

# The mitochondrial pharmacogenomics of haplogroup T: MTND2\*LHON4917G and antiretroviral therapy-associated peripheral neuropathy

JA Canter<sup>1</sup>, DW Haas<sup>2,3</sup>,  
AR Kallianpur<sup>4</sup>, MD Ritchie<sup>1</sup>,  
GK Robbins<sup>5</sup>, RW Shafer<sup>6</sup>,  
DB Clifford<sup>7</sup>, DG Murdock<sup>1</sup>  
and T Hulan<sup>2</sup>

<sup>1</sup>Center for Human Genetics Research, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN, USA; <sup>2</sup>Division of Infectious Diseases, Vanderbilt University School of Medicine, Nashville, TN, USA; <sup>3</sup>Department of Microbiology and Immunology; Vanderbilt University School of Medicine, Nashville, TN, USA; <sup>4</sup>Division of General Internal Medicine and Public Health, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN, USA; <sup>5</sup>Department of Medicine, Massachusetts General Hospital, Harvard University, Boston, MA, USA; <sup>6</sup>Department of Medicine-Infectious Diseases, Stanford University, Stanford, CA, USA and <sup>7</sup>Departments of Neurology and Medicine, Washington University School of Medicine, St Louis, MO, USA

## Correspondence:

Dr JA Canter, Center for Human Genetics Research, 519 Light Hall, Vanderbilt University Medical Center, Nashville, TN 37212, USA.  
E-mail: jeff.canter@vanderbilt.edu

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Peripheral neuropathy (PN) due to mitochondrial injury complicates HIV therapy with some nucleoside reverse transcriptase inhibitors (NRTIs). Variation in the mitochondrial genome may influence susceptibility to NRTI toxicities. Two non-synonymous mitochondrial DNA polymorphisms, MTND1\*LHON4216C (4216C) and MTND2\*LHON4917G (4917G) were characterized in HIV-infected participants exposed to NRTIs in a randomized clinical trial. Among 250 self-identified white, non-Hispanic participants, symptomatic PN ( $\geq$  grade 1) developed in 70 (28%). Both 4216C (odds ratio (OR) = 1.98 (95% confidence interval (CI) 1.05–3.75);  $P = 0.04$ ) and 4917G (OR = 2.93 (95% CI 1.25–6.89);  $P = 0.01$ ) were more frequent in PN cases. These two polymorphisms remained independently associated with PN after adjusting for age, baseline CD4 count, plasma HIV RNA level, and NRTI randomization arm; 4216C (OR = 2.0 (95% CI 1.1–4.0)  $P = 0.04$ ) and 4917G (OR = 5.5 (95% CI 1.6–18.7)  $P < 0.01$ ). When 4917G individuals were excluded from the analysis, the association with 4216C was no longer seen. The mitochondrial 4917G polymorphism may increase susceptibility to NRTI-associated PN.

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## Introduction

Potent antiretroviral therapies have reduced acquired immunodeficiency syndrome (AIDS) morbidity and mortality.<sup>1,2</sup> The nucleoside reverse transcriptase inhibitors (NRTIs) were the first drugs approved to treat HIV infection and are cornerstones of multidrug antiretroviral regimens. These compounds are structural analogs of adenosine, guanosine, cytidine or thymidine. The active intracellular phosphorylated anabolites of NRTIs compete with endogenous nucleotides for incorporation into the proviral DNA causing premature chain termination during reverse transcription.<sup>3,4</sup> Unfortunately, prolonged exposure to NRTIs may cause a number of toxicities. This was first noted when patients treated with zidovudine (ZDV) developed skeletal myopathies with ragged-red fibers, a hallmark of mitochondrial dysfunction.<sup>5</sup> There were also significantly decreased levels of mitochondrial DNA in the muscle and liver tissues of these patients.<sup>4,6,7</sup> These untoward effects are due in part to the

inhibition of mitochondrial DNA polymerase- $\gamma$  by NRTIs.<sup>8,9</sup> Additional toxicities associated with NRTIs include peripheral neuropathy (PN), lipoatrophy, hepatic steatosis and lactic acidosis.<sup>10</sup>

PN can develop in HIV-infected persons during exposure to NRTIs, especially didanosine (ddI) and stavudine (d4T).<sup>11,12</sup> This debilitating complication is characterized by symmetric distal anesthesia and/or painful dysesthesia.<sup>13</sup> PN is also a common finding in inherited mitochondrial disease.<sup>14–16</sup> Elevated blood lactate levels, another sign of mitochondrial dysfunction, were also found in patients developing PN while on NRTIs.<sup>17</sup> Abnormal mitochondria and mitochondrial DNA depletion have also been seen with ddI-associated PN.<sup>18</sup> Similarities between the clinical manifestations of inherited mitochondrial diseases and NRTI toxicities prompted us to look for variations in the mitochondrial genome that may help explain susceptibility to PN among HIV-infected persons.

