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MPV17-related hepatocerebral mitochondrial DNA depletion syndrome (MPV17-NNH) revisited

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ABSTRACT

MPV17-related hepatocerebral mitochondrial DNA depletion syndrome (previously known as Navajo neurohepatopathy) was discovered in children in the Four Corner's region of New Mexico approximately 40 years ago. This disease is associated with a single missense mutation in exon 2 in the *MPV17* gene. The syndrome has now been recognized world-wide. We find that huge quantities of neurotoxins were present in archived nervous tissues from such patients.

Arsenic was increased 18×, cadmium ~10×, cobalt 2.5× and manganese 2.3×; the largest increase was in mercury content 16,000× compared to contemporaneous fresh-frozen normal nervous tissues.

In the Four Corner's region of NM the life span is reduced compared to other parts of the United States and in our patients with MPV17-NNH the average life span was 5.4 years ± 2.7 (SE) years.

We now live in the Anthropocene an epoch characterized by large additions to the biosphere of neurotoxins. The effects of such toxic loads on human health and disease remain to be assessed.

We speculate how such high neurotoxin content in tissues, which is likely to increase during the Anthropocene, may have influenced MPV17-NNH and similar phenotypes in different parts of the world.

Our results imply that selenium supplementation to the diet in the Four Corner's region of NM might be beneficial to normal people and in the management of patients with MPV17-NNH syndrome.

1. Introduction

The first description of children with a previously unrecognized neuropathy in the Four Corners Region of New Mexico (NN) appeared in 1976 [1]. This disease is now named MPV17-related hepatocerebral mitochondrial DNA depletion syndrome (MPV17-NNH).

The clinical features of the disease were widespread anesthesia and painless fractures. Corneal ulcerations, muscle weakness, acral mutilation and absent tendon reflexes were also found. Intellectual function was normal; however, because of cultural and educational barriers the tests were unreliable. Autonomic testing showed extensive paralysis of vasomotor, cardiovascular and thermoregulatory functions in some patients [2]. Sural nerve biopsies showed nearly the total absence of myelinated fibers without evidence of regeneration. Unmyelinated fibers were also affected but regenerative features were present. Additional symptoms and signs included recurrent systemic infections, macronodular cirrhosis of the liver, sexual immaturity, small stature and poor weight gain.

Epidemiological evidence [3] suggested that the disease may run in families; about half the families had more than one affected member. Mean age at death was 10 years. The incidence on the Western part of the Four Corners region was 5 times higher than on the Eastern part [3].

Twenty children with NN were found to have liver disease [4]. The phenotype of this group of patients was not uniform: 1. early onset and progression to liver failure with death before age two; 2. some had onset between 1 and 5 years progressing to liver failure and death within 6 months; 3. nine patients had onset at different ages with progressive neurological symptoms and evidence of liver disease. The three phenotypes led to a name change from NN to neurohepatopathy (NNH) [4]. Similar syndromes were later reported from Egypt [5] Morocco [6] and Iraq [7]. Familial sensory autonomic neuropathy with arthropathy is different from MPV17-NNH. This disease has a different phenotype, and occurs in different families [8]. The molecular aspects of this disease are, as yet, unknown.

Management of the disease is by a multidisciplinary team including specialists in hepatology, neurology, nutrition, medical genetics, and child development. Nutritional support should be provided by a

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dietitian experienced in managing children with liver diseases; prevention of hypoglycemia requires frequent feeds and uncooked cornstarch (1–2 g/kg/dose). Although liver transplantation remains the only treatment option for liver failure, this is controversial because of the multisystem involvement in this disorder and suggestions that 2–3 years after successful transplantation neurological progression is inevitable [4].

Molecular studies on MPV17-NNH patients showed a single missense mutation [9] in exon 2 in the *MPV17* gene (MIM 137960); genetic analyses of unaffected individuals confirmed segregation with the disease which was attributed to a founder effect in the population of the Four Corners region [9]. Additional studies showed that *MPV17* is involved in mtDNA maintenance and in oxidative phosphorylation; it encodes an inner mitochondrial membrane protein and is mutated in infantile hepatic mitochondrial depletion [6]. A role for additional epigenetic modifiers of the disease has also been proposed [9,10,11].

We now live in the Anthropocene. A hallmark of this proposed geological epoch is the documented increase of heavy metals and other neurotoxins in the biosphere [11,12,13] with marked bioaccumulation in some species. However, the human health effects or tissue accumulations of the increasing levels of heavy metals in the biosphere have not been fully elucidated. Evidence suggests, however, that such byproducts of modernity may be linked to epigenetic variations which could play major parts in disease causation [11].

Here we report the heavy metal and neurotoxin content of tissues from patients with MPV17-related hepatocerebral mitochondrial DNA depletion syndrome (MPV17-NNH) and place the results into context with the known contaminations with toxic metals of the biosphere found in the Four Corners region of New Mexico, USA and in other parts of the world where MPV17-NNH occurs.

