

**HIV-1 DRUG RESISTANCE ASSESSMENT IN PEDIATRIC PATIENTS.**

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Key words: HIV-1 genotyping, drug resistance, HIV-infected children

## **ABSTRACT**

Twenty-one vertically HIV-1 infected children were assessed for drug resistance genotyping. Eighteen children presented at least one mutation conferring resistance to the nucleoside reverse transcriptase inhibitors, and seven children presented resistance to the non-nucleoside inhibitors. Among them, two children in whom the therapy had been discontinued two to three years before testing presented K101E, K103N, and G190A mutations conferring resistance to all the non-nucleoside drugs available for treatment. Protease gene mutations conferring resistance to at least two to up to twelve drugs were reported in 12 children. Studies in HIV-infected children have a particular meaning, and with antiretroviral therapy becoming more widely available, surveillance of viral resistance regional levels is relevant for treatment guidelines, supporting the rational use of antiretroviral drugs by treatment programs. Additional larger studies are still required to give more information on HIV-1 drug resistance in pediatric patients.

## **INTRODUCTION**

Studies on antiretroviral resistance in human immunodeficiency virus type 1 (HIV-1) infected children are relevant mainly concerning disease progression and response to antiretroviral therapy. HIV-1 mutant strain emergence is related to therapeutic failure and has hampered the different options available for alternate treatment regimens. Genotypic drug resistance testing is a valuable tool assisting clinical management, and when associated with viral load levels and CD4 T cell counts it may be helpful in determining important therapeutic regimen modifications to achieve an optimum antiviral effect (Viganò et al. 2008). In addition, surveillance of HIV drug resistance emerging in treatment will support implementation of prevention measures.

The decision of when to start the highly active antiretroviral therapy (HAART) in HIV-infected children is quite critical and should consider all the risks and benefits involved and potential treatment compliance. Also, the availability of and access to appropriate regimens, and antiretroviral drug supply continuity must be strictly considered. Several guidelines are available to guide the initiation of therapy, and the recommendations in these guidelines show significant variability. In Brazil, triple antiretroviral therapy is currently recommended for children according to their clinical and immunological status classification, based on Centers for Disease Control and Prevention criteria (1994) adaptation (Ministério da Saúde 2007). The regimens for the initial highly active antiretroviral therapy in Brazilian children include the combination

of two nucleoside reverse transcriptase inhibitors (NRTIs), preferentially zidovudine (AZT) + lamivudine (3TC) or stavudine (d4T) + lamivudine or abacavir (ABC) + lamivudine with one nonnucleoside reverse transcriptase inhibitor (NNRTI) nevirapine (NVP) for children up to 35 months of age and nevirapine or efavirenz (EFV) for children aged or older than 3 years old. Protease inhibitors (PIs) amprenavir (APV), atazanavir (ATV), nelfinavir (NVP), ritonavir (RTV), indinavir (IDV), lopinavir + ritonavir (Kaletra), saquinavir (SQV) are recommended for those regimens presenting therapeutic failure.

We have studied a group of HIV-1 perinatally infected children receiving prolonged antiretroviral treatment and we report in this paper the results concerning the drug resistance mutations profile observed in this group. These mutations were classified according to the current list of resistance mutations proposed by Shafer et al. (2007).

Our study calls attention to the value of resistance testing when monitoring antiretroviral treatment.

## **MATERIAL AND METHODS**

### **Subjects.**

Twenty-one HIV-1 infected children (8 male and 13 female, aging 4 to 15 years old) who were born to HIV-1 infected mothers were enrolled in this study. They have been seen at the Materno-Infantil Department, Hospital Universitário Pedro Ernesto, Rio de Janeiro State University, Brazil for clinical care and clinical category assessment according to Centers for Disease Control and Prevention criteria (1994) adaptation. Inclusion criteria adopted were to be receiving antiretroviral therapy and to be presenting plasma viral loads equal or higher than 1,000 HIV-1 RNA copies/mL. All patients provided an informed written consent obtained from their parents or legal guardians. The Ethics Committee of both institutions participating in this study approved the study protocol.

