Hypothesis: Etiologic and Molecular Mechanistic Leads for Sporadic Neurodegenerative Diseases Based on Experience With Western Pacific ALS/PDC

*Peter S. Spencer**

Department of Neurology, School of Medicine and Oregon Institute of Occupational Health Sciences, Oregon Health & Science University, Portland, OR, United States

Seventy years of research on Western Pacific amyotrophic lateral sclerosis and Parkinsonism-dementia Complex (ALS/PDC) have provided invaluable data on the etiology, molecular pathogenesis and latency of this disappearing, largely environmental neurodegenerative disease. ALS/PDC is linked to genotoxic chemicals (notably methylazoxymethanol, MAM) derived from seed of the cycad plant (*Cycas* spp.) that were used as a traditional food and/or medicine in all three disease-affected Western Pacific populations. MAM, nitrosamines and hydrazines generate methyl free radicals that damage DNA (in the form of O^6-methylguanine lesions) that can induce mutations in cycling cells and degenerative changes in post-mitotic cells, notably neurons. This paper explores exposures to naturally occurring and manmade sources of nitrosamines and hydrazines in association with sporadic forms of ALS (with or without frontotemporal degeneration), progressive supranuclear palsy, and Alzheimer disease. Research approaches are suggested to examine whether these associations might have etiological significance.

LAY SUMMARY

Unknown environmental exposures are thought to be risk factors for non-inherited forms of certain progressive brain diseases, such as sporadic forms of amyotrophic lateral sclerosis (sALS), progressive supranuclear palsy (sPSP), and Alzheimer's disease (sAD). Related progressive and fatal brain disorders coalesce in a single neurodegenerative disease of largely environmental origin (ALS-Parkinsonism-dementia Complex) that has affected three genetically distinct populations residing in islands of the Western Pacific region. Prolonged study of this prototypical neurodegenerative disease has provided invaluable information on the probable environmental cause (specific chemical genotoxins) and molecular mechanisms (unrepaired nerve cell DNA-damage) by which brain degeneration begins, evolves and, years or decades later, clinical signs appear, and progress. This information is used as a foundation to explore whether chemically related genotoxins (nitrosamines, hydrazines) are possible risk factors for sALS, sPSP, and sAD. Methods to test this hypothesis in the field and laboratory are proposed.

INTRODUCTION

There is wide acceptance that environmental exposures contribute to an unknown extent to the genesis of sporadic neurodegenerative disorders. such as amyotrophic lateral sclerosis (sALS), progressive supranuclear palsy (sPSP), and Alzheimer disease (sAD). The nature of these environmental factors, when critical exposures occur, and the molecular mechanisms that propel progressive neuronal demise, are largely unknown. The one exception is Western Pacific ALS where the location of exposure is known, the nature of the culpable chemicals and their molecular mechanisms increasingly understood, and the latency (years to decades) to onset of clinical disease established. It seems logical therefore to draw on this knowledge to develop testable hypotheses about exposures, mechanisms, and course of related neurodegenerative disorders, including sporadic forms of ALS, PSP, and AD.

WESTERN PACIFIC ALS/PDC

Characterization

Three genetically distinct populations in the Western Pacific region have experienced a very high incidence of ALS: Chamorro and other residents of Guam and Rota in the Mariana Islands; Japanese residents in two regions of the Kii Peninsula of Honshu Island, Japan, and Auyu and Jaqai linguistic groups on the island of New Guinea in Papua, Indonesia. These geographic isolates of ALS are associated with a high incidence of atypical parkinsonism (P) and dementia (D), such that they are described as the Western Pacific ALS/P-D Complex (ALS/PDC). Over the past many decades, disease incidence has declined in all three affected populations: for example, whereas in the 1950s, Guam ALS was often familial and 50–100 times more prevalent than anywhere else in the world (1), the disease has now reportedly disappeared from the island (Steele, personal communication). This occurred in concert with the post-World War II acculturation of the Guamanian population to North American practices (2).

Whereas, Western Pacific ALS/PDC was once thought to be genetic and later attributed to gene-environmental interactions (3, 4), it is now clear the etiology of this disease is heavily environmental in origin (2). The clinical phenotypes of ALS/PDC resemble a dose-response regimen in which the largest exposures precipitate motor neuron degeneration at a young age (teenage onward), parkinsonism-dementia with some amyotrophy impacts older subjects, AD-like dementia affects the oldest and putatively least-exposed clinical phenotype, while the pervasive neurofibrillary pathology in clinically normal Guamanians probably reflects the lowest level of exposure to the culpable environmental agent(s) (2).

That ALS/PDC is a single disease entity is generally accepted given the presence in some subjects of overlapping clinical and neuropathological phenotypes.

