearrangements involve the structure of the β3-β4 hairpin loop of HIV-1 RT and are likely to interact with the nucleotide binding process. Because previous reports concerning these rearrangements have been based on few cases, little is known about their prevalence, variability, molecular epidemiology, and clinical significance. We decided to carry out a multicenter, retrospective study in order to document these rearrangements emerging in the context of failure of antiretroviral therapy.

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MATERIALS AND METHODS

RT genotype study. A questionnaire regarding the molecular epidemiology of rearrangements in HIV-1 RT was sent to the virology laboratories belonging to the French Agence Nationale de Recherches sur le SIDA (ANRS) Antiretroviral Resistance study group. Data concerning the amino acid sequence between residues 60 to 71, additional resistance mutations, the treatment history, and the virological and immunological status of the patients were collected each time that a rearrangement was noticed in the RT encoding region. The number of patients with an RT rearrangement was compared, for each laboratory, to the

* Corresponding author. Mailing address: Laboratoire de virologie, Hôpital Pellegrin, Place Amélie Raba Léon, 33076 Bordeaux Cedex, France. Phone: 33 5 56 79 55 10. Fax: 33 5 56 79 56 73. E-mail: bernard.masquelier@chu-bordeaux.fr.

total number of RTI-treated patients who had a documented HIV-1 genotype during the same period.

RT genotypic resistance studies were performed on viral plasma RNA using the ANRS consensus technique. Viral RNA was extracted using a standard guanidium isothiocyanate protocol and amplified in a one-step procedure using the RT-Titan kit (Boehringer) and the primers MJ3 and MJ4 (13). Amplified products were submitted to nested PCR using primers A-35 and Nel-35 (16). Direct population sequencing was performed on purified PCR products using the primers A-20 and Nel-20 (16) with the ABI PRISM DYE termination cycle sequencing Ready Reaction kit with AmpliTaq DNA polymerase (Perkin-Elmer) on an automated DNA sequencer (ABI Model 377; Applied Biosystems; Foster City, Calif.). Sequence alignement was performed with Sequence Navigator software (Perkin-Elmer).

Phenotypic analysis: recombinant virus RTI sensitivity assay. Drug sensitivity assays were performed using a single-cycle recombinant virus assay (RVA) (20).

The HIV RT encoding region was amplified from patient plasma samples by nested RT PCR using the outer primers MJ3 (5′AGT AGG ACC TAC ACC TGT CA 3′) and RT-EXT (5′TTC CCA ATG CAT ATT GTG AG 3′) with inner primers A35 (5′ TTG GTT GCA TAA ATT TTC CCA TTA GTC CTA TT 3′) and RT-IN (5′ TTC CCA ATG CAT ATT GTG AG 3′). The resultant 1,530-bp fragment, extending between codon 93 of the protease-encoding region and codon 503 of RT-encoding region of *pol*, was purified on QiaAmp columns.

The plasmid with a deletion of RT (pSRT) was constructed from pNLenv, a previously described pNL4-3-derived plasmid carrying a near-full deletion of the *env* gene (bp 6343 to 7611) (5). Site-directed mutagenesis was used to alter the sequence to give the unique restriction sites *Sna*B1 at position 3872 and *Nru*I at position 3892. *Bal*I and *Sna*BI were used to remove the RT encoding region (between positions 2618 and 3872), and linearization was achieved using *Nru*I.

Subconfluent 293T cells in T25 flasks were transfected by the calcium phosphate precipitation method with 8 μg of *Nru*I-linearized pSRT, 0.1 μg of VSV-G plasmid (encoding for the vesicular stomitis virus envelope protein), and between 0.5 and 1 μg of the HIV reverse transcriptase PCR product.

The transfection precipitate was washed off the cells after 18 h of incubation, and fresh growth medium was added. After a further 24 h of culture, supernatant was clarified by centrifugation (500 × *g*, 15 min) and transferred to P4 indicator cells (3, 31) that had been preincubated with serial dilutions of RTI, in triplicate wells, for 4 h. The range of drug concentrations used varied between compounds. The level of expression of β-galactosidase in the P4 cell lysates was measured using a colorimetric assay based on the cleavage of chlorophenol red-β-D-galactopyranoside by β-galactosidase. The 50% inhibitory concentration (IC_{50}) was calculated using the median effect equation (4). An RVA index was calculated as the ratio of the IC_{50} for the patient sample to the IC_{50} obtained for pNL4-3 wild-type HIV-1 tested in parallel.

