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

Clifford Qualls [a], Mario Kornfeld [b], Nancy Joste [b], Abdul-Mehdi Ali [c], Otto Appenzeller [d,*]

[a] Department of Mathematics and Statistics, University of New Mexico, Albuquerque, NM 87122, United States
[b] Department of Pathology, University of New Mexico School of Medicine, Albuquerque, NM 87122, United States
[c] Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, NM 87122, United States
[d] Department of Neurology, NMHEMC Research Foundation, Albuquerque, NM 87122, United States

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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.

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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

* Corresponding author at: 361 Big Horn Ridge Drive, NE Albuquerque, NM 87122 USA.
E-mail addresses: clifford.qualls@gmail.com (C. Qualls), mkornfeld@salud.unm.edu (M. Kornfeld), njoste@salud.unm.edu (N. Joste), mehdiali@unm.edu (A.-M. Ali), ottoarun12@aol.com (O. Appenzeller).

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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

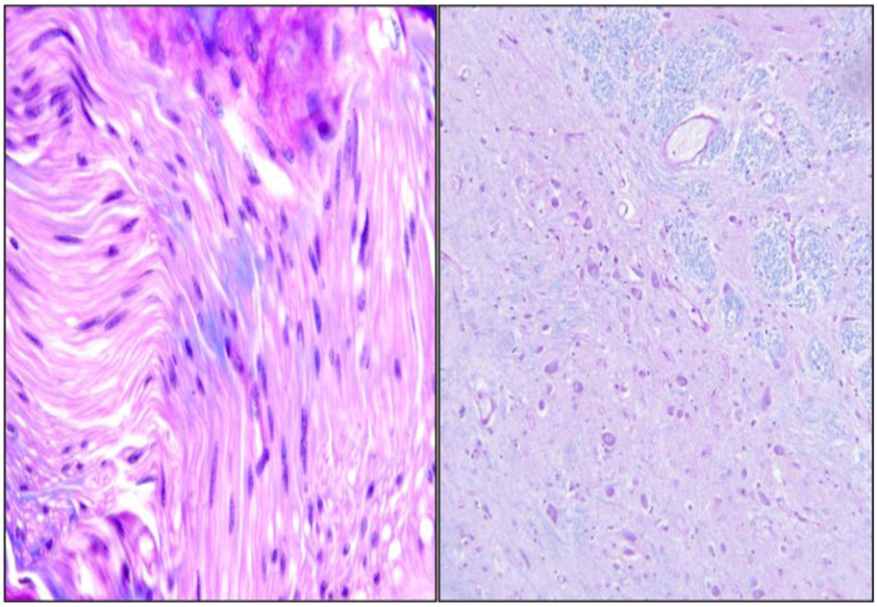

Fig. 1. Sural nerve (left) and pons (right) from a patient with MPV17-NNH; this shows almost the complete absence of myelin in the sural nerve (left × 400) and normal pons (right × 100) (IHC stains-Myelin Basic Protein and Neurofilaments).

parts of the world. We surmise that environmental pollution with neurotoxins, which is likely to increase, may contribute to the shortened life expectancy in the Four Corners region of NM.

3.1. The Anthropocene

The existence of the Anthropocene, a geological epoch, has not yet been generally acknowledged. A ruling in this matter by the International Anthropocene Working Group is expected in 2016. Meanwhile a large literature attesting to the increases in biosphere pollution with heavy metals, including those analyzed in this study in tissues of MPV17-NNH patients, has accumulated [12,13,14]. The Four Corners Generating Station is a coal-fired plant which is sited in Western New Mexico, USA in the Four Corners region. Three units of the plant were permanently shut down in 2010, pollution controls were added but the plant has been in continuous operation since 1964. The Four Corners Generating Station released 563 lbs. of Hg/year amongst other neurotoxins (such as As, Pb, Cd, Co and Mn) in 2005 alone into the environment [12]. This suggests that the increased incidence of MPV17-NNH on the Western part of the Four Corners region [3] may be partly due to the closeness of this part of the region to the power plant site.

3.2. MPV17-related hepatocerebral mitochondrial DNA depletion syndrome phenotype (MPV17-NNH)

The phenotype of this syndrome has been reported from Egypt [5], Morocco, [6], and Iraq, [7] These parts of the world are known to have major metal pollutions [13,14,15]. The implication is that MPV17-NNH is not a unique syndrome limited to the Four Corners region of NM, USA but that it does occur in other areas of the world where similar mutations have been reported and heavy pollution with metals and other neurotoxins has been documented. This environmental pollution is likely to increase in the Anthropocene and by extension MPV17-NNH type syndromes could also arise in parts of the world that have not yet been reported to harbor such patients.

