

## SYMPOSIUM SUMMARY

# Amyotrophic Lateral Sclerosis: Concepts in Pathogenesis and Etiology

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**ABSTRACT:** The ALS symposium in Vancouver was the first of its kind in Canada and was a contribution from both American and Canadian investigators. The main points presented were (1) a definition of what is truly ALS, in the clinical and pathological sense, based on what is called "classical" ALS; (2) how neurons may be cultured to provide a valuable experimental tool; (3) the significance of lipid abnormalities in ALS and the characterization of the ALS-like syndromes produced by hexosaminidase A deficiency; (4) the possible role of autoimmune disease as it may accompany classical ALS and nerve growth factor derived from skeletal muscle; (5) the western Pacific form of ALS as it has been intensely studied and has given rise to two hypotheses on pathogenesis: mineral toxicity caused by secondary hyperparathyroidism and poisoning through ingestion of the cycad seed, and (6) the possible abiotropic interaction of one or many environmental toxins over a lifetime with the aging nervous system, depleting it of its frail reserve of neurons.

**RÉSUMÉ:** La sclérose latérale amyotrophique: des concepts de la pathogénèse et de l'étiologie. Le congrès sur la sclérose latérale amyotrophique, tenu à Vancouver, était le premier de ce genre au Canada. Des chercheurs américains et canadiens y ont contribué. Les thèmes principaux présentés étaient: (1) une définition de la S.L.A. véritable, dans le sens clinique et pathologique à partir du nom «S.L.A. classique»; (2) des méthodes pour cultiver les neurones in vitro afin de fournir un outil expérimental de valeur; (3) la signification des anomalies lipidiques dans la S.L.A. et également les caractéristiques des syndromes qui ressemblent à la S.L.A. provenant de la déficience d'héxosaminidase-A; (4) le rôle potentiel des troubles auto-immunologiques, desquels la S.L.A. peut s'y accompagner, et le facteur de croissance de nerfs extrait du muscle squelettique; (5) les types de S.L.A. du Pacifique occidental qui ont été l'objet d'études approfondies et qui ont donné naissance à deux hypothèses sur la pathogénèse, la toxicité minérale entraînée d'une hyperparathyroïdisme et l'empoisonnement au moyen de l'ingestion du pépin de la cycade, et (6) les effets d'une ou de plusieurs toxines dans l'environnement au cours de toute une vie et en rapport avec le vieillissement du système nerveux qui le laisse dépourvu de sa provision de neurones.

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During the 22nd Canadian Congress of Neurological Sciences, held in Vancouver, B.C., June 24-27, 1987, a special symposium was devoted to amyotrophic lateral sclerosis (ALS). It was the first of its kind to be held in Canada reflecting the interest and expertise that has developed, amongst Canadian neurologists, in this devastating disease. The participants and their topics, in order, were as follows: **Arthur Hudson**, University of Western Ontario, "Clinical evidence for pathogenetically distinct forms of ALS". **Seung Kim**, University of British Columbia, "Tissue culture studies of spinal motor neurons". **Glyn Dawson**, University of Chicago, "The role of gangliosides and hexosaminidase in ALS". **Jack Antel**, Montreal Neurological Institute, "Trophic and immunochemical factors in ALS". **Carleton Gajdusek**, National Institutes of Health, NINCDS, "Physiological changes inducing motor neuron disease: Common and distinguishing features". **Asao Hirano**, Montefiore Medical Center, "Pathological variations and extent of the disease process in ALS". **John Steele**, Guam Medical Center, "Geophysical and nutritional factors in ALS". **Peter Spencer**, Albert Einstein College of Medicine, "Environmental factors

and diseases of the motor neurons". **Andrew Eisen and Donald Calne**, University of British Columbia, "Latent neuroabiotrophies: A clue to ALS".

Additional invited comments were provided by **Marjorie Whiting** and **Leonard Kurland**.

The symposium was opened by Arthur Hudson who outlined the ever increasing varieties of ALS-like motor neuron diseases.<sup>1</sup> The clinically indistinguishable forms of these, using classical sporadic ALS as the benchmark are familial ALS, the Western Pacific forms of ALS, post-encephalitic ALS and a presumably infective form of ALS. The latter two conditions are very rare.<sup>1,2</sup>

One of the important ALS-like disorders, hexosaminidase deficiency, has received increasing attention.<sup>3</sup> Glyn Dawson described the role of gangliosides and hexosaminidases in ALS and ALS-like disorders. A variety of neurological manifestations, including encephalopathy, psychosis, spinocerebellar degeneration, progressive extrapyramidal dysfunction and motor neuron disease, have been described in association with deficiency of one or more of the 3 hexosaminidase isozymes (A, B and S).