Exposure Timing, Type, and Latency

Various studies of population movement from and to areas of high-incidence ALS/PDC have provided critical information on the relationship between the duration of exposure to the disease-associated environment and the latency between the time of exposure termination and the appearance of clinical disease. Study of migrants from Guam to North America showed that risk for neurodegenerative disease could be acquired on-island before the age of 18 and appear clinically up to 34 years after arrival on the U.S. mainland (5). Similarly, Filipino migrants sometimes developed clinical ALS or parkinsonism-dementia years or decades after moving to and arrival in Guam (6). One 3-year-old Japanese subject who migrated from the southern high-risk ALS/PDC area in the Kii Peninsula developed pyramidal signs, parkinsonian symptoms, and mildly impaired cognitive function 73 years later (7), and a member of the U.S. Air Force stationed on Guam for only 4 months was diagnosed with ALS 10–20 years later (2). Taken together, these data suggest that exposure to an environment with high-incidence ALS/PDC is a risk factor for motorsystem disease that appears clinically years or decades after exposure.

While the foregoing suggests the critical environmental exposure can occur in infancy, childhood or adulthood, there is also evidence of developmental perturbation of the brains of some subjects with Guam and Kii-Japan ALS/PDC. A proportion of Guam and Japanese subjects that died with ALS/PDC in late life showed neuropathological evidence of ectopic and multinucleated Purkinje cells (8, 9) similar to those reported in postnatal mice treated with the principal cycad-derived genotoxin (10). The potential significance of these findings for ALS/PDC was discussed in 1987 by Spencer (11) in relation to "slow toxin(s)" in food prepared from incompletely detoxified water-soaked cycad seed formerly eaten on Guam. Thus, the subclinical cerebellar abnormalities found in some Guamanian and Japanese ALS/PDC cases may represent biological markers of early-life exposure to cycad toxins or chemically related substances (see below).

Environmental Agent

The culpable environmental factor(s) appear to be linked to traditional practices that have declined with the advance of modernity in the three Western Pacific populations formerly impacted by high-incidence ALS/PDC. The only significant environmental exposure common to all three geographic isolates of the neurodegenerative disease is to the neurotoxic seed of the cycad plant (*Cycas* spp.) (2), which in various forms was used without detoxication as a traditional medicine. In addition, on Guam and Rota, cycad seed was traditionally an important source of food, especially during periods of shortage. As observed in 1954 by ethnobotanist Marjorie Whiting, preparation (detoxication) is laborious since it involves prolonged soaking of the cycad seed gametophyte in water that must be frequently changed. While a common food component

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Abbreviations: AD, Alzheimer disease; ALS/PDC, Western Pacific amyotrophic lateral sclerosis and parkinsonism-dementia; L-BMAA, beta-*N*-methylamino-L-alanine; DDR, DNA Damage Response; FUS, Fused in Sarcoma; FTD, Frontotemporal degeneration; MAM, Methylazoxymethanol; MFH, *N*-Methyl-*N*-formylhydrazine; MGMT, O^6-Methylguanine methyltransferase; MMH, Monomethyhydrazine; NDEA, *N*-Nitrosodiethylamine; O^6-mG, O^6-Methylguanine; PSP, Progressive supranuclear palsy; STZ, Streptozotocin; TSP-43, TAR DNA-binding protein 43.

at village fiestas during which all-comers (including state-siders, such as U.S. military) were welcome, "only small amounts are given to children because many became ill when they first eat a dish made with cycad starch… Some people can never eat the Federico (cycad): they get a headache even though they like it… When I inquired about the cause of ALS (or, in the vernacular "*leetiko*"), several persons suggested the cycad." People like to eat it and although they know of its toxicity [and] go to considerable lengths to obtain and process it (12). At least one Chamorro adult with ALS/PDC reported exposure to a "bad batch" of cycad on a single occasion (*The Poison that Waits?* BBC, London. 1968. https://vimeo.com/1621281 at 40 minute mark).

In Kii-Japan, cycad seed was prescribed by folk medical practitioners (*kitoshi*) for various ailments (e.g., gonorrhea, dysmenorrhea, neuralgia, abdominal discomfort) and also used to prepare a tonic in a home-based remedy intended to strengthen the body (2). With the post-World War II introduction of allopathic medicine (Ki-Japan, Guam) and American food culture (Guam), the traditional practices declined and gradually disappeared in concert with the risk for ALS/PDC. Similarly, with the Indonesian colonization of West New Guinea, the prevalence of ALS/PDC among the Auyu and Jaqai also declined.

Cycad Chemicals

The major neurotoxic chemical in the seed of *Cycas* spp. is cycasin, the glycone of the genotoxic and neurotoxic agent methylazoxymethanol (MAM). Also present in smaller concentrations is the neurotoxic amino acid beta-*N*-methylamino-L-alanine (L-BMAA) (**Table 1**), a compound that has received much research attention since my group showed in 1987 that large daily oral doses induced a motorsystem disorder in adult primates [reviewed in (13)]. Cycasin appears to be responsible for the locomotor disorder in animals grazing on cycad leaves, and there is strong experimental evidence that MAM is a potent developmental neurotoxin. The concentration in Chamorro-style cycad flour of residual cycasin, but not of L-BMAA, showed a strong statistical relationship with the historical incidence of ALS and P-D among Guam males and females (14). Whether cycasin-MAM alone or with L-BMAA contributes to the etiology of ALS/PDC is unclear but the fact that both substances can act as alkylating agents that induce DNA damage (15, 16) is of singular importance to the present hypothesis, as illustrated by MAM.