Mitochondria are cytoplasmic organelles that have their own DNA.<sup>14</sup> This mitochondrial genome encodes for 13 subunits of the electron transport chain, as well as a complete set of ribosomal RNAs and transfer RNAs.<sup>14</sup> This chain of multisubunit protein complexes is embedded in the inner mitochondrial membrane and produces about 90% of cellular ATP.<sup>19,20</sup> In addition to the key role in cellular energy production, mitochondria are also involved in free radical generation and apoptosis.<sup>15,19,20</sup> The mitochondrial genome is distinct from the nuclear genome and exhibits abundant genetic variation across its 16 569 base pairs.<sup>14,15</sup> Mitochondrial DNA undergoes sequence evolution approximately 10–17 times faster than nuclear DNA.<sup>15</sup> Each mitochondrion contains many copies of this circular, maternally inherited genome.<sup>14,15</sup>

Human mitochondrial genomes have diverged over approximately the last 150 000 years due to natural selection and human migration. The result is many distinct patterns of single-nucleotide polymorphisms in the mitochondrial DNA, called haplogroups.<sup>14–16,21–23</sup> This genetic variation leads to distinctive human mitochondrial electron transport chains. We hypothesize that these haplogroups have different capacities for energy production, free radical generation and apoptosis. Epidemiological evidence for functional differences among haplogroups has been demonstrated previously in studies of neurodegenerative disorders including Parkinson's disease, Alzheimer's disease, Friedreich's ataxia and amyotrophic lateral sclerosis.<sup>24–28</sup>

We previously described an association between haplogroup T and NRTI-associated PN among HIV-infected US participants in a prospective, randomized clinical trial.<sup>29</sup> Mitochondrial haplogroup T is found primarily in people of European descent, hence our analyses focused on Caucasians. Of 137 Caucasians randomized to receive ddI plus d4T, 20.8% of those who developed PN belonged to haplogroup T compared with 4.5% of those who did not develop PN (OR 5.4; 95% confidence interval (CI), 1.4–25.1;  $P=0.009$ ). After adjusting for gender, baseline CD4 count and baseline plasma HIV RNA, only haplogroup T, randomization to ddI plus d4T and older age independently

predicted development of PN.<sup>29</sup> Because the single-nucleotide polymorphism at position 13 368 in the mitochondrial genome that is a marker for haplogroup T happens to be synonymous (not resulting in an amino-acid change), this specific polymorphism is unlikely to alter mitochondrial function and explain susceptibility to NRTI toxicity.<sup>22</sup> We therefore explored in greater detail the underlying genomics of mitochondrial haplogroup T.

Mitochondrial haplogroup T has previously been associated with several other phenotypes. Ruiz-Pesini *et al.*<sup>30</sup> identified an association between haplogroup T and male infertility due to asthenozoospermia. In the same study, Complex I function in haplogroup T was moderately decreased compared with other European mitochondrial haplogroups. Haplogroup T has also been associated with DIAMOAD, a rare disorder comprising diabetes insipidus, diabetes mellitus, optic atrophy and deafness.<sup>31,32</sup> In a study that associated haplogroup T with Parkinson's disease, a link with the non-synonymous, moderately conserved polymorphism at position 4216 was proposed.<sup>33</sup> This MTND1\*LHON4216C (4216C) polymorphism had previously been linked with Leber's Hereditary Optic Neuropathy (LHON).<sup>34,35</sup> This polymorphism substitutes a histidine for tyrosine in the ND1 subunit of Complex I in the mitochondrial electron transport chain. However, 4216C is found in both haplogroup T and haplogroup J. Another non-synonymous polymorphism, MTND2\*LHON4917G (4917G), is found almost exclusively in haplogroup T and has also been associated with LHON. Neither polymorphism is among the three primary LHON mutations, but both have been associated with other neurodegenerative phenotypes.<sup>17,31</sup> The objective of this study was to determine whether these two non-synonymous polymorphisms in the mitochondrial genome, MTND1\*LHON4216C and MTND2\*LHON4917G, are associated with the development of NRTI-associated PN in HIV-infected patients. To accomplish this we further analyzed the same study population in which we first identified an association between mitochondrial haplogroup T and PN.<sup>29</sup>

