Genetic Epidemiology of Amyotrophic Lateral Sclerosis

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Genetische epidemiologie van amyotrofische lateraal sclerose

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Cover: Victor Levasseur “Oceanie”. Paris. 1854. Map shows the Pacific Ocean divided into four regions. 1. Malaisie, 2. Melanesie or Australia, 3. Polynesia and 4. Micronesie. These four divisions were based upon the surveys taken by Capt. Dumont D’Urville during his two circumnavigations in 1822-1825 and 1826-1829 during which he charted the Pacific Islands in great detail. Victor Levasseur, the author of this map, employed a famous French artist, Raimond Bonheur, to draw the wonderful & fanciful scenes surrounding the map including the entrance to a harbor depicted in lower right showing a fort high on a bluff and sailing vessels below. From Levasseur’s “Atlas National Illustre”, published in 1854.

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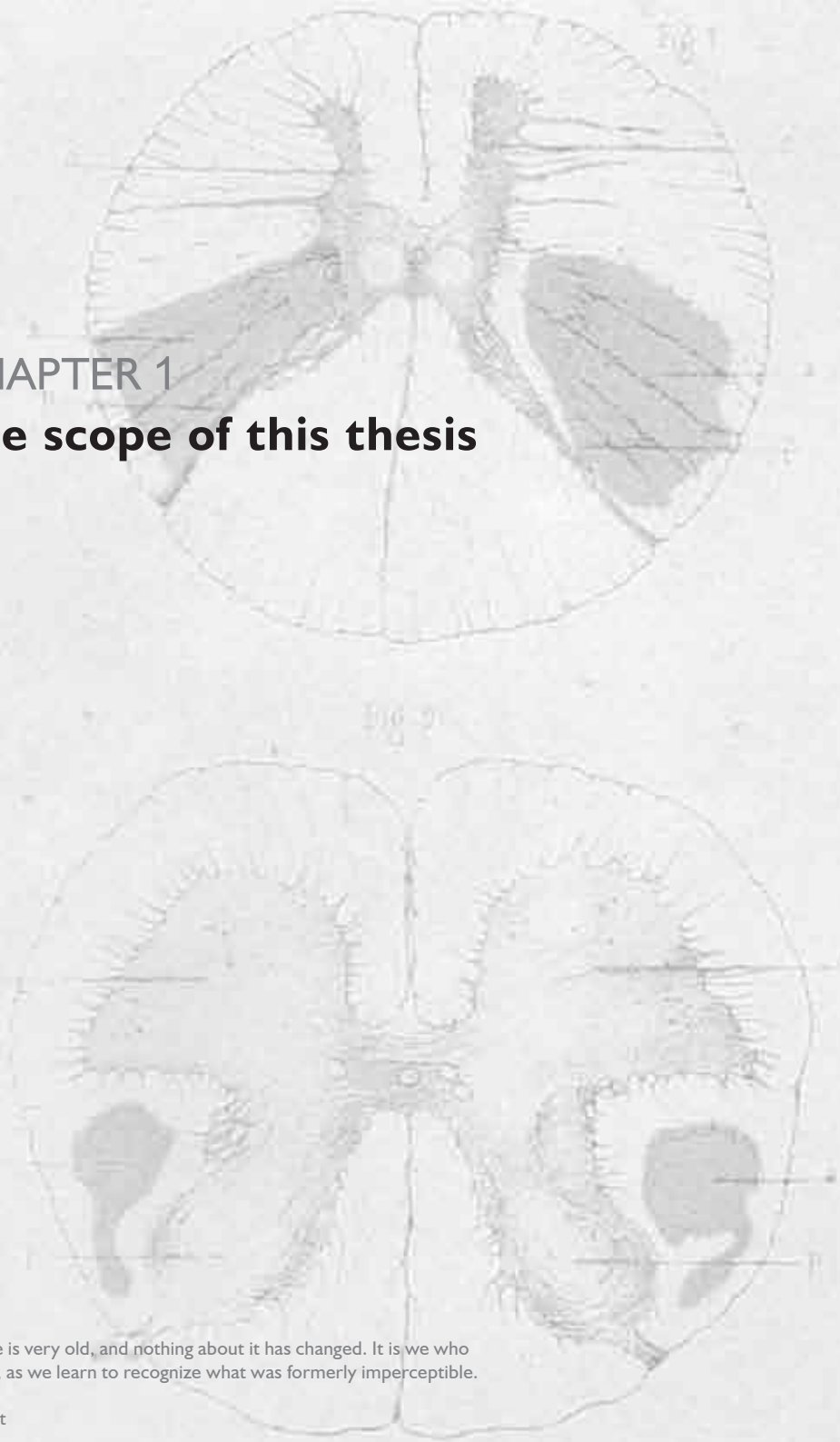

CHAPTER 1
The scope of this thesis

Disease is very old, and nothing about it has changed. It is we who change, as we learn to recognize what was formerly imperceptible.

Charcot

CHAPTER 1
The scope of this thesis

Amyotrophic lateral sclerosis (ALS), dementia and parkinsonism have in common the decrease in nervous system functionality due to loss of neurons through shared or discrete mechanisms. Although the complex etiologies of each of these disorders are not yet fully understood, evidence is increasing that these three neurodegenerative disorders may share distinct neurodegenerative pathways leading to overlapping clinical features and neuropathological characteristics.
The discovery of endemic amyotrophic lateral sclerosis (ALS) in the native Chamorro population of the pacific island Guam in the 1950's, its familial occurrence and the co-occurrence of dementia and parkinsonism led to searches for environmental and genetic causes for these three major neurodegenerative disorders of old age. [1, 2]

In this thesis we describe an epidemiological approach to search for a genetic link between ALS, dementia and parkinsonism in a large group of ALS patients in the New York area. We also studied environmental risk factors that may interact with a genetic susceptibility for ALS.
After an introduction in *Chapter 2,* we present in *Chapter 3* a review of the descriptive epidemiology of ALS and a classification for the major genetic causes of familial ALS and for the genes potentially contributing to the etiology of sporadic ALS, so-called susceptibility genes.
In a case-control study conducted from 1989 to 1991 at the Neurologic Institute of Columbia Presbyterian Medical Center in New York, we collected structured information from the family histories of 151 ALS patients and 140 controls on ALS, dementia and parkinsonism in parents, siblings, and grandparents.

In the first part of this thesis we present the results of the genetic epidemiologic study of familial aggregation of ALS, dementia and parkinsonism. Section *4.1* deals with the results of the analysis of the cumulative incidence of dementia in the relatives of ALS patients and controls and the analysis of the cumulative incidence of parkinsonism in the relatives of ALS patients and controls. In section *4.2* we evaluate the co-occurrence of dementia and parkinsonism in the families of ALS patients and controls.

