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Mitochondrial Genomics and CD4 T-cell Count Recovery after Antiretroviral Therapy Initiation in AIDS Clinical Trials Group Study 384

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Abstract

BACKGROUND—Mitochondrial DNA (mtDNA) variation has been associated with time to progression to AIDS and adverse effects from antiretroviral therapy (ART). In this study, full mitochondrial DNA (mtDNA) sequence data from U.S.-based adult participants in the AIDS Clinical Trials Group (ACTG) study 384 was used to assess associations between mtDNA variants and CD4 T cell recovery with ART.

METHODS—Full mtDNA sequence was determined using chip-based array sequencing. Sequence and CD4 cell count data was available at baseline and after ART initiation for 423 subjects with HIV RNA levels <400copies/mL plasma. The primary outcome was change in CD4 count of ≥ 100 cells/mm³ from baseline. Analyses were adjusted for baseline age, CD4 cell count, HIV RNA, and naïve:memory CD4 cell ratio.

RESULTS—Race-stratified analysis of mtDNA variants with a minor allele frequency >1% revealed multiple mtDNA variants marginally associated ($P < 0.05$ before Bonferroni correction) with CD4 cell recovery. The most significant SNP associations were those tagging the African L2 haplogroup, which was associated with a decreased likelihood of ≥ 100 cells/mm³ CD4 count increase at week 48 in non-Hispanic blacks (adjusted OR=0.17; 95% CI=0.06–0.53; $P=0.002$).

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Potential conflicts of interest:

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CONCLUSIONS—An African mtDNA haplogroup was associated with CD4 cell recovery after ART in this clinical trial population. These initial findings warrant replication and further investigation in order to confirm the role of mtDNA variation in CD4 cell recovery during ART.

Keywords

HIV; CD4 count; Mitochondrial DNA; Pharmacogenetics; Antiretroviral Therapy

Introduction

The CD4⁺ T-lymphocyte count (CD4 count) is a primary determinant of disease progression and opportunistic infection risk among HIV-infected persons. The CD4 count is also a major factor in the decision to initiate antiretroviral therapy (ART) in asymptomatic HIV-infected individuals.¹ There is substantial interindividual variability in the rate and magnitude of CD4 recovery after initiating antiretroviral therapy (ART).² Studies have demonstrated many ART-treated patients failing to attain substantial increases in CD4 count,² and poorer CD4 count responses on ART are associated with disease progression even with adequate virologic responses.^{3,2}

Human genetic variation appears to play a role in CD4 count recovery. An ACTG immunogenetics study suggested possible associations between CD4 count recovery ≥ 200 cells/mm³ after 48 weeks of ART and single nucleotide polymorphisms (SNPs) in several nuclear apoptosis-related genes and cytokine genes.⁴ These results also highlight the possibility that a potentially important host-related factor that influences HIV-infected CD4 cell turnover is regulation of apoptosis.^{5,6}

Within mitochondrial DNA (mtDNA), combinations of SNPs allow for categorization of individuals into haplogroups.⁷ The clinical relevance of haplogroups in particular- and mtDNA variation in general- for disease risk is well described.^{8–14} It is plausible that functional variation in mtDNA would influence T cell dynamics such as apoptosis in response to environmental stressors (e.g. HIV infection and/or ART). We hypothesized that among individuals with control of HIV replication following initiation of ART, variation in mtDNA would influence CD4 count recovery through apoptotic regulatory and/or other mechanisms that modulate the efficiency of CD4 cell proliferation. To more fully characterize relationships between mitochondrial genomics and CD4 count recovery on suppressive ART, we examined full mtDNA sequence from ART-naïve, HIV-infected participants from ACTG 384.

Methods

Study Population

ACTG 384 was a multicenter, double-blind, prospective randomized clinical trial comparing the efficacy of ART regimens consisting of three or four drugs in 980 antiretroviral-naïve adults.¹⁵ Participants were randomized to one of six treatment arms consisting of either didanosine and stavudine or zidovudine and lamivudine combined with either efavirenz, nelfinavir or both. Median follow-up time was 2.3 years. A secondary end point of ACTG 384 was change in CD4 cell count from baseline. CD4 count was measured for all individuals at 48, 96 and 144 weeks and some individuals also underwent specific flow cytometry for proportions of memory and naïve CD4 cells as part of an immunologic substudy.¹⁶ For the purposes of the current study, CD4 count at baseline and weeks 48 and 96 from 530 (54%) participants from ACTG 384 who consented to provide DNA to the ACTG Human DNA Repository (HDR)¹⁷ were considered. Previous publications^{18,19} have reported results from genetic association analysis in the ACTG 384 cohort, and

demonstrated no significant differences between ACTG participants who provided DNA as part of the HDR and those who did not.²⁰