Nucleotide sequence accession numbers. The sequences reported in this study have been assigned the GenBank accession numbers AF315232 to AF315274, AF311177, AF311157, AF311187, AF311159, AF311179, AF311162, and AF311173.

RESULTS

Prevalence of RT rearrangements. In the 10 laboratories participating in the study, 52 rearrangements were reported in the 5′ part (aa 20 to 240) of the RT encoding region from 52 different patients. In the same period (January 1998 to June 1999), 2,152 RT sequences from RTI-treated patients had been documented (prevalence of RT rearrangements, 2.4%). The prevalence in the different centers ranged from 0.8 to 4.5%; this variation was not significant ($P = 0.22$).

Molecular variability of the RT rearrangements. The amino acid variability deduced from the nucleotide sequence of codons 60 to 71 of the RT encoding region is shown in Table 1. The number of inserted residues was 1 (3 samples), 2 (41 samples), 5 (1 sample) or 11 (1 sample). In all cases, insertions were located between aa 68 and 69. In four cases, a deletion of codon 67 was noted. The presence of an 11-aa insertion in one case could be confirmed by sequencing a later sample (data not shown).

The rearrangements also frequently included mutations at positions 67, 68, and 69 in 63, 17, and 78% of the cases, respectively. As previously reported, the molecular changes frequently created serine stretches, with the pattern SSG present at a frequency of 35%.

Associated RTI resistance mutation. The different mutations coding for HIV resistance to NRTI or nonnucleoside RTI (NNRTI) observed in the 52 sequences are shown in Table 1. The major mutations T215Y or T215F (T215Y/F), M184V/I, and K103N were observed in 86, 40, and 25% of the sequences, respectively.

Antiretroviral therapies and virological evolution of the patients. All patients were RTI experienced at the time of sampling for genotype, with a median duration of therapy of 50.5 months; most of them had undergone multiple therapy changes, with a median use of eight drugs and five NRTI. At the time of sampling, the RTI most frequently in use were stavudine (d4T) (55% of the patients), lamivudine (3TC) (44%), and didanosine (ddI) (33%) (Table 1). The antiretroviral therapy comprised at least one protease inhibitor for 69% of the patients. Two patients had stopped antiretroviral therapy at the time of sampling.

At the time of sampling for genotype, the median $CD4^+$ cell count was 124.4/μl (range, 1 to 550) and the median amount of plasma HIV-1 RNA was 4.06 log 10 copies/ml (range, 2.34 to 6.30).

Individual follow-up data concerning 20 patients are shown in Table 2. For some patients, significant decreases of the plasma viral load were observed; this corresponded to the change of therapies, including new protease inhibitors, or to intensification of treatment. In one case, the viral load decreased without any change in treatment, with conservation of an insertion of 11 aa in the RT.

Phenotypic analysis and correlation with RT genotype. Additional frozen plasma samples were available for phenotypic analysis using the single-cycle RVA for 40 of the samples showing a rearrangement in the HIV-1 RT. The RVA index was obtained for five NRTI, zidovudine (AZT), 3TC, d4T, ddI, and abacavir (ABC); and for two NNRTI, nevirapine (NVP) and efavirenz (EFV). HIV-1 variants with an RVA index of <4 were considered sensitive; an RVA index of >10 indicated high-level resistance, while an RVA index between 4 and 10 was considered low-level resistance.

Sensitivity to NRTI. The HIV-1 variants exhibited high-level resistance to a mean of 3.4 ± 1.2 NRTI (median of 4). The pattern of phenotypic sensitivity to the different NRTIs is shown in Fig. 1. The frequency of high-level resistance was greatest for AZT (95%). High-level resistance to d4T was slightly less frequent at 72% (29 of 40). Resistance to d4T was more often observed in samples with a 2-aa insertion than in samples with other rearrangements. In three patients with a 2-aa insertion (patients 10, 38, and 46), high-level resistance to AZT and d4T was detected in the absence of the T215Y/F mutations, which were originally described as crucial for the appearance of a significant degree of resistance to AZT (2, 14, 15); however two of these three samples also contained other thymidine analogue mutations.

