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Impact of Nucleoside Reverse Transcriptase Inhibitors on Mitochondria in HIV-1 Infected Children Receiving HAART

Running head: NRTI and Mitochondrial Toxicity in Children

Akihiko Saitoh[1], Terence Fenton[4], Carmelita Alvero[4], Courtney V. Fletcher[5], Stephen A. Spector[1, 2, 3]

[1] Division of Infectious Diseases, Department of Pediatrics, University of California San Diego, La Jolla, CA, [2]Center for Molecular Genetics and [3]Center for AIDS Research, [4]Center for Biostatistics in AIDS Research, Harvard School of Public Health, Boston, MA, [5]Department of Clinical Pharmacy, University of Colorado Health Science Center, Denver, CO

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3) Corresponding Author: Akihiko Saitoh, M.D.

Division of Infectious Diseases

Department of Pediatrics

University of California, San Diego

9500 Gilman Drive

La Jolla, CA 92093-0672

Telephone: (858) 534-7258

Fax: (858) 534-7411

Email: asaitoh@ucsd.edu

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ABSTRACT

Background: Mitochondrial toxicity induced by nucleoside reverse transcriptase inhibitors (NRTI) has been reported to be responsible for various adverse effects. The relative impact of NRTI on mitochondria of HIV-1 infected children receiving highly active antiretroviral therapy (HAART) is unknown.

Methods: Mitochondrial DNA (mtDNA) levels were quantified longitudinally from peripheral blood mononuclear cells (PBMC) in 31 HIV-1-infected children from PACTG 382 receiving HAART including nelfinavir, efavirenz, and different NRTI with undetectable plasma HIV-1 RNA >2 years.

Results: The median mtDNA levels in PBMC increased from 137 copies/cell at baseline to 179 copies/cell at week 48 ($P = 0.01$) and 198 copies/cell at week 104 ($P < 0.001$). Before initiation of HAART, children who received regimens containing didanosine had persistently lower mtDNA levels compared to those in children without didanosine (106 vs. 140 copies/cell, $P = 0.008$). During HAART, the median mtDNA increase from baseline to week 104 was the lowest in children who received regimens containing didanosine (+26 copies/cell) compared to other regimens (+79 copies/cell) ($P = 0.02$). A multivariate analysis also demonstrated that the use of ddI during HAART was the only NRTI associated with the change in mtDNA levels ($P = 0.007$).

Conclusion: Children receiving didanosine containing antiretroviral regimens have the lowest mtDNA in PBMC and may be at greater risk for long-term adverse effects due to mitochondrial toxicity. This may be of particular importance in resource-limited countries where didanosine is widely used for treatment of HIV-infected children.

INTRODUCTION

The morbidity and mortality associated with HIV-1 infection of children have improved dramatically with the availability of highly active antiretroviral therapy (HAART) (22, 47). Along with the clear benefits of HAART, important adverse effects of antiretrovirals are increasingly being recognized (5). In some patients, these side effects require discontinuing or changing antiretroviral therapy.

In combination with protease inhibitors (PI) or non-nucleoside reverse transcriptase inhibitors (NNRTI), nucleoside reverse transcriptase inhibitors (NRTI) remain the backbone for many HAART regimens. NRTI have been shown to deplete mitochondrial DNA (mtDNA) by selectively inhibiting DNA polymerase γ (28, 30), which is crucial for replication of mtDNA. The depletion of mtDNA causing mitochondrial dysfunction is at least partly responsible for various NRTI-associated adverse effects (21).

The gold standard for the diagnosis of mitochondrial toxicity is examination of biopsy materials from muscle, liver or nerve; however, these biopsies are not practical, especially for vulnerable children. Peripheral blood mononuclear cells (PBMC) are easily obtained from patients and several reports have suggested clinical correlations with mtDNA in PBMC of HIV-infected adults receiving antiretroviral therapy (11, 17, 18, 31, 34). In contrast, no clinical correlations have been identified between the mtDNA levels in PBMC and lipodystrophy, lactate levels or antiretroviral regimens (9, 24, 32, 38, 41, 48). The clinical use of the mtDNA assay in PBMC is still controversial (3, 45); therefore, more research is required to elucidate the importance of the mtDNA levels in PBMC in the clinical setting, especially in children whose sample materials are limited.