### **Antiretroviral therapy.**

The children involved in this study received antiretroviral therapy for periods of time ranging from two years to ten years previous to genotyping assessment. As the observation period of this study ranged from 2005 to 2007, the therapeutic regimens included for most children were prescribed before Brazilian modifications had been introduced in our more recent guidelines. At the time of genotyping assessment, three of

the 21 children were receiving dual therapy, AZT + ddI or AZT + 3TC, and the remaining patients were receiving HAART according to different regimens: 1) AZT, 3TC + NFV or ABC or RTV or EFV or NVP or Kaletra; 2) AZT, ddI + NVP or Kaletra; 3) AZT, TDF, 3TC, RTV; 4) 3TC, d4T + APV or NFV or Kaletra; 5) 3TC, ABC, Kaletra.

**Viral load.**

Plasma viral loads were measured using either commercial kits, the NucliSens HIV-1 QT (NASBA, bioMerieux Inc., Durham, North Carolina) with a lower limit of quantification of 80 copies of RNA/mL or the Quantiplex HIV-1 RNA 3.0 Assay (bDNA, Bayer Diagnostics, Walpole, Massachusetts) with a lower limit of quantification of 50 copies of RNA/mL, according to manufacturer's instructions.

**HIV sequencing.**

HIV-RNA was extracted from plasma samples using a commercial kit (QIAamp Viral RNA purification, QIAGEN Inc., Valencia, CA) and amplified using a commercial HIV-1 genotyping kit (TruGene™, Bayer HealthCare, Tarrytown, NY) resulting in a 1.3 kb amplicon covering the HIV-1 protease gene (codons 1-99) and HIV-1 reverse transcriptase gene (codons 39-244). Sequencing reactions were generated from the amplified cDNA by CLIP™ sequencing (OpenGene™, Bayer HealthCare) and were loaded on an automated DNA sampler and run on 6% acrylamide gel electrophoresis (MicroGene Clipper™, Bayer HealthCare). The assays were base called with GeneObjects software (GeneObjects, Bayer HealthCare), aligned and assembled together with GeneLibrarian software (GeneLibrarian, Bayer HealthCare). The sequences were compared to a database of HIV-1 wild type (HxB2, GenBank K03455). Mutations associated with decreased drug sensitivity were classified according to the consensus statement on antiretroviral drug resistance testing.

**RESULTS**

**NRTIs and NNRTIs Viral Resistance.**

NRTIs and NNRTIs regimen at the time of genotyping assessment, the previous regimen that the children received, and the period of time they were under therapy is presented in Table 1.

**Table 1.** Previous antiretroviral therapy and at the time of genotyping assessment.

<b>Child number</b>	<b>Period of time under therapy</b>	<b>NRTI/NNRTI/PI regimen* previously to genotyping test</b>	<b>NRTI/NNRTI/ PI regimen at the time of genotyping</b>
1	5 years	AZT, ddI	AZT, ddI
2	3 years	AZT, ddI, NFV	AZT, 3TC, Kaletra
3	8 years	AZT, ddI/AZT, 3TC, RTV	AZT, 3TC, NFV
5	7 years	AZT, ddI	AZT, 3TC, NFV
6	3 years	d4T, 3TC, NFV	d4T, 3TC, NFV
7	7 years	AZT, ddI/ AZT, 3TC, NFV/ AZT,ABC,NVP	d4T, 3TC, APV
8	7 years	AZT, ddI	AZT, 3TC, ABC
9	7 years	AZT, ddI	AZT, 3TC, NFV
10	4 years	AZT, ddI/ AZT, 3TC, NVP	AZT, 3TC, ABC
11	5 years	AZT, ddI	AZT, 3TC, RTV
12	5 years	AZT, 3TC/ AZT, NFV	AZT, 3TC, NFV
13	5 years	AZT, 3TC	AZT, 3TC
14	8 years	AZT, ddI/ AZT, 3TC, NFV/AZT, ABC, NVP/TDF, 3TC,LPV <sub>r</sub>	3TC, TDF, EFV, RTV
15	8 years	AZT, ddI	AZT, 3TC, NFV
17	2 years	d4T, 3TC, NFV	d4T, 3TC, NFV
20	No data	No data	AZT, 3TC, EFV
23	10 years	AZT, ddI	AZT, 3TC, RTV
24	3 years	AZT, ddI/ AZT, 3TC	AZT, 3TC, NVP
25	3 years	AZT, ddI, NVP	AZT, ddI, NVP
26	5 years	AZT, 3TC, RTV/ AZT, 3TC, NFV	AZT, 3TC, Kaletra
27	6 years	AZT, ddI	AZT, ddI

\*NRTI = nucleoside reverse transcriptase inhibitors, NNRTI = non-nucleoside reverse transcriptase inhibitors, PI = protease inhibitors

Eighteen among 21 children presented, at least one mutation conferring resistance to the NRTIs. Two 5-year old twins (numbers 12 and 13) and one 15-year old patient (number 26) presented no evidence of resistance. The twins presented F77L or V118I mutations on the HIV-1 reverse transcriptase gene that conferred no evidence of

viral resistance. The 15-year old patient presented no relevant mutations on the HIV-1 reverse transcriptase gene.