High-level resistance to 3TC was seen in 37 (92%) of the samples, including one with an insertion of 11 aa, one with an insertion of two codons, and one with a deletion. Not all high-level resistance to 3TC could be related to the presence of M184V/I mutations, which were present in only 16 (43%) of

TABLE 1. Genotypic and phenotypic patterns of the HIV-1 variants in the study[a]

Patient no.	HIV-1 RNA (copies/ml)	No. CD4+ (cells/μl)	Therapy		RT mutation(s)	Amino acid sequence	
			RTI	PI(s)		60–66	67/68
						VFAIKKK	DS
1	18,700	18	d4T 3TC	RTV SQV	1181 215Y	VFAIKKK	ES
2	94,326	144	d4T 3TC	RTV	1841 215F 219Q	VFAINKK	GS
3	49,400	298	AZT 3TC NVP		69D 7DR 181C 184I 219Q	VFAINKK	GY
4	40,421	548	d4T ddI	IDV	41L 215Y	VFAIKKK	A/DS
5	26,045	311	AZT ddI	NFV	41L 103N 181C 215Y	VFAIKKK	ES
6	221,500	81	ABC ddI	NFV SQV	98C 210W 215Y	IFAIKKK	EN/S
7	47,500	60	d4T 3TC	IDV	41L 44D 118I 184I 215Y	VFVIKKK	SS
8	5,100	172	d4T 3TC	IDV	41L 44D 118I 184I 210W 215Y	VFVIKKK	SS
9	58,600	18	d4T	NFV	41L 74V 118I 190Q 210L 215Y 219Q	VFAIKKK	DS/NS
10	202,653	1	d4T ddI ABC	IDV	41L 181C 215I	VFAIKKK	ES
11	58,800	323	Stop ARV	Stop ARV	41L 181C 210W 215Y 219K/E	VFAIKKK	DS
12	2,009,600	103	d4T 3TC	NFV	41L 210W 215Y	VFAIKKK	ES
13	20,060	114	d4T ddI		41L44D 184I 210W 215Y	VFAIRKK	DS
14	46,650	54	d4T ddI H. Urea		41L 74V 75M 184V 210W 215Y	VFAIKKK	SL
15	23,680	166	AZT 3TC	RTV	41L 210W 215Y	VFAIRKK	DS
16	50,000	34	d4T ddI	NFV SQV	98G 210W 215Y	VFAIKKK	DS
17	272,015	20	d4T ddI NVP	IDV	41L 103N 181C 210W 215Y	VFAIRKK	DS
18	416,096	ND	3TC ABC		108I 184I 210W 215Y	VFAIRKK	QS
19	89,000	157	d4T ABC EFV	APV	41L 74V 181C 103N 190A 210W215Y	VFAIKKK	DS
20	50,000	459	d4T 3TC	IDV	7OR 118I 184V 215F	VFVIKKK	DS
21	247,000	10	ABC NVP	NFV	101Q 190A 210W 215Y	VFA/VIKKK	ES
22	35,000	45	ddI EFV	APV	41L44D 74V 98G 103N 108I 184V 190A 210W 215Y	VFVIKKK	DS
23	652,535	ND	AZT 3TC NVP		41L 44D 74I 103N 184V 190A 210W 215Y	VFVIKKK	DS
24	230,760	50	Stop ARV	Stop ARV	41L 184I 210W 215Y	VFVIRKKK	ES
25	319,000	185	AZT ddI DLV		210W 215F	IFAIKKK	ES
26	22,000	494	AZT ddC		41L 210W 215Y	VFAIKKK	DS
27	530,000	325	AZT	RTV SQV	41L 103N 210W 215Y	VFAIRKK	ES
28	110,000	ND	d4T 3TC	NFV	98G 215Y	VFAIKKK	ES
29	3,230	ND	d4T 3TC	RTV	184V 215Y	VFA/VIRKK	DS
30	4,800	ND	ABC	SQV RTV	None	VFAIKKK	GS
31	512,131	238	AZT 3TC		T215Y	VFAIKKK	V/AS
32	410,876	9	AZT 3TC ddI		41L 74V 118I184V 215F 219Q 103N	VFAINKK	KG
33	200,000	30	d4T ABC		184I	VFAIKKK	GS
34	50,000	98	ABC EFV	RTV SQV	100I 103N 215Y 41L	VFAIKKK	ET
35	800,000	40	ABC EFV	NFV	62V 74V 75I 103N 181C 215F 219E	VFVIKKK	ES
36	380,000	ND	AZT 3TC NVP	IDV	41L 62V 98S 184I 210W 215Y	VFVIKKK	ES
37	850	1,190 (child)	d4T 3TC	RTV	41L 62V 215Y	VFVIKKK	D S/T
38	9,291	550	d4T 3TC	NFV	62V 184V	VFVIKKK	SS
39	128,800	197	d4T 3TC	SQV	41L 62V 98S 184I 210W 215Y	VTVIKKK	ES
40	50,000	25	AZT 3TC EFV	RTV APV	103N 190A 210W 215Y	VFAIKKK	GS
41	50,000	7	ddI ABC NVP		188C 215Y/F	VFAIKKK	DS
42	3,000	495	d4T ddI		215Y	VFAIKKK	GS
43	200,000	14	d4T ddI EFV	RTV	41L 103N 210W 215Y	IFAIKKK	DS
44	55,000	430	d4T 3TC	NFV SQV	62V 184I 215Y	VFVIKKK	ES
45	30,203	456	d4T 3TC	NFV	62V 70R 184V 215F	VFVIKKK	DS
46	4,280	231	d4T ddI		41L 62V 70R	VFVIKKK	DS
47	59,280	376	d4T 3TC	RTV	41L 67N 75L 98S 215Y 219E	VFAIKKK	NN
48	108,840	168	d4T ddI NVP	NFV	41L 74I 103N 118I 181C 190A 210W 215Y	VFAIKKK	DS
49	15,000	310	d4T EFV	SQV	41L 70R 103N 184V 215Y	VFAIKKK	S
50	221	478	AZT 3TC	RTV	41L 184V 215Y 219E	VFAIKKK	S
51	36,320	80	d4T ddI		210W 215F 219E	VFAIKKK	S
52	26,000	232	d4T 3TC	NFV	184V	VFAIKKK	S