Children may be more vulnerable than adults to the adverse effects of antiretrovirals because of the potential negative impact on growth and development with their long-term exposure (33); however, information regarding the frequency and severity of long-term adverse effects in children is limited. In addition, only one cross-sectional study is available that has examined mtDNA levels in PBMC of children receiving antiretroviral therapy (14). This study showed no difference in mitochondrial function and content of PBMC between children with lipodystrophy and those without lipodystrophy.

In order to optimize current and future antiretroviral therapy, there is an urgent need to determine not only which regimens will provide sustained virologic and immunologic benefit, but also those interventions that are least likely to produce long-term toxicity. We investigated the effect of specific NRTI when given in combination with a NNRTI (efavirenz) and a PI (nelfinavir) on mtDNA in PBMC in a cohort of children who achieved sustained virologic suppression.

MATERIALS AND METHODS

Subjects. Thirty-one children (median age: 5.6 years, range: 3.2-16.8 years) were a subset of the Pediatric AIDS Clinical Trial Group (PACTG) study 382 consisting of efavirenz, nelfinavir and 1-2 NRTI. They were selected because they achieved persistent and undetectable plasma HIV-1 RNA (HIV-1 RNA) while receiving HAART for >2 years after the initiation of HAART (44). All 31 patients reached undetectable HIV-1 RNA <50 copies/mL by week 48 and continued to have undetectable HIV-1 RNA up to week 104. Before enrolling in the study, children were PI and NNRTI naïve, but they could be NRTI experienced: one NRTI (6%, n = 2) and two NRTIs (91%, n = 28). One child was antiretroviral naïve (3%, n = 1). NRTI regimens in children during the study were either continued (32%, n =10) or changed (68%, n = 21) based on the clinical judgment of the primary care physicians (Table 1). Informed consent was obtained from study participants and their legal guardians. This study followed the human experimentation guidelines of the US Department of Health and Human Services and the UCSD review board.

Quantitation of mitochondrial DNA and nuclear DNA by real-time PCR. The mtDNA and the nuclear DNA (nDNA) were quantified by real-time PCR using a LightCycler (Roche Applied Science, Indianapolis, IN) as described previously (17). The results are expressed as a ratio of the mean mtDNA level to the mean nDNA level for a given extract in duplicate (mtDNA: nDNA, unit: copies/cell). The ratios were quantified at baseline, weeks 2, 4, 8, 48 and 104 after the initiation of HAART.

Measurement of HIV-1 intracellular DNA, plasma HIV-1 RNA and CD4$^+$ T-lymphocyte counts and percentages. HIV-1 intracellular DNA (HIV-1 DNA) and HIV-1 RNA levels were quantified using the Amplicor monitor HIV-1 DNA assay and the standard and ultrasensitive Amplicor HIV-1 Monitor assay (Version 1.0) (12, 44, 46), respectively (Roche Molecular Systems, Alameda, CA). The absolute numbers and percentages of CD4$^+$ T-lymphocytes were determined in PACTG certified laboratories by flow cytometry.

Physical parameters, lipid profiles and adverse effects. The following data were extracted from the database: 1) body weight and height at baseline, weeks 48 and 104. Body mass index (BMI) was calculated as defined by BMI = weight (kg) / [height (m)]^2 and *z*-scores were used to express the deviation in standard deviation unit, calculated to adjust for age, weight, height and race/ethnicity, 2) lipid profiles including fasting total cholesterol, triglyceride, low density lipoprotein (LDL) and high density lipoprotein (HDL) measured at weeks 112-160 of HAART, and 3) significant adverse effects related to NRTI use (≥ Grade 3).