Mitochondrial DNA Isolation and Sequencing

Isolation of DNA from study participants was by PUREGENE (Gentra Systems Inc., Minneapolis, MN, USA). Full mtDNA sequencing was performed using the GeneChip[®] Human Mitochondrial Resequencing Array v2.0 (Affymetrix, Inc., Santa Clara, CA, USA). From full-sequence data, mtDNA variants were identified using the revised Cambridge Reference Sequence (rCRS).²¹ Haplogroups were assigned using Hernstadt classification,²² and collapsed into higher branch haplogroups for analyses.

Exclusion Criteria

Individuals with HIV-1 RNA ≥ 400 copies/mL at either week 48 (n = 77) or 96 (n = 130) were excluded from analyses for the respective time points. Three (<1%) univariate outliers in CD4 cell count change at week 48 were identified with STATA using the minimum volume ellipsoid method described by Hadi²³ and removed from analyses of week 48 endpoints. Seven (1.3%) participants lacked 96 week CD4 count data and were removed only from week 96 analyses. For the purposes of genetic data quality control (QC) and subsequent analysis, study participants were stratified by race/ethnicity into self-identified non-Hispanic white, non-Hispanic black, and Hispanic groups to prevent potential confounding. Self-described Asian (n = 11) and Native American participants (n = 2) were excluded due to small sample size. We excluded mitochondrial DNA SNPs with minor allele frequency <1% in the stratified racial subpopulation or genotyping completion rate <95%. We excluded samples with genotyping completion rate <90% (n = 4). Genetic quality control was performed with the Whole-genome Association Study Pipeline (WASP) tool (<https://chgr.mc.vanderbilt.edu/plato>) inside of the Platform for the Analysis, Translation and Organization of large-scale data (PLATO)²⁴.

Statistical Analysis

For analyses of binary endpoints in this study, a nested case-cohort design was utilized by adjusting for age.²⁵ The primary outcome was the proportion of subjects with an increase of ≥ 100 CD4 cells/mm³ at week 48. Secondary outcomes included increase of ≥ 100 CD4 cells/mm³ at week 96, increase of ≥ 50 CD4 cells/mm³ at week 48 and 96, increase of ≥ 200 CD4 cells/mm³ at week 48 and 96, and comparison of those participants with an increase of ≥ 200 CD4 cells/mm³ against participants with an increase of <50 CD4 cells/mm³ at week 48 and 96. CD4 count change was also analyzed as a continuous endpoint.

Analyses of the effect of SNPs on CD4 cell count changes utilized the PLINK tool²⁶ (<http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml>) to perform logistic and linear regression. Race-stratified regression analyses were performed, adjusting for clinical covariates previously found to be significantly associated with CD4 cell recovery in ACTG 384,¹⁶ including age, baseline viral load, baseline CD4 cell count, and baseline ratio of naïve-to-memory CD4 cell count. In the primary analysis of ACTG 384, cART regimen was not found to be a significant predictor of CD4 cell recovery following ART initiation and thus was not adjusted for during analysis.¹⁵ Haplogroup analysis was performed in STATA (StataCorp. 2007. *Stata Statistical Software: Release 10*. College Station, TX: StataCorp LP). Statistical association between haplogroup status and CD4 count change from baseline was analyzed in a race-stratified design using a single indicator variable for each haplogroup. With this design, likelihood of CD4 count increase from baseline ≥ 100 cells/mm³ at 48 weeks of follow-up within each haplogroup was compared with the likelihood of ≥ 100 cells/mm³ increase within all other haplogroups. Haplogroup analyses adjusted for the same covariates used in tests of association with individual mtDNA variants.

A Bonferroni multiple-test correction was used to determine the threshold necessary for tests of association to be considered significant. For mtDNA variant analysis, an average of 200 tests were assumed based on the presence of 213, 151 and 216 polymorphisms in the non-Hispanic black, non-Hispanic white and Hispanic self-identified race/ethnicity groups, respectively. For haplogroup analysis, a Bonferroni-corrected threshold was calculated using the 23 haplogroups tested for association with the primary outcome (P threshold $< 2.2 \times 10^{-3}$).