In the 18 children harboring NRTIs-resistant HIV-1 strains, reverse transcriptase gene mutations conferred resistance to at least two to up to eleven drugs. Most importantly, 9 of them presented viral resistance to the reverse transcriptase inhibitors ranging from 8 to 11 drugs.

NNRTIs viral resistance was reported in 7 among the 18 children. Six children presented viral resistance to the all three NNRTIs available to the antiretroviral treatment.

HIV-1 reverse transcriptase gene mutations and the viral resistance observed in these pediatric patients are presented in Table 2

Thymidine analog mutations (TAMs) were detected in 16 among the 18 children. TAM-1 and TAM-2 clusters were also analyzed for them, and the results showed that HIV-1 RT sequence mutations fit TAM-1 pattern for 8 children, whereas TAM-2 pattern was reported for 6 children, and 1 child presented T215Y associated with other mutations than those presented at TAM clusters.

Multi-NRTIs resistance mutations were detected in 4 children and non thymidine analog regimen mutations were additionally observed in 2 children. E44D, V118IV, and H208Y accessory mutations were reported either alone or occurring associated with each other in 12 children. The T69D additional treatment-selected mutation was detected in 4 children and the M184V mutation, associated with increased susceptibility to AZT, TDF, and d4T, was reported in 13 children.

NRTIs resistance mutations occurred most frequently at positions T215F/Y (14 children), M41L (13 children), and M184V (13 children), and the most frequently reported NNRTIs resistance mutations were as follows: A98G (2 children), K101E (3 children), K103N (2 children), V106A (1 child), and G190A (3 children).

NRTIs resistance mutation distribution, according to the Stanford HIV Drug Resistance Database mutation list, is presented in Table 3.

#### **PIs Viral Resistance.**

Protease inhibitors (PIs) regimen at the time of genotyping assessment, the previous regimen that the children received, and the period of time they were under therapy is presented in Table 1.

**Table 2.** HIV-1 reverse transcriptase (RT) gene mutations and viral resistance in HIV-infected pediatric patients.

<b>Child number</b>	<b>RT gene mutations</b>	<b>NRTI and NNRTI* resistance (possible resistance)</b>
1	M41L, D67N, K70R, V118I, T215F, K219Q	AZT, ddI, ddC, d4T, ABC, TDF
2	M41L, D67N, V118I/V, T215Y	AZT, (ddI), (ddC), d4T, (ABC), (TDF)
3	M184V	(ddC), 3TC/FTC
5	M41L, E44D, M184V, T215Y	(AZT), (ddC), 3TC/FTC, (d4T), ABC, (TDF)
6	A62V, K65R, M184V, T215Y	(AZT), (ddI), ddC, 3TC/FTC, (d4T), ABC, TDF
7	M41L, E44D, D67N, M184V, L210W, T215Y	(AZT), ddI, ddC, TC/FTC, (d4T), ABC, TDF
8	M41L, E44D, D67N, T69D, K70R, A98G, M184V, T215F, K219Q	AZT, ddI, ddC, 3TC/FTC, d4T, ABC, TDF, (NVP), (DLV), (EFV)
9	M41L, E44D, D67N, T69D, V118I, M184V, L210W, T215Y	(AZT), ddI, ddC, 3TC/FTC, (d4T), ABC, TDF
10	M41L, D67N, K103N, M184V, H208Y	(AZT), (ddC), 3TC/FTC, (d4T), (ABC), (TDF), NVP, DLV, EFV
11	M41L, E44D, D67N, T69D/N, M184V, T215Y	(AZT), ddI, ddC, 3TC/FTC, (d4T), ABC, (TDF)
12	F77L	No evidence of resistance
13	V118I	No evidence of resistance
14	M41L, K70R, V75M, F77L, K101E, V118I, M184V, G190A, T215F, K219Q	(AZT), ddI, ddC, 3TC/FTC, d4T, (ABC), TDF, NVP, (DLV), (EFV)
15	M184V	3TC/FTC
17	M41L, M184V, T215Y	(AZT), (ddI), (ddC), 3TC/FTC, (d4T), ABC, (TDF)
20	D67N, K101E, G190A, K219Q	(AZT), (d4T), NVP, (DLV), (EFV)
23	A62V, K70R, A98G, V118I, M184V, H208Y, T215C/F, K219Q	(AZT), 3TC/FTC, (d4T), (DLV)
24	M41L, K103N, M184V, T215Y	(AZT), (ddI), 3TC/FTC, (d4T), (ABC), (TDF), NVP, DLV, EFV
25	M41L, T69N, K70R, K101E, V106A, G190A, T215F, K219Q	AZT, ddI, d4T, TDF, NVP, DLV, (EFV)
26	No relevant mutations detected	No evidence of resistance
27	M41L, D67N, V118I, L210W, T215Y	AZT, ddI, d4T, (ABC), TDF