## Results

### Study population

A total of 980 volunteers in the US and Italy were randomized to receive ZDV plus lamivudine (3TC) or ddI plus d4T in combination with efavirenz, nelfinavir, or both in ACTG study 384. Five hundred twenty-six participants (54%) had DNA available for analysis and all were genotyped. Patients with DNA samples were similar to those without genetic material available with regard to age, gender, race, baseline HIV RNA, and baseline CD4 count.<sup>29</sup> We excluded 17 (3.2%) participants from our analyses because they had either a diagnosis of PN or signs consistent with that diagnosis before randomization. Also, because this is a study of polymorphisms associated with haplogroup T, which is found almost exclusively in non-Hispanic white populations, the remainder of the analysis was restricted to

this group ( $N=250$ ) because of insufficient power in the African-American or Hispanic-American groups to make meaningful comparisons. Baseline characteristics of these study participants are presented in Table 1. Of the 250 study participants, 137 (55%) were randomized to receive ddI plus d4T, whereas 113 (45%) were randomized to receive ZDV plus 3TC. Symptomatic PN, defined as  $\geq$  grade 1, developed in 70 (28%) people during the study follow-up period and developed more frequently in participants randomized to ddI + d4T (69 vs 31%,  $P<0.01$ ). The median follow-up time for this study was 2.3 years. Cases with PN were older than the unaffected controls (38 vs 35 years,  $P=0.01$ ). Baseline CD4 lymphocyte counts and HIV-1 RNA levels were not significantly different in cases compared with controls.

*Mitochondrial genotyping results*

As expected, 4216C and 4917G were more frequent in non-Hispanic whites (21.3 and 9.7%, respectively) than in African-Americans (3.9 and 1.3%, respectively). Among the 250 non-Hispanic whites, all were successfully genotyped for 4216C but two (<1%) could not be genotyped for 4917G. The 4917G allele was found almost exclusively in haplogroup T, whereas the 4216C allele occurred in both

haplogroup J and T. Only one individual with the 4917G polymorphism did not also have 4216C polymorphism.

*Analysis of mitochondrial genotypes with PN outcome*

Both 4216C and 4917G were significantly more frequent in cases than controls among non-Hispanic whites (Table 2). 4216C was present in 30% of cases compared with 18% of controls odds ratio (OR) = 1.98 (95% CI 1.05–3.75;  $P=0.04$ ). Twelve (50%) of 24 non-Hispanic Caucasian participants with 4917G developed PN during the study compared with 57 (25%) of 224 participants without this allele (OR = 2.93 (95% CI 1.25–6.89)  $P=0.01$ ). The association was stronger in those initially randomized to ddI plus d4T where the univariate OR for the 4216C allele was 2.5 (95%CI 1.1–5.6,  $P=0.03$ ) and for the 4917G allele was 5.5 (95% CI 1.6–18.7,  $P<0.01$ ).

To determine whether these mitochondrial polymorphisms were independently associated with PN, we adjusted for potential confounders by using a multivariate logistic model that incorporated age, gender, drug randomization arm (ddI plus d4T vs ZDV plus 3TC, as well as blinded efavirenz, nelfinavir, or both), baseline CD4 T-cell count, and baseline plasma HIV-1 RNA concentration. In separate models, both

**Table 1** Baseline characteristics of non-Hispanic white participants from ACTG Study 384 ( $N=250$ )

Variable <sup>a</sup>	Cases (N = 70)	Controls (N = 180)	P-value
Age in years	38 (21–64)	35 (21–64)	0.015
Median (range)			
Female gender (%)	13 (7%)	6 (9%)	0.71
Randomized therapy (%)			
ddI+d4T	48 (69%)	89 (49%)	<0.01
Efavirenz	16 (33%)	22 (25%)	
Nelfinavir	10 (21%)	34 (38%)	
Both	22 (46%)	33 (37%)	
ZDV+3TC	22 (31%)	91 (51%)	<0.01
Efavirenz	7 (32%)	30 (33%)	
Nelfinavir	9 (41%)	27 (30%)	
Both	6 (27%)	34 (37%)	
Baseline HIV RNA ( $\log_{10}$ copies/ml)	5.3 (4.7–5.7)	5.1 (4.4–5.6)	0.11
Baseline CD4 Lymphocytes (cells/mm <sup>3</sup> )	251 (56–429)	305 (114–452)	0.14