DNA Damage From Cycad Chemicals

Experimental animal studies show that single systemic doses of MAM can induce dysplasia of the developing cerebellum, features that are represented in a proportion of Guamanian and Kii-Japan subjects with ALS/PDC (8, 9). The cerebellar pathology appears to result from MAM-induced DNA damage in the form of O^6-methyguanine (O^6-mG) (**Table 1**), which is subject to repair by O^6-mG methyltransferase (MGMT). MAM-induced cerebellar damage is increased in transgenic animals lacking MGMT and decreased in animals overexpressing MGMT (15). The adult human brain often has very low levels of this critically

TABLE 1 | PubChem links for chemical substances in order of their appearance in the text.

Chemical	PubChem URL
Cycasin (MAM-glucoside)	https://pubchem.ncbi.nlm.nih.gov/compound/5459896
Methylazoxymethanol (MAM)	https://pubchem.ncbi.nlm.nih.gov/compound/6433205
Beta-*N*-Methylamino-L-alanine (BMAA)	https://pubchem.ncbi.nlm.nih.gov/compound/105089
O^6-Methylguanine (O^6-mG)	https://pubchem.ncbi.nlm.nih.gov/compound/65275
Streptozotocin	https://pubchem.ncbi.nlm.nih.gov/compound/29327
N-Nitrosodiethylamine	https://pubchem.ncbi.nlm.nih.gov/compound/5921
N-Nitrosoatrazine	https://pubchem.ncbi.nlm.nih.gov/compound/108077
Gyromitrin	https://pubchem.ncbi.nlm.nih.gov/compound/9548611
Monomethylhydrazine (MMH)	https://pubchem.ncbi.nlm.nih.gov/compound/6061
N-Methyl-*N*-formylhydrazine (MFH)	https://pubchem.ncbi.nlm.nih.gov/compound/12962
N-Nitrosodimethylamine	https://pubchem.ncbi.nlm.nih.gov/compound/6124
Formaldehyde	https://pubchem.ncbi.nlm.nih.gov/compound/712
Nitromethane	https://pubchem.ncbi.nlm.nih.gov/compound/6375
Annonacin	https://pubchem.ncbi.nlm.nih.gov/compound/354398
Nitrophenylethane	https://pubchem.ncbi.nlm.nih.gov/compound/118364596
3-Nitrotyrosine	https://pubchem.ncbi.nlm.nih.gov/compound/65124
Phenylhydrazines	https://pubchem.ncbi.nlm.nih.gov/#query=Phenylhydrazines
Pyrazolones	https://pubchem.ncbi.nlm.nih.gov/#query=Pyrazolones

PubChem is a database of chemical molecules, their activities in biological assays, and their health effects in humans and animals. The system is maintained by the National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the U.S. National Institutes of Health (NIH).

important DNA-repair enzyme such that, in MAM-treated *mgmt* ko mouse brain, DNA damage accrues and activates cell signal pathways associated with human neurodegeneration, notably AD and ALS (17). Failure of adult neurons to repair MAM-induced DNA damage is proposed as the trigger that initiates the slow process of post-mitotic neuronal demise. This is consistent with the conclusion from traditional epidemiological reasoning that cumulative DNA damage may contribute to disease onset in ALS (18, 19).

Nitrosamines and Hydrazines

Two classes of chemicals, nitrosamines and hydrazines, have genotoxic mechanisms and clinical effects comparable with those of MAM. Like MAM, both substances are acutely hepatotoxic

and potentially carcinogenic because, in mitotic cells, such as the lining of the gastrointestinal tract, overwhelming DNA damage with limited DNA repair can lead to mutation and uncontrolled cell division. In fact, azoxymethane, which is metabolized to the developmental neurotoxin MAM, is a useful experimental tool to create a model of human colon carcinogenesis (20, 21). By contrast, in the murine brain, post-mitotic neurons accrue MAM-induced DNA damage, with short and long-term effects on cell signal pathways linked to neurodegenerative disease as well as cancer (22, 23).