\*NRTI = nucleoside reverse transcriptase inhibitors, NNRTI = non-nucleoside reverse transcriptase inhibitors, PI = protease inhibitors

**Table 3.** Nucleoside reverse transcriptase inhibitors (NRTIs) resistance mutations distribution according to the Stanford HIV Drug Resistance Database mutation list.

		<b>NRTIs resistance mutations</b>							
		<b>Thymidine analog</b>					<b>Non-thymidine analog</b>		
Codon position		M41L	D67N	K70R	L210W	T215F/Y	K219Q	K65R	V75M
Nr. of children		13	9	5	3	14	6	1	1
		<b>Accessory</b>		<b>Multi-NRTI resistance</b>			<b>Hypersusceptibility</b>	<b>Additional</b>	
Codon position		V118I/V	E44D	H208Y	A62V	F77L	M184V	T69D	
Nr. of children		7	5	2	2	2	13	4	

Nine patients among the 21 included in this study presented no evidence of PIs resistance. Two children presented no PIs included in their antiretroviral regimens at the time of genotyping assessment. In the remaining 7 PIs-exposed patients, polymorphic mutations associated or not associated with L63P were reported at codons K20R, M36I, D60E, A71V, and V77I.

Protease gene mutations conferring PIs viral resistance to at least two to up to twelve drugs were reported in 12 children. HIV-1 protease gene mutations and the viral resistance in the HIV-infected pediatric patients are presented in Table 4.

**Table 4.** HIV-1 protease (PR) gene mutations and protease inhibitor (PI) viral resistance in HIV-infected pediatric patients.

<b>Child number</b>	<b>PR gene mutations</b>	<b>PI resistance (possible resistance)</b>
1	L63P	No evidence of resistance
2	L10F, D30N, L33F, M36L, N88D	NFV, (ATV)
3	L10I, M36I, L63P, L90M	SQV, (IDV), (RTV), NFV, (ATV)
5	L10F, D30N, M36V, L63P	NFV, (ATV)
6	L10I, K20R, D30N, M36V, L63P, A71V, N88D	NFV, ATV
7	D30N, L33F, I54L, L63P, A71T, I84V, N88D, L90M	SQV, IDV, RTV, NFV, APV/FPV, (LPV/r), ATV
8	L63P	No evidence of resistance except for ATV: insufficient evidence
9	G16E, K20I, L33V, M36I, M46I, G73S, L90M	SQV, (SQV/r), IDV, (IDV/r), RTV, NFV, APV/FPV, (APV/r or FPV/r), ATV
10	M36I, L63P	No evidence of resistance
11	L10V, M36I, M46I, I54V, L63P, V82S	SQV, (SQV/r), IDV, IDV/r, RTV, NFV, APV/FPV, APV/r or FPV/r, (LPV/r), ATV
12	M36I	No evidence of resistance
13	M36I, V77I, L89M	No evidence of resistance
14	L10I, K20I, M36I, M46I, I50V, I54V, L63P, A71V, V82A, L89I	SQV, SQV/r, IDV, IDV/r, RTV, NFV, APV/FPV, APV/r or FPV/r, LPV/r, ATV
15	L10I, I13V, D60E, L63P, A71V, G73S, V77I, L90M	SQV, (SQV/r), IDV, (IDV/r), RTV, NFV, (APV/FPV), (APV/r or FPV/r), (LPV/r), (ATV), (ATV/r)
17	K20I, M46I, L63P, T74S, V77I, L90M	SQV, (SQV/r), IDV, (IDV/r), RTV, NFV, APV/FPV, (APV/r) or (FPV/r), (ATV)
20	K20R, D60E, L63P	No evidence of resistance
23	I13V, L24I, L33F, M46L, I54V, L63P, V77I, V82A, L89I	SQV, (SQV/r), IDV, IDV/r, RTV, NFV, APV/FPV, APV/r or FPV/r, LPV/r, ATV, ATV/r, (TPV/r)
24	L63P	No evidence of resistance
25	L63P, A71T	No evidence of resistance
26	L63P	No evidence of resistance
27	M46L, A71V	IDV, (IDV/r), (RTV), APV/FPV, (APV/r)

