Clinical and Pathological Features of Mitochondrial DNA Deletion Disease Following Antiretroviral Treatment

Certain nucleoside analog reverse transcriptase inhibitor (NRTI) antiretroviral drugs used to treat human immunodeficiency virus (HIV) infection lead to accelerated accumulation of somatic mitochondrial DNA (mtDNA) mutations.1 The clinical significance of this observation is unclear but a delayed-onset phenotype would be expected, perhaps years after the relevant drug exposure.

Methods | In our national mitochondrial diagnosis reference service, we have been referred several HIV-infected patients with neuromuscular symptoms where a mitochondrial etiology was suspected clinically, and investigations confirmed the presence of a mitochondrial myopathy, developing years after exposure to potentially mitochondrially toxic NRTIs. In this retrospective case series, we describe the clinical, histochemical, molecular, and imaging findings of the first 4 such patients.

Ethical approval for this study was obtained from the Newcastle and North Tyneside Local Research Ethics Committee, and written consent was obtained from patients.

Results | The clinicopathological characteristics are summarized in the Table. Patient 1 presented with progressive ataxia, with a background of sensorineural deafness and insulin-dependent diabetes mellitus with associated nephropathy requiring continuous ambulatory peritoneal dialysis. At the time of referral, HIV infection was treated with didanosine, lamivudine, and nevirapine for 8 years. Following assessment, didanosine was switched to abacavir. Magnetic resonance imaging of the brain revealed volume loss and periventricular and deep white matter signal change; however, these features were generalized rather than localized to the cerebellum. Proton magnetic resonance spectroscopy findings of the brain were normal. Sequential cytochrome C oxidase (COX)-succinate dehydrogenase (SDH) histochemical reactions revealed 30% COX-deficient fibers with approximately 10% of fibers showing SDH hyperintensity, suggestive of mitochondrial proliferation (Figure). Molecular analyses of skeletal muscle mtDNA showed increased mtDNA copy number and evidence of multiple mtDNA deletions amplified by long-range polymerase chain reaction assays.2 A screen of nuclear genes (POLG, POLG2, PEO1, RRM2B, SLC25A4, and TK2) associated with mtDNA maintenance disorders revealed no mutations.

Patients 2, 3, and 4 presented with myalgia, with or without mildly elevated creatine kinase (patient 2, 564 IU/L; patient 3, 841 IU/L; and patient 4, normal <320 IU/L) to convert serum creatine kinase to microkatal per liter, multiply by 0.0167). All had extensive past antiretroviral exposure including multiple polymerase γ-inhibiting NRTIs.3 Findings from nerve conduction studies revealed mild axonal sensorimotor neuropathy. Cytochrome C oxidase-SDH histochemistry revealed mosaic patterns (15%, 12%, and 1% for patients 2, 3, and 4, respectively) of COX deficiency. Phosphorus magnetic resonance spectroscopy of soleus muscle was performed in patients 3 and 4 and showed significant reduction in the maximal rate of postexercise adenosine triphosphate resynthesis. On molecular analy-

