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Cycads, Flying Foxes, and Brain Disease in Humans

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Introduction

Cycads are seed plants with stout, woody trunks and a crown of large, stiff evergreen leaves existing within tropical and subtropical regions of the world (Chamberlain, 1993). Cycads dominated forest areas from around 250 million years ago to around 65 million years ago and are among the most primitive of today's seed plants; however, all extant cycads evolved within the last 50 million years (Vessey, Pawlowski, & Bergman, 2004). All extant cycads belong to the families Cycadaceae, Stangeriaceae, and Zamiaceae, and they participate in a symbiotic relationship with nitrogen-fixing cyanobacteria (Thajuddin et al., 2010). These cyanobacteria release compounds that, in addition to the cycad's own toxins, can have neurotoxic effects if consumed in sufficient doses. Though they have long been known to be toxic, cycad seeds are eaten by humans after being washed and processed in an effort to remove the toxins. Cycad seeds also make up a significant portion of the diet of some flying foxes. "Flying fox" is a colloquial name referring to a bat of the genus *Pteropus* or the closely related *Acerodon* (De Jong et al., 2013). They are a type of fruit-eating bat and are the largest bats in the world. They are considered a delicacy in some regions and have been threatened by overhunting and deforestation. Several species have gone extinct due to human activity. Flying foxes often share a habitat with cycads, particularly in Old World tropical and subtropical regions such as the Pacific Islands. Heavy consumption of cycad seeds leads to accumulation of toxic chemicals in the tissues of flying foxes. Toxicity resulting from the consumption of cycads and flying foxes is hypothesized to be a cause of significant neurological abnormality in humans including a severe progressive neurodegenerative disease known as the amyotrophic lateral sclerosis and

parkinsonism-dementia complex, or ALS-PDC (Spencer, Lasarev, Palmer, & Kisby, 2010). For much of the twentieth century, incidence of ALS-PDC was far greater in tropical regions in which cycads and flying foxes were common dietary items (Kurland & Mulder, 1954). For example, the Chamorro people of Guam have traditionally consumed significant amounts of cycads and flying foxes. The Chamorro also once had one of the highest known incidence rates of ALS-PDC ever known, up to 200 cases per 100,000 people. The name of this disease reflects the similarity of symptoms and neuropathology found in people suffering from ALS-PDC to that of people afflicted with each of its namesake diseases—amyotrophic lateral sclerosis, abnormal Parkinson's disease, and dementia and Alzhiemer's disease (Kuzuhara et al., 2001). Ethnic groups in which ALS-PDC was once extraordinarily frequent have seen a sharp decline in diagnoses upon reduction in their consumption of cycads and cycad-toxin-biomagnifying flying foxes as they conform to modern life (Monson, Banack, & Cox, 2003).

Interspecies Relationships

There are numerous interspecies interactions that must be considered in order to fully understand the relationships between cycads, flying foxes, and humans and the effects of these interactions on the participants.

Cycads and Cyanobacteria

Cyanobacteria are unusual among prokaryotes in that they possess the capacity to perform photosynthesis—in fact, the prevailing hypothesis on the origin of plant chloroplasts is an early endosymbiotic event in which a cyanobacterium was taken up by a Eukaryotic cell (Raven & Allen, 2003). Most cyanobacteria are also diazotrophic, which means they are able to fix

atmospheric nitrogen (N_2) into a usable form (Fay, 1992). Cyanobacteria are an important source of fixed nitrogen in many ecosystems. They commonly fix nitrogen as free-living organisms or within symbiotic relationships with a variety of fungi and plants (Vitousek et al., 2002). The only known gymnosperms to participate in nitrogen fixation with cyanobacteria are cycads (Thajuddin et al., 2010). It is thought that the relationship between cycads and cyanobacteria began to develop during the time in which cycads dominated the world as early as 250 million years ago.

