NEUROEPIDEMIOLOGY

Neuroepidemiology of amyotrophic lateral sclerosis: clues to aetiology and pathogenesis

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J M Charcot, at La Salpêtrière Hospital in Paris, first identified amyotrophic lateral sclerosis (ALS) from among the heterogeneous group of the spinal muscular atrophies. Between 1872 and 1874, in his Lectures on the diseases of the nervous system (lectures XII and XIII), Charcot masterfully described the clinical and anatomopathological features of the disease that bears his name (Maladie de Charcot).¹ The growing complexity of this field is reflected in the current classification of the spinal muscular atrophies and other disorders of the motor neurons prepared by the World Federation of Neurology (WFN) Research Group on Neuromuscular Disorders.² Likewise, the difficulties in clinically separating cases of ALS from other related forms of the disease for epidemiological studies, for clinical research, and for therapeutic trials, led to the successful meeting in El Escorial, Spain, of a group of experts under the aegis of the WFN to define precise criteria for the clinical, electrophysiological, and neuropathological diagnosis of ALS.³ El Escorial WFN criteria have been recently validated,⁴ and offer a solid foundation for future epidemiological studies of ALS.

Case definition

El Escorial WFN criteria for the diagnosis of ALS require the presence of signs of lower and upper motor neuron damage on clinical examination, and progressive spread of these signs within a region or to other regions, in the absence of electrophysiological and neuroimaging evidence of other disease processes that might explain the clinical signs. Repetition of clinical examination at least every six months is required to assess progression of the disease.

These criteria are stratified clinically in four levels of diagnostic certainty: definite, probable, possible, and suspected ALS. Also, the criteria allow for inclusion of several forms of ALS that present with various other clinical features, or particular genetic or epidemiological characteristics. These include the following types: sporadic ALS—the classic form of the disease, coexistent-sporadic ALS, the ALS related syndromes, and ALS-variants. The last two have provided some of the most interesting clues to the pathogenesis of ALS.

Descriptive epidemiology

It must be mentioned at the outset that, with few exceptions, most epidemiological studies of ALS failed to separate this condition from other forms of motor neuron disease. This was due in part to the absence, until recently, of diagnostic criteria for ALS, and to the fact that even in the latest neurological adaptation of the 10th International Classification of Diseases (ICD-10 NA),⁵ the major sporadic motor neuron diseases of adults (including ALS, progressive spinal muscular atrophy, progressive bulbar palsy, and mixed forms) are coded under a single category (G12-2: disorders of motor neuron of undetermined aetiology). Thus, ALS represents most cases of motor neuron disease in all series (about 80%–90%) and failure to separate other conditions may introduce a 10% error in incidence data and a larger one in prevalence data.⁶ Furthermore, difficulties with clinical diagnoses, incomplete case ascertainment, and subreporting, all adversely affect epidemiological data on ALS.

INCIDENCE, PREVALENCE, MORTALITY

The above caveats notwithstanding, a relatively narrow margin of incidence and prevalence for motor neuron disease has been reported in most of the world (table).

Incidence

Chancellor and Warlow⁷ have recently published a useful review of incidence of motor neuron disease worldwide. The average crude incidence rates for motor neuron disease in several countries ranged from 0.4 to 2.6/100 000/year.⁸ Annual incidence rates for ALS ranged from 0.6 to 1.5/100 000. It is unclear if incidence rates of motor neuron disease lower than 1/100 000/year represent true low disease incidence or limitations in case ascertainment. Most studies showed a trend towards increasing age specific incidence rates with advancing age, beginning at about age 50–59 and reaching a peak at 75 years of age, to decrease again to lower rates at the age of 80 and older. In most studies, motor neuron disease predominated in males, with reported men to women ratios ranging from 1:2:1 to 2:6:1. It has been hypothesised that this male preponderance could be the result of hormone influences or a confounder for putative risk
factors such as trauma, occupational exposure, and physical activity. However, it has also been suggested that the lower ALS rates reported for elderly women may result from incomplete case ascertainment.  