Multiple imputation with the *mice* package²⁷ available in R was used to impute missing values of the naïve-memory ratio conditional on CD4 cell count change, baseline viral load, age and genotype. Imputed values of naïve-memory ratio were not used during analysis due to subsequent discovery of non-random patterns of missingness within the data which violate assumptions of *mice*.

Results

Baseline Demographics

Baseline demographics of study subjects are shown in Table 1. The median age of individuals remaining after QC and exclusion criteria ($n=423$) was 36, with 17% being female, 50% self-identifying as non-Hispanic white, 126 (30%) as non-Hispanic black, and 20% as Hispanic. Median baseline CD4 count was 278 cells/mm³ (interquartile range [IQR] 85-434); log₁₀ HIV-1 copies/mL was 5.0 (IQR 4.3–5.5). Baseline naïve-to-memory cell ratio was available for 66% of 423 participants, with a median of 0.59 (IQR 0.28–1.00) among these participants. No demographic data differed significantly before and after QC exclusions.

Analysis of Change in CD4 Count at Week 48

Median CD4 count increase from baseline was 176 cells/mm³ (IQR 94-273) at 48 weeks and 253 cells/mm³ (IQR 138-387) at 96 weeks. The primary study endpoint focused on differences in mtDNA variation between individuals experiencing an increase of ≥ 100 cells/mm³ (cases) and those with < 100 cells/mm³ change (controls) at 48 weeks of follow up, analyzed separately within strata defined by race/ethnicity. Analysis within the non-Hispanic black (42 controls/82 cases), non-Hispanic white (47 controls/164 cases), and Hispanic (26 controls/59 cases) race/ethnicity stratified groups included tests of association with 213, 151, and 216 SNPs respectively due to the differences in genetic variability between the three race/ethnicity groups. All tests used the major allele as the reference. No associations were significant at the P -value threshold determined by Bonferroni correction for multiple comparisons ($P < 2.5 \times 10^{-4}$) in any of the race/ethnicity groups but analysis revealed multiple marginal associations ($P < 0.05$) in analyses of the non-Hispanic white and non-Hispanic black race/ethnicity groups (Table 3). Figures 1a and 1b display negative log-transformed P -values for tests of association with the primary endpoint in individuals self-identifying as non-Hispanic white and non-Hispanic black, respectively. The association test P -values are plotted with respect to base-pair location of the mtDNA variant along the mitochondrial genome. The most significant associations with CD4 count change at 48 weeks were observed within the analysis of non-Hispanic black participants. Polymorphisms at mitochondrial base pair positions 2789, 7175, 7274, 7771, 9221, 10115, 11914, 13590, 13803, 14566 and 16390 (OR range=0.17–0.24, P -value range=0.002–0.027) define haplogroup L2 and were associated with decreased odds of a CD4 count change of ≥ 100 cells/mm³ in the non-Hispanic black group. Variation at positions 13105 (OR=4.60; 95% CI=1.13–18.70; $P=0.033$) and 15301 (3.64; 1.02–13.06; $P=0.047$) were associated with increased odds of a CD4 count change ≥ 100 cells/mm³ in non-Hispanic blacks. These two polymorphisms define the L2'3'4'6 branch above the L2 haplogroup and associations are to

the allele which is prior to this branch.²⁸ As such, the allele which is present within L2 individuals is associated with reduced odds of a CD4 count change ≥ 100 . Polymorphisms at positions 750 (OR=0.04; 95% CI=0.002–0.60; P=0.02), 7768 (0.10; 0.01–0.80; P=0.03), 8860 (0.02; 0.001–0.68; P=0.03), 14182 (0.13; 0.02–0.92; P=0.04), 15326 (0.02; 0.001–0.68; P=0.03) and 16519 (0.24; 0.08–0.71; P=0.01) were associated with reduced odds of CD4 cell recovery in non-Hispanic white individuals. Polymorphism at positions 750, 8860 and 15326 is representative of the H2a2 haplogroup and is typically rare but is likely represented due to the large number of individuals of the H2 haplogroup (N=26) present in the study.