Table 1
Mean values (±SE) of metal ratios MPV17-NNH/control tissues. Mercury was approximately 16,000 times higher in MPV17-NNH nervous tissue.

Metals	Mean	SE
As	18.2	5.6
Cd	9.8	1.9
Co	2.5	0.72
Mn	2.3	0.51
Pb	0.28	0.09
U	0.30	0.17
Hg	16,300	11,700

3.3. Molecular studies

The MPV17-NNH phenotype is associated with a founder homozygous mutation in Mpv17, a small mitochondrial protein of unknown function, which causes profound depletion of mtDNA, particularly in the liver. Though MPV17-NNH is a genetic disease [9] the consequences of this genetic mutation on phenotypic expression are not clear because they vary with the location from which the disease has been reported.

More than 30 mutations associated with the three phenotypes have been reported to date; a variety of these mutations are found in the hepato-cerebral mitochondrial DNA depletion syndromes starting in infancy [16]. Though not all normal functions of the Mpv17 protein

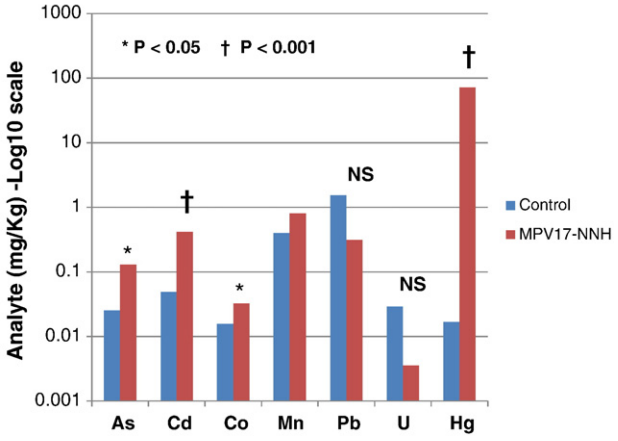

Fig. 2. Metal concentrations in mg/kg in MPV17-NNH samples compared to control nervous tissue from subjects not living in the Four Corners area of NM, showing significantly more metals and neurotoxins in MPV17-NNH tissues (NS—not significant).

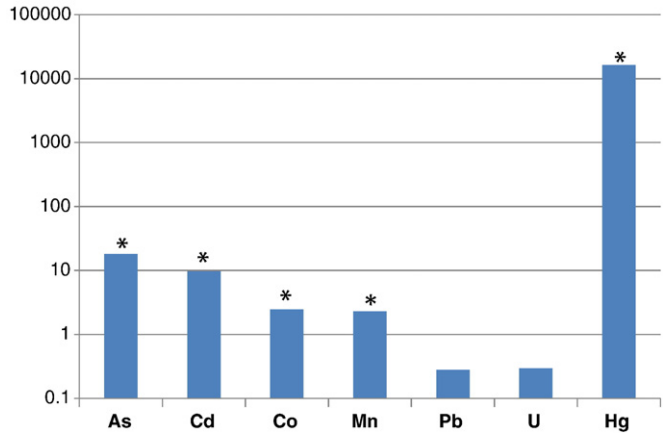

Fig. 3. MPV17-NNH to control concentrations ratio in nerves by metal. There are differences in metal concentrations (ANOVA, $P < 0.001$). Lead and U concentrations are less in MPV17-NNH patients than in controls (for logarithmic transformed values, $P = 0.02$ and $P = 0.06$ respectively) while other metals are significantly higher (all $^{*}P < 0.01$).

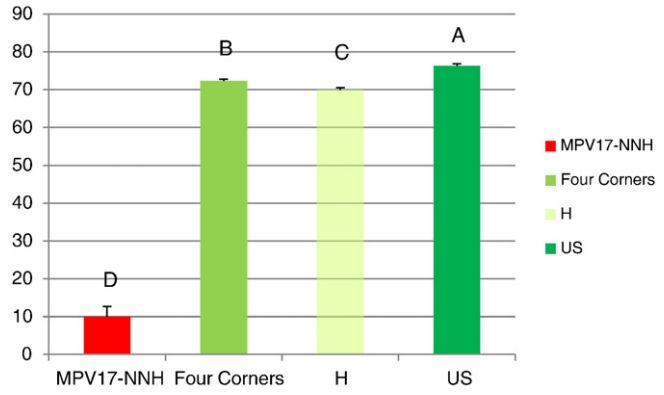

Fig. 5. Lifespan in patients with MPV17-NNH, Four Corners, Huancavelicans (H) and US populations showing adaptation to neurotoxins in heavily polluted environments in Four Corners and H compared to the US population. The Huancavelicans (H) have very low levels of Hg in their hair (well below acceptable levels). Note: lifespans with different letters are significantly different.

are known mitochondria are important in energy production, regulation of cell division and cell growth and in chemical signaling; Mpv17 may also be involved in the metabolism of reactive oxygen species. Not surprisingly because of these functions of Mpv17 the nervous system and the liver are particularly vulnerable in the hepato-cerebral mitochondrial DNA depletion syndromes.

