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Mitochondrial DNA Haplogroups influence lipoatrophy after Highly Active Anti-retroviral Therapy

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Abstract

Although highly active retroviral therapy (HAART) has been extremely effective in lowering AIDS incidence among patients infected with HIV, certain drugs included in HAART can cause serious mitochondrial toxicities. One of the most frequent adverse events is lipoatrophy, which is the loss of subcutaneous fat in the face, arms, buttocks and/or legs as an adverse reaction to nucleoside reverse transcriptase inhibitors (NRTIs). The clinical symptoms of lipoatrophy resemble those of inherited mitochondrial diseases, which suggests that host mitochondrial genotype may play a role in susceptibility. We analyzed the association between mitochondrial haplogroup and severity of lipoatrophy in HIV-infected European American patients on HAART in the Multicenter AIDS cohort Study (MACS) and found that mitochondrial haplogroup H was strongly associated with increased atrophy (arms: $p = 0.007$, OR = 1.77, 95% CI = 1.17–2.69 legs: $p = 0.037$, OR = 1.54 95% CI = 1.03–2.31, and buttocks: $p = 0.10$, OR = 1.41 95% CI = 0.94–2.12). We also saw borderline significance for haplogroup T as protective against lipoatrophy ($p = 0.05$, OR = 0.52, 95% CI = 0.20–1.00). These data suggest that mitochondrial DNA haplogroup may influence the propensity for lipoatrophy in patients receiving NRTIs.

Keywords

lipoatrophy; mitochondrial haplogroup; NRTI; mitochondrial toxicity

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Introduction

Highly active anti-retroviral therapy (HAART) markedly decreases AIDS progression among HIV-infected individuals [1]. However mitochondrial toxicity resulting from the use of specific antiretrovirals used as part of HAART has been linked to several adverse effects including lipodystrophy, peripheral neuropathy, hepatic steatosis, myopathy, cardiomyopathy, pancreatitis, bone-marrow suppression, and lactic acidosis [2–6]. Nearly all of these adverse effects resemble clinical symptoms seen in inherited mitochondrial diseases [7, 8], suggesting that host mitochondrial genotype may play a role in their development. This hypothesis is supported by previous studies of patients receiving antiretroviral therapy for whom mitochondrial DNA haplogroup T was overrepresented among patients with peripheral neuropathy in the ACTG [AIDS Clinical Trials Group] cohort [9, 10], and in five patients with haplogroup J who had higher median limb fat change post-therapy compared to other haplogroups [11].

One of the most common clinical pathologies associated with HAART is lipoatrophy, a physically disfiguring mitochondriotoxicity that occurs in 13–63% of patients on HAART [12–17]. Associated primarily with the thymidine analogue nucleoside reverse transcriptase inhibitors (NRTIs) zidovudine (AZT) and stavudine (d4T), lipoatrophy is the loss of subcutaneous fat from the face, extremities, and buttocks [18]. The distinctive sunken cheeks and wasted appearance can have profound social and psychological impacts for affected persons and can lead to decreased therapy adherence [19–22]. Lipoatrophy has also been observed as a prelude to other health risks such as hypertension [23] and coronary heart disease[24].

Lipoatrophy is caused by a combination of cellular mechanisms including inhibition of mitochondrial gamma-polymerase, depleted mtDNA, acquired mtDNA mutations, and oxidative stress [8, 25–27]. Because mitochondria are critical for energy production and for control of cellular apoptosis, disruption of mitochondrial processes has serious metabolic consequences. Through oxidative phosphorylation (OXPHOS), mitochondria convert calories to ATP, release heat that maintains body temperature, and generate reactive oxygen species (ROS). Many mitochondrial diseases occur when energy production drops below the energetic threshold for a given process in the cell [28–31]. NRTI- induced mtDNA depletion disrupts OXPHOS, likely causing energy deprivation [32]. Compromised ATP production in turn may lower fat production since ATP is needed for triglyceride synthesis in adipocytes [33]. Further, mitochondrial perturbation and oxidative stress can result in the release of apoptosis-inducing factors causing apoptosis of adipocytes and consequent peripheral fat loss [26, 34].

