

# Impact of Human Immunodeficiency Virus Type 1 Subtypes on Virologic Response and Emergence of Drug Resistance among Children in the Paediatric European Network for Treatment of AIDS (PENTA) 5 Trial

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The association between virologic response and human immunodeficiency virus type 1 (HIV-1) subtype was investigated in 113 HIV-1-infected children randomly assigned to receive zidovudine plus lamivudine, zidovudine plus abacavir, or lamivudine plus abacavir in the Paediatric European Network for Treatment of AIDS (PENTA) 5 trial. Symptomatic children ( $n = 68$ ) also received nelfinavir; asymptomatic children ( $n = 45$ ) were randomly assigned to receive nelfinavir or placebo. HIV-1 subtypes A, B, C, D, F, G, H, A/E, and A/G were found in 15%, 41%, 16%, 9%, 5%, 2%, 1%, 5%, and 7% of the children, respectively. Resistance assay failure rates were higher for non-B subtypes than for B subtypes (genotype,  $P = .01$ ; phenotype,  $P = .02$ ). HIV-1 subtype was not associated with virologic response at 24 and 48 weeks after initiation of treatment. No differences were observed in the frequency of development of resistance mutations L90M ( $P = 1.00$ ) and D30N ( $P = .61$ ) in B and non-B viruses. In conclusion, no evidence that subtype determined virologic response to therapy was found.

Human immunodeficiency virus type 1 (HIV-1) can be subdivided into groups (M, N, and O) and subtypes on the basis of genetic relatedness. Subtype B is the most common group M variant within North America, Europe, and Australia; never-

theless, infections with this subtype are only a small fraction of infections occurring throughout the world, where other subtypes, such as A, C, D, E, and recombinant A/E, predominate [1–3].

Genetic variation in the infecting virus may affect a patient's response to antiretroviral therapy. For instance, HIV-1 group O and HIV-2 are inherently resistant to nonnucleoside reverse-transcriptase inhibitors [4, 5]. However, few in vitro or in vivo data are available on drug susceptibility in non-B group M viruses, because antiretroviral therapy has been most widely available in areas of the world where group B viruses predominate. Because the use of antiretroviral therapy is extending into the developing world, information about drug susceptibility in non-B viruses is essential. Furthermore, because of migratory patterns, an increasing proportion of HIV infections within Europe are caused by non-B viruses.

Pretreatment genetic variation in reverse-transcriptase and protease genes of different subtypes may also affect drug-resistance patterns. It is recognized that some genetic changes classified as "secondary" or "accessory" resistance mutations in subtype B viruses are already present at an increased frequency in non-B viruses isolated from samples from antiretroviral-naïve persons [6–8]. This may influence the genetic route to resistance and also has implications for the interpretation of genotypic drug-resistance information.

To date, the limited data available on subtype-specific determinants of antiretroviral response and emergence of resistance primarily have been generated in uncontrolled cross-sec-

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The protocol for the Paediatric European Network for Treatment of AIDS (PENTA) 5 trial (and for obtaining samples for resistance testing) was approved by the ethics committee for each participating center. All primary caregivers gave written consent for the children's participation, and additional written consent was obtained from children, when appropriate, according to age and knowledge of human immunodeficiency virus status.

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tional studies. By contrast, there is a paucity of resistance data available from clinical trials that involve populations infected with a wide variety of subtypes. We have therefore undertaken a substudy to determine the effect of viral subtype on response to therapy and development of resistance by use of samples from children participating in the Paediatric European Network for Treatment of AIDS (PENTA) 5 trial. This trial was designed to evaluate the antiviral activity and safety of zidovudine, lamivudine, and abacavir in dual-nucleoside reverse-transcriptase inhibitor (NRTI) combinations and to evaluate the safety and tolerability of the protease inhibitor (PI) nelfinavir in a group of previously drug-naïve HIV-1-infected children.

## Materials and Methods

**Trial design.** PENTA 5 was a randomized, partially blinded (for nelfinavir) multicenter comparative trial involving children aged 3 months to 16 years who were naïve to antiretroviral therapy (except for therapy received in utero or as perinatal prophylaxis up to 6 weeks after delivery). One hundred twenty-eight previously untreated children were randomly assigned to receive 1 of 3 dual-NRTI regimens—zidovudine plus lamivudine, zidovudine plus abacavir, or lamivudine plus abacavir—between January 1998 and April 1999 [9]. Resistance tests were done on  $\geq 1$  stored samples from 113 of the 128 children (12 children randomized in Brazil and 3 more children from whom no plasma specimens were obtained after trial entry were excluded) [10]. Asymptomatic children ( $n = 45$ ) were also randomly assigned to receive nelfinavir or placebo (part A), and symptomatic children ( $n = 68$ ) all received open-label nelfinavir (part B). The protocol was amended during the recruitment phase of the study: the nelfinavir dosage was changed (from 75–90 to 90–110 mg/kg/day), and the nelfinavir dosing schedule was changed (from 3 to 2 times daily). After interruptions or changes in therapy due to adverse events, inadequate HIV-1 RNA response, or patient request (according to the protocol) were taken into account, the percentages of total child-time in the trial to 48 weeks during which the prescribed NRTI regimen was received were 85%, 86%, and 87% for the zidovudine/lamivudine, zidovudine/abacavir, and lamivudine/abacavir groups, respectively; the percentages for the prescribed nelfinavir or placebo regimens (part A) were 89% and 82%.