The second part of this thesis deals with putative environmental and lifestyle factors

related to the disease. The case control study described in *5.2* was based on the observation of high incidence of ALS and co-occurrence with parkinsonism and dementia in the native population of the pacific island Guam, as described in *5.1.* We found evidence that residence on the pacific island of Guam, during the period of endemic ALS, is - even after decades- a risk factor for ALS in citizens of the Unites States.
In the case control study described in *chapter 4* we also collected information on medical histories (specific infectious diseases, diabetes, autoimmune disorders, surgery and injuries), exposure to animals or to specific environmental toxins (pesticides, aluminum, lead and others), occupational histories, smoking habits and the consumption of alcoholic beverages. The role of each of these exposures was tested in univariable analyses. Further multivariable analysis of a preselection of exposures aimed to control for confounding.
We investigated the interaction of specific environmental exposures with a genetic susceptibility to neurodegeneration by analyzing the interactive effect of each of the alleged environmental risk factors with a family history of ALS, dementia or parkinsonism. The results of this case control study are summarized in section *5.3.* Methodologic issues and a discussion of the results of the epidemiological analyses described in this thesis are presented in *chapter 6*.

During the past decade molecular genetic studies revealed more and more genes involved in mendelian inherited forms of ALS, Alzheimer's dementia, frontotemporal dementia, and parkinsonism. Some of these genetic defects are associated with overlapping clinical and neuropathological features of ALS, dementia and parkinsonism. Furthermore, genetic susceptibilities have also been associated with sporadic ALS but these seem to have smaller effects than the genetic defects in familial ALS and are likely to interact with each other and with environmental risk factors to modulate susceptibility and/or disease phenotype.
At the end of *chapter 6* we offer suggestions for genetic counselling of ALS patients and their families. We conclude with recommendations for further delineation of the neurodegenerative disorders with overlapping features of ALS, dementia and parkinsonism and present our expectations for future research in the complex field of gene-environment interaction studies.

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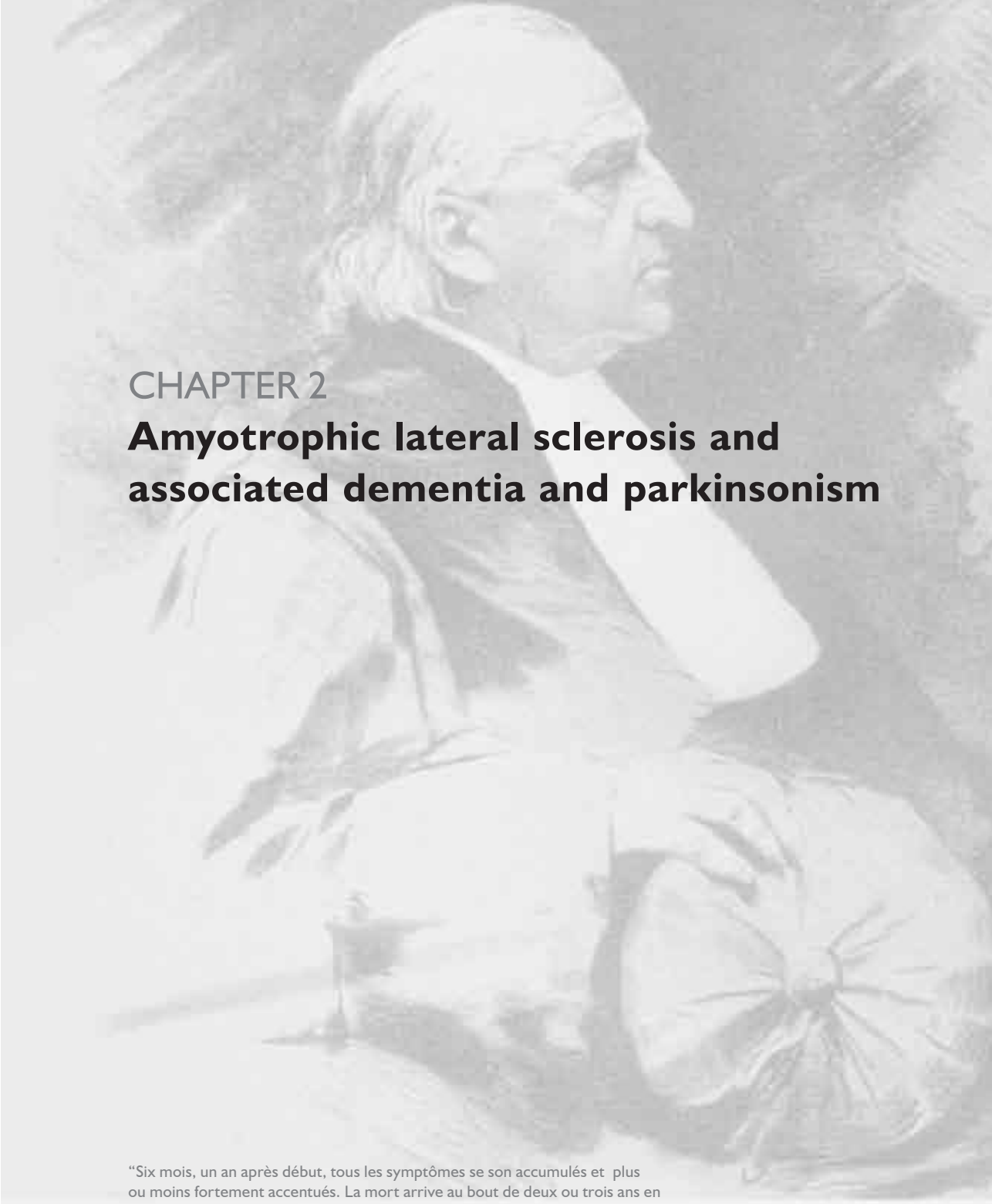

CHAPTER 2

Amyotrophic lateral sclerosis and associated dementia and parkinsonism

"Six mois, un an après début, tous les symptômes se son accumulés et plus ou moins fortement accentués. La mort arrive au bout de deux ou trois ans en moyenne par le fait des symptômes bulbaires."

Charcot (in his lecture summarizing his observations concerning the disease which he called amyotrophic lateral sclerosis; May 18, 1874.)

CHAPTER 2

Amyotrophic lateral sclerosis and associated dementia and parkinsonism

Amyotrophic lateral sclerosis, dementia and parkinsonism are generally viewed as "age-related" (ie, occurring within a specific age range) rather than "ageing-related" disorders (that is, caused by the ageing process itself). [1] Amyotrophic lateral sclerosis generally occurs at a younger age than parkinsonism or dementia. The age-specific prevalence of ALS worldwide (33 per 100 000) is observed at 60 to 75 years. ALS is worldwide responsible for approximately 1 in every 800 deaths, of these less than 10% occur before 40 years of age. [2-5]

The prevalence of Parkinson's disease in industrialized countries is estimated at 0.3% of the general population overall and about 1% in the population older than 60 years and 3.5% in those aged above 85 years. [6, 7]

Dementia is the most common neurodegenerative disorder, affecting about 5% of the population over age 65 years with a lower rate in some ethnic populations. [8, 9] Dementia occurs approximately in 1.5% of individuals at age 65 and prevalence doubles every 4 years to 40% in those 95 years and older.[10] The expected increase of the aged in a growing number of countries, of exposures to a broad range of environmental agents, and improved disease recognition, either at diagnosis or at death, is associated by a rise in the prevalence of neurodegenerative disorders. [11, 12]

It is of great importance to elucidate the molecular pathogenesis of neurodegeneration, because treatment will increasingly be based on the understanding of the pathophysiology. Amyotrophic lateral sclerosis (ALS) belongs to a class of disorders known as motor neuron diseases. Motor neurons arise from the motor cortex and extend from the brain stem throughout the spinal cord, forming the pathway to control voluntary movement. (figure 1) Upper motor neurons carry impulses to lower motor neurons and those innervate muscles. Motor neuron axons are amongst the most unusual cells in the body because of their very large size and their role as the critical link between the motor areas of the brain and the muscles. The loss of these motor neurons causes the muscles under their control to weaken and waste away, leading to paralysis.