Cycads associate with nitrogen-fixing cyanobacteria within specialized roots known as coralloid roots that extend in branches above the ground or at shallow depths. The association of cycads with cyanobacteria within specialized plant structures represent a truly mutualistic form of nitrogen fixation (Vessey et al., 2004). This contrasts with the more common opportunistic type of relationship in which free-living nitrogen-fixing bacteria release fixed nitrogen (usually in the form of ammonia) into the environment, and this environmental nitrogen is subsequently taken up by plants. The endophytic cyanobacteria that infect the coralloid roots of cycads are almost entirely found within the genera *Nostoc* or *Anabaena*, though it is believed that the strains of *Anabaena* participating in nitrogen-fixation symbiotic relationships with cycads would be re-designated as members of *Nostoc* if their taxonomy were to be re-evaluated with improved criteria (Rippka, Deruells, Waterbury, Herdman, & Stanier, 1979). The cyanobacteria are believed to enter the coralloid root through breaks in the root's dermal layers within the soil (Milindasuta, 1975). Several species of cyanobacteria can be found within a single cycad (Lindblad, Haselkorn, Bergman, & Nierzwicki-Bauer, 1989). Cells within the endophyte cavity of the coralloid root possess numerous outgrowths, abundant mitochondria, and many secretory

granules outside the plasma membrane but within the cell wall. These structures grant the cycad the ability to quickly and efficiently exchange nutrients with the nitrogen-fixing cyanobacteria, reflecting the cycad's adaptation to facilitate the symbiotic relationship. Within a single coralloid root, a single species of cyanobacteria will outcompete all others, but different coralloid roots within a single plant can host distinct species (Costa, Paulsrud, & Lindblad, 1999). Like other diazotrophs, cyanobacteria possess a nitrogenase enzyme that ultimately converts atmospheric nitrogen (N_2) into ammonia (NH_3) in a process that requires the cleavage of the triple bond between the two nitrogen atoms in diatomic atmospheric nitrogen (C. Lee, Ribbe, & Hu, 2014). This process requires energy input and is coupled to substantial input of ATP (16 molecules of ATP are required for every molecule of N_2 converted into 2 NH_3) (C. Lee et al., 2014). The relationship between cyanobacteria and cycads is mutualistic in that cyanobacteria obtain ATP to carry out nitrogen fixation and other cellular processes, while cycads obtain a ready source of usable nitrogen.

A consequence of the relationship between cycads and cyanobacteria is the accumulation in cycad tissues of B-methylamino-L-alanine, or BMAA, a neurotoxic non-proteinogenic amino acid secreted by many species of cyanobacteria. While cycads produce toxins of their own, BMAA is the most well-researched of the cycad-involved toxins and has been suggested as a possible cause of ALS-PDC.

Cycads and Flying Foxes

Flying foxes and cycads participate in a typical seed dispersal relationship. Bats are involved in seed dispersal with many fruit-bearing organisms and have been shown to be quite effective seed

dispersers (Medellin & Gaona, 1999). Animal seed dispersal in cycads is not a heavily-researched topic, but bats were one of the first organisms described to disperse cycad seeds (Pijl, 1957). The bats are attracted to brightly colored seeds displayed by the cycad and consume the fleshy, edible outer layer (sarcotesta) before discarding the remainder of the seed elsewhere, still capable of germination. While the sarcotesta contains a relatively small quantity of BMAA, consumption of many seeds and inadvertent consumption of other seed parts leads to the accumulation of BMAA in flying fox tissues. This accumulation results in such biomagnification of BMAA that consumption of a single flying fox by humans could give a dose of BMAA comparable to consumption of 174 to 1,014 kilograms of processed cycad flour (Banack & Cox, 2003).

While cycads and flying foxes participate in a mutualistic interaction, it is unlikely that substantial co-evolution has taken place because neither organism depends on the other. Cycad seeds are only one of many sources of nutrients for flying foxes, and cycad seeds are often dispersed by other animals including possums (Ballardie & Whelan, 1986).

Cycads and Humans

The fact that cycad seeds have been long known to contain toxins has not prevented humans from finding ways to consume them. The Chamorro people of Guam traditionally made tortillas and flour using ground cycad seeds. Other food items such as dumplings and thick soups were also commonly produced from cycad seeds. The Chamorro were aware of the neurotoxic effects of raw cycad seeds and developed methods to remove the toxins. Cycad seeds were washed in water over the course of days or weeks with repeated changing of the wash water. BMAA and