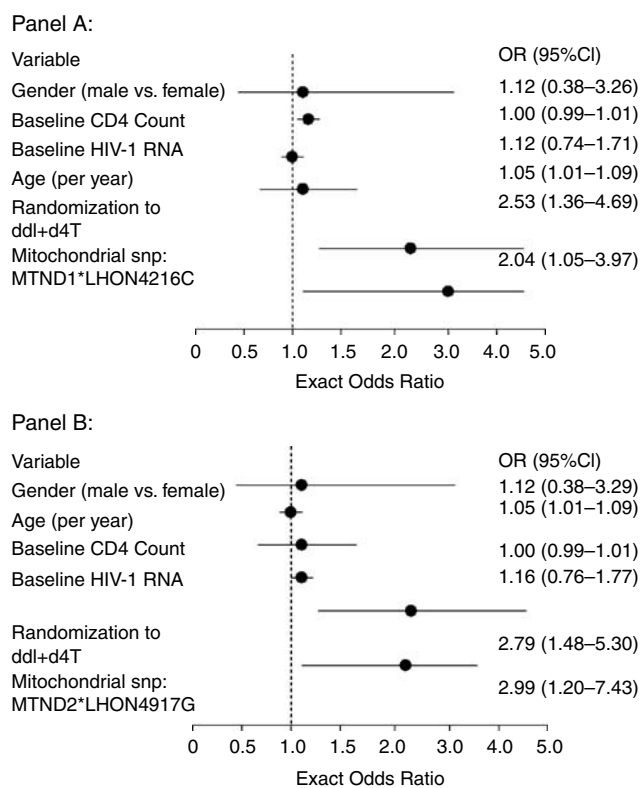
Abbreviations: ddI, didanosine; d4T, stavudine; PN, peripheral neuropathy; 3TC, lamivudine; ZDV, zidovudine.

<sup>a</sup>Fisher’s exact test or Wilcoxon rank-sum test used for comparison between PN cases and unaffected controls.

**Table 2** Frequencies of MTND1\*LHON4216C and MTND2\*LHON4917G genotyping results by PN status

	Cases	Controls	OR (95% CI)	P-value
MTND1*LHON4126C	21 (30%)	32 (17.8%)	1.98 (1.05–3.75)	0.04
MTND1*LHON4126T	49	148	Reference	
MTND2*LHON4917G	12 (17.4%)	12 (6.7%)	2.93 (1.25–6.89)	0.01
MTND2*LHON4917A	57	167	Reference	

Abbreviations: CI, confidence interval; PN, peripheral neuropathy; OR, odds ratio.



**Figure 1** Multivariable analysis of MTND1\*LHON4216C (Panel A) and MTND2\*LHON4917G (Panel B) in relation to peripheral neuropathy (PN). Odds ratio plots from the multivariate analyses of peripheral neuropathy among non-Hispanic white ACTG 384 Study participants. In both cases, the mitochondrial polymorphisms were independent predictors of the PN outcome after adjustment for the listed potential confounders.

4216C and 4917G were independently associated with PN, with adjusted OR 2.0 (95% CI 1.1–4.0,  $P=0.04$ ) and 3.0 (95% CI 1.2–7.4,  $P=0.02$ ), respectively (Figure 1).

Among the subgroup of 53 non-Hispanic whites with the 4216C allele, 12 (52%) of the 23 with the 4917G allele developed PN compared with nine (30%) of the 30 without this allele ( $P=0.10$ ). When those with the 4917G allele are excluded from the multivariate analysis described above, the independent association of the 4216C allele with the PN outcome was no longer seen (OR=1.4, 95% CI 0.6–3.4,  $P=0.44$ ). Therefore, it appears that the 4917G polymorphism may increase susceptibility to the development of NRTI-associated PN.

## Discussion

In this study, we report that a specific allele, 4917G, in the mitochondrial genome that alters the amino-acid sequence of a critically important subunit of Complex I in the electron transport chain was associated with increased risk for the development of NRTI-associated PN. This variation remained a significant independent predictor of

that outcome after controlling for other environmental and demographic risk factors. This, to our knowledge, is the first specific non-synonymous genetic variation related to the development of NRTI-associated PN. The size of the effect detected in this study appears to be considerable for the 10% of the non-Hispanic Caucasian population with the 4917G allele.