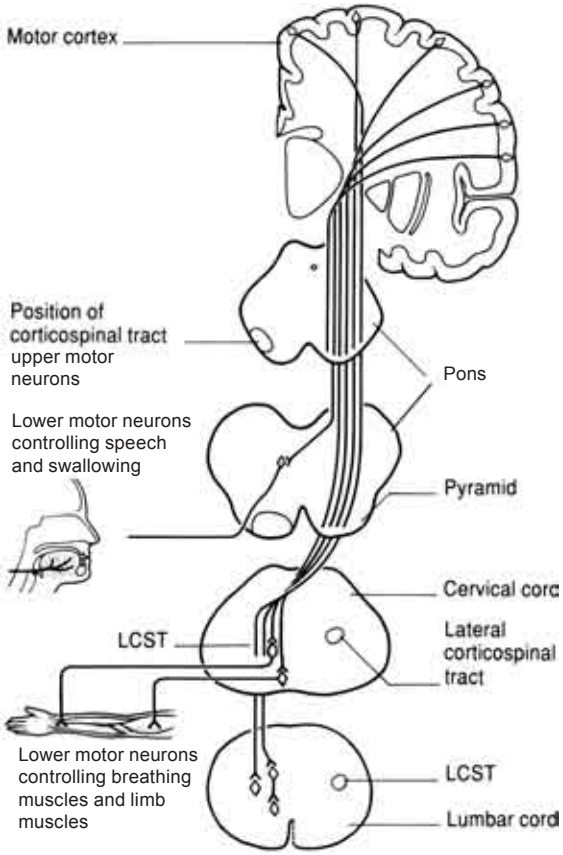

Figure 1. The human motor neuron system

Amyotrophic lateral slcerosis was first accurately described in 1874 by the famous Jean-Marie Charcot (see picture on page 11), at the hospital La Salpêtrière in Paris, who deduced the relationship between the clinical signs and the findings at autopsy. "Amyotrophic" refers to the muscle wasting that signifies disease of the lower motor neurons. "Lateral sclerosis" refers to the hardness to palpation of the lateral columns of the spinal cord in autopsy specimens, where gliosis follows degeneration of the corticospinal tracts. In North America the term "ALS" is used interchangeably with "Lou Gehrig's disease" in memory of the famous baseball player who died of this disease in 1941. (figure 2) In Britain the term "motor neurone disease" referring to the degeneration of the upper and lower motor neurons, is applied. [13]

Figure 2. Lou Gehrig

A considerable increase in mortality from ALS has been observed in the last three decades in Western nations. [14-17] The most significant factor in the geographical variation of ALS mortality is the life expectancy of the populations; the higher the life expectancy of the population concerned the higher the mortality rate of ALS. The rise of ALS paradoxically occurs precisely because populations have overall become healthier and thus more individuals are living up to the ages at which ALS is expressed. [18]

Clinical features of amyotrophic lateral sclerosis

The characteristic feature of amyotrophic lateral sclerosis is the rapidly progressive muscle weakness without sensory signs leading to limb weakness. ALS usually begins in the fifth and sixth decades of life. At onset fatigue, cramps, muscle weakness and wasting of one or more limbs or fasciculation of the tongue (bulbar onset) occur. [19, 20] Bulbar involvement causes difficulties in swallowing and respiration, and is a frequent cause of death in later stages of disease. In a typical patient, the muscles that control eye movement, bowel and bladder function are spared. Features that arise from dysfunction of the upper motor neuron are spasticity, and brisk tendon reflexes (like the extensor plantar responses, Babinski sign). Loss of lower motor neuron results in muscle weakness, painful cramps and muscle fasciculations followed by muscle atrophy and loss of tendon reflexes. When loss of both upper and lower motor neurons occurs spasticity or weakness may predominate before the amyotrophy develops. Pathologically increased reflexes are more common than loss of reflexes. [21]

The prognosis of classic ALS is grave, with death occurring in three to five years after onset of symptoms. However, 10 to 20% of the patients have a milder variant of ALS and may survive for more than 10 years. [22] Prediction of progression and survival is difficult but might be associated with the site of initial clinical involvement and the patient's age at the time of disease onset. Patients in whom bulbar symptoms occur first are considered to have shorter survival times. [23-26]

Currently there is no cure for ALS, the available therapy may prolong survival only. The relentless course is a great burden to patients, relatives and caregivers.

Clinical diagnosis of ALS depends traditionally on signs of progressive limb, bulbar, or respiratory muscle weakness and the physical signs of upper and lower motor neuron deficits. Essential investigations include imaging of the spinal cord, and sometimes brain, to exclude compressive or inflammatory lesions. [21, 27] Currently the El-Escorial World Federation of Neurology Criteria and its more recently revised version, the Airlie House Criteria, are used internationally for the diagnosis of ALS. These criteria grade the likelihood of ALS according to the number of bodily regions involved from clinical examination and electromyography, and require upper and lower motor neuron loss with progression of signs within a body region and to other body regions. [28-30]

According to the El Escorial criteria, ALS, combined with other neurological disorders, such as dementia and parkinsonism, is defined as ALS plus. Electrodiagnostic examination is also important in the diagnosis of ALS. Nerve conduction studies are necessary to exclude conductions block, and electromyography to confirm muscle denervation. [31, 32]

Familial amyotrophic lateral sclerosis

Familial forms with Mendelian inherited ALS are observed in approximately 5 to 10 percent of the patients and are clinically and neuropathologically indistinguishable from sporadic ALS. Genetic analyses over the last decade have identified seven autosomal dominant and three recessively inherited forms of ALS (see *Chapter 3* page 34 , table and Chapter 6, page 124 table). Sporadic ALS has been associated with 'susceptibility' genes because the neurodegenerative effect of these genetic changes probably depends on interactions with other prevalent, low penetrant genetic defects and/or with environmental risk factors (see *Chapter 3* page 34 , table).