The results of a SNP analysis using the entire distribution of CD4 count change as a continuous outcome variable are shown in Supplemental Digital Content Table S1. The majority of mtDNA variants which were found to be significant in analysis of the primary 48 week endpoint remained significant in this analysis with exception of the variant at position 15301 among Non-Hispanic blacks and variants at positions 750, 8860 and 15326 among non-Hispanic whites, all of which were not significant in analyses of continuous CD4 changes at week 48. There were additional polymorphisms which were found to be marginally significant from the analysis of the continuous CD4 count change at week 48 among non-Hispanic black participants, including positions 2768, 3594 and 14178. In addition, many variants were found to be marginally significant among Hispanic participants, although it appears that most of these SNPs are highly correlated.

CD4 Count Change From Baseline Through 96 weeks

Of the 423 individuals who had data for one or more time points after 48 week exclusion criteria, 370 (87%) were included in secondary 96 week analyses. The decreased sample size between 48 and 96 week time points is due largely to exclusion of those participants with an increase in viral load to above 400 copies/mL on study. Results from analysis of 96 week endpoints were similar to results from analysis of the week 48 primary endpoint and results from the analysis of quantitative CD4 count change (data not shown).

Haplogroup Analysis

A total of 23 haplogroups were tested for association with a CD4 count increase ≥ 100 cells/mm³ at 48 weeks of follow-up (A complete listing of haplogroups is shown in Table 2). The Non-Hispanic black participants in this study fall into three major haplogroups, L1 (25%), L2, (32%), and L3 (29%). Analysis of the L2 haplogroup revealed an association with decreased odds (OR=0.17; 95% CI=0.06–0.53; P=0.002) of a CD4 count change of ≥ 100 cells/mm³. This association remains significant after correcting for the number of haplogroups tested in the association analysis ($P < 2.2 \times 10^{-3}$). Among non-Hispanic blacks, those belonging to haplogroup L2 has significantly lower median CD4 T cell increases at study week 48 (96 [IQR 30–252] cells/mm³ vs. 181 [105–285]; P=0.01) and week 96 (177 [120–395] vs. 317 [IQR 173–433]; P=0.05). Haplogroup L2 also remained associated with continuous CD4 count increase at week 48 (adjusted β coefficient= -119.5; 95% CI -187.3, -51.3; P=0.001) after adjustment for covariates noted previously. Haplogroups H1 (13%), H2 (22%), J (13%), K (7%) and U (14%) accounted for the majority of non-Hispanic white participants in this study. Three non-Hispanic white participants were excluded from haplogroup analyses due to inability to assign a haplogroup based on Herrnstadt classification. No tests of association were found to be significant within non-Hispanic whites at the Bonferroni corrected P-value threshold despite the marginal associations observed with polymorphisms indicating the H2a2 sub-haplogroup. Hispanics in our study fell primarily into the A (29%), H2 (11%) and L3 (36%) haplogroups. With such small sample size, statistical power to detect association with Hispanic haplogroups was low and no marginal associations were observed with CD4 count increase.

Discussion

In this study, we examined the effect of mtDNA variation on CD4 cell recovery during ART. Race-stratified analysis of mtDNA variants suggested possible associations between the African L2 haplogroup and reduced magnitude of CD4 cell recovery during ART in non-Hispanic black participants, although no single SNP association withstood correction for multiple testing. Significant correlation structure exists between mtDNA variants however, forming the basis for mitochondrial haplogroups, and an association with the L2 haplogroup was found which is significant after considering the number of haplogroups analyzed. The association was seen in analyses of a discrete outcome of 100 cell/mm³ increase at 48 weeks, and with CD4 count increase as a continuous outcome. It is unlikely that this haplogroup association is the result of an effect of race/ethnicity, as race/ethnicity was not found to be associated with CD4 cell count change in our study or in that of the original immunologic study of ACTG 384.¹⁶ It is also unlikely that associations were related to ART toxicity, virologic failure of treatment, and/or non-adherence, as we only included participants with suppressed HIV-1 RNA at the time point used for analyses.

Associations with other mtDNA SNPs implicate a role for variation not directly related to haplogroup. The role of these variations is not immediately apparent, particularly because the majority of mtDNA variants observed to have a significant association with CD4 count recovery are synonymous (i.e. do not change amino acid sequence). The exception is the 13105 variant, which causes a conservative isoleucine-to-valine change in the ND5 complex I protein. It will be necessary to assess CD4 recovery and mtDNA variation in larger cohorts to replicate these associations, and in model systems to characterize their functional effects.

