The Neurotoxic Effects of Cycads and Metals: A Review

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ABSTRACT

The bioaccumulation of environmental toxins as possible risk factors in the etiology of amyotrophic lateral sclerosis and parkinsonism-dementia complex (ALS/PDC) is studied in three foci of the Western Pacific: Guam, the Kii Peninsula, and West Papua New Guinea. The objective of this study was to evaluate the best evidence on the exogenous causes of ALS/PDC, with emphasis on the role of cycads, iron, and manganese in the Western Pacific foci, by performing a systematic review of major electronic databases using predefined criteria, 68 of which met the selection criteria. Two major environmental hypotheses are associated with this enigmatic disease: the vegetal hypothesis, which focuses on the neurotoxic and genotoxic properties of the cycad, and the mineral hypothesis, which focuses on the neurotoxic properties of metals. Although typically studied independently, environmental data suggests these two hypotheses may, in fact, converge. Epidemiologic research investigating the association between exposure to environmental toxins and ALS/PDC has proven inconclusive. Nevertheless, possible causal links indicate a need for more holistic research to not only better understand ALS/PDC, but also glean new insights regarding the associated neurodegenerative diseases.

Keyterms: amyotrophic lateral sclerosis and parkinsonism-dementia complex; Alzheimer's disease; Parkinson's disease; Western Pacific; cycad; iron; manganese; neurotoxicity

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INTRODUCTION

Amyotrophic lateral sclerosis and parkinsonism-dementia complex (ALS/PDC) is a prototypical, long latency neurodegenerative disease that, during the first decade after World War II, was reported to develop in extraordinarily high frequencies among three geographically and genetically distinct populations in the Western Pacific: the indigenous Chamorro residents of Guam,[1] the Japanese of Honshu Island's Kii peninsula in Japan,[2] and the West Papuan New Guineas of Irian Jaya, Indonesia.[3] The clinical and neuropathological features of this disease are best studied among the Chamorro people of Guam and the Japanese living in the Kii Peninsula of Honshu Island. Least studied, and lacking neuropathological confirmation, is the Auyu and Jakai (Jaqai) linguistic groups in the southern lowlands of West Papua, the Indonesian side within New Guinea. This review explicates the polemical role of plants and minerals in the pathogenesis of ALS/PDC in the three foci of the Western Pacific.

This enigmatic and invariably fatal disease of the Western Pacific is characteristic of classical ALS, Parkinsonism, and Dementia. Although the insidious progression of neurodegenerative diseases is typically due to senescence, the age of onset for ALS/PDC can be as early as adolescence for the ALS phenotype and middle adulthood for the parkinsonian and dementia phenotypes.[4] Those afflicted with this disease experience debilitating symptoms such as cognitive deficits, spasticity, and muscle atrophy leading to a vegetative state and death. Despite the dramatic decline of ALS/PDC incidence, the Western Pacific foci can be a valuable case-study for understanding the etiology of the associated neurodegenerative diseases.

The hallmark biomarker of ALS/PDC is polyproteinopathy, in which multiple proteins aggregate in the brain. In ALS/PDC, the affected brain accumulates a constellation of abnormal intracellular deposits (synuclein, β-amyloid, and transactive response (TAR)-DNA-binding protein 43 (TDP-43))[5] but is dominated by telencephalic (anterior region of the forebrain) neurofibrillary tangles (NFTs), contributing to stark cortical neuron loss.[6] Even though tauopathy, aggregation of tau protein in the brain, is a key characteristic of ALS/PDC, it cannot be distinguished from other neurodegenerative disorders; ALS/PDC has a great degree of heterogeneity. The following examples elucidate this point: (1) the presence of hyperphosphorylated tau[7] and α-synuclein negative inclusions found in ALS/PDC are also seen in frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U), a neuropathological subtype of frontotemporal dementias;[8] (2) tau isoform distribution, commonly associated with ALS/PDC, is observed in cases of Alzheimer's disease (AD);[9] (3) cortical laminar distribution is linked to progressive supranuclear palsy (PSP);[9] (4) possible malfunctioning of TDP-43 proteinopathy is seen in FTLD-U and ALS;[9] and (5) leucine-rich-repeat-kinase 2 (Lrrk2), a gene that, when mutated, is seen in several major neurodegenerative disorders associated with parkinsonism.[10] In combination, these biomarkers convolute the clinical pathological spectrum of ALS/PDC, and, as a result of the heterogeneity, ALS/PDC can only be confirmed by postmortem examination.[4]