other toxins have been shown experimentally to dissociate from the seed as a result of these water washes, although researchers have stated that the use of a less polar solvent such as ethanol would be more effective (Spencer et al., 2010). The Chamorro believed the seeds were safe to eat when "their chickens didn't die after drinking the wash water (Holtcamp, 2012)." Rather than risk losing chickens, some Chamorro stop washing the seeds when the "urine-like smell vanishes (Banack, Murch, & Cox, 2006)." While BMAA has been shown to lead to neurodegeneration and symptoms similar to ALS-PDC in animal models, the hypothesis that BMAA toxicity from consumption of cycad seed-derived food products was briefly contested when it was reported that the Chamorro washing procedure removed a sufficient amount of free BMAA to eliminate the possibility of obtaining a toxic dose through eating seed-derived products (Duncan, Steele, Kopin, & Markey, 1990). However, this conclusion was based on findings that most *free* BMAA was successfully extracted by the washes. It was discovered in 2009 that a substantial amount of additional BMAA exists in protein-bound form within cycad seeds, and this protein-bound BMAA is not removed by Chamorro-like washing patterns; therefore, previous studies likely underestimated the amount of BMAA consumed in cycad seed-derived food products (Cheng & Banack, 2009). Therefore, BMAA toxicity through cycad seed consumption cannot be ruled out as a possible cause of ALS-PDC.

Flying Foxes and Humans

Flying foxes are a traditional food item of several cultures within their territory. Flying fox meat is considered a delicacy in Polynesia and Indonesia (Wheeler, 1979). The Chamorro people of Guam, however, are more invested with flying fox consumption to the point at which "many Chamorros believe that the consumption of flying foxes is central to their cultural identity

(Banack et al., 2006)." The effect of human consumption of fruit bats on fruit bat populations was significantly worsened in the twentieth century. Changes in trade and technology increased the efficiency of flying fox hunting. Essentially, flying foxes were once considered an occasional treat, hunted at a subsistence level using hand nets (Linsley, 1934). They began to be hunted with firearms and traded for profit. In addition to increased popularity and hunting efficiency, other human activity such as deforestation negatively impacted populations of flying fox. Due to cultural pushback, flying foxes were not protected promptly and effectively until after the extinction of some species and endangerment of others (Wheeler, 1979). An illegal flying fox trade persisted for some time, and still exists to some degree, but is limited by a distinct lack of flying foxes to hunt.

The Chamorro people of Guam prepare and eat flying foxes in their entirety. They are strewed with water, vegetables, coconut milk, and MSG, and are then wholly consumed—meat, organs, fur and all (Banack et al., 2006). They are commonly prepared for special occasions and served along with cycad tortillas. Prior to the discovery that many studies had underestimated the amount of BMAA present within washed cycad seeds (Cheng & Banack, 2009), the BMAA hypothesis for ALS-PDC was contested due to the finding that a person could not obtain a sufficient dose to produce symptoms by eating cycad seed-derived foods (Duncan et al., 1990). The BMAA hypothesis was revitalized when it was discovered that flying foxes were shown to contain considerable quantities of either free BMAA, protein-bound BMAA, or both forms of BMAA in all body tissues (Banack et al., 2006). This was true in both raw flying fox tissues and flying fox tissues that were subjected to traditional Chamorro preparation. Flying foxes serve to biomagnify BMAA to such a degree that consumption of a single flying fox provides a

substantially higher dose of BMAA than a person could obtain from directly eating food prepared from cycad seeds (Banack et al., 2006). There are other commonly hunted local fauna such as deer and feral pigs that consume cycad seeds and are also thought to cause BMAA biomagnification, although they are not as well-studied as flying foxes.

Neuropathology

Patients suffering from the amyotrophic lateral sclerosis-parkinsonism dementia complex, or ALS-PDC, present with symptoms and neuropathology that overlap those characteristic of ALS, Parkinson's Disease, and Alzheimer's disease. Currently, a diagnosis of ALS-PDC cannot be confirmed until after death (McGeer & Steele, 2011). The disease's three distinct namesake phenotypes tend to occur at different stages of life. It is uncommon for dementia to occur in affected individuals until they have reached an advanced age, while ALS symptoms can develop in adolescents or young adults. Parkinsonism most commonly appears during adulthood (McGeer & Steele, 2011). It is noteworthy that, like many neurodegenerative diseases, ALS-PDC can manifest quite differently from patient-to-patient. Some experience little to no dementia and are capable of speech and rational thought even after they have become nearly paralyzed due to the disease's effects on motor neurons, while others express dementia-related behavioral and emotional changes prior to significant loss of motor function (Steele, 2005).