Prevalence

Crude prevalence ratios for motor neuron disease showed, in general, a wider range than those for incidence, ranging from 0.8 to 8.5 per 100,000 (table). As in the case of incidence, prevalence ratios lower than 1.5/100,000 may represent regions of lower risk but also raise issues of limitations in case ascertainment, methodological differences, and variations in the reference point for prevalence data, as these diseases have short survival times. Quality of medical care is also reflected in longer survival times and higher prevalence figures. Reported patterns for age and sex specific prevalence seem to be similar to those for incidence.

Mortality

Mortality data from death certificates are readily available and could allow for comparisons of disease frequency. However, there are wide variations in international mortality rates for motor neuron disease, probably representing both differences in quality of the death certificates (subreporting at time of death), failure to clinically identify the disease of interest, and differences in methodology. In industrialised countries, identification of patients with motor neuron disease from death certificates seems to be reasonably accurate (72 and 96% of the cases). None the less, even within industrialised nations, considerable variation in average age adjusted mortality for motor neuron disease has been reported (for ages 40 and over per 100,000 per year), as follows: Sweden (1960–90),14 3.81, Ireland (1978–87)15 3.38, USA (1971, 1973–78)16 2.61, England and Wales (1963–89)17 2.60, Finland (1963–72)18 2.35, France (1968–90)19 2.13, and Italy (1958–87)20 1.51.

Studies of the time trends for mortality from motor neuron disease have shown a tendency towards longitudinal increase in mortality in several countries, particularly in Sweden, Ireland, England and Wales, France, Italy, Norway, Japan, and the USA. This trend has been explained by several factors including better knowledge of the disease among physicians, increased numbers of neurologists, and the so-called Gompertzian effect, or inter-disease competition, whereby better survival from high mortality conditions in old age (stroke and heart disease) would allow the expression of the disease in surviving older patients. An increase in mortality rates for Parkinson’s disease, another neurological disease of elderly people, has also been reported since the 1950s probably as a reflection of increase in the susceptible aged population.

Analytical epidemiology

RISK FACTORS

In a similar manner to Parkinson’s disease, the unknown duration of the incubation period for ALS and the uncertainty of the relevant exposures preclude long term prospective studies comparing exposed and unexposed cohorts. The low frequency of ALS, as well as recall bias, limit case-control studies with appropriate statistical power. Thus no clearly defined risk factors for ALS have been identified.

Case-control studies comparing urban and rural populations in Italy26 found increased risk for ALS among rural populations of lower socioeconomic level, and in occupations involving manual labour and physical activity, mainly farmers (which may indicate exposure to neurotoxic products used in agriculture) and handlers of animal hides or carcasses (a possible surrogate for zoonotic infections, including retroviruses). Also, in community based surveys of motor neuron disease an increased risk has been found in rural populations—reflected in two to seven times more cases found in the agricultural and farmworking populations.26–28 Other less well defined risk factors include exposure to chemical solvents, electrical fields and welding, heavy metals, alcohol consumption, and smoking.7–9

CLUSTERS

Small clusters of non-familial adult onset motor neuron disease have been reported, including cases living in the same block, having similar occupations, or with time clustering of disease onset within a small United States community. In the last, probable chemical exposure was found either from consumption of freshly caught Lake Michigan fish (five of six cases) or from occupational use of chemicals (two of six cases). Analysis of
another perceived cluster of nine cases in a United States community showed this to be a random event.\textsuperscript{38}

An apparent epidemic cluster of motor neuron disease has been described in Sweden, occurring among men living in the county of Skaraborg between 1973 and 1984.\textsuperscript{39,40} Based initially on analysis of death certificates for Sweden in the period 1961–90,\textsuperscript{39} the study then actively searched all cases of motor neuron disease in this community and compared the results with those of the adjacent county of Värmland.\textsuperscript{41} During the period 1973–84, an epidemic-like increase in cases among older men was found with an average annual incidence of 4/100 000 person-years (70 males identified), compared with an incidence of 2·8 for males and 1·6 for females during the entire period 1961–90. These patients were found to have higher age at onset of motor neuron disease and more frequent than expected employment in agriculture.\textsuperscript{40}