Mitochondrial genetic variation has been previously implicated in untoward complications of drug therapy. Increased susceptibility to aminoglycoside ototoxicity has been associated with mitochondrial ribosomal RNA gene mutations.<sup>36</sup> A recent study identified an increased risk for cisplatin ototoxicity associated with European haplogroup J.<sup>37</sup> Our study adds to the growing evidence that variation in the mitochondrial genome has relevance for pharmacogenomics. This should not be surprising given the vital importance of mitochondria in free radical generation, apoptosis and cellular energy production.<sup>38–40</sup>

Although this study suggests a relationship between 4917G and NRTI-associated PN based on epidemiological evidence, the underlying biologic mechanisms remain to be elucidated. Functional alterations in protein subunits of the electron transport chain may result in greater electron leakage and increased formation of reactive oxygen species. Concomitant exposure to NRTIs could make this latent tendency clinically evident. The result may be destruction of critically important biological macromolecules and possibly damage to both mitochondrial and nuclear DNA culminating in mitochondrial dysfunction with, in this case, compromised neuronal function.<sup>38–40</sup> Certain mitochondrial polymorphisms, such as those at position 4917, are associated with late-onset neurodegenerative diseases and their relatively high frequency suggests that they do not severely affect reproductive fitness.<sup>24–28</sup> For this reason, there may have been relatively little selective pressure against these potentially deleterious polymorphisms. The present findings point the way for future studies to quantify the biochemical effects of the 4917G allele, especially as they relate to free radical generation. Replication of our findings in other study populations is needed to validate the observed association. It remains possible that this allele, despite the fact that it results in a non-synonymous change in a key subunit in Complex I, is in linkage disequilibrium with more important causative polymorphisms. Epistatic interactions between loci within the mitochondrial genome and between nuclear and mitochondrial genes should also be investigated further.

Somatic mutations that accumulate in the mitochondrial DNA over the lifetime of a given individual can result in a heteroplasmic mixture of mutant and wild-type DNA.<sup>14,16</sup> Heteroplasmy, this variable proportion of mutant and wild-type mitochondrial DNA, can also affect the clinical presentation of rare familial mitochondrial diseases.<sup>14,16</sup> The polymorphic variations evaluated in this study were homoplasmic, having only single form of the polymorphism present, and represent intrinsic maternal germline variation.

Because mitochondrial haplogroup T is classified by a synonymous single-nucleotide polymorphism at position 13368 in the Torroni/Wallace European haplogroup system, we sought to look elsewhere for the mitochondrial genetic variation that might possibly underlie the potential connection with NRTI-associated PN.<sup>22</sup> 4216C and 4917G emerged as suitable candidate polymorphisms because of their previous association with LHON and other neurodegenerative processes, including PN.<sup>41,42</sup> Case reports of HIV-infected individuals with primary LHON mutations documented onset of the disease phenotype after exposure to antiretroviral therapy.<sup>43–47</sup> These reports suggested that drug exposure unmasked a genetic predisposition to this neurodegenerative process. Non-genetic factors are clearly important in the development of NRTI-associated PN. In this study, age and specific NRTI regimen (ddI + d4T) as expected were major factors influencing susceptibility to PN. Measurement of the exact contribution of each of these factors as well as that of the mitochondrial genetic variations will come from prospective modeling in a validation cohort. However, our analysis took into account several previously reported risk factors for the development of this pharmacologic complication and the 4917G allele remained independently associated with this phenotype.

In summary, a specific variation, 4917G, in the mitochondrial genome may increase susceptibility to NRTI-associated PN. The strength of association suggests that this common allele may help explain the relationship between mitochondrial haplogroup T and NRTI-associated PN. Future pharmacogenomic research into the complications of HIV therapy will need to consider the population frequencies of this polymorphism. As we continue to look for factors that will allow us to prevent drug toxicities and to individualize therapy, we need to remember that humans have two genomes and that both deserve our attention.

## Materials and methods

### Study population ascertainment

Participants from AIDS Clinical Trials Group (ACTG) study 384, a multicenter randomized controlled trial that enrolled volunteers in the United States and Italy between October 1998 and November 1999, were included in this analysis. Eligibility for ACTG 384 required fewer than 7 days of previous antiretroviral therapy and plasma HIV RNA of at least 500 copies/ml. Participants were randomized to receive either ddI plus d4T or ZDV plus 3TC, each in combination with efavirenz, nelfinavir, or both. All NRTIs were open-labeled. Specific details related to ACTG 384 have been published elsewhere.<sup>48</sup> Race and ethnicity were based on self-identification. Human DNA was obtained under ACTG protocol A5128.<sup>49</sup> Studies ACTG 384 and A5128 were approved by institutional review boards at each of the sites and all participants provided written informed consent. The ACTG and Vanderbilt Committee for the Protection of Human Subjects approved this use of DNA.