ALS-PDC and Alzheimer's Disease

A characteristic abnormality in the central nervous system shared by individuals with ALS-PDC and those with Alzheimer's disease is the presence of neurofibrillary tangles (NFTs) in both neurons and glial cells due to aggregation of abnormal tau proteins. Properly functioning tau

proteins serve to stabilize microtubules in neurons of the central nervous system. Tau proteins are expressed at low levels in glial cells as well. In a disease known as a tauopathy, tau proteins function abnormally. Many tauopathies involve the hyperphosphorylation of tau, which inhibits tau binding and stabilization of microtubules and leads to the formation of large tau aggregates known as NFTs (H. Lee et al., 2005). Misfolded tau proteins can induce the aggregation of other tau proteins, so tauopathies are considered to progress in a prion-like manner (Krammer, Schätzl, & Vorberg, 2001). Autopsies reveal that NFTs are found in the cortical areas of individuals who were diagnosed with ALS-PDC to a much higher degree than those who were diagnosed with only ALS. This leads to the conclusion that NFTs in cortical regions of the brain are involved in the parkinsonism-dementia complex in ALS-PDC (Miklossy et al., 2005). Studies have also found deposits of the Alzheimer's disease hallmark β-amyloid deposits within the brains of ALS-PDC-diagnosed individuals (Cox, Banack, & Murch, 2003). In a manner similar to that by which misfolded tau leads to NFT, misfolded amyloid β proteins have a prion-like propagation leading to oligomerization and formation of plaques. β-amyloid plaques are believed to be the most toxic of the plaques that occur in Alzheimer's disease (Zhao, Long, Mu, & Chew, 2012), so their detection in ALS-PDC may contribute to the disease's severity. Furthermore, individuals who displayed more advanced symptoms of dementia showed reduced cortical thickness and reduction in the volume of basal nuclei (Wilson et al., 2004). It should be noted that some ALS-PDC patients do not develop Alzheimer's disease-like symptoms. It is uncertain whether this is more attributable to patients dying before reaching the late-in-life onset of dementia or a different underlying etiology for the non-dementia cases. Mood swings and memory loss are common signs of the dementia complex (Miklossy et al., 2005)

ALS-PDC and Parkinson's Disease

A pathological characteristic of ALS-PDC that is consistent with that of Parkinson's disease is mutation in α-synuclein (Yamazaki et al., 2000). α-synuclein is an abundant soluble protein in the brain whose normal function is not known. Mutation of α-synuclein can lead to aggregation and loss of solubility. Mutant α-synuclein is the primary component of a Lewy body—a mass of insoluble protein aggregates that disrupt cellular function by displacing normal cell components (Schulz-Schaeffer, 2010). Several diseases can result from mutation in α-synuclein mutation and aggregation. These diseases are classified as synucleinopathies. It has been shown both *in vitro* and *in vivo* that synucleinopathies and tauopathies can interact with one another, worsening neurodegeneration in a "deleterious feed-forward loop (Moussaud et al., 2014)." ALS-PDC is an example of a disease expressing both tauopathic and synucleophathic pathology, which may contribute to the severity of the disease. In both ALS-PDC and Parkinson's disease, Lewy body buildup in neurons of the central nervous system is associated with the death of these neurons, although the exact reason that Lewy body accumulation correlates with neuronal death is not yet understood (Kalia & Lang, 2015). While the motor effects of both diseases are highly variable from patient-to-patient, common symptoms include tremors, uncontrollable muscle tension, and spasms. These motor symptoms are primarily the result of neuronal death within the substantia nigra region of the midbrain (Dawson & Dawson, 2003).

ALS-PDC and ALS

The neuropathology and symptoms of ALS are almost entirely present in ALS-PDC. In both diseases, substantial loss of motor neurons can be seen in the spinal cords of autopsied individuals (Morris et al., 2001). A progressive decline in motor capabilities occurs in both

diseases. A biological marker shared by ALS and ALS-PDC is TDP-43 aggregation (Miklossy et al., 2005). Under normal conditions, TDP-43 is a DNA-binding protein that represses transcription of HIV-1 and controls alternative splicing of other genes (Kuo, Doudeva, Wang, Shen, & Yuan, 2009). In a diseased individual, TDP-43 is hyperphosphorylated and otherwise modified to yield "pathogenic TDP-43" which forms toxic aggregations. Cycad consumption is linked to the formation of TDP-43 aggregates through misincorporation of the non-proteinogenic amino acid β-methylamino-L-alanine, present in cycad seeds, during translation (Dunlop, Cox, Banack, & Rodgers, 2013).

