

The H63D Hemochromatosis (*HFE*) Gene Polymorphism and Mitochondrial Haplogroup J are Associated with Limb Fat Changes after Initiation of Antiretroviral Therapy (ART) in ACTG Study A5005s

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ABSTRACT

Background Lipotrophy can complicate ART and may be due in part to nucleoside reverse transcriptase inhibitor (NRTI)-induced mitochondrial dysfunction. Iron modulates both mitochondrial function and oxidative injury. In previous studies, NRTI-induced peripheral neuropathy was associated with European mitochondrial haplogroup T, and chromosomal *HFE* polymorphisms (C282Y and to a lesser extent H63D) were protective. Our objective was to evaluate the relationships between these genetic variants and body fat changes observed in HIV-infected individuals after the initiation of ART.

Methods ACTG 384 randomized ART-naïve subjects to receive didanosine/stavudine or zidovudine/lamivudine in combination with efavirenz and/or nelfinavir. The metabolic substudy A5005s evaluated longitudinal changes in fat distribution by DEXA in a subset of patients at baseline and weeks 16, 32, 48 and 64. Subjects who also consented to participate in the ACTG Human DNA Repository (A5128) had *HFE* C282Y and H63D genotypes and mitochondrial haplogroups determined. Percent change in total body, trunk, and limb fat at week 48 or 64 was compared by the Wilcoxon rank-sum test. We used logistic regression to determine if these variants were associated with lipotrophy (defined as a $\geq 10\%$ limb fat loss at week 48 or 64).

Results Genetic data and either 48 or 64-week DEXA were available from 96 individuals (58% non-Hispanic white, 10% female). Overall median 48 or 64-week change in limb fat was -8.8% (IQR -28.7, +15.6). Among non-Hispanic white participants, 5 haplogroup J individuals had a 26.1% increase in limb fat at week 48 or 64 vs. a 9.7% decrease in non-J individuals (n=50, p=0.07). Haplogroup T was present in 4 individuals and was not associated with fat changes. Among all subjects, *HFE* H63D heterozygotes (nucleotide 187C>G; n=23) had significantly less limb fat loss compared to homozygous wild-type individuals (+6.1% vs. -12.5%; p=0.02) and were less likely to develop lipotrophy (OR 0.31 [95% CI 0.10, 0.95]; p=0.04) after adjustment for age, sex, race, and randomization arm.

Conclusions The common *HFE* H63D polymorphism was associated with protection from significant limb fat loss. There was also a trend toward protection in individuals belonging to mitochondrial haplogroup J. These results suggest that variation in iron metabolism genes and in mitochondrial genes may influence susceptibility to ART-associated lipotrophy. These findings must be validated in larger studies.

BACKGROUND

•NRTIs can interact with human mitochondrial DNA (mtDNA) polymerase- γ to cause mitochondrial toxicities such as lipotrophy.

•mtDNA harbors stable homoplasmic polymorphisms that are maternally inherited, can be used to map prehistoric migration patterns (using haplogrouping), and have implications for disease. (Torroni, et al. *Genetics* 1996; 144: 1835-50; Wallace, et al. *Gene* 1999; 238: 211-30; Van der Walt, et al. *Am J Hum Genet* 2003; 72: 804-11)

•Iron is an important modulator of mitochondrial function and oxidative tissue injury. Common polymorphisms in the *HFE* gene have important and varying effects on iron transport, macrophage function, and inflammatory responses.

•We previously reported an association between European mtDNA haplogroup T and NRTI-associated peripheral neuropathy and between *HFE* gene polymorphisms and peripheral neuropathy during ACTG study 384. (Hulgan, et al. *AIDS* 2005; 19: 1341-9; Kallianpur, et al. *AIDS* 2006; 20: 1503-13)

•The objective of this study was to evaluate relationships between genetic variations in mtDNA and *HFE* and limb fat changes in HIV-infected individuals after initiation of ART.