Statistical analysis. The Wilcoxon matched paired signed rank test was used for the comparison within the subject over time. The Wilcoxon sum rank test was used for the comparison of numerical variables in two independent groups and the Kruskal Wallis test was used for the comparison of numerical variables in more than three independent groups. Correlations between mtDNA levels and various laboratory findings were

calculated using the Spearman's correlation test. Fisher's exact test was used to compare two groups with respect to categorical variables. The multivariate regression analyses on the change in mtDNA levels from baseline to week 104 were performed to evaluate the contribution of covariates including age, and gender (male vs. female), race/ethnicity (African-American vs. others), mtDNA levels at baseline, and the use of ddI during HAART (ddI vs. non-ddI). Of note, the values of mtDNA change were log transformed before calculating the differences because the distribution of untransformed values was skew to lower values. The same multivariate analyses on the change in mtDNA levels from baseline to week 104 were also performed for the use of each NRTI including d4T (d4T vs. non-d4T), ZDV (ZDV vs. non-ZDV), or 3TC (3TC vs. non-3TC) and other covariates.

RESULTS

The impact of NRTI regimens on mitochondrial DNA levels before initiating HAART. Children were divided into two groups based on their use of ddI before enrolling the study: regimens containing no ddI (n = 20) and regimens containing ddI (n = 10) (Table 2). The median mtDNA level was significantly decreased in children who received regimens containing ddI (106 copies/cell, range: 76 - 176 copies/cell) compared to those who did not receive ddI (140 copies/cell, range: 110 - 431 copies/cell, P = 0.008). Notably, patients who received ddI + d4T combination (n = 2) had the lowest mtDNA levels (76 and 92 copies/cell). Of note, the background characteristics among three groups did not differ significantly by age (P = 0.98), sex (P = 0.76), race/ethnicity (P = 0.85), the duration of therapy (P = 0.40), plasma HIV-1 RNA (P = 0.17) or $CD4^+$ T-lymphocyte count (P = 0.20). In addition, the distribution of NRTI switch (ddI containing regimens vs. non-ddI containing regimens) before and after the study was not significantly different (P = 0.68, Table 1). No difference in mtDNA levels at baseline were observed between children who received ZDV and those who did not receive ZDV (P = 0.51), or between children who received d4T and those who did not receive d4T (P = 0.16) before HAART; however, children who received lamivudine (3TC) (n = 21) had higher mtDNA levels compared to those without 3TC (n = 10) (P = 0.003). Of note, among 21 patients who received 3TC, only one patient (4.8%) received ddI, reflecting the difference of the mtDNA levels in children with or without ddI, and not a specific beneficial effect of 3TC.

Mitochondrial DNA levels increase during HAART in children with sustained virologic suppression. Overall, the median mtDNA levels increased from 137 copies/cell [interquartile range (IQR): 115 - 171 copies/cell] at baseline to 179 copies/cell (IQR: 145 - 209 copies/cell) at week 48 (P = 0.01) and 198 copies/cell (IQR: 166 - 290 copies/cell) at week 104 (P < 0.001).

Changes in mitochondrial DNA levels in children receiving didanosine combining HAART regimens are least increased compared to those receiving other NRTI. We next examined the data based on the use of ddI during HAART: 1) children who received ddI (n = 7); and 2) children who did not receive ddI (n = 24). The background characteristics between the two groups were similar for age (P = 0.60), gender (P = 0.56), race/ethnicity (P = 0.20), HIV-1 RNA (P = 0.27), $CD4^+$ T-cell count (P = 0.23), and mtDNA levels at baseline (P = 0.70). The median changes from baseline to week 48 in mtDNA levels in children receiving ddI (+16 copies/cell) was lower than those without ddI (+57 copies/cell) (P = 0.20). Furthermore, the median changes in mtDNA levels from baseline to week 104 in children receiving ddI were significantly lower (+26 copies/cell) than those in children without ddI (+79 copies/cell) (P = 0.02) (Figure 1).

When children were divided based on the use of ZDV, 3TC and d4T during HAART, we found no significant difference in mtDNA changes from baseline to week 48 (P = 0.14 - 0.65). However, at week 104, children receiving ZDV (+101 copies/cell) had the highest mtDNA increases compared to those without ZDV (+52 copies/cell) (P = 0.03, Figure 1). No difference was observed in children receiving 3TC and those without 3TC (+85 copies/cell vs. +51 copies/cell, P = 0.30, Figure 1). In contrast, children

receiving d4T (+51 copies/cell) experienced marginally lower mtDNA increases compared to those who did not receive d4T (+98 copies/cell, P = 0.06, Figure 1).