The mitochondrial genome encodes thirteen proteins that participate in OXPHOS. Variation in these polypeptides may influence energy production efficiency, ROS generation, and levels of apoptosis [35]. Mitochondrial variation has been associated with climate adaptation [28, 36], susceptibility to neurodegenerative disease [37–40], energy deficiency disease [41, 42], longevity [43–45], sperm motility [46, 47], sprint performance [48], and microbial infection [49]. In a previous study, we also showed that specific mtDNA genotypes are associated with AIDS progression in untreated HIV-infected patients[50].

Hence, we sought to determine whether the host mtDNA genotype was associated with propensity for development of lipoatrophy in HIV-infected patients on HAART in the Multicenter AIDS Cohort Study (MACS). We determined the mitochondrial DNA haplogroup of 410 male European American patients who had been assessed for lipoatrophy by clinical examination of fat in the limbs and buttocks, and investigated whether severity of fat loss was associated with mtDNA genotype.

Methods

Cohort

The Multicenter AIDS Cohort Study (MACS) is a United States- based ongoing prospective study of HIV-1 infection in adult (ages 18–70) men who have sex with men (MSM) in Baltimore, Chicago, Pittsburgh, and Los Angeles [51]. This study focused on men who self-reported as "white". White-hispanic men were not included due to different genetic background.

Details of atrophy assessment

The severity of peripheral atrophy was quantified by a standardized physical exam assessment scale which used mild, moderate and severe gradations for each of the affected body areas (arms, legs, face and buttocks). Mild was recorded for atrophic changes that were evident to the MACS clinician upon close inspection; moderate was recorded for changes that were evident without close inspection and severe atrophy was recorded for atrophic changes that were evident to a non-medical person by casual observation. Specific anatomic features noting presence of moderate or greater atrophy were prominence of the nasolabial folds, hollowing of the cheeks ("sunken cheeks"), peripheral venous prominence and apparent bony landmarks. Physical exams were performed during the HAART era between 1999 and 2006.

Genotyping

DNAs were extracted from immortal lymphoblastoid B cell lines for each patient. Six haplotype-tagging SNPs were used initially to classify individuals as mitochondrial macro-haplogroups N, M and L groups. Haplogroups within the Western European (N) subset were further parsed into haplogroups using SNPs in the Mitochondrial Haplogrouping using Candidate Functional Variants (MHCFV) approach as described in Hendrickson et al. (2008), and the subset used in this study to identify the major haplogroups and subgroups within H are shown in Figure 1. Based on the hierarchical nature of the human mtDNA tree we devised a strategy for identifying haplotypes by subdividing the samples using highly conserved polymorphic sites located at key haplogroup branch points. Genotyping was performed using TaqMan Assays-by-Design(SM). Thermocycling conditions were an initial 95°C hold for 3 minutes, followed by 30 cycles of 92°C for 15s, and 56°–62°C annealing for 1 minute depending on primer specificity. Haplogroups were compared against the remaining haplogroups in statistical analyses. Rare, loosely associated haplogroups R*, HV* and JT* were excluded from individual analyses but included in controls.

Statistical analysis

Associations between mitochondrial haplogroup and severity of lipoatrophy were assessed with proportional odds logistic regression (POLR) [52, 53]. All analyses were performed with SAS version 9.1 (SAS Institute, Inc, Cary NC). Sensitivity appeared to vary between the measures of atrophy on arms, legs and buttocks; therefore we analyzed each lipoatrophy assessment separately. Further, we attempted an analysis of the average of these values; however the score test was significant suggesting it violated the model assumptions, likely due to small cell size in the uppermost levels of severity[54]. We used backward selection to test environmental variables to include in our POLR models. Age at HAART initiation ($p = 0.03$), BMI at the time of lipoatrophy assessment ($p < 0.0001$), and AZT and d4T use (both $p < 0.0001$) were all significant at the $p \leq 0.05$. In a previous study, cumulative exposure to NRTIs was associated with decreases in BMI and body circumference over 5 years of follow-up among HIV-infected men in the MACS cohort [55], therefore we used a continuous variable to account to the number of visits (6 month intervals) prior to assessment at which a patient was taking either d4T or AZT.