METHODS

•ACTG study 384 was a treatment strategy trial that 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. (Robbins, et al. and Shafer, et al. *NEJM* 2003; 349)

•A5005s evaluated body composition by dual energy X-ray absorptiometry (DEXA) in a subset of ACTG 384 participants. (Dubé, et al. *AIDS* 2005; 19: 1807-18)

•DNA from ACTG 384/A5005s participants contributing specimens to the ACTG Human DNA Repository was extracted under protocol A5128 as part of New Work Concept Sheet 238.

•Polymorphisms at chromosomal positions 187 (H63D) and 845 (C282Y) in the *HFE* genes, and mtDNA SNPs for Torroni European haplogroup classification (*Genetics* 1996; 144: 1835-50) were determined by 5' nuclease allelic discrimination Taqman™ assays.

•Limb fat loss (lipotrophy) was determined by DEXA and analyzed as either median %change from baseline or dichotomized as $\geq 10\%$ loss of limb fat by week 48 or 64.

•Median %change in limb fat for persons with ≥ 48 -week DEXA data were analyzed using Wilcoxon rank sum test. Odds ratios for the association between the *HFE* H63D polymorphism and lipotrophy were determined using a logistic regression model adjusted for age, race, sex, and randomization arms. Random effects MANOVA was used to assess the overall limb fat changes over time by *HFE* H63D status, adjusted for age, race, sex, and randomization arms.

RESULTS

•105 A5005s participants had DNA available for analysis, 103 of whom also had genotypes available for *HFE* H63D (Table 1).

•94 (91%) of these had complete *HFE* genetic data and paired baseline and 48 or 64 week DEXA data.

•Overall median (interquartile range) 48- or 64-week limb fat change for this group was -8.8% (-28.7, +15.6).

•43/94 (46%) had lipotrophy ($\geq 10\%$ limb fat loss) by week 48 or 64.

•The heterozygous *HFE* amino acid substitution 63H/D (base position 187C>G) was present in 24% of participants, and was less common in blacks (P=0.053) as expected. The major *HFE* variant, 282C/Y (845G>A), was present in 11% of participants.

•Individuals with *HFE* 63H/D had a median %limb fat change of +6.1% (-11.5, +34.8) at week 48 or 64 vs. -12.5% (-35.1, +10.5) among homozygous *HFE* 63H/H individuals (P=0.02; Table 1). Limb fat changes did not differ significantly based on *HFE* C282Y genotype.

Table 1. Baseline characteristics of A5005s participants with DNA and *HFE* H63D genotypes available, total and by *HFE* H63D status.

Characteristic	Total (n=103)	<i>HFE</i> 63H/H (n=78)	<i>HFE</i> 63H/D (n=25)
Age, median (range)	36 (19, 61)	36 (19, 61)	36 (24, 60)
Female sex, n (%)	11 (11)	9 (12)	2 (8)
Race/ethnicity, n (%)			
White, non-Hispanic	57 (55)	39 (50)	18 (72)
Black, non-Hispanic	27 (26)	25 (32)	2 (8)
Hispanic	19 (18)	14 (18)	5 (20)
Baseline CD4 (cells/mm ³)	234 (69, 406)	228 (69, 402)	240 (87, 432)
Baseline HIV RNA (log ₁₀ copies/mL)	5.1 (4.6, 5.8)	5.1 (4.6, 5.7)	5.1 (4.7, 5.9)
Randomization arm, n (%)			
ZDV+3TC	47 (46)	36 (46)	11 (44)
NFV (\pm EFV)	30 (29)	24 (31)	6 (24)
EFV (no NFV)	17 (17)	12 (15)	5 (20)
ddl+d4T	56 (54)	42 (54)	14 (56)
NFV (\pm EFV)	39 (38)	29 (37)	10 (40)
EFV (no NFV)	17 (17)	13 (17)	4 (16)
%change in limb fat, wk 48/64*	-8.8 (-28.7, +15.6)	-12.5 (-35.1, +10.5)	+6.1 (-11.5, +34.8)
Lipotrophy, n (%) ($\geq 10\%$ limb fat loss, wk 48/64)*	43 (46)	37 (52)	6 (26)

Fisher's exact, Chi-squared or Wilcoxon ranksum test P<0.05 for comparisons of *HFE* 63H/H vs. 63H/D, except for race (P=0.053), %change in limb fat (P=0.02), and lipotrophy (P=0.03). Values shown are n (%) or median (interquartile range) except where noted. *Sample sizes for these groups include the 94 individuals with paired data available at baseline and week 48 or 64.