As more genetic defects are identified it becomes more and more clear that ALS phenotypes are part of a broader disease spectrum. Generally, the recessive forms, ALS2 and ALS5, have a juvenile onset, with slow progression and a prolonged survival of many decades. [33-35]Adult onset of disease with a survival of more than ten years belongs to the clinical features of the recessive form of ALS1. [36-40] The broad clinical spectrum of dominant inherited forms, ALS4, ALS6 and ALS8, may also include prolonged survival. In ALS4, mutations of the *senataxin* gene (*SETX*) gene cause a form of ALS featuring

juvenile onset, slowly progressive symmetrical distal limb weakness and amyotrophy combined with pyramidal signs without bulbar involvement. [41-43] The mechanism underlying the clinical variability caused by one mutation in the v*esicle trafficing protein* (VAPB) gene in ALS8 is still unclear. ALS8 is associated with the broad spectrum of clinical features including rapidly progressive adult onset ALS with or without tremor and adult onset spinal muscular atrophy without pyramidal or bulbar signs. [44]

Amyotrophic lateral sclerosis and dementia

Dementia is a prominent feature of ALS. It has been suggested that cognitive decline and/or cortical degeneration occurs in as much as half of the ALS patients. [45, 46] Over the past decade the clinical pattern of ALS-associated dementia has been better delineated, with help of neuropsychological testing, imaging studies, and neuropathological data. The cognitive impairment can precede, accompany, or follow the features of ALS. Psychometric testing has characterized the type of cognitive impairment in most ALS cases as being frontal lobe or frontotemporal lobe type. [23, 47-59]
Familial ALS with dementia occurs in ALS1, ALS6, the ALS-dementia complex mapped to a locus on chromosome 9q21-22, and in a growing number of families with genetic syndromes with or without parkinsonism. [60-63] Similarly, co-morbidity of ALS and parkinsonism, together with neuropathological and neuroimaging evidence of dopaminergic deficit in sporadic and familial ALS patients suggests related or overlapping neuropathologic pathways. [64-68] [69]
The features in ALS patients with dementia and/or parkinsonism may bear clinical similarity to ALS, locally called ‘lytico’, and the parkinsonism-dementia complex, called ‘bodig’, on the Pacific island Guam. A well-known characteristic of these disorders is the intrafamilial variability of the phenotype. [70-75] Within a pedigree, family members can present with different clinical symptoms, which may reflect variation in the distribution and severity of the pathologic process.

Advances in molecular genetic research have delineated several familial forms of ALS with progressive frontal lobe dementia (FTD). One of these forms is the autosomal dominant complex of ALS, FTD and/or parkinsonism, associated with mutations in the *tau* gene. This disorder is referred to as FTDP-17 (because the *tau* gene is located on chromosome 17). Most of the chromosome 17-linked families were found to have disease-causing mutations in the microtubule binding domain coding, mostly associated with overexpression of the 4 repeat isoform of the tau protein, or in splicing regulatory sequences of the *tau* gene. [70, 71, 76] [77, 78] Tau proteins are a group of microtubule associated proteins that play an important role in promoting the assembly and maintaining the structure of microtubules. They are expressed in neurons being particularly abundant

in axons. Alternate mRNA splicing of exons 2,3, and 10 of the *tau* gene results in the expression of six isoforms in the nervous system. The predominant isoforms differ by the presence of either 3 or 4 repeat domains, which results from the exclusion or inclusion of exon 10. Filamentous deposits made of tau constitute a major defining characteristic of several neurodegenerative disorders known as tauopathies. Some of the tauopathies are caused by mutations or polymorphisms in the *tau* gene.[79] Among the various forms of frontotemporal dementia (FTD) approximately 50% of familial FTD is caused by mutations in the *tau*-gene. [80-83] Tauopathies do not seem to play an important role in the clinico-pathological spectrum of ALS-FTD. [49, 82, 84-88]

The occurrence of both major features of Alzheimer pathology, extracellular cerebral amyloid plaques and intraneuronal neurofibrillary tangles is rare in ALS and ALS-dementia. However, plaques without neurofibrillary tangles are known to occur in ALS-dementia. [89] And the hallmark of Guamanian ALS/PDC is abundant neurofibrillary tangles in widespread regions of the central nervous system. [90] These tangles are composed of abnormally modified tau, neurofilaments and other cytoskeleton proteins. [91]
The neurofibrillary tangles in ALS/PDC exhibit very similar biochemical and ultrastructural properties of hyperphosphorylated tau protein and neurofilaments, to Alzheimer's disease but the specific laminar and regional cortical distribution of the neurofibrillary tangles is different from that seen in Alzheimer's disease. Also different is the absence of extracellular plaques. [92-94] The aberrant biochemical processes that hyperphosphorylate normal tau and/or neurofilaments into abnormal filaments in ALS, Alzheimers's disease and ALS/PDC, Guadeloupean parkinsomism complex, and a form of familial FTD linked to chromsome 3 (or in other tauopathies without mutations of the *tau* gene) are not understood. [71, 73, 83, 85, 95-97]
More and more evidence is found that tau pathology also plays a role in forms of parkinsonism. [81, 98-102]

Ubiquitin-positive inclusions seem to be the most important common neuropathologic feature of sporadic and familial ALS, ALS-FTD, primary lateral sclerosis with FTD, and also in sporadic and some familial forms of FTD without *tau* gene mutations. [41, 55, 96, 103, 104] These ubiquitin containing inclusions are negative for α- synuclein or tau. [105-108] The most specific ubiquinated inclusions in ALS, the Bunina bodies, occur in the majority of patients with uncomplicated ALS, ALS/PDC, ALS-FTD, and ALS without clinical signs of FTD or parkinsonism despite frontotemporal atrophy and degeneration of the substantia nigra. [73, 109] The similarities in the molecular pathology of ubiquitinated inclusion bodies in ALS, ALS/FTD with or without parkinsonism, and some forms of familial FTD, strongly suggest related disorders that belong to a clinicopathological spectrum. [49, 105, 109-114 96]

Marked differences in the anatomical distribution of lesions determine the predominance and type of motor and cognitive features in each syndrome. [49, 115]

Amyotrophic lateral sclerosis and parkinsonism

Although the association with parkinsonism may be less apparent than with dementia, post-mortem examination of patients with ALS confirms that the neurodegenerative process may extend from the frontotemporal cortex to the basal ganglia. [49, 116] Parkinson's disease is characterized by resting tremor (classically resembling pill rolling), rigidity, and bradykinesia, which typically responds to dopamine treatment. The neuropathologic hallmarks are loss of dopaminergic neurons and depositions of cytoplasmatic protein aggregates termed Lewy bodies in the sustantia nigra. Lewy bodies are composed of numerous proteins, including α-synuclein, neurofilaments, and ubiquitin. [117] The movement disorder in Parkinson's disease is thought to arise from reduced dopaminergic input to the striatum as a result of nigral degeneration. Parkinsonism is a clinical syndrome with features resembling those of Parkinson's disease, is distinguished from the latter by the presence of additional clinical and neuropathological features. About 15% of patients have first- degree relatives with the disease, typically without a clear mode of inheritance. [118, 119] The main etiology in Parkinson's disease is complex and seems to be determined by several genetic as well as non-genetic factors. [120] In the last few years molecular genetic analysis led to the identification or localisation of eleven loci for rare autosomal dominant and recessive inherited forms of parkinsonism. [121, 122] Missense mutations, duplication and triplication of the α-synuclein gene cause autosomal dominant familial disease. [123, 124] Dementia is observed in patients with the triplication of the *α-synuclein* gene. [125, 126] A-synuclein is a major constituent of Lewy bodies. The mutations and the dose-effect of the *α-synuclein* gene provide a direct link to the neuropathology, suggesting that α-synuclein deposition is relevant to the degeneration of nigral neurons. [126] Moreover linkage of synuclein and tau pathology has been observed in patients with distinct *α-synuclein* mutations and in ALS/PDC patients on Guam, suggesting synergistic interaction that accelerates fibrillization of both proteins. [98, 127]

