## RESEARCH LETTER

### 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. 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 diagnostic 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 mitochondrial 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. 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. 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 postexertional adenosine triphosphate resynthesis. On molecular analy-

### Table. Summary of Clinicopathological Features

<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</td>
<td>COX-deficient fibers (30%), multiple mtDNA deletions</td>
<td>Not performed</td>
<td>Normal (&lt;320)</td>
</tr>
<tr>
<td>2/M/63</td>
<td>25</td>
<td>Zidovudine, zalcitabine, lamivudine, saquinavir, indinavir, didanosine, stavudine, abacavir, efavirenz, ampicrenavir, nelfinavir, lopinavir/r, tenofovir, enfuvirtide, entricatabine, nevirapine, ampicrenavir/r, darunavir/r, maraviroc, and raltegravir</td>
<td>Myalgia</td>
<td>COX-deficient fibers (15%)</td>
<td>Not performed</td>
<td>564</td>
</tr>
<tr>
<td>3/M/48</td>
<td>13</td>
<td>Zidovudine, didanosine, lamivudine, stavudine, ritonavir, nevirapine, indinavir, zalcitabine, abacavir, atazanavir/r, tenofovir, and abacavir</td>
<td>Myalgia</td>
<td>COX-deficient fibers (12%), multiple mtDNA deletions</td>
<td>Impaired Qmax(ATP)</td>
<td>841</td>
</tr>
<tr>
<td>4/M/49</td>
<td>16</td>
<td>Zidovudine, zalcitabine, didanosine, lamivudine, stavudine, saquinavir, nevirapine, indinavir, nelfinavir, abacavir, tenofovir, lopinavir/r, entricatabine, and atazanavir/r</td>
<td>Myalgia</td>
<td>COX-deficient fibers (1%), multiple mtDNA deletions</td>
<td>Impaired Qmax(ATP)</td>
<td>Normal (&lt;320)</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; Qmax(ATP), maximal rate of postexertional adenosine triphosphate resynthesis; fr, ritonavir (boosting dose).

| **Note:** $1$ 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.

$2$ SI conversion factor: To convert CK to microkatal per liter, multiply by 0.0167.

$3$ 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.
exceptions (patients 3 and 4), mtDNA copy number in muscle was normal but large-scale mtDNA deletions were detected (not shown).

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?

Given that mitochondrial disorders presenting in adult life are rare (approximately 1 in 10,000), it seems likely that the patients we described have an iatrogenic disorder caused by earlier exposure to polymerase γ-inhibiting NRTIs. Although we cannot wholly exclude the possibility of a 2-hit model of NRTI exposure and a predisposing nuclear genetic defect of mtDNA maintenance, an extensive search for causative mutations (patients 3 and 4), mtDNA copy number in muscle was normal but large-scale mtDNA deletions were detected (not shown).

In all cases, the observed mtDNA defect comprised mtDNA deletions rather than an mtDNA depletion as reported historically. This argues for the importance of previous rather than current NRTI exposure. 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.

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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.

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COMMENT & RESPONSE

Association Between Vaccines and Neuroinflammation: Time, Risks, and Benefits

To the Editor We read with interest the work by Langer-Gould et al1 about the risk for demyelinating diseases after vaccinations. The major points of the article (no long-term risk for multiple sclerosis [MS] and short-term risk for neuroinflammation for any type of vaccination in younger individuals) suggest various levels of complexities.

Time (that is, age and time of vaccine administration) is important in the interplay between susceptible hosts and environmental factors.2 Previous work on the modeling of non-deterministic processes in MS development (random perturbations and time can amplify the effects of weak genetic and environmental factors)3 may provide a conceptual framework to interpret a timing effect of vaccines on neuroinflammation.

The fact that vaccinations of any type may represent a risk for short-term neuroinflammation is in keeping with previous results showing that, at least for environmental risk factors of viral origin, gene-environment interactions predispose to disease through perturbations that can be pathogen specific but also extensively shared by different viruses.4

A further level of complexity is the potential beneficial effect of the Bacille Calmette-Guérin vaccine in early MS and individuals with a first demyelinating episode,5 which may seem paradoxical considering the supposed relationship between adjuvanticity and autoimmunity. The mechanisms underlying immunomodulatory effects of Bacille Calmette-Guérin in neuroinflammation are not fully defined: the possibility that adjuvant immunotherapy may counteract a pathogenic loop due to Epstein-Barr virus (a known nonheritable risk factor in MS), involving Notch-1 signaling and Epstein-Barr nuclear antigen 2, suggests differences between benign exposure to microbes (as in most vaccinations) and infections in preventing or triggering immunopathology, respectively.

Waiting for the disentanglement of all these levels of complexities, we endorse the authors’ conclusion that no changes to vaccination policy are currently justified (rather, vaccines can even contribute to counteract the deficit of benign exposure to microbes that characterizes westernization and is strongly suspected of having favored the increased incidence of immunopathology in recent decades). 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 pre-disease 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 neuroinflammation.6

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In Reply We thank the authors for their thought-provoking letter regarding our article.7 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.

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