The A isozyme, the only one capable of degrading ganglioside, is composed of  $\alpha$  and  $\beta$  chains whereas the B and S isozymes are composed entirely of  $\beta$  and  $\alpha$  chains, respectively. ALS-like syndromes due to hexosaminidase deficiency have generally been ascribed to  $\alpha$  chain defects but a  $\beta$  locus defect producing hexosaminidase A deficiency also has been reported.<sup>3</sup> Patients with hexosaminidase deficiency are generally much younger and run a more protracted course than what occurs in classical ALS.<sup>4,5</sup>

There is little evidence to the present that ALS is an autoimmune disease. There is, for example, no clearcut association between ALS and other autoimmune diseases. Additionally, significant HLA-disease susceptibility linkage has not been demonstrated and the nervous system lesions that typify classical (sporadic), familial or Western Pacific ALS are not inflammatory. While trials are still being conducted there does not appear to be any response to immunosuppressive therapy.<sup>6</sup> Aspects of disordered immunity in ALS were comprehensively reviewed by Jack Antel.<sup>7</sup> Soluble factors from skeletal muscle and neurotoxins may influence the survival and growth of the lower motor neuron and such factors may have relevance in ALS.<sup>8</sup> Recently, Gurney et al<sup>9</sup>, using immunoblot analysis, reported that ALS serum suppressed botulinum toxin-induced terminal nerve sprouting from rat muscle and suggested the blocking activity was mediated by autoantibodies. The complex inter-relationships between muscle-derived factors and motor neurons, specifically in ALS, remains open and in need of further study.<sup>6</sup>

Growing and maintaining human spinal cord neurons in culture is difficult but clearly has relevance in understanding basic morphological, physiological and immunological neuronal dysfunction in ALS. Seung Kim has successfully developed longterm cultures of fetal human spinal cord explants.<sup>10</sup> Synapses are recognizable after 2 weeks of culture, becoming fully mature after 2 months. At this time neuronal circuitry is well organized and can be correlated with the generation of intracellularly recorded action potentials.<sup>11</sup>

The neuropathology of ALS, described and contributed to largely by Asao Hirano, was presented by Hirano. Loss of anterior horn cells is the most prominent feature but other neurons in some regions such as thalamus can show simple atrophy, pigmentary degeneration and gliosis. The significance of these more extensive changes is not known.<sup>12</sup> Small intracytoplasmic eosinophilic inclusions (Bunina bodies), hyaline bodies and proximal axonal swellings due to neurofilamentous accumulation also typify ALS. Deposition of calcium and aluminum, it has been suggested, may be responsible for some changes.<sup>13</sup> Guamanian ALS differs from classical ALS by having widespread Alzheimer's neurofibrillary changes, most prominent in the hippocampus (usually without concomitant dementia), substantia nigra, locus ceruleus and spinal cord. Neurofibrillary tangles are seen in about 20% of lower motor neurons.<sup>12,14</sup> Neurofibrillary degeneration is also seen in apparently normal Guamanian Chamorros. Whether this implies subclinical disease or is merely a nonspecific response unrelated to the disease is yet to be determined.<sup>15</sup> In this regard abnormal PET scans described in "normal" Guamanians are of interest.<sup>16</sup>

Much of the symposium was devoted to the Western Pacific form of ALS. ALS and related disorders (parkinsonism-dementia (PD) complex) encountered on the Island of Guam is a story that is unfolding in an exciting way and may well result in a

definitive understanding of classical ALS, Parkinson's disease and possibly other chronic neurological diseases of later life. John Steele, who resides on Guam, gave an illustrated account of the island's history and relevant geography. Guam is the largest, southernmost, of the Mariana Islands and became a U.S. territory in 1898. The natives of Guam and the other Marianas (Saipan, Tinian and Rota) are referred to as Chamorros. They are descendents of migrants from the Malay Archipelago (Malaysia, the Philippines and Polynesia) but several centuries of Spanish and U.S. occupation has resulted in a genetic mix of Filipinos, Spaniards, Mexicans, Orientals and Caucasians.<sup>17-19</sup>

When ALS-PD was prominent it was not randomly distributed throughout Guam. There was a high incidence in the southern villages (Umatac and Merizo) and low incidence in the westcentral villages (Piti and Asan).<sup>17-19</sup> Guam, the Kii Peninsula of Japan and West New Guinea are rain forests and it has been suggested that villages with the highest incidence of ALS-PD lie along rivers originating in the coastal plains where millennia of high rainfall has rendered the soil poor in calcium and magnesium<sup>20</sup> (see below). In contrast, those villages lying along rivers originating from central highlands where the soil is richer in calcium and magnesium, have a low incidence of ALS-PD. This geographic association may be true for West New Guinea and the Kii Peninsula of Japan.<sup>21</sup>

Carelton Gajdusek and his colleagues hypothesized that secondary hyperparathyroidism and defects in mineral metabolism underly the high incidence of ALS in the Western Pacific. Low concentrations of calcium and magnesium in conjunction with high concentrations of other minerals as found in drinking water and soil of the 3 geographic foci where ALS-PD have the highest incidence (Guam, Kii Peninsula of Japan and West New Guinea) is viewed as having resulted in the deposition of hydroxyapatite, aluminum, manganese and even silicon in central nervous system neurons.<sup>20,22</sup> This elemental deposition disrupts the neuronal cytoskeleton inducing excessive neurofilament accumulation, in turn accounting for the neurofibrillary tangles characteristic of Guamanian ALS-PD.<sup>12</sup> Disruption of this nature might also be responsible for the hyaline bodies and proximal axonal swellings typical of all forms of ALS.<sup>23</sup> The new application of wavelength-dispersive spectrometry with computer controlled electron beam X-ray microanalysis allows for quantitative imaging of metals in neurons.<sup>20,22</sup> Commencing in early life and long before the onset of clinical disease, the benefits of subsequent adequate dietary calcium and magnesium intake, correcting the secondary hyperparathyroidism, would be unlikely to remove neuronal metallic and hydroxyapatite deposits.<sup>21,24</sup>