We also analyzed the data based on the combination of NRTI that were part of a child's HAART regimen. Subjects were divided into four groups: 1) d4T, 2) ZDV + 3TC, 3) d4T + 3TC, and 4) ddI containing regimens. Although no significant differences in mtDNA change were observed from baseline to week 48 (P = 0.40), at week 104, changes in mtDNA levels significantly differed between children who received ZDV + 3TC (+127 copies/cell, n = 7) compared to those who received ddI containing regimens (+26 copies/cell, P = 0.003, n = 7) or d4T + 3TC (+43 copies/cell, P = 0.008, n = 6) (Figure 2).

Didanosine in multivariate analyses remains an important predictor of change in mtDNA from baseline to week 104. Multivariate regression analyses were performed to assess the impact of ddI on the change in mtDNA from baseline to week 104. The use of ddI during HAART (P = 0.007) and baseline mtDNA levels (P < 0.001) were independently associated with a change in mtDNA from baseline to week 104 when controlling other factors including age, sex, and race/ethnicity (Table 3). The use of ZDV, 3TC, or d4T was not associated with change in mtDNA from baseline to week 104 (P = 0.32, P = 0.40, P = 0.27, respectively). Thus, the use of ddI during HAART was an important determinant of change in mtDNA after controlling for other factors.

Virologic and immunologic responses between children who received ddI as a component of HAART and those who did not receive ddI. The proportions of children

who reached HIV-1 RNA <50 copies/mL within 8 weeks of HAART were similar for those who received ddI (57%) and those who did not receive ddI (50%) (P = 0.78). Median $CD4^+$ T-cell counts in children receiving ddI tended to be lower than those in children who did not receive ddI during HAART: at week 48 (613 vs. 1,054/μL, P = 0.04) and week 104 (751 vs. 1,075/μL, P = 0.12).

Mitochondrial DNA levels and HIV-1 intracellular DNA levels during early stage of HAART. Overall, there was a modest negative correlation between the mtDNA levels and HIV-1 DNA levels (r = -0.29, P < 0.001, n = 173). When the correlation was evaluated at each week, no correlation was seen at baseline (r = -0.10, P = 0.60); however, there were significant negative correlations between the mtDNA levels and HIV-1 DNA levels at week 2 (r = -0.44, P = 0.02) and week 4 (r = -0.43, P = 0.02). No correlations were seen during the rest of study visits; at week 8 (r = 0.19, P = 0.32), week 48 (r = -0.19, P = 0.35) and week 104 (r = -0.25, P = 0.19).

Mitochondrial DNA levels and plasma HIV-1 RNA and $CD4^+$ T cell counts. Although HIV-1 infection itself appears to lower mtDNA content of PBMC in antiretroviral naïve patients (6, 11, 36), in our study, no correlations were observed between the mtDNA and HIV-1 RNA levels at baseline, weeks 2 and 4 (r = -0.29 - -0.34, P = 0.06 - 0.91) when the majority of subjects had detectable plasma HIV-1 RNA. In addition, no correlations were observed between the mtDNA levels and $CD4^+$ T-cell counts at any study visit (P = 0.06 - 0.64).

Mitochondrial DNA levels and physical parameters, lipid profiles, adverse effects and age. No significant correlations were seen between the mtDNA levels and 1) the changes of BMI (P = 0.84) or *z*-score (P = 0.24) at week 48 and the changes of BMI (P = 0.24) or *z*-score (P = 0.68) at week 104; and 2) fasting lipid profiles including cholesterol (P = 0.15), triglyceride (P = 0.68), HDL (P = 0.87) and LDL (P = 0.06) at week 104. Six events in five children were reported as ≥Grade 3 adverse effects including neutropenia (n = 3), hepatitis (n = 2) and thrombocytopenia (n = 1) during HAART. The mtDNA levels in children who experienced adverse effects were not different from the ones who did not experience the adverse effects at each week (P = 0.33 - 0.74). Furthermore, no correlations were observed between age and the mtDNA levels at baseline (P = 0.15), the changes of the mtDNA levels from baseline to week 48 (P = 0.67) and those from baseline to week 104 (P = 0.87).