Children were classified as “nonresponders,” “rebounders,” or “responders” on the basis of HIV-1 RNA response. In responders ( $n = 58$ ), the HIV-1 RNA level decreased to  $<400$  copies/mL before 28 weeks and remained  $<2000$  copies/mL subsequently. In nonresponders ( $n = 21$ ), the HIV-1 RNA level was always  $>400$  copies/mL. In rebounders ( $n = 34$ ), the HIV-1 RNA level fell to  $<400$  copies/mL before 28 weeks but subsequently rebounded to  $>2000$  copies/mL. All baseline samples were assayed for resistance. In addition, samples obtained after baseline from nonresponders and rebounders were assayed for resistance at the time of rebound and/or at 24, 48, and 72 weeks.

**Plasma samples.** Plasma samples were obtained at screening and baseline (weeks  $-2$  and  $0$ ), every 4 weeks until week 24, and then every 8 weeks throughout the trial. Plasma was separated within 6 h and sent to Covance (Geneva) for HIV-1 RNA testing

(see below). Additional plasma was stored at  $-80^{\circ}\text{C}$  and was sent on dry ice to Virco (Mechelen, Belgium) for resistance testing.

**Phenotypic and genotypic resistance testing.** Phenotypic resistance was measured by use of a recombinant virus assay (Antivirogram; Virco). ABI sequencing (Applied Biosystems 373 Nucleotide Sequencer) was used to detect resistance-conferring mutations (VircoGEN; Virco). The recommended lower limit for HIV-1 RNA for these resistance tests was 2000 copies/mL. HIV-1 subtype was determined from the *pol* gene sequence generated by the resistance assay. For both the phenotypic and genotypic assays, all tests that failed initially were repeated; thus, failure rates correspond to failure of 2 tests. Subtype was assigned by comparison of aligned *pol* sequences with prototype subtype sequences in the Los Alamos HIV database (<http://hiv-web.lanl.gov>) or, for samples for which testing had failed, was determined from previous or subsequent assays for the same child, when these were available.

**Plasma HIV-1 RNA levels.** Plasma HIV-1 RNA levels were measured at 2 central laboratories (Covance in Geneva for European centers and Indianapolis for Brazilian centers) accredited by the College of American Pathologists Laboratory Accreditation Program. Samples from weeks  $-2$  and  $0$  were measured using the Roche Amplicor assay, version 1.5 (limit of detection, 400 copies/mL), and subsequent samples were tested with the Roche Ultra-sensitive assay, version 1.5 (limit of detection, 50 copies/mL). Any specimen with a result of  $>40,000$  copies/mL on the Ultrasensitive assay was retested with the standard assay.

**Statistical analysis.** Unadjusted proportions were compared using Fisher's exact test. Plasma HIV-1 RNA results were  $\log_{10}$ -transformed before analysis; 24- and 48-week values were the closest to the nominal week within 8-week windows (interquartile range for actual visit, weeks 23–25 and 47–50, respectively). When plasma HIV-1 RNA levels reached the lower limit of quantification ( $\leq 50$  copies/mL), changes were analyzed by normal interval regression, and values lower than the cutoff level were replaced by the interval in which the true unobserved value could lie (i.e., 0–50 copies/mL), rather than imputing these values by the cutoff level (49 or 50 copies/mL) [11]. All logistic regression models used to compare subtypes were adjusted for NRTI group and nelfinavir receipt (in part A or B) because of marginal imbalances in the randomization that resulted from the small subject population [9]. Models used to compare the change in  $\log_{10}$  HIV-1 RNA level across subtypes were additionally adjusted for age, baseline HIV-1 RNA level, and baseline CD4 cell percentage [9]. Effects of factors and heterogeneity across subtypes were assessed by use of Wald tests.  $P < .05$  was considered to be statistically significant;  $P$  values between .05 and .10 provided marginal evidence against the null hypothesis.