Nitrosamines

There is strong evidence for the neurotoxic potential of the nitrosamine-related compound streptozotocin (STZ: 2-deoxy-2-(3-methyl-3-nitrosoureido-D-glucopyranose) (**Table 1**), the cytotoxicity of which is mainly due to DNA alkylation, including O^6-methylguanine, 7-methylguanine, 3-methyladenine and 7-methyladenine in liver, kidney, intestine, and pancreas, but little methylation of brain DNA (24). However, intracerebroventricular injection of STZ produces effects that resemble molecular, pathological, and behavioral features of AD, including increased amyloid-β protein and neurofilament expression, increased phosphorylation of tau protein, and neuronal loss in hippocampus and cerebral cortex (24–28). Rats treated with STZ show a reduction in alpha and gamma motor neurons innervating soleus and medial gastrocnemius muscles, together with the areas of corticospinal tracts serving the trunk, hindlimb and, to a lesser extent, the forelimbs (29–31). It is not clear if loss of motor neurons represents a direct effect of STZ or whether it results from a chronic diabetic state induced in the animals by the toxic effect of the nitrosamine derivative on pancreatic beta-islet cells.

The direct neurotoxic property of N-nitrosamines has been demonstrated in the case of the food and water contaminant N-nitrosodiethylamine (NDEA). Treatment of post-mitotic rat cerebellar neurons (48 h) *in vitro* produced dose-dependent increases in DNA damage and oxidative stress, similar to the effects of STZ treatment and AD neurodegeneration (32). The authors of this study pointed out that nitrosamines such as NDEA readily form in meat and fish that have been preserved or flavored with nitrites, and nitrosamines can form in the gastric acidic environment from ingested nitrites and nitrates (33–35). They suggest that the expanded use of nitrites and nitrates in foods and agricultural products over the past 30–40 years may have contributed to the growing prevalence of AD and possibly other forms of neurodegeneration (32). Exposure to nitrosamines with DNA adduct formation also occurs during inhalation of tobacco smoke, which is sometimes identified as a risk factor for both ALS (36–38) and AD (39, 40) as well as cancers (41).

Nitrosamine exposure might also be relevant to the high incidence, younger age of onset, and clustered appearance of ALS among soccer players in Italy (42–46) and elsewhere (47, 48). For Italian professional soccer players aged 45 years and younger, the ALS rate (1959–2000) was 4.7 times higher than the general population, and the average age was 43 years at onset compared with 63 years. Herbicides used on playing fields were among the factors linked to the high rates of ALS

among Italian soccer players (45). Herbicides such as atrazine, which was used in Europe between 1960 and 2004, when it was banned because of persistent groundwater contamination, were employed to control weeds during both the soccer and off season (49). Atrazine can react with nitrite to form a potentially toxic nitrosamine product, N-nitrosoatrazine (**Table 1**) (50, 51). Since nitrosamines are present in rubber products (52), nitrosamines are also liberated from recycled rubber crumb used in artificial turf (53, 54). Additionally, since nitrosamines are associated with leather products (see below), contact with leather soccer balls and footwear provide other potential opportunities for exposure to these toxic substances. Professional soccer now employs synthetic leather balls.

Hydrazines

The second group of compounds related mechanistically to MAM are hydrazine compounds. Hydrazine is used in agricultural chemicals (pesticides), chemical blowing agents, pharmaceutical intermediates, photography chemicals, boiler water treatment for corrosion protection, textile dyes, and as fuel for rockets and spacecraft. These neurotoxic compounds are also associated with some poisonous mushrooms that are eaten and sometimes precipitate acute neurotoxic illness.

Hydrazones (such as gyromitrin: acetaldehyde N-methyl-N-formylhydrazone) that generate monomethylhydrazine (MMH) upon hydrolysis are components of certain wild fungi, notably False Morels (*Gyromitra*, *Helvella*, *Verpa* spp.) (**Table 1**). Consumption of *Gyromitra* spp. can trigger acute gastrointestinal (nausea, vomiting, diarrhea) and neurotoxic effects (headache, vertigo, ataxia, fever, muscle fasciculation, seizures, coma) (55). MMH forms hydrazones with pyridoxal phosphate, which reduces production of the inhibitory neurotransmitter (GABA) via decreased activity of glutamic acid decarboxylase (56). Additionally, N-methyl-N-formylhydrazine (MFH), which is produced during MMH metabolism, undergoes cytochrome P450-regulated oxidative metabolism and, via reactive nitrosamide intermediates, leads to the formation of DNA-damaging methyl free radicals (57). As with MAM and nitrosamines, the O^6-methylation of guanine can cause liver and kidney lesions (58–63) and tumor formation (62). The risk of long-term adverse effects may be greater in individuals with genetic slow acetylation rates because decreased detoxication (acetylation) of MFH would result in larger amounts of MMH formed from gyromitrin (64, 65).

Since hydrazines and MAM induce the same type of DNA damage, both potentially with the involvement of formaldehyde (18, 66), it is hypothesized that single or repeated exposure to methyl free-radical-generating hydrazines might trigger long-latency neurodegeneration culminating in ALS or a related brain disease. Four sources of information are consistent with this hypothesis and thus require detailed research exploration. Two involve potential links to False Morel mushrooms and two to engine fuels, both of which are associated with hydrazines. The information available on these topics is scattered, far from complete, and is offered here with the intent of stimulating new avenues of investigation of possible risk factors for sporadic

neurodegenerative disorders, the causes of which have so far defied explanation.