DISCUSSION

We have shown that the ddI containing HAART regimens were associated with the greatest degree of mtDNA suppression in PBMC when compared to regimens not containing ddI in HIV-1 infected children who sustained undetectable HIV-1 RNA during HAART.

To our knowledge, the current study is the first longitudinal evaluation of mtDNA levels in PBMC of HIV-1 infected children receiving HAART. We demonstrated a gradual increase in mtDNA levels in PBMC of children who sustained undetectable HIV-1 RNA during HAART, which is also supported by the data in adults (8). Our current study was able to control for several important factors compared to other longitudinal studies (17, 24, 34, 38, 41, 48), and cross-sectional studies (9, 11, 15, 18, 32), in adults that could potentially affect the mtDNA levels in PBMC. First, we selected children with sustained undetectable HIV-1 RNA during HAART decreasing that likelihood that patients with detectable HIV-1 RNA can significantly reduce the mtDNA content of PBMC (6, 11, 36). Second, although the majority of previous studies have shown the lack of association between the mtDNA levels in PBMC and lipodystrophy (9, 11, 14, 24, 32, 38, 41, 48), children in our study did not have obvious symptoms or signs of lipodystrophy. Third, of previously published studies, our study has the longest observation period of patients receiving HAART up to 104 weeks. Finally, although the effects of PI and NNRTI combining with NRTI on mtDNA levels in PBMC are unknown, our cohort of children received the same combination of a PI (nelfinavir) and a NNRTI

(efavirenz) with only the NRTI being different. Thus, having controlled for PI and NNRTI background, we were able to differentiate the effects of specific NRTI on mtDNA levels.

Clinically, d4T and ddI are known to be associated with higher incidence of adverse effects in HIV-1 infected patients receiving antiretroviral therapy. Cross-sectional studies in adults have suggested that subjects receiving d4T in combination with ddI experience low mtDNA levels in PBMC compared to those receiving other regimens (11, 18). This clinical observation is supported by *in vitro* studies illustrating the inhibition of DNA polymerase-γ associated with mitochondrial toxicity is differentially affected by NRTI: d4T = ddI >> 3TC > tenofovir > ZDV (25, 28). In our current study in children, ddI was the most important determinant of mitochondrial toxicity as defined by the lowest mtDNA levels before and during HAART. These findings were also confirmed by multivariate analyses controlling other confounding factors including d4T use.

In contrast, mtDNA levels in children receiving ZDV containing regimen (ZDV + 3TC) were significantly increased compared to those in children receiving other NRTI regimens. Similarly, although the duration of therapy is only six weeks for infants receiving ZDV to prevent mother-to-child transmission of HIV-1, mtDNA has been observed to increase in PBMC in infants who are exposed to ZDV perinatally (1, 16). The mechanisms of increase in mtDNA are still unknown, but in our *in vitro* data, mtDNA levels in human skeletal muscle myoblasts treated with ZDV show the highest increase compared to untreated cells or the cells treated with different NRTI when the

cells were incubated for two days (43). These findings are also consistent with an increase in mtDNA levels observed with ZDV in other *in vitro* models including human hepatoblastoma (HepG2) (20) and human skeletal muscle cells (2). In total, these results strongly suggest that ZDV upregulates genes encoding mtDNA. Because ZDV has been reported to cause significant mitochondrial toxicity in different *in vitro* models (23), this may reflect a compensatory response to mitochondrial dysfunction caused by a different pathway. Importantly, an increase in mtDNA does not necessarily mean an increase in mitochondrial function; several studies have shown inhibition of respiratory function by NRTI independently through mtDNA polymerase γ (29), oxidative stress (26), and increases in mtDNA and mitochondrial mass by oxidative stress (27, 51). In addition, NRTI including 3TC, abacavir (ABV) and emtricitabine (FTC) and the nucleotide reverse transcriptase inhibitor, tenofovir, which are known to be less toxic to mitochondria, have been shown to increase in mtDNA levels in HepG2 cells (20), human skeletal muscle cells (2), and lymphoblasts (40). Further studies are needed to be delineated the mechanisms associated with changes in mtDNA levels by NRTI, mitochondrial function, and the clinical significance of an increase in PBMC mtDNA.