[a] RTI and PI, antiretroviral therapy at the time of detection of an RT rearrangement; 60 to 71, amino acid sequence of the HIV-1 variants between positions 60 and 71 in the RT. Phenotypic results are expressed as the RVA index for each drug tested. ND, not determined; PI, protease inhibitor; ddC, zalcitabine; APV, amprenavir; ARV, antiretroviral therapy; IDV, indinavir; NFV, nelfinavir; RTV, ritonavir; SQV, saquinavir; DLV, delavirdine; H. Urea, hydroxyurea.

the variants with an RVA index of >10 for 3TC. Another mutational pattern which has been described as involved in 3TC resistance (E44D/A and/or V118I mutations with M41L and T215Y changes) (10) was present in seven cases in conjunction with 184V/I mutations and in three cases with the 184M wild-type genotype with RVA indices for 3TC of 15 (1-aa insertion), >25 (2-aa insertion), and 2 (11-aa insertion). High-level resistance to ABC was noted in 25 of 40 patients (62%). In these 25 samples, the M184V/I mutations were present in only 7 cases and fewer than three NRTI resistance mutations were detected in 11 cases.

ddI phenotypic sensitivity displayed a different pattern, with only 15% (6 of 40) of samples showing high-level resistance and 40% (16 of 40) showing low-level (median increase, sev-