ALS and Fungal Hydrazines
France

Mushroom consumption has been identified as a risk factor for a cluster of 12 ALS patients, one with ALS-parkinsonism, in a morel-consuming community in Savoie in the French Alps (67, 68). Between 1991 and 2003, five long-term residents (including 2 spouses) in a population of 200 subjects developed ALS. Seven other ALS cases spent part of their life in the area. Half of the 12 cases consumed local mushrooms, including morels. While True Morels (*Morchella* spp.) are highly prized as a delicacy in Europe and beyond, it is often difficult to distinguish anatomically between True and False Morels (69, 70). As described above, False Morels are a source of neurotoxic and DNA-damaging MMH. Almost half of the ALS cases had an acute intoxication with "morels" in their medical history (68).

Finland

The birth location of a cluster of ALS subjects in Finland corresponds to a region of False Morel consumption. A single significant cluster of 227 ALS cases was identified in southeast Finland using data on the place of birth (*circa* 1920) (71, 72). The cluster involved a population of half a million subjects residing in parts of the provinces of Kuopio, Mikkeli, and Pohjois-Karjala, as well as parts of present-day Russian Karelia. Whereas, the prevalence of multiple sclerosis was 52.5% lower, ALS rates were 225% higher among Finnish WWII evacuees from Karelia (18 per 100,000) compared with non-evacuees (8 per 100,000). As noted by the authors, these data speak against a genetic etiology for ALS and for one or more environmental factors that made the evacuees more liable to develop motor neuron disease later in their lives (73, 74).

During and after the Finnish Winter War with Russia (November 30, 1939–March 12, 1940), there was a mass migration of Karelians (*circa* 400,000 persons) to Finland and particularly to the southeast (Itä-Suomi) where there is a strong mushroom-eating culture, including MMH-generating *Gyromitra esculenta*, among Karelians. Between 1914 and 1945, one quarter of the number of acute poisonings attributed to *G. esculenta* occurred in southeastern Finland (75). Notably, at that time, dried, or once-boiled fresh specimens were considered safe to eat, in contrast to the extensive washing and double-boiling procedure later recommended by the Finnish Food Authority which, in 2019, stated that *G. esculenta* is not to be eaten by pregnant and breastfeeding women and children because of "residues of the toxin gyromitrin despite processing" (https://www.ruokavirasto.fi/en/private-persons/information-on-food/instructions-for-safe-use-of-foodstuffs/safe-use-of-foodstuffs/). Consumption of mushrooms increased during wartime because of marked food shortages. Mushroom consumption by Finnish "families of workers and functionaries" peaked between 1943 and 1944 when, in Kuopio for example, an amount of 165.5 g/week per consumer unit was 5 times greater than mean consumption in 1941 and 1945 (75).

Given the varied phenotypes of Western Pacific ALS/PDC, including an AD-like form, it is noteworthy that Finland is also reported to have the highest death rate from dementia in the world, which has been suggested to result from AD-related environmental factors, including L-BMAA generated by cyanobacteria in the Gulf of Finland and in the country's many freshwater lakes (76). A possible contribution from the unique practice of consuming wild and commercially available False Morels is another possible subject for investigation.

Mid-West, USA

There is a high prevalence of ALS in the mid-West (77), where cooking and eating False Morels is practiced (70, 78). The highest number of historical reports of acute MMH poisoning has occurred in Michigan (79). While environmental exposures linked to ALS have been studied in Michigan (77, 80), and in a small ALS focus near Lake Michigan WI in which familial cancer and frequent fish consumption were more often reported in cases than controls (81), the question whether sALS is linked to food use of wild mushrooms and mushroom poisoning has not been explored.

ALS and Hydrazine Fuels

Hydrazines have been used in hypergolic fuels to power spacecraft for over 50 years (82). In the Titan II Nuclear Missile program, the toxic breakdown products of hydrazine and unsymmetrical dimethylhydrazine (UDMH) (including *N*-nitrosodimethylamine and formaldehyde) persisted for 6 weeks or longer in the missile silos after spills or leaks (**Table 1**) (83, 84). Ground fueling of spacecraft with hydrazine mixtures requires a crew of five using protective gear, supported by 20 specialists (84). Hydrazine fuels appear to be of past and ongoing interest to the NASA Ames Research Center in relation to the Pioneer 10 and future Mars space probes, respectively (85–89). NASA Ames lists five papers researching hydrazine sulfate and ammonium hydrazinium sulfate between 1993 and 1998 (90). In 2016, an apparent cluster of ALS cases was reported at the Ames Research Center, at Moffett Federal Airfield in California's Silicon Valley. Several of the ALS cases worked together, and seven of the 8 employees who had worked in buildings 240, 244, or 245 on the north side of the Ames campus had contracted ALS post-2000 (91, 92). Given that ~2,500 people worked at the Ames campus, the number of ALS cases was clearly in excess. One subject was diagnosed with ALS at the age of 55, 21 years after beginning work at the Ames Research Center (92). Whether there is any relationship between ALS and exposure to hydrazine or other chemicals at this site has yet to be investigated.