## Results

Of the 113 children from whom samples were available for resistance assay, 32, 39, and 42 were randomly assigned to the dual-NRTI regimens zidovudine/lamivudine, zidovudine/abacavir, and lamivudine/abacavir, respectively. Twenty-four children received nelfinavir and 19 received placebo in the part A comparison.

One hundred ninety resistance tests were undertaken in these 113 children (111 tests at baseline and 79 after baseline), with

**Table 1.** Relationship between human immunodeficiency virus type 1 (HIV-1) subtype and assay failure rate for 113 HIV-1-infected children.

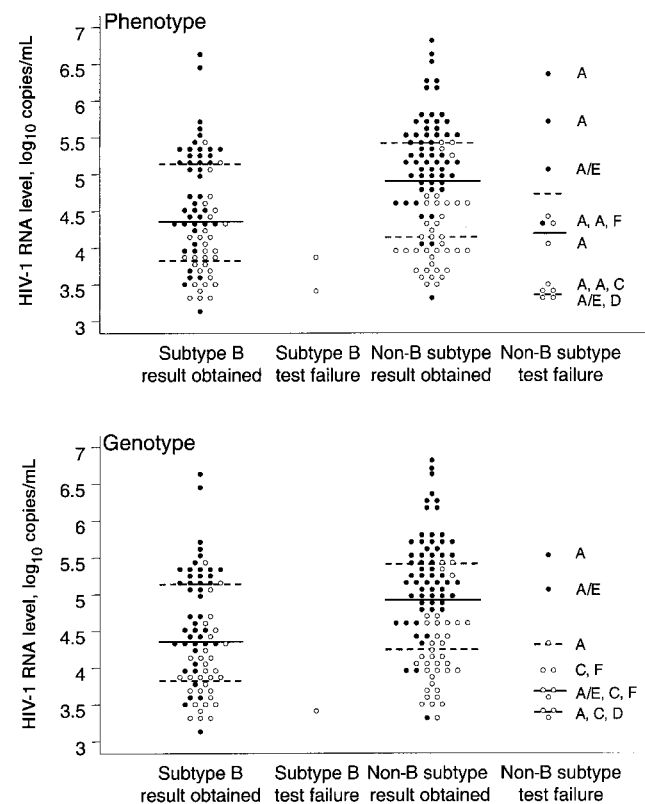
HIV-1 subtype	No. of tests with no result (% of total tests)		Total no. of tests (no. of children tested)
	Phenotypic	Genotypic	
A/E	2 (22)	2 (22)	9 (5)
A/G	0	0	10 (8)
A	7 (22)	3 (9)	32 (16)
B	2 (3)	1 (1)	76 (44)
C	1 (3)	3 (10)	31 (17)
D	1 (7)	1 (7)	15 (10)
F	1 (12)	2 (25)	8 (5)
G	0	0	3 (2)
H	0	0	1 (1)
Unknown	2 (40)	5 (100)	5 (5)
Total	16 (8)	17 (9)	190 (113)

an overall assay failure rate (no result obtained) of 8% (16 tests) and 9% (17 tests) for phenotypic and genotypic repeat testing, respectively (8 samples had no result for both phenotypic and genotypic assays) (table 1). Unadjusted assay failure rates varied substantially across subtypes for both phenotype ( $P = .02$ ; Fisher's exact test) and genotype ( $P = .06$ ; Fisher's exact test) (table 1). However, the assay failure rate depended both on HIV-1 RNA level and on subtype (figure 1) and, as expected, decreased as HIV-1 RNA level increased. One or more samples with subtype A, A/E recombinant, or F and an HIV-1 RNA level of >10,000 copies/mL failed genotypic or phenotypic resistance tests (figure 1). Overall, when the analysis was adjusted for  $\log_{10}$  HIV-1 RNA load as well as for NRTI regimen and receipt of nelfinavir in part A or part B, and allowing for the fact that >1 resistance test was done on samples from some children [12], there was a 6.0-fold increase in the odds of phenotypic assay failure for children infected with non-B subtype viruses, compared with those infected with B subtype viruses (95% confidence interval [CI], 1.31–27.3;  $P = .02$ ) and a 17.5-fold increase in the odds of genotypic assay failure (95% CI, 1.82–169.1;  $P = .01$ ). The odds ratio (OR) for each log increase in HIV-1 RNA was 0.33 (95% CI, 0.14–0.79;  $P = .01$ ) for phenotypic and 0.17 (95% CI, 0.04–0.80;  $P = .02$ ) for genotypic assay failure. Although the odds of failing resistance tests varied across the non-B subtypes, there was no strong, consistent statistical evidence for heterogeneity ( $P = .02$  for phenotype and  $P = .89$  for genotype), although the numbers of individual samples from non-B subtypes were small.