Based on these results, an extensive case-control study of motor neuron disease was performed,\textsuperscript{41} showing that the highest odds ratio (OR) for the disease (\(OR = 15–18\), lower limit 95% confidence interval (95% CI) 2·8) corresponded to the combination of heredity (family history of neurodegenerative or thyroid disease), male sex, and exposure to solvents. On the basis of these studies, Gunnarsson\textsuperscript{42} postulated that motor neuron disease may occur in a genetically susceptible population subset. These people may have defective detoxification leading to the neurodegenerative process precipitated by different agents after an induction period of one to two decades.\textsuperscript{14,42}

\textbf{TRAUMA}

"Lou Gehrig's disease" identifies ALS in the United States after the famous New York baseball player who died in 1941, aged 37, of this disease. Some case-control studies have found increased risk associated with physical activity, as well as with previous mechanical, chemical, surgical, and electrical trauma.\textsuperscript{7–9} However, no safe inferences can be drawn from most of these studies. The evidence in favour of previous remote trauma as a risk factor for ALS has been recently reviewed,\textsuperscript{43} only to be strongly criticised and basically disallowed due to serious methodological shortcomings.\textsuperscript{44}

\textbf{Aetiological studies}

By contrast with the largely inconclusive results of analytical epidemiology, some clinical observations included in the group of the \textit{ALS related syndromes} and \textit{ALS-variants} have provided important information. Among those explored are the \textit{ALS related syndromes} occurring in association to toxins (heavy metals, organic pesticides),\textsuperscript{1} endocrine disorders (hyperthyroidism, hyperparathyroidism, hypogonadism, etc),\textsuperscript{2} immune or lymphoproliferative disorders,\textsuperscript{45,46} and infections, in particular polio,\textsuperscript{47,48} and retroviruses such as HTLV-I,\textsuperscript{49–51} found with high prevalence in some areas of Japan, the Caribbean, and Latin America.

The \textit{familial ALS (FALS)-variants} have also contributed to the pathogenesis of ALS. Recently, patients with chromosome 21 associated FALS were found to possess dominantly inherited mutations in the gene that encodes copper-zinc superoxide dismutase (CuZnSOD).\textsuperscript{52} This enzyme defect resulted in high catalytic activity of the peroxidase reaction, inducing in cell cultures H2O2 mediated apoptosis or programmed neuronal death which could be inhibited by copper chelators, offering a novel therapeutic approach with chelators and antioxidants.\textsuperscript{52}

Demonstration of abnormally high concentrations of glutamate and aspartate in plasma and CSF of patients with ALS,\textsuperscript{53–56} led to subsequent research on excitotoxicity—that is, the effect of excitatory amino acid neurotransmitters on neuronal death in neurodegenerative diseases.\textsuperscript{57} Controlled clinical trials of riluzole,\textsuperscript{58} an agent that alters glutamate release and protects against glutamate toxicity, resulted in its recent approval for treatment of ALS.

\textbf{Geographic aspects}

\textbf{GEOGRAPHIC VARIANTS}

Peculiar geographic ALS-variants include early onset forms of ALS. The \textit{Madras form}, described in India, begins between 10 and 30 years of age and is characterised by bulbar-pontine involvement, distal atrophy of the limbs, deafness, and a chronic benign course.\textsuperscript{59} It resembles the chromosome 2 associated juvenile FALS form described in Tunisia.\textsuperscript{60} These variants provide evidence in favour of the clinical and genetic heterogeneity of ALS.