<table>
<thead>
<tr>
<th>Patient No./ Sex/Age, y</th>
<th>Duration of Diagnosed HIV, y</th>
<th>Lifetime ART History</th>
<th>Clinical Features</th>
<th>Mitochondrial Abnormalities*</th>
<th>31P-MRS</th>
<th>Serum CK, IU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/55</td>
<td>9</td>
<td>Zidovudine, lamivudine, efavirenz, didanosine, and nevirapine</td>
<td>Ataxia COX-deficient fibers (30%), multiple mtDNA deletions</td>
<td>Not performed</td>
<td>Normal (&lt;320)</td>
<td></td>
</tr>
<tr>
<td>2/M/63</td>
<td>25</td>
<td>Zidovudine, zalcitabine, lamivudine, saquinavir, indinavir, didanosine, stavudine, abacavir, efavirenz, amprenavir, nelfinavir, lopinavir/ritonavir, tenofovir, enfuvirtide, entricitabine, nevirapine, amprenavir/ritonavir, darunavir/ritonavir, maraviroc, and raltegravir</td>
<td>Myalgia COX-deficient fibers (15%)</td>
<td>Not performed</td>
<td>564</td>
<td></td>
</tr>
<tr>
<td>3/M/48</td>
<td>13</td>
<td>Zidovudine, didanosine, lamivudine, stavudine, ritonavir, nevirapine, indinavir, zalcitabine, abacavir, atazanavir/ritonavir, tenofovir, and abacavir</td>
<td>Myalgia COX-deficient fibers (12%), multiple mtDNA deletions</td>
<td>Impaired Q_{max}(ATP)</td>
<td>841</td>
<td></td>
</tr>
<tr>
<td>4/M/49</td>
<td>16</td>
<td>Zidovudine, zalcitabine, didanosine, lamivudine, stavudine, saquinavir, nevirapine, indinavir, nelfinavir, abacavir, ritonavir, lopinavir/ritonavir, entricitabine, and atazanavir/ritonavir</td>
<td>Myalgia COX-deficient fibers (1%), multiple mtDNA deletions</td>
<td>Impaired Q_{max}(ATP)</td>
<td>Normal (&lt;320)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ART, antiretroviral therapy; CK, creatine kinase; COX, cytochrome C oxidase; HIV, human immunodeficiency virus; M, male; mt, mitochondrial; 31P-MRS, phosphorus magnetic resonance spectroscopy; Q_{max}(ATP), maximal rate of postexercise adenosine triphosphate resynthesis; ritonavir (boosting dose).

S1 conversion factor: To convert CK to microkatal per liter, multiply by 0.0167.

* Respiratory chain biochemistry was not performed because health and safety regulations did not permit homogenate preparation using muscle from HIV-infected individuals in our diagnostic laboratory.
In all cases, the observed mtDNA defect comprised mtDNA deletions rather than an mtDNA depletion as reported historically.\(^5\) This argues for the importance of previous rather than current mtDNA depletion as reported in the most severely affected case failed to find a cause. We cannot wholly exclude the possibility of a 2-hit model of earlier exposure to polymeraseγ–inhibiting NRTIs. Although the frequency of COX-deficient fibers in patient 4 was low, the presence of somatic mtDNA mutations and abnormal muscle bioenergetics suggests he also had a mild mitochondrial myopathy. What is the likely cause of these findings?

Given that mitochondrial disorders presenting in adult life are rare (approximately 1 in 10 000),\(^4\) it seems likely that the patients we described have an iatrogenic disorder caused by polymericase γ–inhibiting dideoxynucleoside analogs are particularly worthy of further investigation.

**Discussion** | We describe 4 adults with treated HIV infection and evidence of mitochondrial dysfunction. Patients 1, 2, and 3 had significant levels of COX-deficient skeletal muscle fibers consistent with mitochondrial myopathy. Although the overall frequency of COX-deficient fibers in patient 4 was low, the presence of somatic mtDNA mutations and abnormal muscle bioenergetics suggests he also had a mild mitochondrial myopathy. What is the likely cause of these findings?

In all cases, the observed mtDNA defect comprised mtDNA deletions rather than an mtDNA depletion as reported historically.\(^5\) This argues for the importance of previous rather than current NRTI exposure.\(^4\) Therefore, we suggest that in HIV-infected patients presenting with neuromuscular symptoms, the possibility of an acquired mitochondrial defect should continue to be considered in those patients with a relevant treatment history. Those patients with historical exposure to the polymerase γ–inhibiting dideoxynucleoside analogs are particularly worthy of further investigation.

**Author Affiliations:** Wellcome Trust Centre for Mitochondrial Research, Institute of Genetic Medicine, Newcastle University, Newcastle-upon-Tyne, England (Payne, Gardner, Horvath, Chinnery); Department of Infection and Tropical Medicine, Royal Victoria Infirmary, Newcastle-upon-Tyne, England (Payne); Wellcome Trust Centre for Mitochondrial Research, Institute of Neuroscience, Newcastle University, Newcastle-upon-Tyne, England (Blakely, Taylor); Royal Derby Hospital, Derby, England (Maddison).