In a recent study of adults, d4T and ddI use was associated with fat mtDNA depletion, whereas ddI exposure was the only observed association with mtDNA depletion in PBMC (10). In an *in vitro* model, the thymidine analogues including d4T and ZDV, but not ddI, decreased mitochondrial activity and cell survival in adipose tissue (4). In contrast, ddI decreased mtDNA content significantly in neuronal cells (19), human hepatoma cell lines (37, 50), and human skeletal muscle cells (2) whereas no significant mtDNA change was observed with d4T, ZDV, or 3TC. In our preliminary

studies using human skeletal muscle myoblasts, ddI significantly decreased mtDNA as well as mtRNA levels (43). Summarizing currently available data, the effects of NRTI on mitochondria seem to differ in different tissues. Therefore, the evaluation of mtDNA in future studies should include the possible anatomical sites where adverse effects are potentially greatest.

Our findings also showed a negative correlation between mtDNA and HIV-1 DNA levels in PBMC during the early stages of HAART. Although our data clearly showed that ddI containing regimens can alter mtDNA levels, the negative effect of ddI may be masked by the beneficial effect of decreasing intracellular virus during the early stage of HAART. Our finding that the mtDNA levels no longer correlated with the HIV-1 DNA levels during prolonged HAART, suggests that at steady state the use of specific NRTI has a greater impact on the mtDNA levels than relatively small differences in HIV-1 DNA levels. Interestingly, the correlations between the mtDNA and the HIV-1 DNA levels were not observed at baseline, which likely reflects the failure of mono or dual NRTI therapy to suppress viral replication in study participants.

Although improved growth is usually associated with effective HAART in HIV-1 infected children (35, 49), the changes in the mtDNA levels in PBMC did not correlate with changes in BMI or *z*-score, suggesting that the degree of mitochondrial toxicity did not effect growth over the 104 weeks of effective HAART. However, no measurement of peripheral or visceral fat was performed to determine the presence or absence of

lipodystrophy in this study; therefore, the ability of mtDNA in PBMC to predict lipodystrophy was not possible.

We appreciate that there are several potential limitations of the current study. First, the number of children evaluated is relatively small, a larger cohort may help to define the impact of low mtDNA levels on HIV-infected children. Second, we quantified the mtDNA levels in PBMC containing different cells at different stages of activation which has been reported to change the levels of mtDNA (7). Third, it has been suggested that platelet contamination in PBMC could be a potential confounding factor for mtDNA levels (13), which was not investigated in the current study. Finally, although we showed that ddI significantly affects mtDNA levels in PBMC in children, it is still unknown how well the mtDNA levels in PBMC reflect the mtDNA levels at other anatomical sites *in vivo*. In addition, information regarding the mtDNA levels in PBMC in normal children is limited (16, 42). Thus, future research should focus on the impact of mtDNA levels in PBMC on physical growth, peripheral or visceral fat, muscle mass, toxicity and neurodevelopmental progress in children.

In conclusion, we have shown that didanosine has a significant impact on mtDNA levels found in PBMC of children with sustained virologic suppression during HAART. Our findings that ddI is associated with the greatest suppression of mtDNA are of particular importance in resource-limited countries where didanosine is a common component to antiretroviral therapy (39, 52). A prospective study to elucidate the long-term clinical significance of mtDNA of children and the NRTI regimens causing the least mitochondrial toxicity is urgently needed.

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FIGURE LEGENDS

Figure 1 . Changes in mitochondrial DNA levels from baseline to week 104 between children who received specified nucleoside reverse transcriptase inhibitors (NRTI) and those who did not receive specified NRTI during highly active antiretroviral therapy (HAART). The squares represent median values of the mitochondrial DNA (mtDNA) changes from baseline to week 104. The bars indicate interquartile ranges of mtDNA changes from baseline to week 104. ddI: didanosine, ZDV: zidovudine, 3TC: lamivudine, d4T: stavudine. The Wilcoxon sum rank test was used for the comparison of mitochondrial DNA levels in two groups.