Mutations in the *parkin, DJ-1 and PINK1* genes cause autosomal recessive inheritance of parkinsonian disorders. [128-130] *Parkin*-linked cases (PARK2) generally exhibit selective loss of nigral degeneration in absence of Lewy bodies, even though the clinical features of patients bearing *parkin* mutations are often indistinguishable from idiopathic Parkinson's disease. [131, 132] Parkin is an E3 ubiquitin ligase , targeting and promoting degradation of α-synuclein. The absence of Lewy bodies in PARK2 shows that parkin loss of function may cause degeneration by a mechanism other than aberrant protein accumulation. [121, 133, 134] The most recent identified and the most frequent cause of familial parkinsonism (PARK8)

is associated with mutations in the *LRRK2* gene, coding for the dardarin protein (from the Basque word dardara, which means tremor, referring to the Basque origin of the first affected families). [135] In PARK8 patients a wide spectrum of nigral pathology is found: absence of other distinctive pathologic features ('pure nigral degeneration'); brainstem Lewy bodies, typical of Parkinson's disease; widespread brainstem and cortical Lewy bodies, consistent with Lewy Body dementia; or absence of Lewy bodies but presence of neurobibrillary tangles, consistent with a tauopathy like FTDP-17. [63, 69, 135-138] Clinical features of PARK8 includes parkinsonism with dementia or amyotrophy or both, suggesting that the function of the *LRRK2* gene may be central to the pathogenesis of several major neurodegenerative disorders associated with parkinsonism. [69]

Thus, the clinical, pathological, and genetic heterogeneity associated with familial ALS, dementia and parkinsonism suggest that multiple pathogenetic events may result in neurodegeneration, which may produce variably ALS, dementia and/or parkinsonism, along with variable pathologic features. A central challenge for research will be to identify these multiple pathogenic mechanisms and their points of molecular convergence.

With the current study we aimed to find support for a shared genetic susceptibility to the three major neurodenegerative disorders, dementia, parkinsonism and ALS. In that view, new genetic studies should be directed at finding genetic defects in families presenting with familial aggregation of these three disorders. This approach might become as successful as the genetic research in families with either one of these disorders. Indeed, the results of the genetic studies of either one the disorders more and more point in the direction of genetic causes for overlapping neurological phenotypes.

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CHAPTER 3

Genetic epidemiology of amyotrophic lateral sclerosis

Review article

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CHAPTER 3
The genetic epidemiology of ALS

Introduction

Amyotrophic lateral sclerosis (ALS) is a late onset, rapidly progressive and ultimately fatal neurological disorder. The disease, also referred to as motor neuron disease or Lou Gehrigs disease, is caused by a progressive loss of motor neurons in the brain and spinal cord. It usually begins focally and then spreads. There is concurrent involvement of corticospinal (upper) and spinal (lower) motor neurons. The diagnosis is based on the El Escorial criteria.[1] Involvement of the lower motor neurons results in muscle denervation of the affected muscles, loss of tendon reflexes and fasciculations, followed by muscle atrophy. Degeneration of corticospinal motor neurons causes additional focal spasticity. Clinical experience indicates that before spreading the disease begins with fatigue, cramp, muscle weakness and wasting of one or more limbs or fasciculation of the tongue (bulbar onset). Losses of reflexes are less common symptoms than pathologically increased reflexes.[2] The disease progresses for an average of 3-5 years, leading to paralysis and premature death. Massive conglomeration of neurofilaments in motor neurons is observed in both familial and sporadic ALS. Animal studies have shown that motor neuron dysfunction precedes the onset of symptoms and that compensatory mechanisms are successful in maintaining motor functions until more than 50% of motor units have been lost, at which point symptoms appear and the number of motor units declines rapidly.[3, 4] The progressive proportional increase of the aged in many populations will be associated by an increase of age-related diseases such as ALS. [5-8] Despite more than a century of research, there is currently no cure and the available therapy may prolong survival only by a few months. Familial forms of the disease occur in approximately ten percent of the patients. Three genes and linkage to four additional gene loci have been identified so far for monogenic ALS. The majority of the patients however have multifactorial ALS which are likely to be due to complex interaction between genes and environmental factors. This review focuses on the genetic background of ALS.

Epidemiology

The crude prevalence of ALS is estimated at 4-6 per 100,000 population. The prevalence of ALS increases with age, reaching a peak in the 60-75 age group at 33 per 100,000

population for men and 14 per 100,000 population for women (figure 1).[9-11] On average, a male preponderance of 1.5 to 1 is reported worldwide, but this is less pronounced after the age of 70 years. Male preponderance occurs only in sporadic ALS and is still unexplained.[12, 13] The incidence rate of ALS is 1-3 per 100,000 person years and increases with age.[9, 10, 14] A peak incidence rate is observed in the 60-75-age group of 10.5 and 7.4 per 100.000 men and women, respectively (figure 2). Ethnicity data from the U.S. show lower mortality rates from ALS in nonwhites than whites (ratio of 1:1.6), but with age and sex differences similar to whites.[15] There is a latitude-related increase in ALS, with geographical age adjusted incidence rates ranging from 2 in Israel (32^0) to 8 in the northern Scandinavian countries ($>60^0$).[16-19] Geographical differences of ALS world-wide are probably the result of regional differences in ascertainment, regional differences in genetic susceptibility and/or exposure to environmental factors.