PIs resistance most important mutations were observed at codons M46IL (6 children), L90M (5 children), and D30N (4 children). Hypersusceptibility I50V and I54L mutations were detected in one child each.

Accessory mutations were frequently reported: L63P and M36IVL polymorphic accessory mutations were respectively detected in 16 and 10 children, and L10F and T74S nonpolymorphic accessory mutations were respectively detected in 2 children and 1 child.

Uncommon residues were registered for L89MI mutations, and we could not meet criteria for their classification according to the Stanford HIV Drug Resistance Database mutation list. Therefore, these mutations will be discussed later on.

The frequency of PIs resistance, accessory, additional, and hypersusceptibility mutations, observed in this group of pediatric patients, is presented in Table 5.

**Table 5.** Protease inhibitors (PIs) resistance mutations and polymorphisms distribution according to the Stanford HIV Drug Resistance Database mutation list.

		<b>PIs resistance mutation</b>									
Codon position		L24I	D30N	M46IL	I54V	G73S	V82SA	I84V	N88D	L90M	
Nr. of children		1	4	6	3	2	3	1	3	5	
		<b>Accessory polymorphic</b>									
Codon position		L10IV	I13V	G16E	K20RI	M36IVL	D60E	L63P	A71VT	V77I	
Nr. of children		5	2	1	5	10	2	16	6	4	
		<b>Accessory non-polymorphic</b>			<b>Hypersusceptibility</b>			<b>Additional</b>			
Codon position		L10F	T74S	I50V	I54L			L33V			
Nr. of children		2	1	1	1			1			

## DISCUSSION

In this study, all of the 21 HIV-1 infected children harbored virus strains presenting at least one polymorphic mutation or antiretroviral resistance mutation in HIV-1 protease or reverse transcriptase genes. Resistant strains to nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and protease inhibitors were respectively reported in 18, 7, and 13 children. This distribution slightly differed from that observed in a previous study conducted by us (Simonetti et al. 2003).

Among the 18 children presenting NRTIs resistance, reverse transcriptase (RT) gene mutations were reported occurring most frequently at codons T215F/Y (14 children), M41L (13 children), and M184V (13 children).

Mutations at codons 41 and 215, together with other RT mutations at codons 67, 70, 210, and 219 are termed thymidine analog resistance mutations (TAMs). They occur gradually under the selection pressure of thymidine analogues, either zidovudine or stavudine, and can promote resistance to almost all nucleoside and nucleotide analogues, hampering antiretroviral regimens management (Shafer et al. 2000; Hu et al. 2006). In our study, 17 among the 18 children presenting viral resistance to the NRTIs were receiving zidovudine or stavudine regimens at the time of genotyping assessment.

Several studies have suggested that TAMs are clustered in two distinct groups. TAM-1 cluster encompasses mutations that occur together with T215Y, including M41L, D67N, and L210W whereas mutations that occur together with T215F, including D67N, K70R, and K219Q constitute the TAM-2 cluster (Hanna et al. 2000, Marcelin et al. 2004). TAMs division in two clusters has important clinical meaning as viruses resistant to zidovudine carrying TAM-1 mutations commonly are cross-resistant to didanosine and tenofovir, whereas those carrying TAM-2 mutations usually are susceptible to them (Marcelin et al. 2005). In our study, the 8 children clustered as TAM-1 presented cross-resistance to didanosine and tenofovir. However, for those 6 children clustered as TAM-2, our results showed that only 2 children were susceptible to didanosine and tenofovir. Among the remaining 4 children presenting resistance to didanosine and tenofovir, 3 were receiving regimens containing these drugs, acting as selective pressure on the emergence of resistant viral strains.

