

# Non-synonymous Mitochondrial DNA (mtDNA) Polymorphisms and Peripheral Neuropathy during Nucleoside Reverse Transcriptase Inhibitor (NRTI) Therapy in AIDS Clinical Trials Group (ACTG) Study 384

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## ABSTRACT

**Background** Peripheral neuropathy (PN) is a common complication of NRTI therapy for HIV infection and may involve mitochondrial dysfunction due to inhibition of mtDNA polymerase- $\gamma$ . We previously described an association between mitochondrial haplogroup T and PN among white participants in ACTG 384, a study which randomized antiretroviral therapy-naïve subjects to initiate therapy with didanosine (ddI) plus stavudine (d4T) or zidovudine plus lamivudine in combination with efavirenz and/or nelfinavir. Since the defining polymorphism for haplogroup T (G13368A) is synonymous (does not change an amino acid), we explored two non-synonymous polymorphisms, T4216C and A4917G, that have previously been linked with both haplogroup T and human neurodegenerative diseases.

**Methods** We analyzed mtDNA from ACTG 384 participants who contributed specimens to the ACTG Human DNA Repository under protocol A5128. Non-synonymous polymorphisms at positions T4216C and A4917G were determined by 5' nuclease allelic discrimination Taqman™ and Eclipse™ assays, respectively. PN cases were identified by patient-reported symptoms and/or physical examination findings. Controls did not develop symptomatic PN during the study. Participants with PN at baseline were excluded. Logistic regression was used to calculate odds ratios.

**Results** Of the 250 self-identified white, non-Hispanic subjects from ACTG 384 who were included in this analysis, T4216C and A4917G were present in 53 (21%) and 24 (10%), respectively. Of the 24 individuals with A4917G, all but one also had T4216C. Symptomatic PN ( $\geq$ grade 1) developed in 70 (28%) of these subjects during study follow-up, 48 (69%) of whom received ddI+d4T at randomization. Both T4216C (OR=2.5; 95% CI 1.1-5.6;  $P=0.03$ ) and A4917G (OR=5.5; 95% CI 1.6-18.7;  $P=0.006$ ) were more frequent among those with PN than among those without PN. In separate models adjusting for age, randomization arm, and baseline CD4 lymphocyte count and plasma HIV-1 RNA level, T4216C and A4917G remained independent predictors of PN.

**Conclusions** T4216C and A4917G, two non-synonymous mtDNA polymorphisms, were frequent among white ACTG 384 participants, and were associated with the development of PN among those individuals randomized to receive ddI+d4T. These polymorphisms are known to alter amino acids in mitochondrial Complex I subunits and are associated with other human neurodegenerative diseases. Further studies to validate these associations are warranted.

## BACKGROUND

•NRTIs can interact with human mtDNA polymerase- $\gamma$  to cause mitochondrial toxicities such as PN.

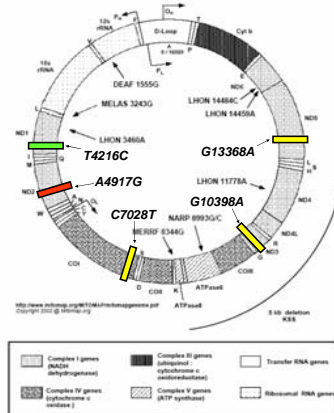
•mtDNA (Figure 1) is prone to somatic mutations (heteroplasmy) *de novo* and from environmental exposures (including NRTIs).

•mtDNA also harbors stable germ-line (homoplasmic) polymorphisms that are maternally inherited, can map prehistoric migration patterns (via haplogroups), and have implications for disease. Torroni, et al. *Genetics* 1996; 144: 1835; Wallace, et al. *Gene* 1999; 238: 211-30; Van der Walt, et al. *Am J Hum Genet* 2003; 72: 804-11

•We previously reported an association between European mitochondrial haplogroup T and PN during ACTG study 384. Hulgan, et al. *AIDS* 2005; 19: 1341-9

•The signature SNP for haplogroup T (G13368A) is synonymous, but non-synonymous SNPs are associated with haplogroup T and with neurodegenerative diseases.

•We explored two previously characterized non-synonymous mtDNA SNPs in the ACTG 384 population to determine associations with PN during NRTI therapy.



**Figure 1.** Map of the human mitochondrial genome with some of the known pathogenic mutations noted. Yellow bars denote approximate locations of SNPs that define the T haplogroup. Green and red bars indicate approximate locations of the two non-synonymous SNPs described in this study.

MITOMAP: A Human Mitochondrial Genome Database. <http://www.mitomap.org>, 2005.

## METHODS

•ACTG study 384 randomized 980 volunteers in the U.S. and Italy between October 1998 and November 1999 to receive ZDV+3TC or ddI+d4T in combination with efavirenz, nelfinavir, or both. Participants could substitute NRTIs (3TC for ddI; d4T for ZDV) for intolerance. Follow-up continued for up to 3 years.

•mtDNA from ACTG 384 participants who contributed specimens to the ACTG Human DNA Repository was extracted under protocol A5128.

•Polymorphisms at positions T4216C and A4917G were determined by 5' nuclease allelic discrimination Taqman™ and Eclipse™ assays, respectively.

•PN cases were identified by patient-reported symptoms and/or physical examination findings. Controls did not develop symptomatic PN during the study.