### Definition of clinical outcome: development of PN

Signs, symptoms and diagnoses were recorded at study entry and at each study visit during ACTG study 384 using a symptom distress self-report questionnaire that included pain and neuropathy. PN was graded according to the Division of AIDS (National Institutes of Health, Bethesda, MD, USA) Table for Severity of Adult Adverse Experiences as described elsewhere.<sup>29</sup> Cases in this study developed new PN of at least grade 1 severity during participation in ACTG 384. Controls were participants who did not develop signs and symptoms of PN. Participants with signs and/or symptoms of PN before randomization were excluded from the analyses.

### Genetic analyses (DNA analysis)

DNA was isolated from whole blood using PUREGENE (Gentra Systems Inc., Minneapolis, MN, USA). Genotyping was performed with the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc., Foster City, CA, USA) using the 5' nuclease allelic discrimination Taqman assay. The mtDNA MTND1\*LHON4216C polymorphism was detected using the same ABI sequence detection system. Primer and probe sequences are as follows: TaqMan MGB probe for the T allele: 6-FAM-AGCATTACTTA TATGATATGTC; TaqMan MGB Probe for the C allele: VIC-TAGCATTACTTATATGACATGTC; 4216 forward primer, TCCTATGAAAAAAGCTCCTACCACTCA; 4216 reverse primer, GCTGGAGATTGTAATGGGTATGG. The mtDNA MTND2\*LHON4917G polymorphism was detected by using a MGB Eclipse Probe (Nanogen, Bothell, WA, USA) because of a polymorphism at position 4818. This sequence is available upon request. Genotypic data were analyzed using ABI Sequence Detection System version 2.1 software and confirmed by visual inspection of the plots. Genotypes were classified as undetermined if PCR amplification failed with the specified sets of probes and primers.

### Statistical analysis

Mitochondrial genotype frequencies were compared between cases and controls using Fisher's exact test or Pearson's  $\chi^2$  tests. The MTND1\*LHON4216C genotype frequencies were calculated as the proportion of cases or controls that carried T or C alleles and in the case of MTND2\*LHON4917G, the A or G alleles. These polymorphisms are generally homoplasmic. Continuous variables were compared using Student's *t*-test or Mann-Whitney *U* as appropriate. Tests for statistical significance were two-sided with an  $\alpha$ -level of 0.05. Multivariable logistic regression was used to assess relationships between independent variables and outcome. Effect sizes for associations are measured as OR with 95% CIs. Stata 9.0 (College Station, TX, USA) was used for all statistical analyses.

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## Duality of interest

The authors have no duality of interest to disclose.