TABLE 1—*Continued*

Amino acid sequence			RVA index for drug						
69	70/71	Comment	AZT	3TC	d4T	ddI	ABC	EFV	NVP
T	KW	Wild type							
SG	KW	Insertion 1 aa	183	15	9.0	2.8	10.1	0.6	1.0
TT	RW	Insertion 1 aa	9	>339	5.3	4.4	18.3	0.7	0.7
SD	RW	Insertion 1 aa	ND	ND	ND	ND	ND	ND	ND
SSG	KW	Insertion 2 aa	>1,805	>245	55.1	3.4	>9	0.5	1.7
SSG	KW	Insertion 2 aa	>1,805	>245	66.7	8.0	>9	6.8	>463
SSC	KW	Insertion 2 aa	>1805	>245	67.8	3.7	>9	3.9	9.0
SCT	KW	Insertion 2 aa	297	>245	68.1	3.0	>9	0.7	0.9
SCT	KW	Insertion 2 aa	131	>245	27.4	5.6	6.3	0.2	1.0
SCA	KW	Insertion 2 aa	>301	>25	>28	6.2	21.1	>68	>947
SVG	KW	Insertion 2 aa	>301	>25	>28	7.4	>24	23.9	>947
SSG	KW	Insertion 2 aa	>301	>25	8.9	5.1	>24	9.1	20.4
SSG	KW	Insertion 2 aa	>301	>25	20.6	5.4	>24	1.5	5.8
SSG	KW	Insertion 2 aa	>301	>25	>28	4.7	15.2	0.2	0.4
AVG	KW	Insertion 2 aa	>30.1	>25	>28	10.7	>24	1.2	2.5
STT	KW	Insertion 2 aa	ND	ND	ND	ND	ND	ND	ND
SSG	KW	Insertion 2 aa	ND	ND	ND	ND	ND	ND	ND
SSA	KW	Insertion 2 aa	>1805	>245	67.7	2.5	>9	91.0	>1,463
SSA	KW	Insertion 2 aa	ND	ND	ND	ND	ND	ND	ND
SST	KW	Insertion 2 aa	169	4	1.8	2.1	7.2	>209	>2,894
SSS	RW	Insertion 2 aa	36	>339	1.3	1.3	12.2	0.3	0.4
SES	KW	Insertion 2 aa	1,999	>339	32.3	11.7	32.4	43.5	>2,894
SSK	KW	Insertion 2 aa	3,745	>339	9.7	2.7	>48	1.2	13.0
SSG	KW	Insertion 2 aa	ND	ND	ND	ND	ND	ND	ND
SST	KW	Insertion 2 aa	ND	ND	ND	ND	ND	ND	ND
SMT	KW	Insertion 2 aa	ND	ND	ND	ND	ND	ND	ND
SST	KW	Insertion 2 aa	>1,805	46	7.3	2.5	7.5	0.4	0.4
SSA	KW	Insertion 2 aa	>1,805	>245	20.3	3.1	>9	0.8	1.6
SSG	KW	Insertion 2 aa	>1,805	>245	17.1	5.1	>9	0.7	0.9
SSS	KW	Insertion 2 aa	168	>245	11.9	2.3	7.2	0.5	1.1
SSG	KW	Insertion 2 aa	ND	ND	ND	ND	ND	ND	ND
SSG	RW	Insertion 2 aa	>1,805	>245	26.8	4.3	5.6	0.6	1.4
STT	RW	Insertion 2 aa	>1,805	>245	32.4	5.1	8.1	>137	>1,463
SVG	KW	Insertion 2 aa	ND	ND	ND	ND	ND	ND	ND
SVT	KW	Insertion 2 aa	>3,397	>339	53.9	10.8	>48	>209	>2,894
SSG	KW	Insertion 2 aa	>1,805	>245	35.3	2.6	5.7	>137	>1,463
SVT	KW	Insertion 2 aa	758	>245	203.2	5.9	7.7	1.0	0.1
SSA	KW	Insertion 2 aa	ND	ND	ND	ND	ND	ND	ND
SSG	KW	Insertion 2 aa	1,214	>245	11.2	1.6	5.0	1.1	2.1
SVT	KW	Insertion 2 aa	1,129	>245	37.8	3.2	6.5	0.6	0.7
SSG	KW	Insertion 2 aa	>3,397	>339	19.6	4.4	>48	76.1	262.5
SSG	KW	Insertion 2 aa	ND	ND	ND	ND	ND	ND	ND
SIG	KW	Insertion 2 aa	>3,397	>339	10.5	2.4	17.9	0.3	0.3
SSG	KW	Insertion 2 aa	>586	>619	155.8	18.4	>19	1.1	2.5
SSG	KW	Insertion 2 aa	>586	>619	>382	22.6	>19	1.0	0.8
SVS	RW	Insertion 2 aa	>586	>619	321.7	40.2	>19	0.4	0.7
SVA	RW	Insertion 2 aa	>3,688	>346	14.8	6.1	9.2	1.0	1.0
ITT	RVMGK	Insertion 5 aa	>301	>25	24.7	5.8	18.3	1.0	3.8
TTE	GKKDSTRWRKI	Insertion 11 aa	168	2	2.8	1.3	4.1	38,3	>947
G	RW	Deletion	15	>619	2.1	6.8	0.8	>229	5,147.0
G	RW	Deletion	ND	ND	ND	ND	ND	ND	ND
G	RW	Deletion	1,029	9	0.9	0.8	5.1	0.2	0.2
G	KW	Deletion	4	>339	1.5	2.3	2.7	0.5	0.3

enfold) resistance. The mutation L74V, usually associated with resistance to ddI (25), was present in six samples, three of which remained sensitive to ddI.