Genomic imprinting is independent of classic Mendelian inheritance. Many human diseases involve genomic imprinting, that is, they are epigenetic. Because metals in the biosphere have profound epigenetic impacts on neurologic and other diseases it is likely that MPV17-NNH may, in addition to the demonstrated genetic factors, also depend in part on the abundance of toxic metals absorbed by such patients from the biosphere [17].

3.4. Heavy metals in tissues of MPV17-NNH patients and in controls

There were enormous levels of Hg (mean 16,000× increase over controls) in the tissues of patients with MPV17-NNH. The large variations (SD) in tissue Hg content were the results of including liver tissue (one pre-transplantation cirrhotic liver) in our analysis. We also found high levels of other neurotoxins in MPV17-NNH nervous tissues when compared to tissues from subjects not living in the Four Corners region. Arsenic, Cd, Co and Mn were all significantly increased in MPV17-NNH nervous tissue; surprisingly Pb and U were higher in MPV17-NNH tissue but the increase was not statistically different from controls. This may be due to the differential storage of these metals after metabolic absorption. For example lead is stored in bone and teeth which were not available for analysis whereas U is excreted through the kidney and only minute quantities are stored in human tissues (for a 70 kg human only 100–125 μg/g of tissue) [18].

All metals analyzed in this study were also present in varying amounts in our control tissues. This we attribute partly to geochemical enrichment of the biosphere, that is the enrichment with metals attributed to geological events such as plate collisions and volcanic eruptions or local enrichment attributed, for example, to fungal activity in the soil and also to anthropogenic enrichment which occurs worldwide [19]. Importantly, also dietary enrichment depending especially on fish intake, could contribute to the accumulation of neurotoxins in otherwise normal people [20].

We compared lifespan during the European Renaissance of Pb and Hg intoxicated individuals in Italy [21] and compared this to lifespan in patients with Pb and Hg intoxication who died in the Four Corners region of NM, USA with MPV17-NNH in the 20th century. The average survival with heavy metal loads in Italy was significantly longer than in the Four Corners region of NM. Thus living in that region appears to shorten survival of patients with MPV17-NNH.

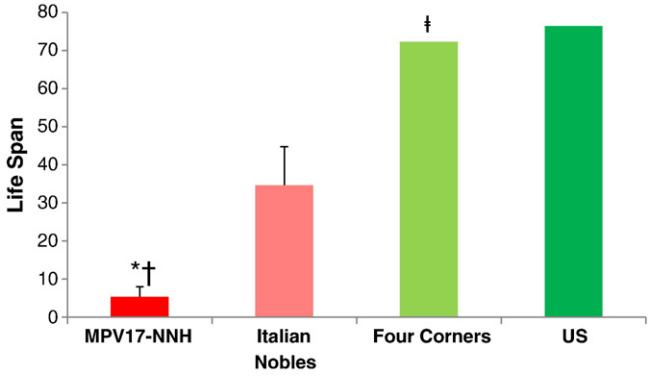

Fig. 4. Lifespan of MPV17-NNH patients; (geometric mean = 5.4 years ± 2.7 SE) and Italian nobles (geometric mean = 34.7 ± 10.1 SE) with Hg and Pb poisoning compared to lifespan of the general population in the Four Corners region (72 years) and the general US population (76 years). *Patients with MPV17-NNH neuropathy and Italian nobles with Hg and Pb poisoning differ from the general population of the Four Corners and the US ($P = 0.006$). †Patients with MPV17-NNH neuropathy also differ from the Italian nobles, $P < 0.001$. ‡Four Corners general population lifespan compared to US lifespan differ, $P < 0.001$.