However, subtype and ethnicity were highly confounded: 42 (95%) of the 44 children infected with subtype B HIV-1 were white, compared with 7 (11%) of the 64 children infected with non-B HIV-1. Genotype data were not obtained for 5% of samples from white children, compared with 10% of samples from black children and 22% of samples from children in other ethnic groups (6 children with one black and one white parent, 2 Asian children, 1 Romanian child, and 1 North African child) ( $P = .05$ ; Fisher's exact test). Phenotype data were not ob-

tained for 4%, 12%, and 11% of samples, respectively ( $P = .07$ ; Fisher's exact test). Furthermore, 4 of the 5 children for whom HIV-1 subtype could not be identified because all of their resistance tests failed were nonwhite.

*Association between baseline HIV-1 RNA levels and viral subtype.* The most common HIV-1 subtypes were A (15%), B (41%), and C (16%) (table 2). HIV-1 RNA levels at the time of resistance testing were higher among children with non-B subtypes than among children with B subtypes (figure 1;  $P = .005$ , by the Wilcoxon rank sum test), without adjustment for confounding factors. Furthermore, this analysis includes results of testing done both at baseline and after the initiation of antiretroviral therapy and might, therefore, reflect differences in baseline values and/or response to antiretroviral therapy according to subtype. However, there was still significant variation in baseline  $\log_{10}$  HIV-1 RNA level between specific subtypes ( $P = .01$ ) after adjustment for baseline CD4 cell percentage alone (allowing a nonlinear effect) [13]; subtype B was associated with the lowest baseline HIV-1 RNA level at the same baseline CD4 cell percentage. After adjustment for randomization in part A versus part B, in utero antiretroviral therapy, and age at randomization, in addition to baseline CD4 cell percentage, and stratification for



**Figure 1.** Human immunodeficiency virus type 1 (HIV-1) RNA by subtype and status of resistance test. *Solid line*, within-group median; *dashed lines*, interquartile range; *solid circles*, results of resistance tests done at baseline; *open circles*, results of resistance tests done after baseline.

**Table 2.** Effect of viral subtype on virologic response to antiretroviral therapy in 108 human immunodeficiency virus type 1 (HIV-1)-infected children.

Factor	No. of children	Change in HIV-1 RNA level, mean log <sub>10</sub> copies/mL (SE)	
		From randomization to week 24	From randomization to week 48
Effect of NRTIs			
ZDV + 3TC	31	-1.27 (0.37)	-1.78 (0.43)
ZDV + ABC	35	-1.89 (0.36)	-2.14 (0.42)
3TC + ABC	42	-2.10 (0.40)	-2.70 (0.46)
Effect of subtype in addition to that of NRTIs <sup>a</sup>			
A/E and A/G	13	-0.06 (0.43)	-0.02 (0.50)
A	16	+0.04 (0.39)	+0.49 (0.44)
B (reference)	44	—	—
C	17	-0.10 (0.37)	+0.17 (0.42)
D	10	-0.36 (0.45)	-0.30 (0.52)
F, G, and H	8	-0.01 (0.49)	+0.41 (0.60)
Heterogeneity, <i>P</i>		.99	.93

NOTE. Data are from 108 children infected with viruses for which the subtype was known; the week 24 HIV-1 RNA measurement was missing for 1 child (subtype A), and the week 48 measurement was missing for 1 child (subtype G). Analysis was adjusted for age, baseline HIV-1 RNA level, baseline CD4 cell percentage, NRTI group, and nelfinavir receipt in part A or B. Similar results were obtained when the analysis was restricted to children receiving nelfinavir. 3TC, lamivudine; ABC, abacavir; NRTI, nucleoside reverse-transcriptase inhibitor; ZDV, zidovudine.

<sup>a</sup> Compared with the reference category (subtype B). The effect of subtype was assumed to be common across all NRTI groups.

country of randomization (United Kingdom [ $n = 43$ ; 15% subtype B], Italy [ $n = 37$ ; 81% subtype B], or other country [ $n = 33$ ; 25% subtype B]), to avoid potential confounding, baseline HIV-1 RNA levels in children infected with subtype B virus appeared to be 0.24 log<sub>10</sub> lower than levels in those infected with non-subtype B virus ( $P = .05$ ).