## References

- Palella Jr FJ, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* 1998; **338**: 853–860.
- Mocroft A, Ledergerber B, Katlama C, Kirk O, Reiss P, d'Arminio Monforte A et al. Decline in the AIDS and death rates in the EuroSIDA study: an observational study. *Lancet* 2003; **362**: 22–29.
- Kakuda TN. Pharmacology of nucleoside and nucleotide reverse transcriptase inhibitor-induced mitochondrial toxicity. *Clin Ther* 2000; **22**: 685–708.
- Dagan T, Sable C, Bray J, Gerschenson M. Mitochondrial dysfunction and antiretroviral nucleoside analog toxicities: what is the evidence. *Mitochondrion* 2002; **1**: 397–412.
- Gertner E, Thurn JR, Williams DN, Simpson M, Balfour Jr HH, Rhame F et al. Zidovudine-associated myopathy. *Am J Med* 1989; **86**: 814–818.
- Lewis W, Copeland WC, Day BJ. Mitochondrial DNA depletion, oxidative stress, and mutation: mechanisms of dysfunction from nucleoside reverse transcriptase inhibitors. *Lab Invest* 2001; **81**: 777–790.
- Walker UA, Bauerle J, Laguno M, Murillas J, Mauss S, Schmutz G et al. Antiretroviral therapy with didanosine, stavudine and zalcitabine is associated with depletion of mtDNA in the liver. *Antivir Ther* 2003; **8**: L15–L16.
- Lewis W, Day BJ, Copeland WC. Mitochondrial toxicity of NRTI antiviral drugs: an integrated cellular perspective. *Nat Rev Drug Discov* 2003; **2**: 812–822.
- Genschenson M, Nguyen VT, St Claire MC, Harbaugh SW, Harbaugh JW, Proia LA et al. Chronic stavudine exposure induces hepatic mitochondrial toxicity in adult *Erythorhynchus patas* monkeys. *J Hum Virol* 2001; **4**: 335–342.
- Moyle G. Clinical manifestations and management of antiretroviral nucleoside analog-related mitochondrial toxicity. *Clin Ther* 2000; **22**: 911–936.
- Browne MJ, Mayer KH, Chafee SB, Dudley MN, Posner MR, Steinberg SM et al. 2',3'-didehydro-3'-deoxythymidine (d4T) in patients with AIDS or AIDS-related complex: a phase I trial. *J Infect Dis* 1993; **167**: 21–29.
- Kelleher T, Cross A, Dunkle L. Relation of peripheral neuropathy to HIV treatment in four randomized clinical trials including didanosine. *Clin Ther* 1999; **21**: 1182–1192.
- Keswani SC, Pardo CA, Cherry CL, Hoke A, McArthur JC. HIV-associated sensory neuropathies. *AIDS* 2002; **16**: 2105–2117.
- DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. *N Engl J Med* 2003; **348**: 2656–2668.
- Wallace DC, Brown MD, Lott MT. Mitochondrial DNA variation in human evolution and disease. *Gene* 1999; **238**: 211–230.
- Wallace DC. Mitochondrial diseases in man and mouse. *Science* 1999; **283**: 1482–1488.
- Brew BJ, Tisch S, Law M. Lactate concentrations distinguish between nucleoside neuropathy and HIV neuropathy. *AIDS* 2003; **17**: 1094–1096.
- Dalakas MC, Semino-Mora C, Leon-Monzon M. Mitochondrial alterations with mitochondrial DNA depletion in the nerves of AIDS patients with peripheral neuropathy induced by 2',3'-dideoxycytidine (ddC). *Lab Invest* 2001; **81**: 1537–1544.
- Wallace DC. Mitochondrial DNA sequence variation in human evolution and disease. *Proc Natl Acad Sci USA* 1994; **91**: 8739–8746.
- Papa S. Mitochondrial oxidative phosphorylation changes in the life span. Molecular aspects and pathophysiological implications. *Biochim Biophys Acta* 1996; **1276**: 87–105.
- Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J et al. Sequence and organization of the human mitochondrial genome. *Nature* 1981; **290**: 457–465.
- Torroni A, Huoponen K, Francalacci P, Petrozzi M, Morelli L, Scozzari R et al. Classification of European mtDNAs from an analysis of three European populations. *Genetics* 1996; **144**: 1835.
- Herrnstadt C, Howell N. An evolutionary perspective on pathogenic mtDNA mutations: haplogroup associations of clinical disorders. *Mitochondrion* 2004; **4**: 791–798.
- van der Walt JM, Nicodemus KK, Martin ER, Scott WK, Nance MA, Watts RL et al. Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. *Am J Hum Genet* 2003; **72**: 804–811.
- van der Walt JM, Dementieva YA, Martin ER, Scott WK, Nicodemus KK, Kroner CC et al. Analysis of European mitochondrial haplogroups with Alzheimer disease risk. *Neurosci Lett* 2004; **365**: 28–32.
- Shoffner JM, Brown MD, Torroni A, Lott MT, Cabell MF, Mirra SS et al. Mitochondrial DNA variants observed in Alzheimer disease and Parkinson disease patients. *Genomics* 1993; **17**: 171–184.
- Giacchetti M, Monticelli I, De Biase L, Pianese L, Turano M, Filla A et al. Mitochondrial DNA haplogroups influence the Friedreich's phenotype. *J Med Gen* 2004; **41**: 293–295.
- Mancuso M, Francesca LC, Rocchi A, Tessitore A, Muglia M, Tedeschi G et al. Could mitochondrial haplogroups play a role in sporadic amyotrophic lateral sclerosis? *Neurosci Lett* 2004; **371**: 158–162.
- Hulgan T, Haas DW, Haines JL, Ritchie MD, Robbins GK, Shafer RW et al. Mitochondrial haplogroups and peripheral neuropathy during antiretroviral therapy: an adult AIDS clinical trials group study. *AIDS* 2005; **19**: 1341–1349.
- Ruiz-Pesini E, Lapena AC, Diez-Sanchez C, Perez-Martos A, Montoya J, Alvarez E et al. Human mtDNA haplogroups associated with high or reduced spermatozoa motility. *Am J Hum Genet* 2000; **67**: 682–696.
- Hoffman S, Bezold R, Jaksch M, Kauffhold P, Obermaier-Kusser B, Gerbitz KD. Disease relevance of the so-called secondary Leber hereditary neuropathy mutations. *Am J Hum Genet* 1997; **60**: 1539–1542.
- Hofmann S, Bezold R, Jaksch M, Obermaier-Kusser B, Mertens S, Kauffhold P et al. Wolfram (DIDMOAD) syndrome and Leber hereditary optic neuropathy (LHON) are associated with distinct mitochondrial DNA haplotypes. *Genomics* 1997; **39**: 8–18.
- Ross OA, McCormack R, Maxwell LD, Duguid RA, Quinn DJ, Barnett YA et al. mt4216C variant in linkage with the mtDNA T1 cluster may confer a susceptibility to mitochondrial dysfunction resulting in an increased risk of Parkinson's disease in the Irish. *Exp Gerontol* 2003; **38**: 397–405.
- Torroni A, Petrozzi M, D'Urbano L, Sellitto D, Zeviani M, Carrara F et al. Haplotype and phylogenetic analyses suggest that one European-specific mtDNA background plays a role in the expression of Leber hereditary optic neuropathy by increasing the penetrance of the primary mutations 11778 and 14484. *Am J Hum Genet* 1997; **60**: 1107–1121.
- Orth M, Schapira AH. Mitochondrial involvement in Parkinson's disease. *Neurochem Int* 2002; **40**: 533–541.
- Fischel-Ghodsian N. Genetic factors in aminoglycoside toxicity. *Pharmacogenomics* 2005; **6**: 27–36.
- Peters U, Preisler-Adams S, Lanvers-Kaminsky C, Jurgens H, Lamprecht-Dinnesen A. Sequence variations of mitochondrial DNA and individual sensitivity to the ototoxic effect of cisplatin. *Anticancer Res* 2003; **23**: 1249–1255.
- Smeitink J, van den Heuvel L. Human mitochondrial complex I in health and disease. *Am J Hum Genet* 1999; **4**: 1505–1510.
- Robinson BH. Human Complex I deficiency: clinical spectrum and involvement of oxygen free radicals in the pathogenicity of the defect. *Biochim Biophys Acta* 1998; **1364**: 271–286.
- De Benedictis G, Rose G, Carrieri G, De Luca M, Falcone E, Passarino G et al. Mitochondrial DNA inherited variants are associated with successful aging and longevity in humans. *FASEB J* 1999; **13**: 1532.