The hypersusceptibility M184V mutation was frequently observed among the pediatric patients included in this study. It increases viral susceptibility to zidovudine, tenofovir, and stavudine partially reversing T215Y-mediated resistance (Shafer et al.

2000, Whitcomb et al. 2003). However, it causes high-level in vitro resistance to lamivudine (higher than 100-fold) and emtricitabine. It is usually selected on abacavir-containing regimens (Miller et al. 2000) and less commonly with ddI (Winters et al. 1997) causing low-level in vitro resistance (about 2-fold resistance) to each of them.

The accessory mutations V118IV, E44D, and H208Y detected in 12 children in our study, occur with TAMs and are associated with susceptibility reductions to multiple NRTIs. In particular, V118IV occurs with increased frequency in persons receiving multiple NRTIs causing low-level resistance to lamivudine and to other NRTIs when present with E44AD and more TAMs. In relation to the H208Y mutation, there is a strong association between H208Y and NRTIs experience, mainly in persons with HIV-1 harboring multiple NRTI resistance mutations (Nebbia et al. 2007). All of the 12 children with these accessory mutations showed a viral resistance profile compatible with the one mentioned, except for one child (number 13), who was receiving a zidovudine and lamivudine-containing regimen at the time of genotyping assessment, and presented the single V118IV mutation conferring no evidence of resistance. This child was one of the twins who were receiving a continuous antiretroviral therapy for a period of time lasting for 5 years. His brother (child number 12) also presented a singular profile. He was receiving a zidovudine, lamivudine, and nelfinavir-containing regimen at the time of genotyping assessment, and he reported the single F77L mutation conferring no evidence of resistance.

In relation to NNRTIs, we observed in our study 7 children harboring viral strains with mutations conferring resistance to delavirdine (one child) or to all three drugs, nevirapine, delavirdine, and efavirenz.

Three children were receiving nevirapine or efavirenz at the time of genotyping assessment, and presented specific mutations conferring intermediate to high-level resistance to all three drugs.

Two other children presented the A98G mutation. It occurs in about 1% of NNRTI-naïve persons and causes low-level nevirapine resistance. The children presenting this mutation were NNRTI-naïve, but instead of nevirapine-resistance they presented low-level resistance to either all three drugs or just to delavirdine.

The remaining two children were not receiving NNRTIs at the time of genotyping assessment, but received nevirapine-containing antiretroviral regimens two to three years before testing. One of them presented the K103N mutation, and the other one presented K101E and G190A mutations conferring resistance to all three drugs in both

children. This is possibly explained because NNRTIs resistance mutations, once selected, are often detectable in plasma for months to years (Joly et al. 2001, Soriano 2006).

Among the 12 children presenting PIs resistance, PR gene mutations were reported occurring most frequently at codons M46IL (6 children), L90M (5 children), and D30N (4 children).

Mutations at position 46 contribute resistance to each of the PIs, decreasing susceptibility to IDV, NFV, FPV, LPV, ATV, TPV, and possibly SQV and DRV when present with other mutations. It has been commonly reported during therapy with IDV, RTV, APV, and NFV (Condra et al. 2000). The 6 children presenting M46IL mutation were receiving NFV or RTV-regimens at the time of genotyping assessment except for one child (number 27) who had never received PIs-containing regimens. In addition to the M46L mutation, she presented A71V polymorphism that occurs in 1-2% of untreated persons but which becomes much more frequent in persons receiving PIs.

The L90M mutation confers resistance to NFV, SQV, ATV, and IDV (Drona et al. 2001). When present with other mutations it also compromises the activity of FPV, LPV, and TPV. All the 5 children harboring this mutation showed an important HIV-1 strain multi-drug resistant profile, ranging from five to twelve PIs.

Uncommon residue was registered for L89MI mutation. L89M was observed in two PI-naïve children with no evidence of PIs resistance. This mutation is a naturally occurring polymorphic substitution which may lead to early development of drug resistance in some infected patients (Abecasis et al. 2005; Sanches et al. 2007). On the other hand, the L89I mutation was reported in two children who were receiving RTV-regimen and presented multi-drug viral resistance.

Further analyses are still required to provide insights and give more information on the role of polymorphic substitutions and uncommon mutations in viral drug resistance in pediatric HIV-1 infected patients.

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