Etiology

The three most researched cycad toxins that have been named as potential causes for ALS-PDC are: β-methylamino-L-alanine, or BMAA; methylazoxymethanol, or MAM, which is derived from cycasin; and β-sitosterol β-D-glucoside (BSSG). The most support in scientific literature has been provided for BMAA, although there have been times in which BMAA was largely rejected as a potential cause before being reinvigorated by new findings. While BMAA is thought to have multiple modes of neurotoxicity, all three of these toxins share one commonality: toxin-involved overstimulation of glutamate receptors leads to neuronal cell death. The potential neurotoxic effects of the three toxins should not be viewed as alternative hypotheses for the true one cause of ALS-PDC. It is possible that all these toxins, along with other factors such as genetic predisposition and age of exposure, contribute to the etiology of the disease and make individuals more susceptible to ALS-PDC.

BMAA

β-methylamino-L-alanine, or BMAA, is the most studied of the neurotoxins in cycad-related neurodegeneration. Structurally, BMAA differs from the normal, proteogenic protein alanine from which it is derived only by the addition of a methylamino group on the side chain. This chemical substitution results in a distinct change in chemical properties—while the side chain of alanine is small, nonpolar and hydrophobic, the side chain of BMAA has polar and basic properties. As a common cyanobacterial neurotoxic secretion, BMAA is not limited to cycad roots; it has been found in contaminated seafood and drinking water supplies as well (Holtcamp, 2012). BMAA is a non-proteinogenic amino acid, meaning it is not one of the twenty amino acids that can be used by organisms to synthesize a polypeptide chain during protein synthesis. Although DNA transcription and mRNA translation are highly controlled mechanisms with specialized regulatory systems that help to prevent the addition of such abnormal amino acids to a polypeptide sequence, BMAA and other incorrect amino acids may be included in a protein at low error rates. For somatic cells, this is largely inconsequential because significantly affected cells can undergo apoptosis and be replaced before the abnormal proteins can create large-scale problems. However, neurons do not undergo such cellular replication. Abnormal proteins such as those resulting from BMAA inclusion can accumulate in these cells. For this reason, neurons are especially susceptible to the effects of BMAA toxicity. There are several neurotoxic effects that have been linked to BMAA exposure, leading to the hypothesis that BMAA exposure from cycad and flying fox consumption is to blame for the increased rate of ALS-PDC in the Chamorro people of Guam and other populations.

One mode of BMAA neurotoxicity involves improper inclusion of BMAA in polypeptide chains leading to improper protein folding. This can result in the exposure of normally interior hydrophobic amino acids, which are attracted to exposed hydrophobic amino acids in other misfolded proteins. These intermolecular attractions between hydrophobic amino acids in misfolded proteins result in the formation of small protein aggregates. Much like the propagation of prion proteins in prion diseases, abnormal proteins containing BMAA can induce the aggregation of other proteins. BMAA inclusion in polypeptides ultimately results in the formation of large, toxic protein aggregates that may disrupt cellular function (Krammer et al., 2001). The hypothesis that BMAA-induced misfolding was the cause of its neurotoxic effects was criticized due to the high specificity of tRNA limiting the potential for a non-proteinogenic amino acid such as BMAA to be incorporated into a polypeptide chain. However, it was later discovered that BMAA can be mistaken for L-serine by tRNA synthetase (Dunlop et al., 2013), giving it the potential to be misincorporated into proteins at a higher rate than previously thought. One specific protein that is particularly affected by BMAA misincorporation is TDP-43. As previously discussed, TDP-43 aggregates are often found in post-mortem brain examinations of people who died of amyotrophic lateral sclerosis (Igaz et al., 2011).

BMAA's role in neurotoxicity is not limited to improper incorporation into polypeptide chains. Direct exposure to unbound BMAA leads to substantial loss of motor neurons in spinal cord culture due to overstimulation of glutamate receptors (Rao, Banack, Cox, & Weiss, 2006) . Glutamate is an excitatory neurotransmitter whose overexpression can lead to toxic effects in neurons. Glutamate-mediated excitotoxicity is characteristic of other neurodegenerative diseases including Alzheimer's disease (Hynd, Scott, & Dodd, 2004). BMAA-associated acute motor

neuron loss was prevented in cultures including NBQX, a glutamate receptor antagonist. In motor neurons, but not the remainder of spinal neurons, glutamate receptor overstimulation by BMAA induced a toxic increase in production of reactive oxygen species. Extended BMAA exposure depletes glutathione, an important antioxidant, which may worsen the effect of the upregulated reactive oxygen species on cells (Rush, Lie, & Lobner, 2012). Glutamate receptor overstimulation also leads to a substantial rise in intracellular calcium ion concentration (Rush et al., 2012), which has been shown to correlate with neuronal degeneration (Hartley, Kurth, Bjerkness, Weiss, & Choi, 1993). It is possible that the loss of spinal motor neurons resulting from overstimulation of glutamate receptors by BMAA plays a role in the ALS-like symptomology and pathology seen in most cases of ALS-PDC.