Results

HIV-1 infected Caucasian men have an increased prevalence of lipoatrophy [56]; therefore the European American men on HAART in the MACS represent a high-risk group. We successfully genotyped 536 patients who self identified as “white” and had a Western European, or “N”, mitochondrial macro-haplogroup. Individuals who had L or M macro-haplogroups (found in Africa and East Asia) were excluded from the study, and those within the N haplogroup were further parsed into N haplogroups H, T, IWX, J, T, V, and U. A complete clinical data set for all variables used in the final analyses of lipoatrophy and mitochondrial haplogroup association was available from 410 of these patients.

Clinical characteristics of study participants are shown in Table 1. Age at HAART initiation, BMI at the time of lipoatrophy assessment, and cumulative AZT and d4T exposure were all significantly associated with an increased incidence of lipoatrophy, consistent with previous studies[33] (Table 2). Age was only strongly significant in for atrophy in the legs according to our models, but because of its known importance in previous studies, we included it in all models. We also evaluated whether tenofovir, which has been reported to cause lipodystrophy in a small percentage of patients [57], or nelfinavir were associated with lipoatrophy, but found no associations between their use and lipoatrophy in the MACS.

Mitochondrial haplogroup H was strongly associated with significant increases in extremity lipoatrophy (arms: $p = 0.007$, OR = 1.77, 95% CI = 1.17–2.69; legs: $p = 0.03$, OR = 1.54, 95% CI = 1.03–2.31) (Table 3). We also observed a trend for increased lipoatrophy in the closely related V haplogroup ($p = 0.07$ OR = 2.59, 95% CI = 0.93–7.26). The phylogenetic tree of the major haplogroups is shown in Figure 1. In contrast, weak significance suggesting a protective effect against lipoatrophy were observed with haplogroup T ($p = 0.05$). No significant associations were observed for haplogroup J, which is closely related to T, however, odds ratios were consistently protective.

Because BMI is a confounding variable during atrophy assessment but is also biologically related to atrophy, we repeated the analysis without BMI as a covariate in the model. Results were generally the same but with slightly weaker associations observed. The association between haplogroup H and increased arm atrophy remained significant ($p = 0.021$, OR = 1.60, 95% CI = 1.07–2.38), but associations with buttock and leg lipoatrophy became non-significant (p-values of 0.15 and 0.08 respectively). The association between haplogroup T and buttock lipoatrophy diminished to borderline significance ($p = 0.07$). All other results were non-significant.

Haplogroup H is composed of 6 distinct subhaplogroups (H1-H6) which are separated by SNPs 3010 G>A in 16S rRNA (non-coding), 1438 A > G in the 12S gene (consensus), 6776 C > T in the Cytochrome Oxidase I (synonymous), 4024G > A in ND1 (T240A), 4336 C > T in TQ (tRNA), and 3915 A > G in NDI (synonymous) as shown in Figure 1b. The haplogroups defined by 3010, 4336, and the H* (the remaining unclassified H mtDNA) haplogroup demonstrated significant associations with lipoatrophy in the same direction as the H haplogroup. The other haplogroups occurred infrequently (< 4%) in our sample; therefore any lack of association may simply be a consequence of their low prevalence and lack of power (for genotypes with frequency ~4%, power is only 13% for a OR=1.5 at $\alpha=0.05$).

Lipoatrophy and lipo-accumulation may arise via different mechanisms because they represent extremes in metabolism and are often independent [22]. In our patients, the presence lipo-accumulation in the back of the neck, known as a dorsocervical fat pad or “buffalo hump”, was correlated with lipoatrophy with a Pearson correlation coefficient of 0.2 ($p < 0.0001$). We saw a trend for T to be protective against the presence buffalo hump ($p = 0.06$, OR = 0.30, 95% CI = 0.09–1.04), but no other statistically significant associations were observed.