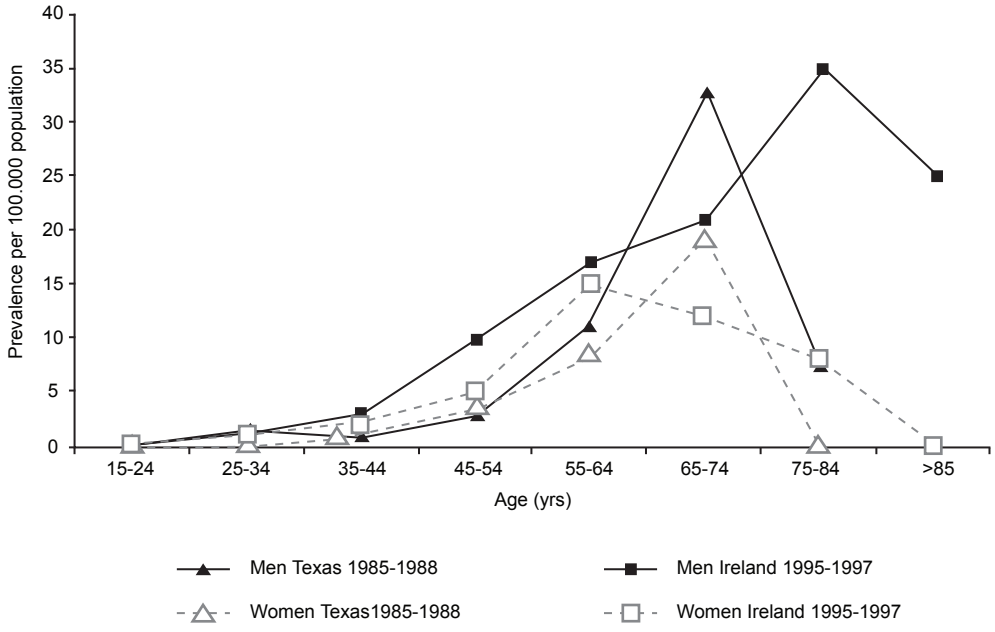

Figure1. Age-specific prevalence of amyotrophic lateral sclerosis (ALS) in men and women from Harris County, Texas(9) and Ireland (10).

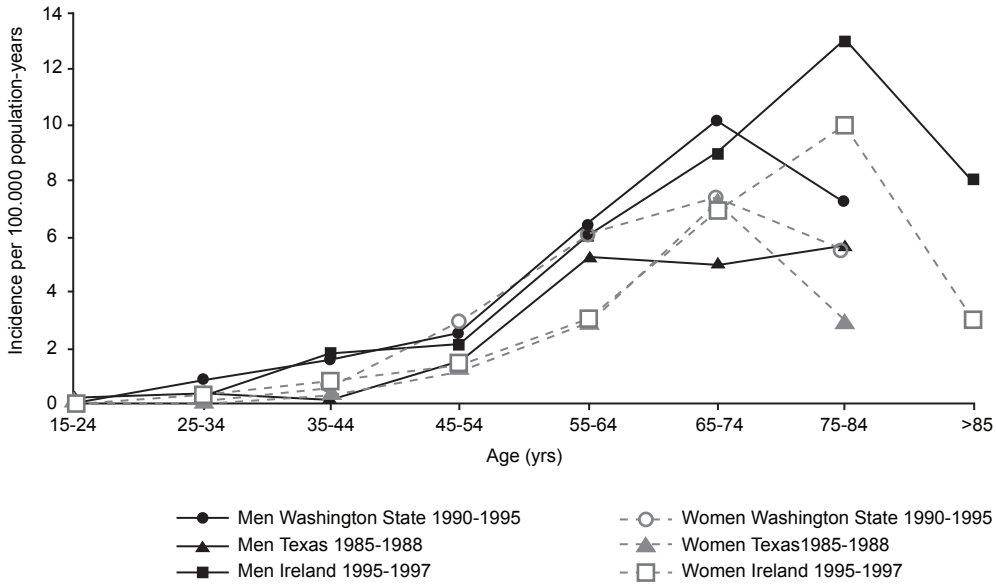

Figure 2. Age-specific incidence of amyotrophic lateral sclerosis (ALS) in men and women for Washington State (14), Harris County, Texas (9) and Ireland (10).

Four well studied geographical areas with a high prevalence of ALS (clusters) are known. In the Chamorro population of the western Pacific islands Guam and Rota two neurologic conditions are frequent: ALS, locally known as 'lytico', and the parkinsonism-dementia complex (PDC) of Guam, locally known as 'bodig'. Lytico and bodig may co-occur in patients and families.[20] The strong suspicion of an identical aetiology has not been proven.[21] The crude prevalence rate of ALS on Guam declined from 100 in the 1950's to 50 and 25 per 100.000 population for men and women, respectively, in the 90'ies.[22-24] Two areas of the Japanese Kii peninsula show a similarly high prevalence of ALS, [25,26] as well as a population of the western coast of Papoea New Guinea, Irian Jaya (Indonesia).[27] At the same pacific latitude, a fourth high incidence area is found in an isolated tribe at Anguru on Groote Eylandt in the Gulf of Carpentaria (North Australia).[28] In these endemic areas in the South Pacific, ALS accounts for about one in ten deaths. Also in Guadeloupe, in the French West Indies, an unusual high frequency of atypical parkinsonism occasionally accompagnied by ALS occurs, reminiscent of the one described in Guam.[147]
Smaller clusters of ALS, related to professional or residential proximity, have been reported from the same apartment building (n=3) [30], a school (n=3) [31], a group of farm-rangers in South-Dakota (n=4)[32, 33 105], among mail carriers (n=3) in a small town [34] or a small

community (6 cases of ALS between 1975 and 1983) near lake Michigan, Wisconsin, USA.[35, 36] Conjugal ALS has been reported not only in Guam,[37] but also in four couples outside the Western Pacific.[38-41] These clusters of ALS may have been caused by common exposure or transmission of a causal agent, or alternatively may reflect coincidence. Analysis of small clusters is usually complicated because of lack of data on the total population of origin. Nevertheless, these clusters kept the discussion alive about potential environmental and genetic causes of ALS.

Environmental risk factors

The environmental risk factors of neurodegeneration in ALS were largely unknown until recently. The endemic occurrence of ALS such as in the high-risk areas in the Pacific, could not be fully explained by genetic factors,[29,42, 43] and evoked an animated discussion on gene-environment interaction. Cycad nuts, mineral content of the drinking water and the soil content became suspected as long-term acting environmental neurotoxic risks. Attention especially focused on the fruits of the locally growing cycad palms, as vividly described by Oliver Sacks in 'Cycad Island ',[44-47] also because geographical incidence rates for ALS are strongly correlated with concentrations of cycasin in traditional food prepared from the toxic seed of the cycad plants.[24, 45] The pathogenic effect of cycasin seems to be due to a decrease of neuronal DNA-repair, persistent upregulation of *tau* mRNA expression, and enhancement of excitatory neurotoxicity.[48]

Other putative environmental risk factors for ALS include a history of trauma to the brain and spinal cord, strenuous physical activity, and exposure to radiation, electrical shocks, welding or soldering materials, paint- or petroleum industry and the dairy industry. However, none of the exogenous risk factors for ALS has been reported consistently. Although malignancies were reported in 10% of patients with motor neuron disease in a population study,[49] this association remains unexplained. Long-latency viral infections are another potential etiology of ALS.[50, 51] Enterovirus nucleic acids have been detected by reverse transcriptase-PCR in 15/ 17 spinal cords of sporadic ALS patients in a French study, but not in more than 20 spinal cords examined in other studies.[52-56]

In conclusion, exogenous risk factors are inconsistently reported in ALS. This might indicate that these risk factors do as such not cause the pathology. Alternatively, it might reflect a complex interaction between several environmental factors and specific genetic susceptibilities.