Hydrazine has also been used to fuel the emergency power units (EPU) of the NASA Space Shuttle, the U-2 spy plane, the International Space Station (see also Note Added in Proof), and the General Dynamics F-16 fighter jet. A 70% aqueous solution (H70) of hydrazine powers the F-16 EPU, which provides emergency electrical and hydraulic power in the event of engine failure. Pilots and ground maintenance crews can be exposed to hydrazine vapors during operations (93–96). F-16s were used by the USA between 25 January and 28 February 1991 in the Desert Storm combat phase of the Gulf War. Military personnel, notably

Air Force personnel, who were deployed to the Gulf Region during the Gulf War period, experienced a greater post-war risk of ALS (and younger age of onset) than those who were not deployed to the Gulf (97). For example, one pilot who flew 44 F-16 combat missions over Iraq during Desert Storm was diagnosed with ALS at the age of 37 (98). Whether these associations are circumstantial or otherwise, they offer a new avenue to explore the etiology of early-age onset of ALS among military personnel who served in the Gulf War.

Hydrazine and nitromethane were used to fuel racing cars, funny cars and dragsters in the 1960s (99, 100). An Internet search reveals several individuals with a history of motor racing who developed ALS (101–106).

Genotoxins and PSP and ALS

PSP in Guadeloupe

Typical ALS and, in older subjects, upper and lower motor neuron signs in combination with supranuclear palsy, parkinsonism, and dementia, have been described in the Guadeloupe archipelago (107). A significant number has a syndrome resembling progressive supranuclear palsy (PSP) (108, 109), which has also been reported in New Caledonia and on Guam (110–112). The disorders in Guadeloupe and New Caledonia are associated with regular food and beverage use of the fruit of Annonaceae, such as the sour sop (*Annona muricata*). Annonaceae contain the acetogenin annonacin (**Table 1**), a mitochondrial Complex 1 inhibitor with neurotoxic properties (113). There is also evidence that prolonged (12 months) oral ingestion of commercially available *A. muricata* juice aggravates cerebral tau phosphorylation in wild-type and tau-transgenic mice. The brains of these animals showed increased reactivity of 3-nitrotyrosine (**Table 1**), which suggests oral intake of sour sop juice promotes the generation of reactive nitrogen species (114). Conceivably, this might arise from the presence in Annonaceae of nitrophenylethane (**Table 1**) (115) which has a terminal O=N=O group that, as with nitrite, can react readily with amines and amides to form *N*-nitroso compounds (116). The possibility that MAM-like nitrosamines contribute to the etiology of environmental PSP may merit investigation see below).

PSP in France

Proposed as the most compelling case for an environmental etiology of PSP is the report of a cluster of classical cases in northern France (117). Cases were found in the adjacent French towns of Wattrelos and Leers, the former location throughout most of the 20th century of numerous textile-dyeing and leather-tanning plants. Area residents raised fruits and vegetables for home consumption and for sale at local markets. While the authors of this report tentatively attributed the cluster of PSP to metals present in soil—more likely to arsenic than chromium, arsenicosis and peripheral neuropathy, not PSP, usually result from chronic arsenic exposure. However, it is noteworthy that arsenic can upregulate nitrosamine metabolism and increase DNA lesions of the type associated with MAM (118).

The textile dyeing and finishing industry has utilized thousands of chemicals and is considered to have been one of the most environmentally polluting industries. A large amount of water is used to wash dyed and printed textiles and to clean printing screens and dyeing vessels. Since contaminated water has been discharged directly into water bodies, hazardous chemicals can readily enter soil (119). Among chemicals used in the dyestuff industry are phenylhydrazines that produce heterocylic coupling components such as pyrazolones (**Table 1**) (120). The preferred hydrazines are those containing two hydrocarbon groups bound to the same nitrogen atom, such as *N*,*N*′-dialkylhydrazines (121). Hydrazine is a reducing agent for many transition metals and some semimetals, including arsenic (122), and plants and fungi can bioconcentrate metal and non-metal substances in soil and water, including arsenic (122–126).

Leather tanneries have also used dimethylamine sulfate, a *N*-nitrosodimethylamine precursor, as a depilatory agent in the hide process (127). Thus, workers in the textile and leather industries may be exposed to nitrosamines and hydrazines. Research is therefore needed to explore the possibility that genotoxic chemicals previously used in the textile and leather industries are risk factor for sporadic forms of PSP. The textile industry in Israel (128) and the leather industry in Britain have been associated with an increased risk for ALS (129, 130), and "textile, garment, and related trade workers" were prominent among the higher incidence of ALS among female workers (131).