Genetic studies posit that ALS/PDC does not follow Mendelian patterns of inheritance.[1] Rather, it follows an irregular, multifactorial autosomal dominant mode of inheritance[11] with incomplete penetrance.[12] The familial nature of ALS/PDC indicates that one or more genes may be responsible; however, attempts to identify a causative gene have yet to be successful.[9] Since NFTs are the most prominent biomarker of this disease, a genetic study of Guam focused on the gene that encodes for microtubule-associated protein tau (MAPT). Two independent single nucleotide polymorphisms (SNPs), variations of a single base pair in a DNA sequence, within the MAPT region confer a risk of susceptibility by a recessive, cis-acting mechanism; however, the polymorphisms only increase the risk in combination with other genetic and environmental factors.[13]

Methods

A computer literature search of the PubMED/MEDLINE, Google Scholar, and Mendeley databases was conducted to find relevant literature on ALS/PDC in the Western Pacific foci with respect to cycad and mineral neurotoxicity. The main search terms were ALS/PDC, cycad, iron, manganese, Guam, Kii Peninsula, West Papua New Guinea, and neuro# (the symbol is used for identifying all words starting with neuro, e.g. neurodegenerative, neurotoxic, and neuropathological). The literature found to

satisfy the following criteria was included in the review: (a) examination of at least one risk factor of ALS/PDC in at least one of the Western Pacific foci; (b) discussion of cycad or mineral neurotoxicity; and/or (c) discussion of the biomarkers of ALS/PDC. The following literature was excluded: (a) language other than English, (b) small sample size, and/or (c) ALS/PDC not being the central focus.

Results

Environmental Aspects

Although genetic factors may be linked to ALS/PDC, the decline in prevalence of these disorders over a short period argues for a gene-environmental interaction in which exogenous or environmental factors may contribute to the pathogenesis of ALS/PDC. Many environmental risk factors have been examined over the past years, including exposure to animals, fish poisoning, and mineral deficiencies; however, no relationship has been definitively identified. There are two major environmental hypotheses regarding ALS/PDC that are typically researched separately: the vegetal hypothesis and the mineral hypothesis.

1. Vegetal Hypothesis

The vegetal hypothesis focuses on a common, etiological factor to all three ALS/PDC foci: the exposure to traditional foods and medicines derived from the cycad plant.[14] Major cycad neurotoxins correlated with a high incidence of ALS/PDC include methylazoxymethanol β-D-glucoside (cycasin), and its aglycone methyl-azoxymethanol acetate (MAM), β-N-methylamino-L-alanine (BMAA), and β-oxalylamino-L-alanine (BOAA).[15] The toxic part of cycasin is the active ingredient that is released as MAM by enzymatic processes occurring in digestion; thus, cycasin only exerts a toxic effect when it is ingested.[16]

The most affected population, due to consumption of cycads and inhalation of cycad pollen, was the indigenous Chamarro of Guam. They used fresh cycad seed cover to relieve thirst and dried seed cover as a confection;[17] however, the most studied and common traditional food of the Chamarro is a flour called fadang made from the seed. Although the preparation of the flour includes successive washings of cycad ovules to reduce the content of cycad toxins, a study revealed large concentrations of cycasin were still present in the flour which, though not lethal, did induce acute illness in children likely due to the hepatotoxic properties of cycasin.[18] Additionally, the consumption of flying foxes, or fruit bats, in the diets of the Chamorros has been proposed to cause ALS/PDC due to the bats' substantial consumption of cycad seeds and bioaccumulation BMAA.[19] It should be noted, however, that flying foxes are not part of the diet of Japanese or New Guinean subjects at risk for ALS/PDC. Moreover, cycads have been observed to produce pollen with high concentrations of cycasin and BMAA.[20] The respiratory system is another potential entry path for cycad toxins: the pollen contacts the nasal epithelium and can be transported to brain tissue to induce neurotoxic effects.[21] A recent study confirmed that intranasal administration of MAM in mice caused elevated mitogen-activated protein kinases (MAPKs) and increased caspase-3 activity, which are linked to the tau aggregation and neuronal cell death that is characteristic of ALS/PDC.[22]

The medicinal use of cycads through prolonged subcutaneous or repeated oral application of raw cycad seed is common to all three foci of the Western Pacific. The cycad seed has been used as a topical treatment for skin lesions,[23, 24] but such use undoubtedly declined as man-made pharmaceuticals were introduced. The use of the cycad seed for oral medicine was practiced in Japanese folk medicine in the Kii Peninsula until the 1980s, with prescriptions written by practitioners and filled by pharmacies.[24] It should also be noted that the Fore people, outside of the ALS/PDC foci, living in the south-eastern Papua New Guinea, were exposed to cycad toxins by chewing the fleshy cycad seed cover and spitting the contents into food which precipitated kuru, a neurodegenerative disease with tau pathology.[5]