**HIV-1 RNA response to antiretroviral therapy.** Overall, the virologic response to therapy was best in the lamivudine/abacavir group and poorest in the zidovudine/lamivudine group [9]. Thus, among the 113 children for whom resistance tests were done, mean log<sub>10</sub> HIV-1 RNA levels had decreased by 1.72, 2.05, and 2.61 log<sub>10</sub> copies/mL (estimated in the absence of nelfinavir) at 48 weeks in the zidovudine/lamivudine, zidovudine/abacavir, and lamivudine/abacavir groups, respectively, after adjustment for baseline factors (global  $P = .03$ ; results were very similar to those for all 128 children [9]). There was no evidence that any specific non-B HIV-1 subtype was associated with poorer virologic response, either in terms of a change in HIV-1 RNA level between baseline and 24 or 48 weeks or in the proportion of children with HIV-1 RNA levels <400 or <50 copies/mL at both 24 and 48 weeks, in an analysis that included all children (table 2) or that was restricted to those receiving nelfinavir. Nor was there any evidence of a difference between B and non-B subtypes in the relative efficacy of the dual-NRTI combinations ( $P = .66$  and  $P = .96$  at 24 and 48 weeks, respectively). Similarly, there was no evidence that the presence of specific protease polymorphisms at baseline was associated with poorer virologic response in all children (table 3) or in those receiving nelfinavir.

**Association between patterns of resistance mutations and viral subtype.** No child had viruses with primary resistance muta-

tions in protease or reverse transcriptase at baseline. Of 40 non-responders and rebounders from whom follow-up samples were available, the primary PI-resistance mutations that emerged included L90M, D30N, N88D, and N88S. The absolute numbers of these mutations were small, and no differences were observed between subtype B and non-B viruses in the emergence of L90M (2 of 18 vs. 3 of 22 children;  $P = 1.00$ ) or D30N (1 of 18 vs. 3 of 22 children;  $P = .61$ ) (table 4). As expected, the most prevalent NRTI-resistance mutation was M184V, which was detected in at least 1 sample from a nonresponder or a rebounder for each HIV-1 subtype other than A/G (for subtype B vs. non-B, 11 of 18 vs. 15 of 22 children, respectively;  $P = .74$ ). L74V, 215Y/F, K65R, K70R, and Y115F mutations were observed at lower frequencies than M184V, and the small numbers preclude any conclusion being drawn about subtype specificity. Of note, however, although 45%, 43%, and 36% of children were infected with subtype B in the zidovudine/lamivudine, zidovudine/abacavir, and abacavir/lamivudine groups, respectively ( $P = .68$ ), the development of K65R in 3 children receiving lamivudine/abacavir was observed only in subtype B viruses (for subtype B vs. non-B, 3 of 18 vs. 0 of 22 children, respectively;  $P = .08$ ; among children receiving abacavir, 3 of 12 vs. 0 of 11 children;  $P = .22$ ) (table 4).

**Impact of viral subtype on secondary PI-resistance mutations.** We assessed the prevalence of mutations in the protease gene (compared to the consensus sequence for subtype B virus) at positions 10, 20, 36, 71, and 77. Of the 173 samples assayed for which a genotypic test result was obtained, 125 (72%) yielded virus with  $\geq 1$  of these secondary PI-resistance mutations. Only 1 other sample yielded virus with any other secondary PI-resistance mutation without the presence of 1 of

**Table 3.** Effect of protease polymorphisms on virologic response to antiretroviral therapy in 104 human immunodeficiency virus type 1 (HIV-1)-infected children.

Factor	No. of children	Change in HIV-1 RNA level, mean log <sub>10</sub> copies/mL (SE)	
		From randomization to week 24	From randomization to week 48
<b>Effect of NRTIs</b>			
ZDV + 3TC	28	-1.05 (0.38)	-1.84 (0.46)
ZDV + ABC	34	-1.53 (0.38)	-1.96 (0.45)
3TC + ABC	42	-1.80 (0.39)	-2.54 (0.46)
<b>Effect of polymorphism in addition to that of NRTIs<sup>a</sup></b>			
No polymorphism (reference)	25	—	—
M36I without L10V/I	45	-0.64 (0.31)	-0.37 (0.37)
L10V/I without M36I	8	-1.03 (0.51)	-0.17 (0.60)
M36I and L10V/I together	12	-0.08 (0.42)	-0.47 (0.50)
V77I	16	-0.78 (0.36)	-0.91 (0.43)
K20M/R	11	-0.40 (0.41)	-0.27 (0.49)
A71T/V	4	+0.66 (0.64)	+0.39 (0.77)
Heterogeneity, <i>P</i>		.54	.85
Interaction between M36I and L10V/I, <i>P</i> <sup>b</sup>		.09	.95

NOTE. Data are from 104 children infected with viruses for which the baseline polymorphisms were known. Analysis was adjusted for age, baseline HIV-1 RNA level, baseline CD4 cell percentage, NRTI group, and nelfinavir receipt in part A or B. Similar results were obtained when the analysis was restricted to children receiving nelfinavir. 3TC, lamivudine; ABC, abacavir; NRTI, nucleoside reverse-transcriptase inhibitor; ZDV, zidovudine.