Limitations of our data include small sample sizes which may have impaired our ability to detect associations in other racial/ethnic populations, use of self-identified race/ethnicity instead of genetic ancestry for stratification, missing data on naïve-to-memory cell ratio, and an incomplete understanding of the biologic rationale for the observed genomic associations. There are multiple potential mechanisms through which variation in mtDNA could alter CD4 cell count. The role of the mitochondrion as an apoptotic regulator has been increasingly recognized, and its specific importance in HIV-infected CD4⁺ lymphocytes has been studied.²⁹ In a recent study, PBMCs from HIV-infected long-term non-progressors demonstrated less mitochondrial dysfunction and mitochondrially-mediated apoptosis.³⁰ Published data on the effects of specific mtDNA variation on apoptosis include a murine model of mtDNA depletion³¹ and small studies of patients with mtDNA tRNA point mutations showing increases³² or no increases³³ in TUNEL-positive staining in muscle fibers. An additional study in fibroblasts demonstrated massive ROS-induced apoptosis in the presence of a mtDNA point mutation.³⁴

Hendrickson, et al. reported associations between European haplogroups J and U5a and increased pre-ART progression to AIDS and CD4 count <200 cells/mm³ among a Caucasian, HIV-infected cohort; 97% of whom were male.³⁵ These findings are also consistent with a role for mtDNA variation in CD4 T cell dynamics, but different associations in our study may be due to a smaller population of persons of European descent and/or different phenotypes: disease progression *prior to* ART and CD4 count recovery *during* ART may differ, even if similar mechanisms (e.g. apoptosis regulation) play a role in both. The current study provides insight into the contribution of mitochondrial genomics to CD4 count recovery in individuals of non-European descent. Further study in larger populations is necessary to more definitively determine the role of mtDNA variation in CD4 T cell dynamics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Kitahata MM, Gange SJ, Abraham AG, et al. Effect of early versus deferred antiretroviral therapy for HIV on survival. *N Engl J Med.* 2009; 360:1815–1826. [PubMed: 19339714]
2. Gutierrez F, Padilla S, Masia M, et al. Clinical outcome of HIV-infected patients with sustained virologic response to antiretroviral therapy: long-term follow-up of a multicenter cohort. *PLoS One.* 2006; 1:e89. [PubMed: 17183720]
3. Grabar S, Le MV, Goujard C, et al. Clinical outcome of patients with HIV-1 infection according to immunologic and virologic response after 6 months of highly active antiretroviral therapy. *Ann Intern Med.* 2000; 133:401–410. [PubMed: 10975957]
4. Haas DW, Geraghty DE, Andersen J, et al. Immunogenetics of CD4 Lymphocyte Count Recovery during Antiretroviral Therapy: An AIDS Clinical Trials Group Study. *J Infect Dis.* 2006; 194:1098–1107. [PubMed: 16991084]
5. Petit F, Arnoult D, Viollet L, et al. Intrinsic and extrinsic pathways signaling during HIV-1 mediated cell death. *Biochimie.* 2003; 85:795–811. [PubMed: 14585547]
6. Varbanov M, Espert L, Biard-Piechaczyk M. Mechanisms of CD4 T-cell depletion triggered by HIV-1 viral proteins. *AIDS Rev.* 2006; 8:221–236. [PubMed: 17219737]
7. Wallace DC, Brown MD, Lott MT. Mitochondrial DNA variation in human evolution and disease. *Gene.* 1999; 238:211–230. [PubMed: 10570998]
8. van der Walt J, Nicodemus KK, Martin E, et al. Mitochondrial Polymorphisms Significantly Reduce the Risk of Parkinson Disease. *Am J Hum Genet.* 2003
9. Autere J, Moilanen JS, Finnila S, et al. Mitochondrial DNA polymorphisms as risk factors for Parkinson's disease and Parkinson's disease dementia. *Hum Genet.* 2004; 115:29–35. [PubMed: 15108120]