Genetic risk factors

Genetic risk factors for ALS have been studied extensively. Familial aggregation of ALS,

with an age-dependent but high penetrance, is a major risk factor for ALS. Familial ALS (FALS) is clinically and genetically heterogeneous with multiple autosomal dominant and recessive forms. Molecular genetic analysis of FALS has led to the discovery of several genes involved in ALS (table 1). These ALS genes, which we will refer to as 'major genes' cause ALS with a clearly monogenic inheritance pattern. They may either predominantly lead to ALS (ALS1-ALS6) or cause multisystem neurodegeneration (tauopathies and ALS with dementia and parkinsonism) with ALS as an occasional symptom.
Other genes, that we will call 'susceptibility genes', may trigger the cascade of neurodegeneration or may act as susceptibility factors for neurodegeneration in interaction with environmental risk factors or other (genetic) risk factors.
This review presents a tentative classification of the "major" and "susceptibility" genes for ALS. Subsequently we will discuss current insights in gene-gene and gene-environment interaction in ALS.

Major genes

In this review, we first discuss the monogenic forms of ALS showing clear mendelian inheritance. Seven different gene loci for familial ALS (FALS) have been reported, and three major genes (*SOD1, alsin and tau*) have been identified (Table 1).
The difficulty of clinically distinguishing ALS from other forms of motor neuron disorders, especially in patients who lack the classical signs of each disease, is underscored by the occasional finding of a deletion in one of the genes for spinal muscular atrophy, the survival motor neuron gene *SMN1*[57] and the centromeric *SMN2* [58], and the neuronal apoptosis inhibitory protein gene *NAIP* in patients diagnosed with sporadic and familial ALS.[59-62] Furthermore, about 2% of the male patients diagnosed with ALS actually have Kennedy disease (SBMA, X-linked spinobulbar muscular atrophy) caused by a trinucleotide expansion in the androgen receptor.[63, 64] Autosomal dominant inheritance of FALS occurs in approximately 5- 10% of ALS patients and is clinically and neuropathologicaly indistinguishable from sporadic ALS.[65] Dominant FALS is characterized by large intra- and interfamilial variability of age of onset and progression. Incomplete penetrance has been observed in pedigrees [66-69] and confirmed by mutation analysis.[70]
Autosomal dominant FALS is genetically heterogeneous; currently two genes, one on chromosome 21 (*SOD1* gene) and one on chromosome 17 (*tau* gene) and three additional loci (one on chromosome 18 and two on chromosome 9) are known (Table 1).
Before linkage analysis and molecular diagnosis of ALS through molecular analysis became available, autosomal recessive FALS was considered very rare. However, currently already three forms of recessive ALS are recognized, and the genes for two of those, *SOD1* (ALS1) and *alsin* (ALS2) have been identified. Altogether the now established forms of FALS account for probably 20-30% of FALS.

Table 1. **Genetics of Amyotrophic Lateral Sclerosis (ALS)**

Classification	Gene	Localization	Inheritance	Ref.
Major genes				
ALS1	*SOD1*	21q22	AD/AR	73,75
ALS2	*alsin*	2q33-34	AR	126,127
ALS3		unkown	AD	
ALS4		9q34	AD	128
ALS5		15q12-21	AR	129
ALS6		18q21	AD	131
FTDP	*tau*	17q21.2	AD	132, 138
FTD		9q21-22	AD	155
Susceptibility genes				
Neurofilament heavy chain	*NF-H*	22q12.2		187
Neurofilament light chain	*NF-L*	8p21		191,193
Peripherin	*PRPH*	12q12-13		182
Glutamate transporter	*EAAT2*	11p13-12		215
Glutamate receptor	*AMPA*	5p33		207
Apolipoprotein E	*ApoE*	19q13.2		219
Ciliairy neurotrophic factor	*CNTF*	11q12.2		230
Debrisoquine hydroxylase	*CYP2D*	22qq13.1		233
Apurinic apyrimidinic endonuclease	*APEX*	14q11-12		236
Mitochondrial DNA	*COX*			246
Manganese superoxide dismutase	*SOD2*	6q25		248
P2 Blood Group	*P2*	22q11		262

AD, autosomal dominant; AR autosomal recessive; SOD, superoxide dismutase; FTD, frontotemporal dementia-ALS complex; FTDP, frontotemporal dementia and parkinsonism complex.

ALS1 (*SOD1*)

In 1991 the first ALS gene (ALS1) for an autosomal dominant form of FALS was mapped to chromosome 21q.[71] Two years later the cytosolic copper-zinc superoxide dismutase (*SOD1*) gene was shown to be the ALS1 gene.[72] SOD1 is a 153- amino acid metalloenzyme with high expression in nervous tissue, liver and erythrocytes. The enzyme catalyses the conversion of the superoxide radical anion (O_2^-) to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). Presently, over 90 different mutations in all five exons of this gene have been identified.[65] All these *SOD1* mutations are associated with autosomal dominant FALS, except two, D90A and D96N that can cause both dominant and recessive ALS.[73-76]
SOD1 mutations have been detected in 195/916 families showing autosomal dominant ALS, suggesting that *SOD1* is implicated in ± 21% (range 13-84 %) of dominant FALS.[65, 72, 77-83] Additionally, 112 of 946 apparently sporadic ALS patients or about 12% (range 2.5-23%) was heterozygous for a *SOD1* mutation.[65, 78, 84, 85] The mutation pattern in sporadic ALS is similar to FALS.[86, 87] Sporadic ALS due to one *SOD1* mutation may represent a new dominant mutation, or incomplete penetrance of a dominant mutation in the parents. Some of the *SOD1* mutations occur as recurrent mutations or as founder mutations sometimes having a worldwide distribution. The A4V mutation, the most frequent *SOD1* mutation overall, is yet only found in patients from North America.[73, 86]
Most mutations in the *SOD1* gene associated with FALS behave as dominant traits. However, the D90A mutation can also be recessively inheritated. This missense mutation involving an A to C transversion giving rise to substitution of aspartic acid by alanine at codon 90 can cause ALS both in the homozygous state or in the compound heterozygous state with the D96A mutation.[76, 88] The recessive inheritance of the D90A mutation has been reported mainly in Scandinavian families.[74, 80, 84] In other parts of the world the D90A mutation is a rare cause of recessive FALS and usually causes ALS in the heterozygous state.[75, 82, 85, 86, 89, 90] In the Northern Scandinavian population homozygotes for this mutation show a uniform disease phenotype with insidious onset (mean age at onset: 44 years, range 20-94): the slowly ascending paresis begins distally in the lower extremities and has relatively slow progression (mean survival: 14 years).[82, 84] In the population of northern Sweden and Finland this type of ALS1 is responsible for 9.6% (44/451) of ALS,[80, 91] and a tenfold increase of the allele frequency of the D90A mutation in this population was found (1 to 2 %).[84, 92] A study of 28 recessive D90A pedigrees showed that 20 families shared the same founder haplotype,[93] whereas several founders were detected in 8 families with the dominant D90A mutation Therefore, it was suggested that there might be a protective factor that is tightly linked to the *SOD1* mutation in the genetically rather homogenous northern Scandinavian population and reduces susceptibility to ALS, which would also explain the very long disease duration in the Scandinavian patients.
The majority of *SOD1* mutations are missense mutations and there is no clear correlation