<sup>a</sup> Compared with reference category (no polymorphism) in a multivariate model containing all 5 polymorphisms.

The effect of polymorphisms was assumed to be common across all NRTI groups.

<sup>b</sup> No other interactions were significant (*P* < .20 was considered to be significant).

these 5 mutations (M46I alone). At baseline, 79 (76%) of 104 children with genotypic results had ≥1 of these secondary PI-resistance mutations (table 5). Non-B subtypes were significantly more likely to have secondary PI-resistance mutations at baseline (*P* < .001; 2 × 3 Fisher’s exact test), of which M36I was the most prevalent (82% of children). Conversely, this mutation was least prevalent among subtype B viruses and was present in only 7 (16%) of 43 children at baseline. The secondary mutation most prevalent among subtype B viruses at baseline was V77I, which was present in 9 (21%) of 43 children.

We then assessed the emergence of these secondary mutations after the initiation of therapy. Overall, the proportion of strains that acquired these mutations was very low. Most striking was the emergence of V77I in 4 of 17 subtype B viruses that were wild type at this position at baseline (table 6). However, only 2 of the 4 children infected with these viruses had taken nelfinavir before the V77I mutation was acquired (1 child in part B and 1 child who was assigned to receive nelfinavir in part A). These children were both nonresponders who developed other secondary PI-resistance mutations during nelfinavir therapy (A71V and M46I, respectively). The other 2 children had received placebo plus NRTIs only, and V77I was observed at the time of HIV-1 RNA rebound at weeks 23 and 30, respectively, which suggests that this mutation was already present within the plasma quasi species at baseline, although at undetectable levels.

**Discussion**

In view of the extensive and increasing genetic diversity of HIV-1 worldwide, which is reflected in the number of different

subtypes and recombinant strains, an assessment of the clinical implications of variation has become increasingly important. Recently, data have been presented on the impact of subtype on the natural history of HIV-1 disease. Thus, seroconverters in Thailand who were infected with subtype E virus were reported to have higher virus loads than those with subtype B [14]. Furthermore, a study from Uganda suggested that HIV-1 disease caused by subtype A virus may progress more slowly than disease caused by subtype D [15]. However, as the availability of antiretroviral therapy increases worldwide, it is also essential to assess the response of different HIV-1 subtypes to these therapeutic interventions. Of 113 children in the PENTA 5 trial from whom samples were available for resistance assays, 59% were infected with HIV-1 of a non-B subtype, which reflects the epidemiology of pediatric HIV-1 infection in the centers in Europe that recruited children to the study. This heterogeneity allowed us to explore HIV-1 subtype determinants of response to therapy and the emergence of resistance.

Our most important observation is that viral subtype does not appear to influence response to the antiretroviral regimens used in the present study (table 2). The most-represented non-B subtypes in our study were A, C, and D; similar reductions in HIV-1 RNA level were observed for all subtypes. Previous data on this issue have focused on a comparison between subtype B and non-B viruses [16], generally because of the small numbers of persons infected with non-B subtypes. This approach is inadequate for discerning subtype-specific determinants of virologic response, because there is no evidence that all non-B viruses behave similarly. However, in our study, there may also be low power to detect heterogeneity in virologic

**Table 4.** New primary protease and reverse-transcriptase mutations occurring after baseline in 40 human immunodeficiency virus type 1 (HIV-1)-infected children who experienced antiretroviral treatment failure.

HIV-1 subtype	Total no. of children	No. (%) of children with given protease mutation				No. (%) of children with given reverse-transcriptase mutation					
		L90M	D30N	N88D	N88S	M184V	T215Y/F	K65R	L74V	K70R	Y115F
A/E	2	0	0	0	0	1 (50)	1 (50)	0	1 (50)	0	0
A/G	1	0	0	0	0	0	0	0	0	0	0
A	8	0	0	0	0	4 (50)	0	0	0	1 (12)	0
B	18	2 (11)	1 (6)	0	0	11 (61)	3 (17)	3 (17)	4 (22)	1 (6)	2 (11)
C	7	2 (29)	1 (14)	1 (14)	1 (14)	6 (86)	0	0	1 (14)	0	1 (14)
D	2	1 (50)	1 (50)	1 (50)	0	2 (100)	0	0	0	1 (50)	0
F	1	0	1 (100)	0	0	1 (100)	0	0	0	0	0
G	1	0	0	0	0	1 (100)	0	0	0	0	0
Total	40	5 (12)	4 (10)	2 (5)	1 (2)	26 (65)	4 (10)	3 (8)	6 (15)	3 (8)	3 (8)
<i>P</i> <sup>a</sup>		1.00	.61	.49	1.00	.74	.31	.08	.38	1.00	.58

NOTE. No child had L90M, N88D/S, D30N, M184V, T215Y/F, K65R, L74V, K70R, or Y115F at baseline. Some children had >1 primary protease or reverse-transcriptase mutation.