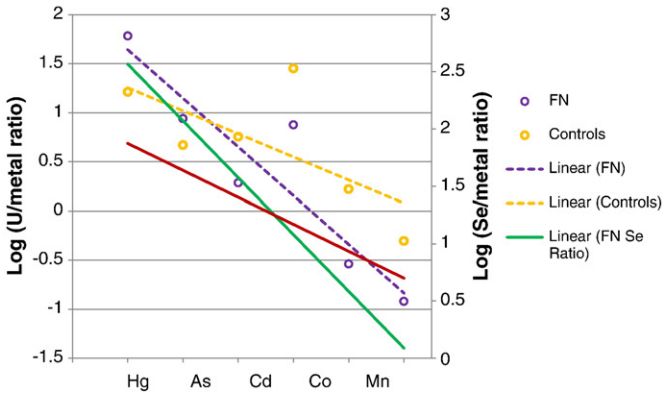

Fig. 6. Control samples including neural tissue for selenium and uranium to all other metals. Red line for Se ratios; dashed orange line for U ratios both in non-neural tissues. The uranium ratios are nearly proportional to the selenium ratios for all chemicals shown. In neural control tissues (green line (FN) for Se and purple (dashed line) for U the ratios are also nearly proportional. We used uranium as a proxy for, selenium in statistical analyses (see S1).

Huancavelica is a highly polluted town in the high Andes (Peru) sitting at the foot of a hill of cinnabar (mercury sulfide) which has been mined for millennia. We have analyzed tissues from present day Huancavelica residents and found that they showed signs of adaptation to the high levels of mercury pollution in the environment in which they live [22].

Similar findings have been reported for Argentineans living in the high Andes in highly contaminated arsenic environments [23] and in free ranging whales who roam oceans with very high concentrations of Hg in the water [24]. Thus preliminary evidence suggests that long term human and animal survival in highly toxic environments may depend to some degree on evolutionary adaptation.

3.5. Heavy metal interactions in biology

Mercury and selenium interact in biology and are correlated across samples on a global scale [24]. This interaction is primarily through an equimolar detoxifying effect of selenium on mercury [24]. Different biotas have stable ratios of metals concentration in tissues such as the Se/Hg ratios [24]. Uranium and selenium also interact extensively in biota. This is evident in bacteria [25] and yeast [26].

We selected uranium as a proxy for selenium in our statistical analyses (see S1) because this metal is non-toxic to humans if stored in tissues (<0.005% risk) in contrast to an expected indirect risk of 0.2%–3% if inhaling the radioactive inert gas radon [27].

In our control non-nervous tissues from individuals not living in the Four corners region, uranium and selenium concentrations were proportional (Fig. 6). Therefore we substituted uranium for selenium in our nervous tissues from MPV17-NNH patients (Fig. 7). Remarkably a highly significant difference in slopes ($P < 0.001$) supports the suggestion that in this model uranium is a good substitute for selenium. This also implies that MPV17-NNH patients have practically no mercury-detoxifying mechanism in their tissues [24]. Not surprisingly, people living in the Four Corners region but not afflicted by the MPV17 mutation can cope with the heavy metal loads and remain clinically normal.

We conclude that the huge quantities of mercury found in tissues from patients with MPV17-NNH compared to controls are not detoxified by natural mechanism unlike in people living in the same environment without obvious harm. We have compelling but circumstantial evidence which suggests that metals in the biosphere of the Four Corners region of NM, USA may contribute to shortened life-span in that region of the population in general and of patients with MPV17-NNH in particular.

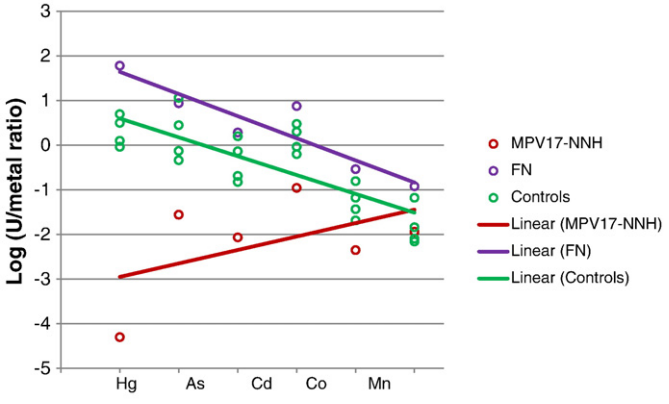

Fig. 7. Uranium:metal ratios in controls (green circles), control nervous tissue (FN = purple circles) and in MPV17-NNH tissues (red circles). A statistically significant difference in slopes (red) ($P < 0.001$) is shown. Control = non-neural tissues from subjects not living on the in the Four Corners region of NM.

3.6. Study limitations

We had insufficient archived material from MPV17-NNH patients for the analysis of selenium levels.

Conflict of interest

All authors declare no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ensci.2016.01.004.

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