Cycasin & MAM

Unlike BMAA, cycasin is secreted by cycads themselves and not by a cyanobacterial endophyte. Cycasin is an azoxyglucoside that is readily hydrolyzed through metabolism to form glucose and methylazoxymethanol, or MAM. MAM is linked to carcinogenicity, hepatotoxicity, and neurodegeneration in humans (Wilson, Khabazian, & Wong, 2002). One-time exposure to MAM has been shown in animal models to lead to substantial DNA damage in the form of O6-methyldeoxyguanosine lesions (Esclaire et al., 1999). As the name implies, these lesions involve improper methylation of guanine nucleotides at the O6 position of the nitrogenous base. This increases the likelihood of G:C-A:T mutations in DNA replication. It is believed that this occurs when DNA polymerases mistake the O6-methylguanine as an adenine nucleotide due to the conformational similarity of O6-methylguanine to adenine (Kisby et al., 2011).

The link to O6-methylguanine-induced DNA replication errors readily explains the carcinogenicity of MAM; if mutations continue to occur in certain signaling or replication mechanisms, uncontrolled cell proliferation could result and lead to tumor formation. It is less obvious how the DNA damage induced by MAM affects neurons of the central nervous system that do not undergo mitosis. Convincing evidence toward the etiology of ALS-PDC resulting from MAM toxicity was first presented in 2011, when unrepaired O6-methylation of neuronal DNA in young animal models was linked to activation of signaling pathways associated with neurodegeneration (Kisby et al., 2011). One such pathway involves upregulation of two distinct glutamate receptors. As discussed in previous sections, glutamate-mediated excitotoxicity is linked to neurodegeneration. These findings suggest that exposure to MAM through consumption of cycad-derived foods in early life may contribute to the development of the neurodegenerative ALS-PDC.

BSSG

Isolation of cycad seed compounds reveals that cycads contain numerous sterols (Marler & Shaw, 2009). One of these sterols, β-sitosterol β-D-glucoside (BSSG), has been shown in multiple animal models to have substantial neurotoxic effects (Wilson et al., 2002; Wilson, 2005). β-sitosterol (BSS) is a widely distributed sterol that was never considered as a candidate for explaining cycad neurotoxicity due to its presence in many food items that are not linked to neurodegenerative disease, such as vegetable oil and nuts (Anderson & Nabenhauer, 1924). BSSG is a derivative of BSS that is rare in most organisms, but present in cycads. A 2002 study revealed that, when neurodegeneration was triggered in animal models through cycad consumption, the most toxic compound by some measures was BSSG; BMAA and MAM were

only found to be present in trace amounts (Wilson et al., 2002). The neurotoxicity in this study was quantified by measuring depolarizing field potentials leading to lactate dehydrogenase release. Lactate dehydrogenase is a known marker for cell turnover and has been described previously as useful for quantification of glutamate-mediated neuronal death (Koh & Choi, 1987). It is noteworthy that BSSG was found to be more toxic than BMAA and MAM by a marker of neurotoxicity that is based upon glutamate-mediated neurotoxicity, considering that this is a proposed mechanism of neurotoxicity for both of these compounds as well (as discussed in previous sections). However, these studies took place prior to the finding that the methodology of previous studies likely underestimated BMAA quantities in tissues.

Genetic Considerations

ALS-PDC was quickly ruled out as a genetic disorder. While most cases occurred in certain populations, there are documented cases of people outside these populations contracting the disease in the same geographic location. For example, a Caucasian man contracted ALS-PDC after spending several years in Guam (Waring & Annegers, 1994). The fact that non-natives can develop ALS-PDC in the same geographic location led to the conclusion that ALS-PDC is primarily caused by environmental factors. However, while genetics are not the primary cause of ALS-PDC, there are genetic factors that have been linked to increased or decreased susceptibility to the disease.