\textbf{GEOGRAPHIC ISOLATES}

The extraordinary frequency of ALS and parkinsonism-dementia complex in remote areas of the western Pacific remains an intensively studied but yet unsolved major problem in neuroepidemiology. These high incidence geographic isolates occur in the Mariana Islands—mainly Guam and Rota,\textsuperscript{61–64} the Kii peninsula of Japan,\textsuperscript{65} and the west Irian region of New Guinea,\textsuperscript{66} resulting in rates of incidence, prevalence, and mortality 50 to 100 times larger than those found elsewhere in the world (table 1).

\textbf{Guamanian motor neuron disease}

In 1956, the United States National Institute of Neurological Disorders and Stroke (NINDS) established on Guam a registry for cases of motor neuron disease and parkinsonism-dementia complex. Originals are kept locally, but complete copies of medical records, death certificates, and epidemiological and pathology reports were deposited and inventoried at the Neuroepidemiology Branch, NINDS, National Institutes of Health in Bethesda, Maryland. Using this case registry, we analysed the epidemiological temporal pattern of occurrence of motor neuron disease on
Guam from 1941 to 1985, to define the duration of the latency period for the disease, the most recent years of meaningful risk, and the critical age for acquiring the disease.67 Likewise, we investigated the geographic occurrence at onset of disease,64 using election districts—the smallest defined areas with population information available from 1956 to the end of 1985.

Chamorro family histories were traced taking into account that Guam was a Spanish colony until 1899 and, therefore, married Chamorro women follow the complex surnames customs used in Spain. This factor could have influenced previous efforts to find a familial pattern of inheritance on Guam.69 70

Finally, we undertook a correlation analysis to investigate the relation between these demographic, geographic, and familial data with elements of the two main causal hypotheses—that is, cycad neurotoxins71 and mineral content (low calcium, high aluminium) in water and soil.72

EPIDEMIOLOGY OF GUAMANIAN MOTOR NEURON DISEASE

A group of 407 Chamorros with motor neuron disease (255 men (63%) and 152 women) were included in the analysis; in 171 (44%) the diagnosis was confirmed by pathology. The men to women ratio was 2:2. The median age at onset was 48 (range 19–84) years with a median duration of the disease from onset to death of about 4 (range 0-2–24-8) years. Average age adjusted annual incidence rates peaked at 179/100 000 for men in 1959–61 and 61/100 000 for women in 1956–58. Since then, a steady downward pattern has occurred (figure). From 1941 to the end of 1985, the median age at onset increased for both sexes (from 42 to 56 years for men, and from 42 to 55 years for women) but the men to women ratio declined from 2:5 to 1:5.

We calculated the observed ratios for number of cases of motor neuron disease occurring in birth cohorts from 1886 to the end of 1950. The ratios peaked for both sexes in those born between 1901 and 1910 and declined in the birth cohorts after 1930–35. By the end of 1987 no Chamorro born after 1949 had developed motor neuron disease.

The latency (or incubation) period is defined as the time interval between acquisition and onset of motor neuron disease and was inferred based on analysis of cases in migrants to and from Guam who subsequently developed motor neuron disease. The longest period was 34 years, found in a Chamorro born on Guam who resided overseas for that length of time, returned to Guam, and then developed motor neuron disease. The shortest incubation period was three years, found in a Filipino who lived in Guam 36 months before onset of motor neuron disease. In general, over the time exposure period on Guam to acquire motor neuron disease has increased, or the latent period has increased, or both have increased.67

In view of the clear decline of incidence of motor neuron disease on Guam, we analysed the most recent years of meaningful risk for acquiring motor neuron disease, based on migrants to Guam. There were six Filipino men and three United States white men who arrived to the island in the late 1940s (seven), 1956 (one), and 1963 (one) and developed motor neuron disease. Despite a large Filipino migration to Guam since 1960 no new cases of motor neuron disease have occurred in these migrants. Likewise, among Chamorro migrants who left Guam between 1934 and 1966 there were 41 cases of motor neuron disease, by contrast with only six cases of the disease among later migrants, despite a much larger emigration after 1966. Thus 1960 to 1966 seem to be the most recent years of meaningful risk for acquiring motor neuron disease on Guam.