Sensitivity to NNRTI. Fourteen patients had a phenotypic assay with high-level resistance to NVP and/or EFV, explained in 13 cases by the presence of mutations related to NNRTI resistance in the RT encoding region (K103N, Y181C, or G190A). In one case (patient 9), no explicative mutation was found, but the mutation G190Q was detected. The remaining 26 (65%) samples were sensitive to NNRTI.

DISCUSSION

This study enabled us to describe HIV-1 RT rearrangements in a large cohort of HIV-1-infected, NRTI-treated patients. The rearrangements were present in 2.4% of the patients. We confirm the results of previously published studies, based on smaller numbers of patients, which reported prevalences of RT rearrangements of between 0.5 and 2.7% (1, 27, 28). Despite this low prevalence, future studies will be necessary to assess the evolution of the incidence of such rearrangements. A ge-

TABLE 2. Follow-up of 20 patients of the study[a]

Patient no.	Pattern 69, baseline	Antiretroviral therapy	Time of follow-up (weeks)	Δ $\log_{10}$ HIV-1 RNA	Pattern 69, follow-up
3	S-D	AZT 3TC **APV**	8	0.25	ND
6	S-SG	ABC **NVP** NFV SQV	36	−0.82	S-ST/S
9	S-CA	d4T **3TC RTV SQV EFV**	40	0.36	S-CA
10	S-VG	**Megahaart**	10	−2.26	ND
12	S-SG	2 months wash-out	35	−0.47	S
13	S-SG	3 months wash-out	13	−0.26	ND
15	S-TT	**ddI EFV NFV SQV**	64	−2.06	ND
16	S-SG	**AZT 3TC EFV RTV** SQV	8	0.11	ND
17	S-SA	d4T **3TC APV**	24	0.22	ND
18	S-SS	**d4T** 3TC **NFV**	4	−1.01	ND
21	S-ES	ABC NFV NVP	16	0.48	ND
23	S-SG	AZT 3TC **ABC** NVP **SQV**	24	−0.32	ND
25	S-MT	**d4T 3TC IDV**	28	−3.19	ND
26	S-ST	**d4T 3TC RTV**	8	−2.03	ND
29	S-SS	d4T 3TC **IDV**	28	0.76	ND
30	S-SG	ABC SQV RTV	22	1.68	ND
31	S-SG	AZT 3TC **RTV**	36	−0.93	S-SG
47	Insertion 5 aa	**ddI ABC EFV**	24	0.12	ND
48	Insertion 11 aa	d4T ddI NVP NFV	24	−0.74	Insertion 11 aa
52	Deletion	d4T 3TC NFV	24	0.07	ND

[a] Δ $\log_{10}$ HIV-1 RNA, changes of the HIV plasma viral load from the baseline. Antiretroviral therapy, the drugs which were absent at baseline appear in bold (can be recycled from previous regimens). Megahaart, 8 antiretroviral drugs combined with hydroxyurea. Pattern 69, genotype at codon 69 and rearrangements. ND, genotype not determined. RTV, ritonavir; SQV, saquinavir; IDV, indinavir; NFV, nelfinavir.

notypic study on 400 antiretroviral therapy-naive patients included during the same period in France did not detect any RT rearrangement (9), suggesting that RTI are responsible for the selection of these mutational patterns.