**Corresponding Author:** Brendan A. I. Payne, PhD, FRCPath, Wellcome Trust Centre for Mitochondrial Research, Institute of Genetic Medicine, Newcastle University, International Centre for Life, Central Parkway, Newcastle-upon-Tyne NE1 3BZ, England (brendan.payne@ncl.ac.uk).

**Author Contributions:** Dr Payne had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Payne, Chinnery.

**Acquisition, analysis, or interpretation of data:** All authors.

**Drafting of the manuscript:** Payne, Chinnery.

**Critical revision of the manuscript for important intellectual content:** All authors.

**Obtained funding:** Payne, Taylor, Chinnery.

**Administrative, technical, or material support:** Taylor.

**Study supervision:** Payne, Chinnery.

**Conflict of Interest Disclosures:** None reported.

**Funding/Support:** Dr Payne was funded by the Medical Research Council (United Kingdom). Dr Taylor receives support from the Wellcome Trust Centre for Mitochondrial Research (096919Z/11/Z), the Medical Research Council (United Kingdom) Centre for Translational Muscle Disease Research (G0601943) and the UK National Health Service Highly Specialised Rare Mitochondrial Disorders of Adults and Children Service. Dr Chinnery receives support from the Wellcome Trust (101876/Z/13/Z and 096919Z/11/Z), the Medical Research Council (United Kingdom) Centre for Translational Muscle Disease Research (G0601943), European Union FPT Treat Iron-Related Childhood-Onset Neuropathogenesis, and the National Institute for Health Research Newcastle Biomedical Research Centre based at Newcastle-upon-Tyne Hospitals National Health Service Foundation Trust and Newcastle University.

**Role of the Funder/Sponsor:** The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Depletion of muscle mitochondrial DNA in AIDS patients with clinically isolated syndromes or in relatives of patients with presymptomatic immunopathology, respectively. However, vaccine procedures are potentially capable of activating (auto)immune effectors in patients with MS with sustained disease activity, as well as in individuals at risk (eg, in persons with radiologically isolated syndromes or in relatives of patients with presymptomatic state). These conditions may be disclosed by a prevaccination contrast-enhanced magnetic resonance imaging of the brain and may deserve a postponement of vaccination even in case of subclinical signs of immunopathology. 6

Giovanni Ristori, MD
Rosella Mechelli, PhD
Marco Salvetti, MD

Author Affiliations: Centre for Experimental Neurological Therapies, S. Andrea Hospital-Site, Department of Neuroscience, Mental Health, and Sensory Organs (NESMOS), Sapienza University, Rome, Italy.

Corresponding Author: Marco Salvetti, MD, Faculty of Medicine and Psychology, Centre for Experimental Neurological Therapies, S. Andrea Hospital-Site, Department of Neuroscience, Mental Health, and Sensory Organs (NESMOS), Sapienza University, Via di Grottarossa 1035-1039, 00189 Rome, Italy (marco.salvetti@uniroma1.it).

Conflict of Interest Disclosures: None reported.


In Reply We thank the authors for their thought-provoking letter regarding our article. 4 To clarify, our data do not support the suggestion that vaccinations should be withheld or postponed from those individuals who have relatives with multiple sclerosis (MS) or radiologically isolated syndrome until brain imaging is performed as the risk for developing the first symptoms of MS 6 weeks after vaccination was not increased. In addition, we actually recommend the flu vaccine for our patients with MS because influenza illness is more likely to result in an MS relapse than influenza vaccination. 2 We agree with the authors that vaccination in the midst of an MS relapse is not advised.

Annette Langer-Gould, MD, PhD

Author Affiliation: Kaiser Permanente Southern California, Research and Evaluation, and Neurology, Pasadena, California.

Corresponding Author: Annette Langer-Gould, MD, PhD, Kaiser Permanente Southern California, Research and Evaluation, and Neurology, 100 S Los Robles, 2nd Floor, Pasadena, CA 91101 (annette.m.langer-gould@kp.org).

Conflict of Interest Disclosures: None reported.