Figure 2. Changes in mitochondrial DNA levels from baseline to weeks 8, 48 and 104 based on their nucleoside reverse transcriptase inhibitor (NRTI) regimens during highly active antiretroviral therapy (HAART). The squares represent median values of the mitochondrial DNA (mtDNA) changes from baseline to week 104 in children who received 1) stavudine (d4T), 2) zidovudine (ZDV) + lamivudine (3TC), 3) d4T + 3TC, and 4) didanosine (ddI) containing regimens. The bars indicate interquartile ranges of mtDNA changes from baseline to week 104. The Wilcoxon sum rank test was used for the comparison of mitochondrial DNA levels in two groups.

Table 1. Nucleoside reverse transcriptase inhibitors (NRTI) regimens before and during the study

	NRTI regimens during the study						
NRTI regimens before the study	Non-ddI containing regimens			ddI containing regimens			Total
	d4T	ZDV + 3TC	d4T + 3TC	ddI + d4T	ZDV + ddI	ddI + 3TC	
Non-ddI containing regimens							
ZDV + 3TC	7 (23%)	2 (7%)*		4 (13%)			13 (42%)
d4T + 3TC	2 (7%)		5 (16%)*				7 (23%)
ddI containing regimens							
ZDV+ ddI	1 (3%)	2 (7%)			2 (7%)*		5 (16%)
ddI + d4T		2 (7%)					2 (7%)
ddI	1 (3%)		1 (3%)				2 (7%)
ddI + 3TC						1 (3%)*	1 (3%)
Naïve		1 (3%)					1 (3%)
Total	11 (35%)	7 (23%)	6 (19%)	4 (13%)	2 (7%)	1 (3%)	31 (100%)

d4T: stavudine, ZDV: zidovudine, 3TC: lamivudine, ddI: didanosine
* Children who did not change NRTIs regimens before and during HAART (n = 10, 32%).

Table 2. Mitochondrial DNA levels in subjects based on their nucleoside reverse transcriptase inhibitors (NRTI) regimens before initiation of HAART

NRTI Regimens	No. of Subjects (%)	Median mtDNA levels at baseline (copies/cell) (range)	P-value*	Median duration of NRTI therapy (weeks) (range)	P-value**
Non-ddI containing regimens	20 (65%)	140 (110 - 431)	0.008	57 (8 - 285)	0.40
ZDV + 3TC	13 (42%)	170 (116 - 238)		79 (23 - 285)	
d4T + 3TC	7 (23%)	127 (110 - 431)		53 (8 - 101)	
ddI containing regimens	10 (32%)	106 (76 - 176)		85 (7 - 274)	
ZDV + ddI	5 (16%)	104 (76 - 124)		103 (7 - 274)	
ddI + d4T	2 (7%)	85 (78 - 92)		63 (52 - 73)	
ddI alone	2 (7%)	157 (137 - 176)		79 (24 - 138)	
ddI + 3TC	1 (3%)	149		49	
Naïve	1 (3%)	137		0	

ZDV: zidovudine, 3TC: lamivudine, d4T: stavudine, ddI: didanosine, mtDNA: mitochondrial DNA

* Comparison in the mitochondrial DNA levels between children with non-ddI containing regimens and those with ddI containing regimens before initiation of HAART.

** Comparison in the duration of therapy between children with non-ddI containing regimens and those with ddI containing regimens before initiation of HAART.