Discussion

We examined the genetic association between six major European mitochondrial DNA haplogroups and clinical severity of lipoatrophy in 410 patients receiving HAART in the MACS cohort. We found a significant association between haplogroup H and increased risk for lipoatrophy among men graded on lipoatrophy presence and severity in legs, arms and buttocks. We also observed a borderline significant association between the presence of haplogroup T and protection against lipoatrophy. In the context of previous studies of fat accumulation conducted in the ACTG cohort, we did not observe a significant association between the presence of haplogroup J and protection against fat loss as reported by Hulgan et al. 2008; however odds ratios in our study suggest J may be protective against lipoatrophy.

We recently investigated the relationship between mitochondrial haplogroups and rate of progression to AIDS in untreated patients infected with HIV where we observed an association between the J haplogroup and accelerated AIDS progression, as well as associations between certain U haplogroups and IWX and progression to disease [50]. Although the effects of HIV-virus and drugs would likely influence mitochondrial function through different mechanisms, it was important to determine whether mtDNA haplogroup risk associated with disease progression in untreated patients were later affiliated with adverse events in patients on HAART. The data in the present study suggest that risk associated in untreated patients is not a factor in risk for lipodystrophy in patients on NRTIs. In patients on HAART in the present study, no significant associations were found between J and lipoatrophy despite the strong association between J and accelerated AIDS progression in untreated patients. Further, in the present study, we found a strong associations between haplogroup H and increased risk of lipoatrophy, however, we saw no over-all association between haplogroup H and progression in untreated patients, and one group within H (H3, which contains a mutation 6776 C>T) was found to be protective against AIDS progression and death in transfusion patients. These data suggest the risk of drug-toxicities associated with different mitochondrial haplogroups in patients on NRTIs is not effected by and independent of associations between mitochondrial haplogroups and disease progression in untreated patients.

Although the mechanism by which lipoatrophy develops during NRTI exposure has not been elucidated, one of the proposed mechanisms is that the release of apoptosis-inducing factors by damaged mitochondria cause apoptosis of adipocytes and lead to peripheral fat loss [26, 34]. Variation in mtDNA among haplogroups may influence energy production efficiency, ROS generation, and levels of apoptosis. H in particular is thought to be tightly coupled to the production of ATP and consequently more ROS, while J and T are partially uncoupled, thus produce less ATP and therefore less ROS [35]. In patients with the H haplogroup, oxidative stress and subsequent apoptosis could be exacerbated by increased baseline ROS production compared to other mitochondrial haplogroups. Further, oxidative stress has also been proposed as a principle mechanism operative in NRTI-related mitochondrial toxicity that leads to mtDNA depletion and subsequent mitochondrial energetic deficiencies [27]. Again, the threshold effect of increased ROS production in the tightly coupled H haplogroup may worsen this effect. On the other hand, haplogroups J and T, which are uncoupled, had less atrophy, consistent with this hypothesis. However, the consistency in the protection of the T haplotype against both lipoatrophy and the presence of “buffalo hump” ($p=0.07$) appears to support the hypothesis that fat loss and accumulation are part of a single syndrome [58, 59], and a better understanding of the pathology of NRTI-related lipodystrophy, as well as additional genetic studies are needed to elucidate a mechanistic relationship between mitochondrial haplogroup and altered fat distribution.

One other potential explanation for the observed associations involves consideration of the strong phylo-geographic structure of mitochondrial haplogroups. Because of this phenomenon,

it is possible that the associations observed in our study are correlated with background nuclear genetic effects that are distinctive between geographically separated populations. However, population stratification analysis using 304 autosomal markers in a previous study[50], and ongoing work in our lab based on a genome scan did not reveal significant geographic structure in the mitochondrial haplogroups associated with lipoatrophy. Regardless, it will be important to repeat these associations in populations of different ethnic background.