•Median %change in limb fat at 48/64 wks in the four randomization-drug/genotype groups were: -15% (ddl+d4T/63H/H); -10% (ZDV+3TC/63H/H); -2% (ddl+d4T/63H/D); and +10% (ZDV+3TC/63H/D)

•In a multivariable logistic regression model adjusted for age, sex, and randomization arms (Table 2), 63H/D individuals were less likely to develop lipotrophy (OR 0.31 [95% CI 0.10, 0.95]; P=0.04).

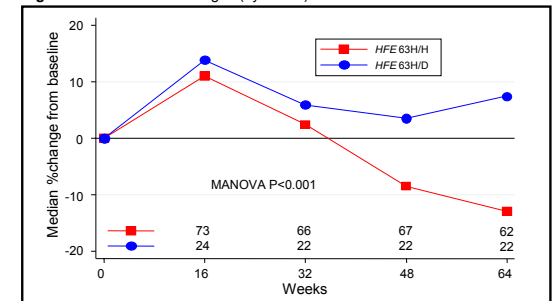
•63H/D individuals also had less limb fat loss over the 64-weeks of follow-up by MANOVA after adjusting for baseline factors (P<0.001; Figure). This was true in race- and NRTI randomization arm-stratified analyses (P<0.001 for both; data not shown).

•4 non-Hispanic white individuals belonged to mtDNA haplogroup T, and this haplogroup was not associated with limb fat changes. 5 non-Hispanic white individuals belonged to haplogroup J, and tended to have less limb fat loss than the 50 non-haplogroup J individuals (+26% [+22.5, +37.0] vs. -9.7% [-26.4, +4.2]; P=0.065).

Table 2. Univariate and adjusted odds ratios (OR) of potential predictors of lipotrophy.

Characteristic	Univariate OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Age (per year increase)	1.00 (0.96, 1.05)	0.88	1.00 (0.95, 1.05)	0.97
Sex (female vs. male)	2.12 (0.52, 8.77)	0.30	3.65 (0.68, 19.76)	0.13
Race/ethnicity (white, non-Hispanic vs. other)	0.94 (0.42, 2.13)	0.89	0.95 (0.38, 2.36)	0.91
NRTI randomization arm (ddl+d4T vs. ZDV+3TC)	2.09 (0.91, 4.78)	0.08	1.94 (0.78, 4.83)	0.15
Randomization to any NFV (yes vs. no)	2.63 (1.05, 6.60)	0.04	2.63 (0.98, 7.08)	0.055
<i>HFE</i> 63H/D (yes vs. no)	0.32 (0.11, 0.92)	0.03	0.31 (0.10, 0.95)	0.04

Figure. Median limb fat changes (by DEXA) from baseline over 64 weeks of therapy.



CONCLUSIONS

•Among these clinical trial participants, a common *HFE* gene polymorphism (H63D) was associated with less limb fat loss by DEXA after initiating ART containing 2 NRTIs, and was independent of the NRTI randomization arm.

•A trend toward less limb fat loss was also observed in non-Hispanic white individuals belonging to European mitochondrial haplogroup J, which has been found to be protective in other degenerative phenotypes.

•Effects on iron metabolism, macrophage function, inflammation and/or reduced susceptibility to NRTI-mediated mitochondrial toxicity are possible explanations for these associations.

•Further studies are needed to validate and explore the mechanisms underlying these findings.