Table 3. Multivariate analyses of mitochondrial DNA changes from baseline to week 104

Covariates	Regression Coefficient ± S.E.	P-value
Constant	2.3000 ± 0.5491	0.0003
Age (years)	0.0071 ± 0.0117	0.5509
Sex (male)	0.0403 ± 0.0811	0.6235
Race (African-American)	0.1406 ± 0.0793	0.0884
Baseline mtDNA (log copies/cell)	-1.0121 ± 0.2534	0.0005
Didanosine use	-0.2782 ± 0.0946	0.0070

S.E.: Standard Error, mtDNA: mitochondrial DNA

Figure 1

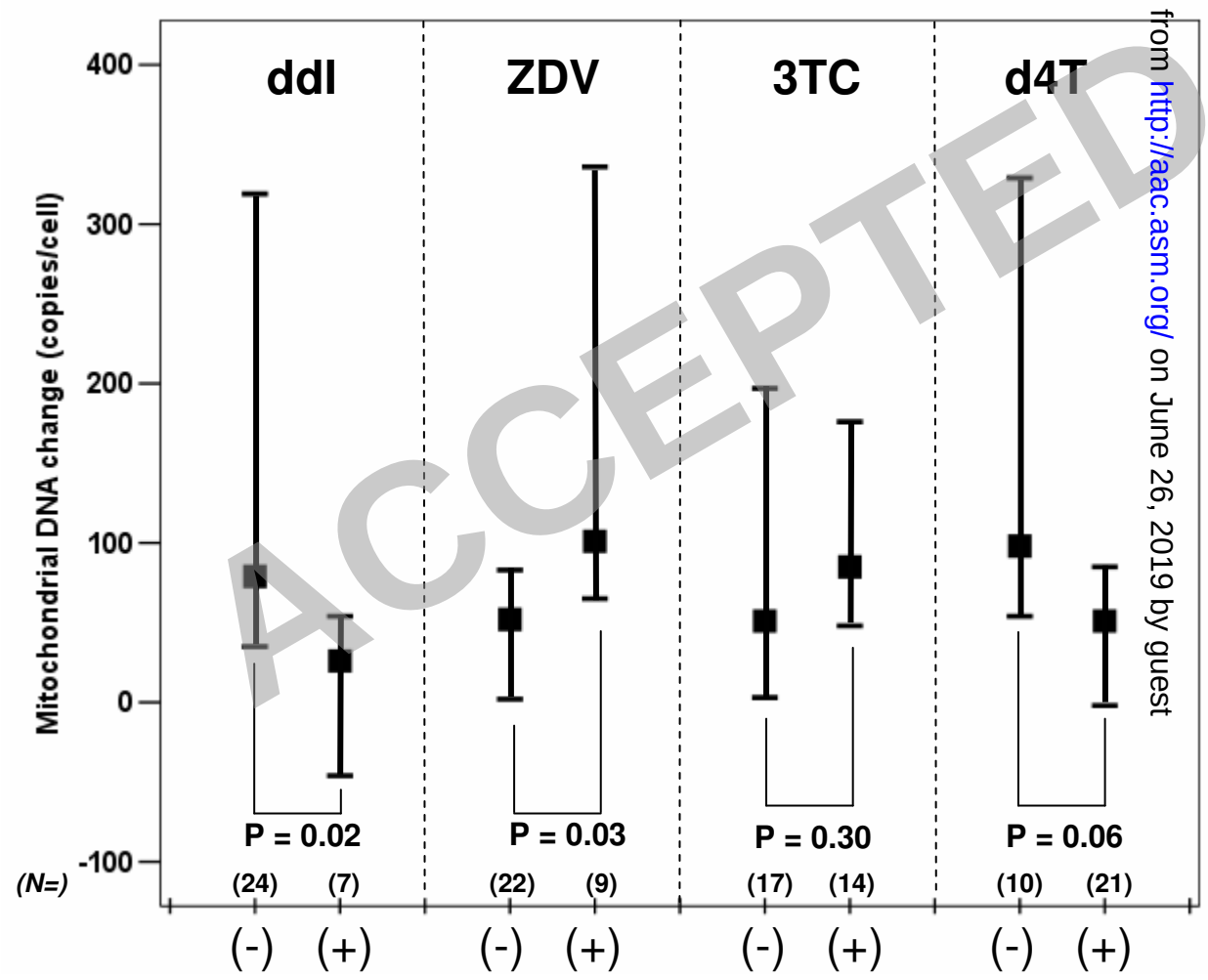

Figure 2