10. Castro MG, Huerta C, Reguero JR, et al. Mitochondrial DNA haplogroups in Spanish patients with hypertrophic cardiomyopathy. *Int J Cardiol.* 2006; 112:202–206. [PubMed: 16313983]
11. De BG, Rose G, Carrieri G, et al. Mitochondrial DNA inherited variants are associated with successful aging and longevity in humans. *FASEB J.* 1999; 13:1532–1536. [PubMed: 10463944]
12. Niemi AK, Hervonen A, Hurme M, et al. Mitochondrial DNA polymorphisms associated with longevity in a Finnish population. *Hum Genet.* 2003; 112:29–33. [PubMed: 12483296]
13. Canter JA, Robbins GK, Selph D, et al. African mitochondrial DNA subhaplogroups and peripheral neuropathy during antiretroviral therapy. *J Infect Dis.* 2010; 201:1703–1707. [PubMed: 20402593]
14. Hulgán T, Haubrich R, Riddler SA, et al. European mitochondrial DNA haplogroups and metabolic changes during antiretroviral therapy in AIDS Clinical Trials Group Study A5142. *AIDS.* 2011; 25:37–47. [PubMed: 20871389]
15. Robbins GK, De GV, Shafer RW, et al. Comparison of sequential three-drug regimens as initial therapy for HIV-1 infection. *N Engl J Med.* 2003; 349:2293–2303. [PubMed: 14668455]
16. Gandhi RT, Spritzler J, Chan E, et al. Effect of baseline- and treatment-related factors on immunologic recovery after initiation of antiretroviral therapy in HIV-1-positive subjects: results from ACTG 384. *J Acquir Immune Defic Syndr.* 2006; 42:426–434. [PubMed: 16810109]
17. Haas DW, Wilkinson GR, Kuritzkes DR, et al. A multi-investigator/institutional DNA bank for AIDS-related human genetic studies: AACTG Protocol A5128. *HIV Clin Trials.* 2003; 4:287–300. [PubMed: 14583845]
18. Kallianpur AR, Hulgán T, Canter JA, et al. Hemochromatosis (HFE) gene mutations and peripheral neuropathy during antiretroviral therapy. *AIDS.* 2006; 20:1503–1513. [PubMed: 16847405]
19. Moutsier AA, Ritchie MD, Shafer RW, et al. Multilocus genetic interactions and response to efavirenz-containing regimens: an adult AIDS clinical trials group study. *Pharmacogenet Genomics.* 2006; 16:837–845. [PubMed: 17047492]
20. Hulgán T, Haas DW, Haines JL, et al. Mitochondrial haplogroups and peripheral neuropathy during antiretroviral therapy: an adult AIDS clinical trials group study. *AIDS.* 2005; 19:1341–1349. [PubMed: 16103764]
21. Anderson S, Bankier AT, Barrell BG, et al. Sequence and organization of the human mitochondrial genome. *Nature.* 1981; 290:457–465. [PubMed: 7219534]
22. Herrnstadt C, Elson JL, Fahy E, et al. Reduced-median-network analysis of complete mitochondrial DNA coding-region sequences for the major African, Asian, and European haplogroups. *Am J Hum Genet.* 2002; 70:1152–1171. [PubMed: 11938495]
23. Hadi AS. Identifying Multiple Outliers in Multivariate Data. *Journal of the Royal Statistical Society.* 1992; 54:761–771.
24. Grady BJ, Torstenson E, Dudek SM, et al. Finding unique filter sets in plato: a precursor to efficient interaction analysis in gwas data. *Pac Symp Biocomput.* 2010:315–326. [PubMed: 19908384]
25. Little J, Sharp L, Khoury MJ, et al. The epidemiologic approach to pharmacogenomics. *Am J Pharmacogenomics.* 2005; 5:1–20. [PubMed: 15727485]
26. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007; 81:559–575. [PubMed: 17701901]
27. van Buuren, S.; Oudshoorn, C. mice: Multivariate Imputation by Chained Equations library. 2010.
28. van Oven M, Kayser M. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Hum Mutat.* 2009; 30:E386–E394. [PubMed: 18853457]
29. Garg H, Blumenthal R. HIV gp41-induced apoptosis is mediated by caspase-3-dependent mitochondrial depolarization, which is inhibited by HIV protease inhibitor nelfinavir. *J Leukoc Biol.* 2006; 79:351–362. [PubMed: 16330530]
30. Peraire J, Miro O, Saumoy M, et al. HIV-1-infected long-term non-progressors have milder mitochondrial impairment and lower mitochondrially-driven apoptosis in peripheral blood mononuclear cells than typical progressors. *Curr HIV Res.* 2007; 5:467–473. [PubMed: 17896966]
31. Wang J, Silva JP, Gustafsson CM, et al. Increased in vivo apoptosis in cells lacking mitochondrial DNA gene expression. *Proc Natl Acad Sci U S A.* 2001; 98:4038–4043. [PubMed: 11259653]