Our results would no longer be significant if a conservative Bonferoni correction was performed, however we did see more significant associations than would be expected by chance, which implies that the mitochondrial genotype has at least a moderate effect on lipoatrophy. We also realize that lipoatrophy is difficult to assess, and therefore the results could potentially mask a stronger relationship. An indication that this may be the case is the very strong association between low BMI and severe atrophy. This inverse correlation may reflect the slowed diagnosis of moderate to severe atrophy in those with very high BMIs and hence very high peripheral fat depots.

Although many mechanisms are likely involved in the development mitochondrial dysfunction and subsequent lipoatrophy, this study demonstrates that mitochondrial haplogroup may be an important genetic factor in the development of lipoatrophy associated with NRTI treatment in HIV-infected persons.

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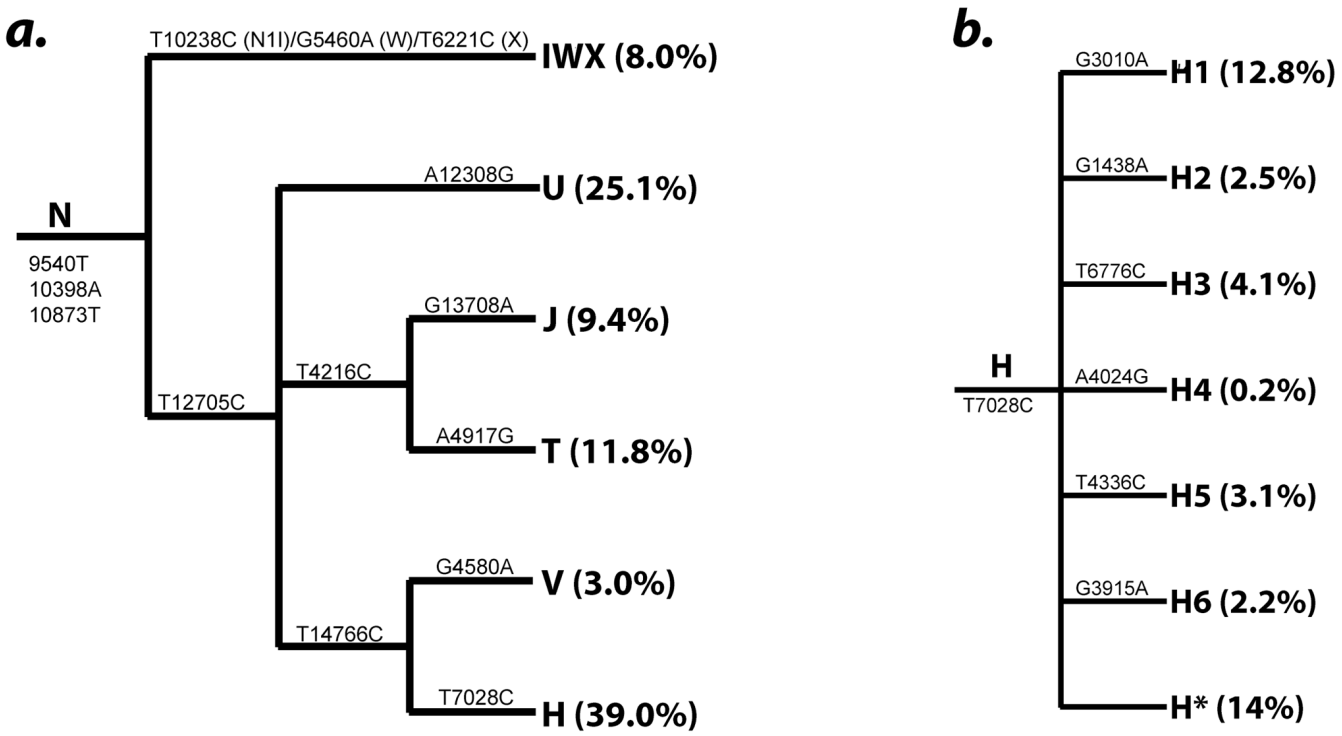

Figure 1.
Relationships between the European mtDNA haplogroups surveyed in this study and the SNPs used to identify them: (*a*.) Macro-haplogroup N, and (*b*.) a detail of the subhaplogroups within haplogroup H and the SNPs supporting each branch.