DNA Damage and Repair

The foregoing shows that natural and synthetic hydrazines, nitrosamines and MAM are genotoxic chemicals that damage DNA and may be associated with neurodegenerative diseases (ALS, PSP, AD), as well as with cancer. DNA damage and repair has been critical to understanding the pathogenesis of various cancers and, in recent years, the subject has occupied increasing attention in relation to brain aging and progressive neurodegenerative disorders, including ataxia-telangiectasia, Huntington disease, Parkinson disease, AD and ALS (132) (see also Note Added in Proof).

The cellular DNA damage response (DDR) system (133, 134), which comprises at least 450 proteins, is responsible for initiating DNA repair or, if DNA damage is too severe, instructing the cell to cease growth or die. DDR proteins include Fused in Sarcoma (FUS), which is involved in DNA repair and, in mutant form, accumulates in familial ALS. FUS has marked functional similarities to TAR DNA-binding protein 43 (TDP-43), which has recently been shown also to participate in DNA repair (135). Pathological forms of TDP-43 characterize the motor neurons of almost all cases of familial ALS with frontotemporal degeneration (FTD) and approximately half of FTD cases (136, 137). TDP-43 inclusions are also present in the tau-dominated polyproteinopathy of Western Pacific ALS/PDC (138, 139), in particular in the spinal cord, limbic, and cortical regions of Guam cases (140, 141). In sum, therefore, components of the DDR response are prominent in both genetic and environmental forms of these neurodegenerative disorders. In addition to the various types of DNA damage and plasticity associated with ALS (142), evidence from Western Pacific ALS/PDC suggests that CNS O^6-mG DNA damage, its specific repair enzyme MGMT, and other targets of genotoxic alkylating agents (143) merit increased research attention in this and related neurodegenerative diseases.

TESTING THE HYPOTHESIS

This hypothesis proposes that exposure to natural or manmade nitrosamines and hydrazines, acting via a specific pattern of poorly repaired neuronal DNA damage induced by methyl free radicals (144–148), may be potential risk factors for some sporadic forms of ALS, PSP, and AD. The latency of years or decades that intervenes between exposure to an environmental agent capable of inducing neurodegeneration and the appearance of clinical disease makes proof of causation exceptionally difficult. Four possible research approaches may be useful.

The first involves extensive assessment of lifetime total chemical exposure (exposome) using investigative techniques that go far beyond those typically employed in epidemiological instruments. Targeting individual cases who develop disease at a very young age has proved invaluable in past investigations of ALS/PDC because the duration of potential exposure is relatively short and living caregivers and relatives can provide information on exposures that occurred *in utero* and during infancy and early childhood of the disease-affected subject. Detective research of this type was crucial in first linking ALS in Guam and Kii-Japan to oral exposure to cycad seed (13, 149).

Secondly, a multidisciplinary research approach is needed to understand how methyl free radicals derived from alkylating agents generate O^6-mG lesions that might trigger neurodegenerative disease. The low levels of the O^6-mG DNA repair enzyme MGMT in human brain tissue, and its epigenetic regulation (150–152), appear to be of central importance in neuronal degeneration induced by alkylating agents such as MAM and related compounds. MGMT is a nuclear protein that transfers the alkyl group from the O^6 position of guanine, thereby restoring guanosine to its undamaged state, while the alkylated "suicide" protein is subsequently ubiquitinated and then degraded by the proteasome (151, 152). However, alkylated O^6-guanine lesions in rat brain genomic DNA are removed more slowly than those in liver or kidney (153–155), and MAM-induced brain DNA damage can modulate cellular pathways associated with neurodegenerative disease (17, 22, 23) and induce degeneration in non-cycling cells (156) as well as terminally dividing neurons. The amount and persistence of unrepaired DNA damage in neurons, a reflection of genotoxin dosage and DNA-repair efficiency, predictably would determine the rate of differential neuronal loss, the duration of the latency period and, potentially, the eventual clinical disease phenotype.

A third research approach concerns the possibility that metabolic differences are important in the expression of neurodegenerative disease linked to the alkylating genotoxins discussed here. For example, genetic control of the rate of metabolic acetylation (fast, intermediate, slow), a subject relevant to ALS (157), should modulate individual susceptibility to hydrazines. Similarly, aldehyde dehydrogenase (*ALDH2*), which is linked to AD risk (158), metabolizes formaldehyde, a common and potentially genotoxic metabolite of MAM and L-BMAA (17, 19).

Finally, the availability of identified single-cell sequencing provides an opportunity to determine whether DNA damage accrues in neurons vs. glial cells in sporadic neurodegenerative diseases (e.g., sALS, sPSP, sAD), and the relationship between DNA damage, gene expression, and abnormal protein deposition (159). This approach could be applied to Western Pacific ALS/PDC as well as related sporadic neurodegenerative disorders.