- 41 Brown MD, Sun F, Wallace DC. Clustering of Caucasian Leber hereditary optic neuropathy patients containing the 11778 and 14484 mutations on an mtDNA lineage. *Am J Hum Genet* 1997; **60**: 381–387.
- 42 Torroni A, Petrozzi M, D’Urbano L, Sellitto D, Zeviani M, Carrara F *et al*. Haplotype and phylogenetic analyses suggest that one European-specific mtDNA background plays a role in the expression of Leber hereditary optic neuropathy by increasing the penetrance of the primary mutations 11778 and 14484. *Am J Hum Genet* 1997; **60**: 1107–1121.
- 43 Luke C, Cornely OA, Fricke J, Lehrer E, Bartz-Schmidt KU. Late onset of Leber’s hereditary optic neuropathy in HIV infection. *Br J Ophthalmol* 1999; **83**: 1204–1205.
- 44 Luzhansky JZ, Pierce AB, Hoy JF, Hall AJ. Leber’s hereditary optic neuropathy in the setting of nucleoside analogue toxicity. *AIDS* 2001; **15**: 1588–1589.
- 45 Mackey DA, Fingert JH, Luzhansky JZ, McCluskey PJ, Howell N, Hall AJ *et al*. Leber’s hereditary optic neuropathy triggered by antiretroviral therapy for human immunodeficiency virus. *Eye* 2003; **17**: 312–317.
- 46 Shaikh S, Ta C, Basham AA, Mansour S. Leber hereditary optic neuropathy associated with antiretroviral therapy for human immunodeficiency virus infection. *Am J Ophthalmol* 2001; **131**: 143–145.
- 47 Warner JE, Ries KM. Optic neuropathy in a patient with AIDS. *J Neuroophthalmol* 2001; **21**: 92–94.
- 48 Shafer RW, Smeaton LM, Robbins GK, De Gruttola V, Snyder SW, D’Aquila RT *et al*. Comparison of four-drug regimens and pairs of sequential three-drug regimens as initial therapy for HIV-1 infection. *N Engl J Med* 2003; **349**: 2304–2315.
- 49 Haas DW, Wilkinson GR, Kuritzkes DR, Richman DD, Nicotera J, Mahon LF *et al*. A multi-investigator/institutional DNA bank for AIDS-related human genetic studies: AACTG Protocol A5128. *HIV Clin Trials* 2003; **4**: 287–300.