•Participants with PN at baseline were excluded.

•Odds Ratios were determined using logistic regression models.

•Separate models were used for each of the two SNPs.

## RESULTS

•250 non-Hispanic white ACTG 384 participants had DNA available for analysis (Table).

•248 had genotypes determined at both T4216C and A4917G (two participants [ $<1\%$ ] could not be determined at position 4917).

•Symptomatic PN ( $\geq$ grade 1) developed in 70 (28%) of these subjects during ACTG 384 follow-up, 48 (69%) of whom were randomized to receive ddI+d4T.

•T4216C and A4917G were present in 53 (21%) and 24 (10%) participants, respectively.

•22/53 (42%) T4216C and 23/24 (96%) A4917G participants belonged to haplogroup T.

•Both SNPs were present in 23/248 (9%) participants, all but one of whom belonged to haplogroup T.

•Both T4216C (univariate OR [95% CI]= 2.0 [1.0-3.8];  $P=0.04$ ) and A4917G (2.9 [1.2-6.9];  $P=0.01$ ) were more frequent in participants with PN than those without (Table).

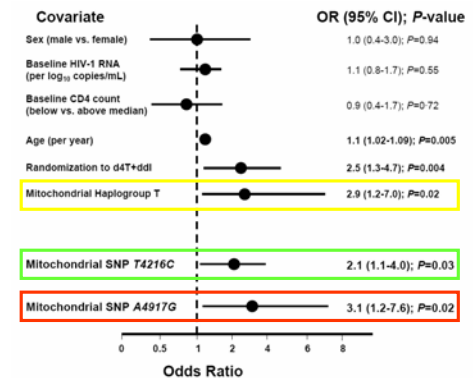
•These associations were also seen among the subgroup of participants randomized to ddI+d4T (2.5 [1.1-5.6];  $P=0.03$  and 5.5 [1.6-18.7];  $P=0.006$ , respectively).

•In separate multivariate models adjusting for age, sex, randomization arm, and baseline CD4 lymphocyte count and plasma HIV-1 RNA level, T4216C and A4917G remained independent predictors of PN (Figure 2).

**Table.** Baseline characteristics of non-Hispanic white ACTG 384 participants with DNA available.

Characteristic	Total (n=250)	Controls (n=180)	PN cases (n=70)
Age in years, median (range)	36 (19-64)	35 (19-62)	38 (21-64)*
Female sex	19 (8)	13 (7)	6 (9)
<b>Antiretroviral therapy at randomization</b>			
ddI + d4T	137 (55)	89 (49)	48 (69)*
efavirenz	38 (28)	22 (25)	16 (33)
nelfinavir	44 (32)	34 (38)	10 (21)
efavirenz + nelfinavir	55 (40)	33 (37)	22 (46)
ZDV + 3TC	113 (45)	91 (51)	22 (31)
efavirenz	37 (33)	30 (33)	7 (32)
nelfinavir	36 (32)	27 (30)	9 (41)
efavirenz + nelfinavir	40 (35)	34 (37)	6 (27)
Baseline HIV RNA (log <sub>10</sub> copies/mL)	5.2 (4.5-5.6)	5.1 (4.4-5.6)	5.3 (4.7-5.7)
Baseline CD4 lymphocytes (cells/mm <sup>3</sup> )	290 (87-440)	305 (114-452)	251 (66-429)*
<b>Mitochondrial Haplogroup T</b>	24 (10)	12 (7)	12 (17)*
<b>Mitochondrial SNP T4216C</b>	53 (21)	32 (18)	21 (30)*
<b>Mitochondrial SNP A4917G</b>	24 (10)	12 (7)	12 (17)*

\*Fisher's exact or Wilcoxon ranksum  $P<0.05$  for comparison of PN cases vs. controls. Participants with DNA available were more likely to have been randomized to d4T+ddI, but otherwise did not differ from other ACTG 384 participants (data not shown). Values shown are n (%) or median (interquartile range) except where noted.



**Figure 2.** Odds Ratio plot for multivariate analyses of peripheral neuropathy among non-Hispanic white ACTG 384 participants.

The top group of covariates were included in a single model which controlled for the factors shown.

The A4216C and A4917G results were derived from separate multivariate models. Both models were adjusted for sex, baseline HIV-1 RNA and CD4 count, age, and randomization to ddI+d4T vs. ZDV+3TC.

## DISCUSSION AND CONCLUSIONS

•The non-synonymous mtDNA polymorphisms T4216C (Tyr→His) and A4917G (Asn→Asp), were frequent among non-Hispanic white ACTG 384 participants, were closely linked to each other and to the T haplogroup, and were associated with the development of PN independent of other putative risk factors.

•These SNPs alter amino acids in mitochondrial Complex I subunits and are associated with other human neurodegenerative diseases, including Leber's Hereditary Optic Neuropathy (LHON). Exacerbation of LHON has been reported in HIV-infected individuals after exposure to NRTIs (Luzhanski, et al. *AIDS* 2001; 15: 1588-9, Mackey, et al. *Eye* 2003; 17: 312-17).

•Studies to validate these associations, identify SNPs associated with PN in other racial/ethnic groups, and elucidate functional effects of these SNPs *in vitro* and *in vivo* are needed.