Despite extensive research on the cycad, no conclusive association between ALS/PDC and plant or animal toxins has become evident. A study on cycad-derived products such as *fadang,* flying foxes, and topical medicine as possible risk factors for dementia, mild cognitive impairment (MCI), and ALS/PDC found no significant relationship between the consumption of flying foxes or topical medicine, but did find a significant odds ratio (OR), which provides a measure of the strength of association,[25] for picking, processing, and eating fadang in young

adulthood for any of the neurodegenerative diseases present in the native population of Guam.[26] Although starch-making from cycads was prevalent in the Mariana Islands, ALS/PDC was found to be concentrated only in certain villages on Guam such as Umatac, Merizo, and Inarajan; however, the BMAA content of cycad samples from Umatac contained no significant differences relative to the controls.[27] Additionally, a survey of the Hohara area of Nasei-cho, one of the foci in the Kii Peninsula, showed no relationship between cycad use and neurological disease.[28] Furthermore, the consumption of cycads is not remarkable because aboriginal groups in Australia historically prepared food from carefully detoxified cycad seed ovules, and Japanese living in the Ryukyu Islands employed fermentation techniques to eliminate cycad toxins without precipitating neurological disease.[29] Thus, the vegetal hypothesis by itself appears to lack scientific support, suggesting the role of other possible risk factors.

2. Mineral Hypothesis

Environmental data from the Western Pacific endemic foci of ALS/PDC supports the interactions between essential and neurotoxic metals and contributes to what is known as the mineral hypothesis. Although ALS/PDC is possibly associated with a constellation of metals,[30, 31, 32, 33] this review focuses on the bioaccumulation of iron (Fe) and manganese (Mn) from the environment in bulk central nervous system (CNS) tissue of patients in the ALS/PDC foci. Metals and trace elements play salient roles in the CNS; however, clinical disease may result from deficiencies and excesses of such essential minerals, and nonessential trace elements may also induce neurological disease through excessive exposure.[34] Thus, iron and manganese may be causally implicated in ALS/PDC in the Western Pacific foci based on the bio-accumulation of neurotoxic minerals in the soil, drinking water, and vegetation.

A. Iron

Iron is integral to many biological functions: it has a role in many enzymes involved in oxidative and amino acid metabolism, it has an effect on dopamine D2 receptor function, and it interacts with other neurotransmitters such as gamma-aminobutyric acid (GABA)[35] and glutamate.[36] Iron deposition in the brain is most prominent in the globus pallidus, red nucleus, substantia nigra pars reticulate, putamen, caudate, and the dentate nucleus, but is found in white matter and cortex as well.[37] Trace amounts of these deposits are minimal at birth and gradually increase for the first three decades of life after which they tend to stabilize until about the sixth decade of life, and then insidiously increase.[38] Excessive iron deposition is associated as a putative factor in the pathogenesis of neurodegenerative disorders, most notably AD and Parkinson's disease (PD).[39]

The neurotoxic effects of iron may result from iron catalyzing the production of reactive oxygen species (ROS) through the Fenton and Haber-Weiss reactions, provoking oxidative stress.[40, 41] Moreover, the products of these reactions can continuously form organic free radicals, spawning a self-perpetuating neuronal death cascade that is "continuously propagated" by excess free iron.[42]

A neutron activation analysis (NAA), a non-destructive technique for simultaneously determining the concentrations of trace elements in a sample,[43] of iron and zinc (Zn) in gray and white matter of the frontal and occipital regions in Guam patients with ALS/PDC indicated an increase of iron in gray and white matter and a decrease of zinc in gray matter, relative to controls, coupled with an excess of bioavailable aluminum (Al) and deficiency of calcium (Ca).[44] However, this result conflicts with the findings of another study of Guamanian patients with ALS/PDC: eight metals in formalin-fixed brain tissue were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS),[45] revealing that for all metals, the concentrations tended to be higher in gray matter than in white matter, and finding no significant differences between the patients and the control groups for iron.[30] Even though the sample sizes of both studies are small, the contradictory results of the two studies suggest other risk factors are at play in the precipitation of ALS/PDC, in addition to iron concentration.

In an environmental field study, samples of soil, water, and vegetation were obtained from three southern villages of Guam with high incidences of neurodegenerative disease — that is, Umatac, Merizo, and Inarajan — to investigate any abnormal mineral concentrations and whether they could be linked to ALS/PDC. The study indicated higher levels of iron, among other compounds, in these villages

than in the disease-free north of Guam. Specifically, elevated levels of iron were found in the red laterite top soils along the western side of Guam,[46] in the river water at Merizo, and in the vegetation around Umatac and Merizo.[31]

The soil, drinking water, and vegetation were also analyzed for mineral imbalances in the Kii Peninsula. An analysis of seven metal concentrations in the environment of the Hohara area reported elevation of iron and manganese in the drinking water of Iseji, one of the five sub regions of Hohara, relative to that of Uchikawame and Nankohdai, two control areas remote from the focus.[47] In contrast, in another study, a chemical analysis by neutron activation of Guam and Kii Peninsula water sources found no significant difference in iron content;[48] however, a constellation of factors, such as a deficiency in suitable controls, may result in inconsistencies across studies that may be related to the incidence of ALS/PDC in the foci.