<sup>a</sup> Comparison between children with B and children with non-B subtypes; Fisher's exact test.

response across specific non-B subtypes in individual analyses because of the large number of non-B subtypes found. In particular, assuming an SD of 1.15 in the change in  $\log_{10}$  HIV-1 RNA level from baseline (as observed in PENTA 5), the inclusion of 108 children would provide at least 97% power to detect a difference of 1.5  $\log_{10}$  copies/mL between any 2 of 6 equally sized subgroups (using a global  $\chi^2$  test for which 2-sided  $\alpha = .05$ ) but only 65% and 40% power to detect differences of 1  $\log_{10}$  and 0.75  $\log_{10}$  copies/mL, respectively. Thus, our study has relatively high power to rule out the possibility that little or no reduction in HIV-1 RNA occurred in children infected with any particular subtype following initiation of antiretroviral therapy. Much larger prospective studies are required to demonstrate smaller subtype-specific differences in therapy response. We encourage investigators with appropriate data to undertake such analyses and to present results for specific HIV-1 subtypes so that meta-analysis will be possible.

Of note, the resistance assays used in our study were less

able to generate a genotypic result from non-B viruses than from subtype B viruses, and this was not solely determined by differences in the virus load. This is consistent with observations of other resistance assays for genotype made in a European setting [17]. These findings are not surprising, because the polymerase chain reactions involved in resistance assays have been validated on subtype B variants. Indeed, the early HIV load assays also showed similar differences in the efficiency of polymerase chain reaction amplification [18–20]. Because an increasing number of resistance tests are likely to be undertaken on individuals with diverse viral subtypes, it is important that diagnostic companies and reference laboratories continually reevaluate their assays to maintain optimum performance.

Similarly, our understanding of the genetic basis of HIV drug resistance is largely based on subtype B viruses [21–23]. Many key resistance-associated mutations in reverse transcriptase and protease exert their effect by altering highly conserved motifs of these proteins, and it would be expected that these changes

**Table 5.** Secondary protease mutations at baseline in 104 drug-naive human immunodeficiency virus type 1 (HIV-1)-infected children.

HIV-1 subtype	Total no. of children	No. (%) of children with given no. of mutations			No. (%) of children with given protease mutation				
		0	1	$\geq 2$	M36I	L10V/I	V77I	K20M/R	A71T/V
A/E	4	0	2 (50)	2 (50)	4 (100)	2 (50)	0	1 (25)	0
A/G	8	0	4 (50)	4 (50)	8 (100)	3 (37)	0	1 (12)	0
A	15	0	10 (67)	5 (33)	15 (100)	3 (20)	0	2 (13)	1 (7)
B	43	21 (49)	17 (40)	5 (12)	7 (16)	8 (19)	9 (21)	0	3 (7)
C	16	2 (12)	12 (75)	2 (12)	12 (75)	0	2 (12)	2 (12)	0
D	10	1 (10)	8 (80)	1 (10)	4 (40)	1 (10)	4 (40)	1 (10)	0
F	5	0	1 (20)	4 (80)	5 (100)	2 (40)	0	4 (80)	0
G	2	0	1 (50)	1 (50)	2 (100)	1 (50)	1 (50)	0	0
H	1	1 (100)	0	0	0	0	0	0	0
Total	104	25 (24)	55 (53)	24 (23)	57 (55)	20 (19)	16 (15)	11 (11)	4 (4)
<i>P</i>			<.001 <sup>a</sup>		<.001 <sup>b</sup>	1.00 <sup>b</sup>	.27 <sup>b</sup>	.002 <sup>b</sup>	.30 <sup>b</sup>

NOTE. Data are from 104 children for whom genotypic information was available at randomization. Two children (subtypes B and C) had V179D at randomization, and 6 children (subtypes 4B, C, and G) had A098S/G at randomization, all in addition to 1 of the 5 secondary mutations shown above.

<sup>a</sup> Comparison for B and non-B vs. 0, 1, and  $\geq 2$  mutations;  $2 \times 3$  Fisher's exact test.

<sup>b</sup> Comparison between children with B and children with non-B subtypes; Fisher's exact test.

**Table 6.** New secondary protease mutations occurring after baseline in 40 human immunodeficiency virus type 1 (HIV-1)-infected children who experienced antiretroviral treatment failure.