32. Mirabella M, Di GS, Silvestri G, et al. Apoptosis in mitochondrial encephalomyopathies with mitochondrial DNA mutations: a potential pathogenic mechanism. *Brain*. 2000; 123 (Pt 1):93–104. [PubMed: 10611124]
33. Sciacco M, Fagiolari G, Lamperti C, et al. Lack of apoptosis in mitochondrial encephalomyopathies. *Neurology*. 2001; 56:1070–1074. [PubMed: 11320180]
34. Geromel V, Kadhon N, Cebalos-Picot I, et al. Superoxide-induced massive apoptosis in cultured skin fibroblasts harboring the neurogenic ataxia retinitis pigmentosa (NARP) mutation in the ATPase-6 gene of the mitochondrial DNA. *Hum Mol Genet*. 2001; 10:1221–1228. [PubMed: 11371515]
35. Hendrickson SL, Hutcheson HB, Ruiz-Pesini E, et al. Mitochondrial DNA haplogroups influence AIDS progression. *AIDS*. 2008; 22:2429–2439. [PubMed: 19005266]

Fig. 1.

Fig. 2.

Table 1

Baseline demographics for those individuals with DNA in the ACTG Human DNA Repository, for those who passed QC and exclusion criteria, and for case and control groups for the primary analyses.

	Participants with DNA (N=530)	Eligible Participants Passing QC (N= 423)	Increase ≥ 100 cells at week 48 (N=308)	Increase <100 cells at week 48 (N=115)	P-value ^d
Median age in years (range)	36 (17–72)	36 (17–72)	35 (17–72)	40 (20–65)	0.005
Female sex- N (%)	93 (18)	74 (17)	51 (17)	23 (20)	0.47
Race/ethnicity- N (%) ^b					
Non-Hispanic White	258 (49)	212 (50)	165 (54)	47 (41)	0.06
Non-Hispanic Black	163 (31)	126 (30)	84 (27)	42 (37)	
Hispanic	96 (18)	85 (20)	59 (19)	26 (23)	
Asian and Native American	13 (2)	0	0	0	
Randomized NRTIs-N (%) ^b					0.51
ddl+d4T	280 (53)	217 (51)	161 (52)	56 (49)	
ZDV+3TC	250 (47)	206 (49)	147 (48)	59 (51)	
Randomized NNRTI/PI- N (%) ^b					0.27
Efavirenz	166 (31)	133 (31)	98 (32)	35 (30)	
Nelfinavir	173 (33)	133 (31)	98 (32)	35 (30)	
Efavirenz+nelfinavir	191 (36)	157 (37)	112 (36)	45 (39)	
CD4 count/mm ³ -median (IQR)	275 (85–429)	278 (85–434)	261 (67–396)	342 (190–472)	0.001
Naïve-to-memory CD4 ⁺ lymphocyte ratio- median (IQR)	0.58 (0.26–1.00) ^c	0.59 (0.28–1.00) ^d	0.62 (0.32–1.09)	0.46 (0.18–0.72)	0.003
HIV-1 log ₁₀ RNA copies/mL plasma-median (IQR)	5.00 (4.37–5.55)	4.95 (4.32–5.53)	5.13 (4.54–5.64)	4.57 (4.03–5.15)	<0.001

^a For participants who passed quality control, differences in baseline demographics are compared between those with a CD4 count increase of ≥ 100 cells/mm³ at 48 weeks (cases for primary endpoint) and those with a CD4 count increase of <100 cells at 48 weeks (controls). P-values from unadjusted Wilcoxon rank-sum, Fisher's exact or Chi-squared tests.

^b Totals may not equal 100% due to rounding.

^c N= 359;

^d N=281

3TC= lamivudine; ddl= didanosine; d4T= stavudine; IQR= interquartile range; NRTIs= nucleoside reverse transcriptase inhibitors; NNRTI= non-NRTI; PI= protease inhibitor; QC= quality control; ZDV= zidovudine.