Probably the biggest surprise of the symposium was when Steele, in collaboration with Donald McLachlan of the University of Toronto, reported that calcium and magnesium concentrations found in soil and water (for example, in Umatic, which has the highest incidence of ALS-PD) are the same or even higher than elsewhere in Guam. While this, of course, does not preclude the possibility that low concentrations of calcium and magnesium were formerly present and have normalized with the gradual increased acculturation and westernization of the area, it raises the possibility of there being other explanations for the dramatic and potential disappearance of ALS-PD on Guam.<sup>21,24</sup> A strong alternative proposal was put forth by those speakers who view the cycad seed as important in the cause of ALS (Spencer, Steele, Kurland and Eisen).

Over 20 years ago Marjorie Whiting suggested that ALS-PD on Guam was due to intoxication from the seed of the cycad plant (*cycas circinalis*).<sup>25,26</sup> A staple in the Guamanian diet prior to westernization was fadan, a flat bread not unlike Mexican tortilla. It is prepared from the cycad plant by grinding the dried nut to a fine pulp. Native Guamanians recognized from the early 1800's that the nut was poisonous and potentially lethal, inducing "lytico" (from the Spanish paralytico). In preparing fadan, the nut is washed in water for several days to remove the toxin before grinding. It was deemed safe if chickens fed the absorbent water survived. Leonard Kurland agreed that the cycad nut was important in the etiology of ALS<sup>27</sup> and suggested that methylazoxymethanol (MAM) was a likely toxic ingredient. This substance is toxic and carcinogenic. However, feeding it to animals induces a cerebellar syndrome quite unlike human ALS-PD complex.<sup>28</sup> Because of this the cycad story temporarily lost appeal as the possible cause of Guamanian ALS-PD complex. Most agreed that an environmental agent(s) was important and attention was turned to mineral deficiency or poisoning.

The cycad story has, recently, been vigorously reactivated through the efforts of Peter Spencer and his colleagues. It has been recognized for some time that lathyrism, a motor system syndrome characterized by spastic paraparesis, resulted from excessive consumption of the chickling pea.<sup>29,30</sup> The active chemical is  $\beta$ -N-oxalylamino-L-alanine (BOAA). Oral infusions of this excitotoxic amino acid, a potent glutamate agonist, induces a corticospinal deficit in macaques that is very similar to human lathyrism.<sup>31,32</sup>  $\beta$ -N-methylamino-L-alanine (BMAA), another excitotoxic amino acid closely related to BOAA,<sup>33</sup> also induces a motor system disorder in primates.<sup>32</sup> BMAA is present in *cycas circinalis* and is a candidate in the pathogenesis (or cause) of Guamanian ALS-PD complex.<sup>31,34</sup> Whiting and Kurland's original observations that Guamanian ALS-PD complex was the result of environmental toxicity, specifically from ingestion of "faden" prepared from the cycad nut may, therefore, have been vindicated. While requiring further proof it is evident, at least, that the offending chemical would be BMAA, not MAM, as originally thought.

Can the concepts proposed in Guamanian ALS be applied to the classical form of ALS? Calne and colleagues<sup>35</sup> have recently hypothesized that several neurological disorders could result from interaction between prior toxic exposure and aging. Andrew Eisen closed the symposium with an account on the development of this hypothesis. ALS, Parkinsonism's disease and Alzheimer's disease characteristically become clinically overt in later life when one might believe that the aging process "is beginning". Indeed, it has been suggested that premature aging may be the basis for ALS.<sup>36</sup> The evidence, however, points to a possible onset as long as 30 or more years before these diseases become clinically overt. This was shown by Garruto et al<sup>37</sup> who described development of ALS in 28 Chamorro migrants. The patients had spent their childhood and youth in Guam and migrated to the United States, Japan, Germany or Korea from 1 to 34 years before developing clinical ALS. In effect, their having left Guam appears to have made no difference.

Eisen proposed that if various nerve cell populations naturally decline in number at different ages (for example, the neurons of the extraocular motor nuclei having a normally longer life expectancy than those of the substantia nigra) then exposure to an environmental toxin, virus or other noxious

agent early in life might significantly alter the regression slope representing cellular attrition. Subclinical cellular loss upon reaching a critical level, depending on the affected cell population, would become clinically apparent at different times.<sup>38</sup> This has, to some extent, been demonstrated in the case of the substantia nigra.<sup>39</sup> Such a mechanism could have major importance in the pathogenesis of all forms of ALS.

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