Population, HIV-1 subtype ( <i>n</i> )	Secondary protease mutations, <i>n/N</i> (%) of children <sup>a</sup>				
	M36I	L10V/I	V77I	K20M/R	A71T/V
All children (40)					
A/E	—	0/1	0/1	0/1	0/1
A/G	—	—	0/1	0/1	0/1
A	—	0/6	0/7	0/6	0/6
B	1/15 (7)	0/13	4/17 (24)	0/17	1/15 (7)
C	0/2	0/6	0/6	0/6	0/6
D	0/1	0/2	0/1	1/2 (50)	1/2 (50)
F	—	0/1	0/1	—	0/1
G	—	—	—	0/1	0/1
Total	1/18 (6)	0/29	4/34 (12)	1/34 (3)	2/33 (6)
Children who received nelfinavir before resistance testing (29)					
A/G	—	—	0/1	0/1	0/1
A	—	0/6	0/7	0/6	0/6
B	0/11	0/11	2/13 (15)	0/13	1/12 (8)
C	0/2	0/5	0/5	0/5	0/5
D	0/1	0/2	0/1	1/2 (50)	1/2 (50)
F	—	0/1	0/1	—	0/1
G	—	—	—	0/1	0/1
Total	0/14	0/25	2/28 (7)	1/28 (4)	2/28 (7)

NOTE. Data are from children for whom both baseline genotype determinations and at least 1 genotype determination after baseline were available. Children who had each specific mutation at baseline were excluded.

<sup>a</sup> *n/N*, no. of children in whom a specific new mutation occurred/no. of children without specific mutation at baseline from whom follow-up samples were available. —, *N* = 0.

would be common across all subtypes. On the other hand, some mutations exert more subtle influences on the 3-dimensional structure of the protein. Because genetic differences between the subtypes in the *pol* gene are well recognized (and, indeed, allow subtyping of viruses to be undertaken effectively [24]), it would not be surprising if some differences in this class of drug-resistance mutations were observed between subtypes.

Our analysis of samples obtained from children in the PENTA 5 trial allowed us to address only mutations associated with zidovudine, lamivudine, and abacavir within reverse transcriptase and nelfinavir within protease genes. As would be expected, the M184V mutation was most commonly observed. This mutation sits within the highly conserved active site of reverse transcriptase [25], and no differences in the prevalence of the mutation have been observed between subtypes [26]. Other NRTI-resistance mutations emerged at a lower frequency, which precluded our drawing any firm conclusions about subtype distribution. It is of interest that the only 3 instances of the K65R mutation within reverse transcriptase that occurred in abacavir-treated children were all in subtype B viruses. However, because this mutation has previously been observed in subtype C viruses [27], we cannot suggest that it is unique to subtype B viruses. Rather, there may be a differential tendency for its emergence in response to abacavir between viral subtypes. With regard to nelfinavir-resistance mutations, we noted changes at positions 30, 88, and 90 of the protease gene, in accordance with established data on the genetic basis of nelfinavir resistance. The precise source of nelfinavir resistance (either mutation D30N or mutation L90M) is important, because viruses with the

D30N mutation alone remain susceptible to other PIs in vitro. Similarly, viruses with the N88S mutation appear to be hypersusceptible to amprenavir. However, the low number of subjects with viruses encoding such mutations precluded any examination of subtype differences.

By contrast, major differences were observed between subtypes in the prevalence of secondary PI-resistance mutations. The M36I protease mutation was extremely common in all subtypes other than subtype B before antiretroviral therapy was started, as described elsewhere [6, 7], and K20I/R was also more common in non-B than in B viruses [6, 27], although this was rarely selected for by treatment. The V77I mutation was identified in subtypes B, C, D, and G, although not in any of the 27 patients infected with subtype A or with subtype A/G or A/E recombinants. The presence of this mutation both as a pre-existing polymorphism and as a nelfinavir-selected secondary mutation has been documented elsewhere [28].

Finally, our data on the differences in frequency of secondary resistance mutations have implications for the use of routine genotypic assays to guide therapeutic decision-making for treatment of patients infected with non-B viruses. Many of the currently available commercial and noncommercial interpretation systems for drug resistance-associated mutations include the secondary PI-resistance mutations among those contributing, in one form or another, to reduced drug susceptibility. However, it is becoming clear that some mutations, such as M36I in protease, may represent the wild-type consensus sequence for a number of non-B subtypes. On the one hand, the preexistence of such variants may predispose a patient to treatment failure,

as was suggested by Perno et al. [29] with regard to a population infected with subtype B. On the other hand, these data cannot necessarily be extrapolated to non-subtype B viruses, and we found little evidence that their presence alters response to PIs. More work is required to clarify this issue.

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