We were also able to calculate the critical age of exposure for acquiring the disease on Guam. Based on data from Chamorro migrants, high risk exposure only in childhood failed to cause motor neuron disease, whereas high risk exposure during adolescence led to all the known cases. During the years of meaningful risk three to 10 years of exposure during adulthood may have led to development of motor neuron disease among some non-Chamorro migrants to Guam. The recent increase in latency or exposure periods and the decline in incidence could indicate a possible dose-response relation (lower levels of exposure to causative agents would result in longer latency periods).

GEOGRAPHIC DISTRIBUTION OF GUAMANIAN MOTOR NEURON DISEASE

Familial aggregation notwithstanding, we categorised the Guam districts as high, medium, and low risk areas. The two southern districts of Inarajan and Umatac showed average annual age adjusted incidence rates of motor neuron disease that were significantly higher than the overall rate. Geographic patterns of incidence were significantly related to the number of persons per household (Spearman rank correlation coefficient (r) = 0·64), median income per family (r = 0·52), and years of education (r = 0·48), indicating (for
men only) higher incidence in lower socioeconomic groups. Over time, high incidence of motor neuron disease persisted longer in these same southern districts, especially for men. This has been attributed in part to the geographical isolation and poverty of this region, particularly in Umatac. By contrast with central districts of Guam where economic development and westernisation advanced more rapidly after the end of the second world war, the south retained longer a subsistence economy and a traditional Guamanian diet, culture, and lifestyle.73

CYCAD FLOUR: CYCASIN AND β-N-METHYLAMINO-L-ALANINE (BMAA)
At the district level, we calculated Pearson correlation coefficients for reported mean concentrations (µg/g) of two cycad flour neurotoxins, cycasin (methyl-azoxymethanol β-D-glucoside) and BMAA (β-N-methylamino-L-alanine) in cycad flour samples from nine Guam districts.74 We demonstrated for men and women a highly significant correlation (P = 0.00002, r = 0.98) between average annual age adjusted incidence rates of motor neuron disease and cycasin but not BMAA.48

GEOCHEMICAL ELEMENTS
Geochemical factors were correlated by using reported mean concentrations (ppm) of selected chemical elements in water and soil samples.75 We found a statistically significant correlation (P = 0.0002, r = 0.77 for men, r = 0.69 for women) for high water iron content in samples from 18 districts, and with silicon concentrations in water (for men only).48 Among soil elements, only cobalt and nickel were significant for men and women with motor neuron disease.48

FAMILIAL OCCURRENCE OF GUAMANIAN MOTOR NEURON DISEASE
Family history was available for 303 Chamorros (187 men, 116 women) who developed motor neuron disease in Guam between 1956 and 1985. Positive family history of documented motor neuron disease was found in 108 (36%), including affected parents in 18, 78 in sibs, and 13 in offspring. Of those with a positive family history, 19 (17.6%) had affected spouses suggesting a high risk of developing motor neuron disease among spouses. There were 160 offspring producing couples (born before 1921) who had a total of 1335 children of whom 11 (0.85%) were known to have developed motor neuron disease by 1985. There were eight couples where both partners were affected by the disease. They had a total of 69 children of whom two (2.9%) had developed the disease by 1985. The risk of motor neuron disease in susceptible sibships was about 4.9–15.5% or about seven to 28 times greater than the general population lifetime risk (seven times higher in high rate districts and 28 times higher in low rate districts). These values are lower than expected with Mendelian inheritance, and considering the decline of incidence rates with time, polygenic inheritance alone is also unlikely.

GUAMANIAN MOTOR NEURON DISEASE REVISITED: A UNIFYING HYPOTHESIS
Reappraisal of Guamanian ALS data is consistent with a process resulting from genetic susceptibility precipitated by exposure to environmental or exogenous agents during adolescence.