One major finding of our study is the extensive heterogeneity of the rearrangements. Because of this heterogeneity, we decided to document the phenotypic sensitivity to RTI of the different variants, using an RVA. Insertions of 1, 2 (majority in our study), and 5 aa after residue 69 were associated with high-level phenotypic resistance to AZT, 3TC, d4T, and ABC. This phenotype was, in some cases, found in the absence of the different mutations reported to be involved in high-level resistance to these four drugs. In contrast, the 11-codon insertion, found surprisingly in one patient, was not shown to be involved in resistance to NRTI; the later evolution of the viral load, decreasing without any treatment change, was in concord in this case with the phenotype. Similarly, none of the three deletions with an RVA available in our study were linked to phenotypic resistance to NRTI. Recent studies have used site-directed mutagenesis to determine the specific phenotypic impact of the rearrangements; Larder et al. (17) reported that the 69S-SS insertion (mutation T69S with an insertion of two serines) did not code for MDR by itself but contributed to MDR in the presence of AZT resistance mutations. Inversely, 69S-SA and 69S-SG patterns were found to code for MDR even in absence of other genotypic changes (29). In a recent report (30), Winters et al. described the frequent association of 3-bp deletions in the β3-β4 region of the RT gene with the Q151M mutation. Using site-directed mutagenesis, they showed that the deletions could code for MDR in the presence

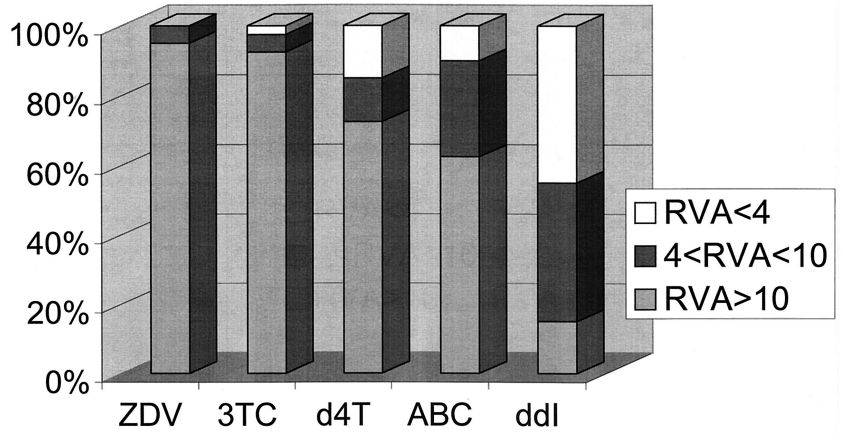

FIG. 1. Phenotypic sensitivities to NRTIs of 40 HIV-1 variants with an RT rearrangement. Phenotypic results are expressed for each drug as percentages of variants divided into three groups: RVA of <4, phenotypic sensitivity; RVA of >4 and <10, low-level resistance; RVA of >10, high-level resistance. ZDV, zidovudine.

of the T215Y mutation. These findings are not confirmed by our RVA results; this can be explained by differences in the phenotypic assays used, as well by the genetic background of the viruses, which can be present in recombinant viruses but not in isolates obtained by site-directed mutagenesis. Other studies involving greater numbers of isolates will be necessary to clarify the role of deletions in MDR.

One intriguing finding of this study is the relatively low level of phenotypic resistance to ddI induced by the rearrangements. In previous studies, assays using peripheral blood mononuclear cell cocultured virus isolates, or recombinant viruses, have also failed to detect high-level phenotypic resistance to ddI, even in the presence of the L74 V mutation (6, 18). It is thus difficult to know whether these data represent a true sensitivity to ddI or an inadequacy of such in vitro tests to measure resistance to ddI. Standardization of the interpretation of RVAs, including the definition of the threshold values for sensitivity or resistance, seems to be particularly crucial for this drug.

Our study thus provides correlates between genotype and phenotype for a wide variety of RT rearrangements. This information is of particular importance at a time when genotypic assays are becoming more widely used in the management of antiretroviral treatment failure. MDR is potentially a major limitation to antiretroviral efficacy. Some individual follow-up in our study suggested that therapy intensification could have some impact on the viral load in plasma. However, 30% of all included patients were infected by HIV-1 quasispecies that were resistant to both NRTI and NNRTI, and most of the variants also displayed major resistance mutations for protease inhibitors (data not shown). In such patients with a probable resistance to most of the inhibitors presently available, genotype-guided treatments have few chances to be effective. Alternative strategies, including treatment interruption strategies and/or the use of new drugs targeting other stages of HIV replication, should be evaluated in this context.

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