Table 2

Haplogroup distributions, overall and by race/ethnicity

Haplogroup ^a	Total (N=423)		Non-Hispanic White (N=212)		Non-Hispanic Black (N=126)		Hispanic (N=85)	
	N	%	N	%	N	%	N	%
A	31	7.33	2	0.94	4	3.17	25	29.41
H	10	2.36	9	4.25	1	0.79	0	0
H1	30	7.09	28	13.21	0	0	2	2.35
H2	59	13.95	46	21.7	4	3.17	9	10.59
H3	8	1.89	8	3.77	0	0	0	0
I	4	0.95	3	1.42	1	0.79	0	0
I1	2	0.47	2	0.94	0	0	0	0
I2	1	0.24	1	0.47	0	0	0	0
J1	25	5.91	21	9.91	2	1.59	2	2.35
J2	7	1.65	6	2.83	0	0	1	1.18
K	1	0.24	1	0.47	0	0	0	0
K1	10	2.36	8	3.77	0	0	2	2.35
K1a	1	0.24	1	0.47	0	0	0	0
K2	7	1.65	7	3.3	0	0	0	0
L1/L2	4	0.95	1	0.47	2	1.59	1	1.18
L1a	7	1.65	0	0	7	5.56	0	0
L1b	14	3.31	1	0.47	10	7.94	3	3.53
L1c	15	3.55	0	0	14	11.11	1	1.18
L2a	28	6.62	0	0	25	19.84	3	3.53
L2b	19	4.49	1	0.47	15	11.9	3	3.53
L3	42	9.93	3	1.42	16	12.7	23	27.06
L3b	20	4.73	2	0.94	12	9.52	6	7.06
L3e	11	2.6	1	0.47	8	6.35	2	2.35
T1	6	1.42	5	2.36	1	0.79	0	0
T2	2	0.47	2	0.94	0	0	0	0
T2b	7	1.65	5	2.36	1	0.79	1	1.18
U	1	0.24	1	0.47	0	0	0	0

Haplogroup ^a	Total (N=423)		Non-Hispanic White (N=212)		Non-Hispanic Black (N=126)		Hispanic (N=85)	
	N	%	N	%	N	%	N	%
U2	3	0.71	3	1.42	0	0	0	0
U4	7	1.65	7	3.3	0	0	0	0
U5	4	0.95	4	1.89	0	0	0	0
U5a	6	1.42	6	2.83	0	0	0	0
U5a1	8	1.89	8	3.77	0	0	0	0
U5b	1	0.24	1	0.47	0	0	0	0
U6	3	0.71	0	0	2	1.59	1	1.18
U9	3	0.71	2	0.94	1	0.79	0	0
V	4	0.95	4	1.89	0	0	0	0
W	4	0.95	4	1.89	0	0	0	0
W3	2	0.47	2	0.94	0	0	0	0
X1	3	0.71	3	1.42	0	0	0	0
No-calls ^b	3	0.71	3	1.42	0	0	0	0

^aHaplogroups assigned using methods described by Herrnstadt, et al.²²

^bSubjects unable to be classified.

Significant SNP results ($P \leq 0.05$) from adjusted logistic regression analysis of discrete outcome $\geq$ or < 100 cell/mm³ increase in CD4 count at week 48.

Table 3

Base position	Gene/Region	MAF	Race/ethnicity	OR	95% CI	P-value
2789	rRNA	0.194	NHB	0.24	0.07, 0.85	0.03
7175	CO1	0.194	NHB	0.24	0.07, 0.85	0.03
7274	CO1	0.187	NHB	0.23	0.07, 0.82	0.02
7771	CO2	0.194	NHB	0.24	0.07, 0.85	0.03
9221	CO3	0.315	NHB	0.17	0.06, 0.53	0.002
10115	ND3	0.306	NHB	0.17	0.06, 0.52	0.002
11914	ND4	0.268	NHB	0.24	0.07, 0.76	0.02
13105	ND5	0.333	NHB	4.60	1.13, 18.70	0.03
13590	ND5	0.312	NHB	0.19	0.06, 0.58	0.004
13803	ND5	0.187	NHB	0.22	0.06, 0.78	0.02
14566	ND6	0.194	NHB	0.24	0.07, 0.85	0.03
15301	CYB	0.369	NHB	3.64	1.02, 13.06	0.05
16390	CR	0.311	NHB	0.19	0.06, 0.58	0.004
750	rRNA	0.029	NHW	0.037	0.002, 0.60	0.02
8860	ATP6	0.053	NHW	0.023	0.0008, 0.68	0.03
15326	CYB	0.024	NHW	0.023	0.0008, 0.68	0.03
16519	CR	0.053	NHW	0.24	0.08, 0.71	0.01

CI= confidence interval; CR= control region; MAF= minor allele frequency; NHW= non-Hispanic white; NHB= non-Hispanic black; OR= odds ratio; SNP= single nucleotide polymorphism. See figure legend for gene/region abbreviation listing.

Models adjusted for age, baseline viral load, baseline CD4 cell count, and baseline ratio of naive-to-memory CD4 cell count.