FAMILIAL FACTORS
Early in the studies of motor neuron disease on Guam a dominant pattern of inheritance was suggested to explain the familial aggregation.69 However, it soon became clear that the epidemiology of the disease was inconsistent with Mendelian inheritance.62,64,70,76 In view of recent progress in the understanding of FALS, it is conceivable that a susceptibility gene could be present among the Chamorro.

We found that the risk of development of motor neuron disease in susceptible sibships was up to 28 times greater than the general population lifetime risk.48 Unfortunately, to date no genetic studies to define chromosome linkage or known gene products (Cu/ZnSOD deficiency, hexosaminidase A/B deficiency) have been carried out on Guam.

ENVIRONMENTAL FACTORS
Regarding the nature of the exogenous agents, the epidemiological evidence favours, with modifications, the two current causal hypotheses—that is, cycad excitotoxins57 and geochemicals.72

Cycas circinalis
There was a highly significant correlation between average annual age adjusted incidence rates of motor neuron disease on Guam and flour content of cycasin—one of the postulated exogenous toxins of Cycas circinalis. Most of the experimental work on cycas neurotoxicity was based on chronic BMAA intoxication in monkeys, inducing clinical and pathological features reminiscent of Guamanian ALS-parkinsonism.41,74,77 Our epidemiological evidence would also provide support for cycas neurotoxicity as an aetiological agent in Guam.78

Aluminium and iron
The alternative geochemical hypothesis surmised that low concentrations of calcium and magnesium along with increased aluminium in the soil and water caused Guamanian ALS.72 This was based on the finding of accumulation of calcium, aluminium, and silicon in neurofibrillary tangle bearing neurons in the brains of patients with Guamanian ALS-parkinsonism-dementia complex.73 Juvenile primates fed a low calcium diet with or without supplemental aluminium showed neuropathological changes suggestive of those of Guamanian ALS, including neurofibrillary tangles.80 Intracisternal injections of aluminium chloride in rabbits produced a chronic
encephalopathy with impaired axonal transport and inclusions that resembled those of ALS. 81

We found no correlation of motor neuron disease with calcium, magnesium, or aluminium content in soil and water, as previously reported. None of the less, a significant correlation did exist with iron concentrations in water samples. 82

Iron has been recently implicated in neurodegenerative disorders—mainly Parkinson’s disease, 83,84 because of its ability to generate free radicals and to cause oxidative stress. 85 Also, the protein lactotransferrin, which transports iron and other metals, is present in the neuropathological lesions of some degenerative disorders, including most prominently Guamanian ALS-parkinsonism-dementia complex. 86 Lactotransferrin is strongly immunoreactive with Betz cell and with other neurons of the motor cortex affected in ALS, 87 suggesting that increased deposition of iron could be of pathogenetic importance in motor neuron disease by inducing oxidative cytotoxicity.

As in the case of familial ALS associated with deficiency of Cu/Zn superoxide dismutase (SOD1) and in mice transgenic for mutant SOD1, a perturbation in free radical metabolism, perhaps through membrane lipid peroxidation, could be important in motor neuron cell death. 88 In the experimental model in mice, vitamin E, riluzole, and gabapentin influenced the progression of the disease.89

Conclusions

Epidemiological evidence on Guam favours the hypothesis of a genetic predisposition to develop motor neuron disease after exposure to environmental excitotoxins or chemicals—in particular, cyclic neurotoxins and iron. This view essentially agrees with the postulated pathogenesis of motor neuron disease based on epidemiological studies of an outbreak of motor neuron disease in Sweden. 42 The nature of the genetic susceptibility is unclear but pharmacogenetic polymorphism leading to deficient detoxifying capacity has been suggested. 43 In turn, this might induce release of free radicals, oxidative cytotoxicity, and apoptosis leading to neuronal death.

Geographic isolates of neurodegenerative diseases constitute invaluable “experiments of nature”, the lessons of which we are just beginning to discern.

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