NOTE ADDED IN PROOF

A mixture of UDMH (1,1-dimethylhydrazine) and nitrogen tetroxide is the propellant used for thruster firings on the International Space Station (ISS). The Spacecraft Maximum Allowable Concentration (SMAC) for human exposure (based on alterations of "red blood cell mass") is set at 4 ppm (for 1 h), 0.3 ppm (24 h), 0.02 ppm (30 days) and 0.004 ppm (168 days) (91, 160, 161). The SMAC for methylhydrazine is 0.002 ppm (based on nasal toxicity) at all timepoints from 1 h to 180 days (162). Hydrazine levels in the ISS workspaces were not reported in the NASA Twins Study, which attributed many of the findings to increased exposure to radiation and other stressors (163, 164). The study employed multiple assays to compare the health of a pair of 50-year-old male monozygotic (identical) twin astronauts, one of whom worked in the ISS for 340 days (3.21.15 to 3.21.16) while the other (control) remained earthbound. Significant responses of the twin while working in the ISS included blood cell (CD4, CD8, LD) genomic instability and lengthening of telomeres, the chromosomal termini that maintain genomic integrity by preventing inappropriate DNA-damage responses. Although the expression of >90% of in-space modulated genes returned to normal postflight, persistent (at least 6 months) changes were found in (a) the expression of a subset involved with immune function and DNA repair, and (b) the DNA damage from chromosomal inversions, increased number of short telomeres, and attenuated cognitive function that was acquired while in space. Telomere maintenance resulting in lengthening (recorded in flight) is frequently activated in tumors of mesenchymal or neuroepithelial origin (165), and telomere shortening (found post-flight) has been associated with long-term exposure to particulate air pollution, certain chemicals including *N*-nitrosamines) decreased telomerase activity and increased cellular aging (166, 167). Ionizing radiation has a bell-shaped dose-response relationship with telomere shortening at low dose (166). Mission-associated mutations of cell-free DNA were found in blood, and increases were noted in biomarkers of inflammation and in serum folate levels, which were correlated with inflight telomere lengthening (163, 164). Tetrahydrofolate, the active form of folate, can damage DNA via metabolic decomposition to formaldehyde (66, 168, 169), the common metabolite of a DNA-damaging hydrazine [60] and of both BMAA and MAM, a radiomimetic substance (17, 19, 170, 171).

Unknown are the cause(s) and significance of the 6-month persistent changes in the twin astronaut who returned to Earth after one year of ISS service. For the following reasons, the possibility of continuous inflight exposure to extremely low levels of UDMH might be considered, along with the astronaut's gene acetylation status. Hydrazine toxicity is concentration-dependent and multiple low doses are cumulative. People with a slow acetylator genotype have less functional *N*-acetyltransferase and therefore do not clear hydrazine from

the body as rapidly as fast acetylators; they accumulate higher circulating levels of hydrazine and are probably more susceptible to its short- and long-term toxic effects. Hydrazine reduces liver glutathione, inhibits catalase activity, and increases oxidized glutathione, followed by generation of free radicals (methyl, acetyl, hydroxyl, and hydrogen), increased reactive oxygen species, oxidative stress, and increased lipid peroxidation. Data on the neurological effects of hydrazine(s) are limited (172–175): the acute neuroexcitatory effect (seizures) of ingestion of False Morel mushrooms (see above) result principally from the reaction of hydrazine species with pyridoxal 5-phosphate (activated form of pyridoxine) to form a hydrazone that reduces the inhibitory neurotransmitter GABA by decreasing the enzyme activity of glutamic acid decarboxylase (176); however, hydrazines, such as 1,1-dimethylhydrazine that damage tissue (liver, kidney, CNS?) form a reactive nitrosamide which generates methyl free radicals that produce DNA damage (61, 64) (O^6- and N^7-methylguanine adducts (177) which, if unrepaired, are (like MAM) pro-mutagenic in cycling cells and, presumably, trigger degeneration in terminally dividing and post-mitotic nerve cells (17, 19, 22). Of note, male and female hamsters with year-long intermittent inhalation exposure to hydrazine (0.25, 1.0, and 5.0 ppm) showed "pathologic changes characteristic of degenerative disease, including amyloidosis."

Notably, "Using amyloidosis as a criterion, a no-effect level was not achieved in hamsters (178)." "Exposure-related amyloidosis, hemosiderosis, testicular senile atrophy, and bile duct hyperplasia appeared to effect age-related degeneration (162)." The CNS of these animals is not described and may not have been examined.

AUTHOR CONTRIBUTIONS

This paper was developed solely by the author and draws on nearly 40 years of collaborative research on ALS/PDC and related disorders. For most of this time-span, I have had the privilege of working in the field and/or laboratory with key research colleagues, notable among which are Valerie Palmer and Glen Kisby, respectively, with whom I have co-authored many papers relevant to the present topic.

ACKNOWLEDGMENTS

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