Apolipoprotein E (ApoE) is a protein in mammals that is involved in fat and cholesterol metabolism. It is produced by the liver for somatic tissues and produced by astrocytes for tissues of the central nervous system (Liu, Kanekiyo, Xu, & Bu, 2013). The ApoE that is secreted by

astrocytes is the primary carrier of cholesterol in the central nervous system (Puglielli, Tanzi, & Kovacs, 2003). ApoE is heavily researched as a risk factor for Alzheimer's disease (Liu et al., 2013). In some populations, those who express the E4 variant of E4 carry 10 to 30 times the risk for late-onset Alzheimer's disease compared to the general population. In his 2005 dissertation, Wilson (2005) published experiments on two considerations of the potential role of ApoE in ALS-PDC. The first experiment sought to determine whether the presence or absence of ApoE affected neurodegeneration in cycad-fed mice. He found that ApoE knockout mice did not show the motor deficits that the ApoE wild type mice showed after being subjected to the same cycad-rich diet. The second compared neurodegeneration in cycad-fed mice expressing different isoforms of ApoE: human E2, E3, and E4 isoforms, as well as mouse wild-type ApoE. This revealed that cycad-fed mice expressing human ApoE4 were particularly susceptible to motor deficits, while those expressing E2 were somewhat protected (although still affected). The results of these experiments indicate that ApoE plays some role in generating neurotoxicity from cycad toxins, and humans expressing the E4 variant of ApoE may be more susceptible. The exact mechanism is not known, although Wilson speculates that the cholesterol-carrying ApoE may contribute to entry and metabolism of BSSG, a neurotoxic sterol found in cycad seeds and discussed previously.

Another genetic factor that has been suggested to affect susceptibility to ALS-PDC is variation in the microtubule associated protein tau gene, *MAPT,* which codes for the production of tau proteins. Alternative splicing of *MAPT* results in the potential for multiple forms of tau to be produced by the *MAPT* gene (Goedert, Wischik, Crowther, Walker, & Klug, 1988). *MAPT* variation has been shown to increase susceptibility to other neurodegenerative diseases such as

progressive supranuclear palsy (Conrad et al., 1999). A 2007 study comparing individuals diagnosed with ALS-PDC to control groups identified two independent sites of genetic variation within the *MAPT* gene that are associated with an increased risk of developing the disease (Sundar et al., 2007).

Areas of Interest for Future Research

Findings based on molecular data revealed that all extant cycads evolved within the last 50 million years. Because all extant cycads possess coralloid roots to facilitate symbiosis with cyanobacteria, it is possible that these structures helped these extant cycads to survive extinction events, while any cycads that lacked coralloid roots died out. It is difficult to find literature on the evolutionary origin of coralloid roots. If possible, future morphological and molecular studies should be performed on cycad fossils to attempt to discover the point in cycad evolutionary history at which coralloid roots are first seen.

More studies should be performed on biomagnification in mammals outside of flying foxes. If cycad seed-eating feral pigs, deer, or possums accumulate BMAA or other toxins within their tissues, consumption of these wild-caught animals should be avoided.

As the exact mechanisms of toxicity are elusive for all neurodegenerative diseases, further studies should continue to seek to find the exact manner in which ALS-PDC and other diseases progress. Markers such as NFT, β-amyloid plaques, Lewy bodies, and TDP-43 aggregations are evidently associated, but it is not certain exactly how they cause toxicity or whether they are

actually toxic or simply a by-product of unknown toxic mechanisms. Studies to identify other genes that affect susceptibility to ALS-PDC should also be performed.

Conclusion

Cycads are ancient seed plants with a rich fossil history. Their association with nitrogen-fixing cyanobacteria contributes to some, but not all, of their well-known toxicity. Flying foxes and other cycad-fruit-eating mammals serve to biomagnify cycad toxins due to the accumulation of these toxins within the mammals' tissues. Consumption of cycad-derived foods and flying foxes allows the effects of cycad toxins to manifest in humans and other animals. The interactions between cycads, cyanobacteria, flying foxes, and humans may lead to a severe neurodegenerative disease known as the amyotrophic lateral sclerosis and parkinsonism-dementia complex, or ALS-PDC. Incidence of ALS-PDC has decreased accordingly with a decrease in cycad and flying fox consumption among populations that previously expressed extraordinarily high incidence rates of the disease.

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