2. Materials and methods

We analyzed 13 archived, formalin-fixed, tissue-blocks. We deparaffinzed the blocks containing 9 sural nerves and 4 muscle samples obtained by biopsy from patients with clinical diagnoses of MPV17-NNH who were born and had lived in the Four Corners area. Similarly archived pons and proximal femoral nerve were obtained from 1 patient autopsy. Permissions for biopsy and for the autopsy were obtained from parents and/or relatives according to the usual standards of ethical medical practice current ~40 years ago.

We also analyzed 9 blocks of non-nervous tissues from individuals who never lived in the Four Corners region of New Mexico and did not have MPV17-NNH. These tissues were also obtained ~40 years ago. Both MPV17_NNH and control non-nervous tissues archived for the same period were obtained from the same hospital archives. Additionally we used brain and nerve tissue (fresh frozen, stored at −80 °F.) obtained from a brain bank (NeuroBioBankUniversity of Maryland, Baltimore, MD.) from the frontal, parietal, occipital and midbrain region from older individuals who had lived in the Eastern parts of the United States. Pooled sural nerves and pooled muscle tissues from patients with MPV17-NNH were analyzed. The autopsy material was analyzed separately. The non-nervous tissues were also analyzed in separate runs.

The paraffin was removed from the tissue by melting @65 °C. The tissue was then placed at room temperature into xylene for 1 h using the agitation stir-bar. Subsequently, tissues were placed into decreasing alcohol concentrations (100%, 95%, 80% and 50%) for 30 min each using the agitation stir-bar for each step; finally the tissues were placed into distilled water.

We used inductively coupled plasma-optical emission spectroscopy (ICP-OS, Perkin Elmer, Optima 5300DV) for As, Cd, Co, Mn, Pb, Se and U and flow injection mercury sampler (FIMS) for mercury analysis. We digested samples with acids and transferred the samples into 15 ml glass tubes. The system was optimized using mercury optical alignment and manganese view touch alignment. For FIMS the system was optimized and calibrated for Hg, using a blank and 3 calibrated standards, that were diluted sequentially in order to achieve a linear calibration curve. A set of quality control check samples (Initial Calibration Blank Verification "ICBV", Verification "ICV", and Continuing Calibration Verification ("CCV") were measured to verify and validate calibration and data quality. Data were reduced, verified, validated, and reported in mg/kg of elemental metals.

2.1. Statistical analyses

The overall analysis of the metal concentration profile between MPV17-NNH and control tissue samples is a 2-way analysis of variance (ANOVA). Post hoc comparison for each metal concentration between MPV17-NNH and controls follows Fisher's least significant difference strategy; concentrations were log-transformed to equalize variances. As an alternative metal ratios of concentrations for patients to control nerve tissue are reported as mean values ± standard error (SE) and the metal ratios across the profile of metals were compared by 1-way ANOVA. Post hoc comparison for each metal concentration ratio to one compares between MPV17-NNH and controls; concentration ratios were log-transformed to equalize variances. The overall analysis of life spans of different populations is also a 1-way ANOVA with post hoc pair-wise comparisons following Fisher's least significant difference strategy. P values ≤ 0.05 were considered statistically significant. In the analyses of samples from MPV-17patients we substituted uranium for selenium (see, supplemental on line material (S1)).

2.2. Results

The pathological features found in sural nerves and central nervous system blocks ~40 years after they were obtained are illustrated in Fig. 1.

Ratios (mean ± SE) of metal content in mg/kg in nervous tissues from MPV17-NNH to control nervous tissues from subjects who had not been living in the Four Corners region of New Mexico are given in Table 1.

Statistical analyses of the concentrations of metals in MPV17-NNH and control tissues are shown in Fig. 2. The concentrations of metals in MPV17-NNH tissue compared to controls, not from the Four Corners area of NM, were significantly higher. Although uranium and lead levels were higher in MPV17-NNH tissues than in controls these quantities did not reach statistical significance.

Fig. 3 shows the ratios of metal content for each metal analyzed in MPV17-NNH samples to controls not living in the Four Corners area of NM.

Fig. 4 shows comparisons of life spans of Hg and Pb intoxicated people who died from metal poisoning during the Renaissance (~600 years ago) in Italy. The average survival in Italy was 36 ± 9 years whereas in MPV17-NNH on the Western part of the Four Corners region was 9.3 ± 3.1 years ($P = 0.006$ for geometric means). Higher levels of both Hg and Pb were found in the tissues from the MPV17-NNH patients compared to tissues from Italy (Pb $P = 0.03$ and Hg $P = 0.01$) (Fig. 5).

Fig. 6 shows the control samples including neural tissue for selenium and uranium to all other metals.

3. Discussion

Over the course of some 40 years since its original description the disease has changed names seven times reflecting the studies carried out as more sophisticated techniques became available. Technical advances have also transformed this disease from a localized problem to a disease with worldwide incidence.

Here we document the metal content in MPV17-NNH tissues. An observational study such as this cannot prove that the neurotoxins we found in patient's tissues are causally related to the pathogenesis, nor to the symptoms of the disease. We speculate how such high neurotoxin content in tissues, which is likely to increase during the Anthropocene, may have influenced MPV17-NNH and similar phenotypes in different