Table 1

Basic characteristics of European American patients in the MACS cohort in this study. Clinical visits for each patient were approximately 6 months apart.

Variable	Mean (sd)
Age at HAART	43.8 (7.0)
Baseline BMI	24.9 (3.5)
Baseline HIV-1 RNA (copies/mL)	87507 (193956)
Baseline CD4 (cells/mL)	365.5 (298.12)
AIDS prior to HAART	20.0%
Weight[1] (kg)	78.8 (13.3)
Visits on AZT[2]	7.2 (6.3)
Visits on d4TD4T[2]	6.2 (5.6)
Total Visits[2]	32.0 (7.5)

[1] at time of atrophy assessment

[2] prior to assessment visit

sd, standard deviation.

Table 2

Odds ratios (OR), 95% confidence interval, p-values (p), and population frequency for covariates included in the mtDNA haplogroup models for severity of lipoatrophy in three anatomical sites.

mtDNA haplogroup	Buttocks		Legs		Arms	
	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)
Age	0.17	1.02 (0.99–1.05)	0.03	1.03 (1.00–1.06)	0.06	1.03 (1.00–1.06)
AZT visits	<0.0001	1.06 (1.03–1.09)	<0.0001	1.07 (1.04–1.10)	<0.0001	1.07 (1.04–1.10)
D4T visits	<0.0001	1.07 (1.04–1.10)	<0.0001	1.08 (1.04–1.11)	0.001	1.05 (1.02–1.09)
BMI	<0.0001	0.44 (0.33–0.59)	<0.0001	0.52 (0.40–0.68)	<0.0001	0.41 (0.30–0.55)

All tests were done using Proportional Odds Logistic Regression (POLR) models with lipoatrophy scored as 0=no atrophy, 1=mild, 2=moderate, and 3=severe. BMI at the time of the assessment was scored as underweight(<18.5), normal (≥18.5 to <24.9), overweight (24.9<BMI<29.9), and obese (BMI ≥29.9).

Table 3

Odds ratios (OR), 95% confidence interval, p-values (p), and population frequency for major European mtDNA haplogroups with severity of lipoatrophy in three anatomical sites.

mtDNA haplogroup	Freq(%)	Buttocks		Legs		Arms	
		p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)
IWX	**8.0**	0.10†	1.75 (0.89–3.42)	0.38	1.35 (0.69–2.65)	0.51	1.27 (0.63–2.55)
U	**25.1**	0.33	0.79 (0.49–1.27)	0.40	0.82 (0.52–1.30)	0.14	0.69 (0.42–1.12)
H	**39.0**	**0.10**	1.41 (0.94–2.12)	**0.03**	**1.54 (1.03–2.31)**	**0.007****	**1.77 (1.17–2.69)**
V	**3.0**	**0.07**	**2.59 (0.93–7.26)**	0.13	2.2 (0.79–6.22)	0.23	1.94 (0.66–5.66)
J	**9.4**	0.52	0.80 (0.40–1.59)	0.43†	0.76 (0.39–1.50)	0.30	0.68 (0.33–1.41)
T	**11.8**	**0.05**	**0.52 (0.2–1.00)**	0.12	0.61 (0.33–1.14)	0.43	0.77 (0.41–1.46)

All tests were done using Proportional Odds Logistic Regression (POLR) models with lipoatrophy scored as 0=no atrophy, 1=mild, 2=moderate, and 3=severe. The age at of the patients at the beginning of treatment, BMI at the time of the assessment [scored as underweight(<18.5), normal (≥18.5 to <24.9), overweight (24.9<BMI<29.9), and obese (BMI ≥29.9)], and number of visits with AZT and d4T use were included as covariates in the model. N=410. Rare, Loosely associated haplotypes R*, HV* and JT* were excluded from individual analysis and therefore the total of frequencies shown is 96.3%. Likewise, NIb, W, and X were not analyzed in individual analyses due to low sample size.

† POLR model had a significant score test, which suggests the model assumptions were violated, likely due to small cell size.

** Indicates p-value remained significant when BMI was removed from the POLR model.