B. Manganese

Manganese is another essential metal that is important for many physiological processes such as carbohydrate metabolism, calcium absorption, defense against free radicals, and is an important cofactor in several enzymes integral for neuronal and glial cell function and enzymes involved in neurotransmitter synthesis and metabolism, namely dopamine, GABA, and glutamate.[49, 50, 51] Despite its vital role in a multitude of biological functions, excessive manganese exposure is associated with several neurodegenerative diseases, including ALS, PD, Manganism (manganese poisoning, an analog of PD), and AD.[52]

The highest concentrations of manganese occur in the basal ganglia, more specifically, the same deep-brain nuclei associated with iron deposition.[53, 54, 55, 56] Due to the similarity of iron and manganese, both metals are interdependent and can use the same transporters.[57] Moreover, the neurotoxic effects may transpire from the interactions between iron and manganese: an *in vivo* study indicated elevated manganese exposure facilitated unidirectional influx of iron from the blood to the cerebrospinal fluid (CSF) in rats, thus by increasing free iron levels, manganese may elicit iron-induced oxidative stress and cause oxidative damage to neurons.[58, 59] Additionally, excess manganese has been linked to decreased function of dopamine, glutamate, and GABA which can induce neurological disease.[60]

By neutron activation analysis, a study of Guam and Kii Peninsula ALS-PDC analyzed samples of water, soil, plants, CNS tissue, and cattle hair. The study found a higher content of manganese in the spinal cord than in any other CNS tissue and, concurrently, reported a generally high content of manganese in the river and drinking water of Guam, particularly in Inarajan and in the tap water of Agana. In addition, while the water samples from the Kii Peninsula have about the same content of manganese as the rivers in the Kinki District and the rest of Japan, the residences of a few patients showed a relatively high content of manganese in their drinking water. Furthermore, the study found elevated manganese levels in the soil taken from both foci, and significantly high manganese content in the hair of cattle living in the Kii Peninsula.[61]

The same environmental study of soil, water, and vegetation in three high incidence villages of Guam — Umatac, Merizo, and Inarajan — found elevated manganese levels, along with iron and other metals, in the top soils and in the vegetation around Umatac and Merizo. The results suggest that the elevated levels of iron and manganese, besides other metals, in the soil could cause enhanced levels of magnetic susceptibility in Southern Guam which may be a key to understanding the pathogenesis of ALS/PDC.[31]

Samples of soil, water, and vegetation were also studied in the Kii Peninsula in which the concentrations of seven minerals were analyzed. The study found significantly higher manganese levels in the paddy field soils of Iseji and higher manganese and iron levels in Iseji drinking water, relative to the two control areas Uchiwakame and Nankohai. Furthermore, the study found higher manganese intake in Iseji local rice consumers than in imported rice consumers from the same area and in three control areas (Kirihara, Uchiwakame, and Nankohai), and higher manganese content on a dry-weight basis in boiled rice in Iseji than in Kirihara. Regardless of the types of samples collected, the manganese content was always more elevated in Iseji than in the control areas.[47]

In West Papua New Guinea, the primitive Auyu and Jakai (Jaqai) populations lacked manufactured products due to their isolation and primitive technology.[62] The high ALS/PDC incidence in this focus was hypothesized to be associated with low concentrations of calcium and magnesium (Mg) in their drinking water,[62] which is also seen in other foci.[63, 64] However, ALS prevalence in these sessile populations declined without any known change in their source of drinking water.[3] This finding conflicts with a study of the Kii Peninsula that showed an increase in ALS incidence due to a change in sources of drinking water.[65] Contradictory evidence may suggest the role of other risk factors and calls for further investigation.

Concluding Remarks

Although there are studies focusing on the possible risk factors in the three foci of the Western Pacific, they are often insufficient in suitable controls, and, in some cases, sample size to show that the cycads and the levels of metals are causally related to the high incidence of ALS/PDC. While these studies tend to treat the vegetal and mineral hypotheses as mutually exclusive, it may be more beneficial to consider an intersectional relationship. For instance, the vegetal hypothesis focuses on cycads, yet, the Western Pacific foci is known to be a manganese-rich environment (as is plainly evident in the aforementioned studies), especially as it relates to Guam,[66] with elevated levels of iron and low levels of calcium and magnesium present, suggesting possible mineral interdependency[57, 59] and antagonism,[67, 68, 47] respectively. Thus, for the reasons discussed *supra*, further investigation of cycads and metal neurotoxicity, in combination, in all three foci of the Western Pacific, is warranted, and could be beneficial in further understanding the etiology and underlying mechanisms of the enigmatic ALS/PDC endemic.

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