with the clinical expression of the disease (genotype-phenotype correlation).[65, 80, 86] This inter- and intrafamilial variability precludes precise predictions on the course of the disease. Clinical characteristics age at onset and survival vary greatly between and among specific mutations. The phenotypic variability extends from rapidly progressive disease with only lower motor neuron signs to a very slowly progressing disease with upper and lower motor neuron signs.[70, 94-96] Typically, in one family with dominant ALS with a D90A mutation of the *SOD1* gene, manifestations ranged between juvenile focal amyotrophy of the arm to classical symptoms of ALS in another relative.[75] Even non-penetrance was recently found for some *SOD1* mutations, including the I113T, V4G, A76T, G21L, A101A and G16S mutations,[69, 97, 98] although penetrance of most *SOD1* mutations may seem high (80% at age 85 years) in families ascertained for familial ALS.[66]

Some of the *SOD1* mutations often are associated with 'early onset' (G37R, L38V), 'shorter survival' (A4V) or relatively 'benign' forms of ALS (G37R, G41D, G93C, and H46R).[70, 79, 83, 86, 99-101] However, these associations must be interpreted with caution when used to give a prognosis in individual cases. Onset of disease with progressive bulbar paralysis seems quite rare in ALS1 patients (reported once in a case with a deletion of the *SOD1* gene).[102] Sometimes extreme differences in survival within some families with ALS have important implications for genetic counseling in these families, and raises fundamental biological questions concerning the existence of modifying properties of additional genetic or environmental risk factors.

It is unclear how *SOD1* mutations cause ALS. As the level of residual SOD1 enzyme activity does not correlate with disease expression in ALS patients, the loss of function of SOD1 is not a likely cause of motor neuron degeneration.[103] This is corroborated by the observation that knockout mice models have no ALS phenotype.[104-106]Actually, dominant *SOD1* mutations are thought to act as gain-of-function mutations inducing mutant SOD1 proteins that are selectively toxic to motor neurons. The mutant proteins might be responsible for the intracellular aggregation of neurofilamentous protein in neurons and astrocyts, that coincides with disease onset and increases as disease progresses.[107,108] Studies of transgenic mice carrying mutated *SOD1*[109] suggest that variation in expression of neurofilament influences selective vulnerability of motor neurons and slows down the toxicity of mutant SOD1 (see: neurofilament gene); other evidence suggest that abnormalities in the neurofilament organization may not be essential for SOD1- mediated ALS.[110,111]

Other toxic effects of *SOD1* mutations may involve increased vulnerability to excitotoxic mechanisms by selective inactivation of the glutamate transporter EAAT1,[112-114] or by mitochondrial degeneration.[115] Recent work on *SOD1*-transgenic mice has shown that mutant SOD1 proteins may exert their toxic properties by decreasing calcineurin activity (CaN; an enzyme regulating neuronal excitability by controlling the activity of ion channels and the release of neurotransmitter).[116,117]

Neurofibrillary depositions and Lewy body-like inclusions belong to the pathological spectrum of ALS1.[86,118] Neurofilamentous inclusions in motor neurons may occur in patients with the A4V and H48Q mutations. Also I113T mutation (which is the second most common *SOD1* mutation) seems to predispose to extensive intraneuronal neurofilament depositions and multisystem distribution of intraneuronal neurofilament accumulation.[95,102,118-121] Immunohistochemical co-localization of SOD1 in neuronal Lewy body-like hyaline inclusions (LBHIs) in the spinal cords of ALS1 patients indicates that SOD1 is a component of LBHI.[122] Co-localization of copper chaperone (CCS) and SOD1 suggests a specific interaction of SOD1 and CCS in the formation of LBHIs in ALS.[123]

ALS2 (*ALSIN*)

Autosomal recessive FALS was first described in a large inbred family in Tunisia showing linkage to the chromosomal region 2q33-34. Symptoms occur in the first or second decade of life, and include progressive spasticity of the limbs and the facial and pharyngeal muscles. Survival is relatively long (range from 15 to 20 years).[124]
Recently, the gene for ALS2 was identified in Tunesian and Kuwaiti families and named *alsin* or *ALS2*.[125-127] Alternative splicing of this gene produces a short and a long transcript. Deletions affecting both transcripts result in the ALS2 phenotype, whereas homozygous deletions in exon 9 and affecting only the short form of the protein cause juvenile primary lateral sclerosis (JPLS). JPLS is even more rare than ALS2, and its phenotypic presentation consists of slowly progressive spastic paraparesis and muscle weakness of the oro-facial and ocular muscles.
Expression of the mouse orthologe of alsin was found in neuronal cells throughout the brain and spinal cord. Because alsin has protein domains with similarities to pleckstrin and guanine-nucleotide exchange factors (GEFs) known to activate GTPases, alsin may act as a regulator/activator of GTPases, and modulate microtubuli assembly, membrane organization and trafficking in neurons.

ALS3

The majority of dominant FALS has not yet been assigned to any given locus and was lumped into the designation ALS3.[71]

ALS4

A second form of autosomal dominant FALS (ALS4) has been localized to a locus on chromosome 9q34 in an extended family with juvenile onset (mean age at onset 17 years)

and slow progression without bulbar involvement. However the ALS4 gene has not yet been identified. Patients initially show difficulty in walking, followed by weakness and wasting of muscles of the hand and distal lower extremities. Significant proximal weakness occurs in the 4th or 5th decade, with loss of useful hand function by the 6th decade. Neuropathological evaluation disclosed degeneration extending to the dorsal roots, dentate nucleus, nucleus gracilis, and inferior olivary nucleus.[128]

ALS5

This form of autosomal recessive FALS is clinically similar to ALS2.[129] ALS5 may be the most prevalent form of recessive ALS, and was identified in several ethnic groups (North-African, South-Asian, and European). The ALS5 gene was mapped to chromosome 15q, but the gene remains to be identified.[130]

ALS6

Only recently, a third locus for autosomal dominant FALS was mapped to chromosome 18q21 in a large European family with classic adult-onset ALS.[131]

Tauopathies

ALS1, ALS4 and ALS6 represent pure forms of FALS. However, ALS can also be part of a multisystem neurodegeneration including the tauopathies and the combination of ALS with dementia and parkinsonism. Mutations in the tau gene are associated with a clinical phenotype that includes frontotemporal dementia, Pick's disease, corticobasal degeneration, and familial progressive supranuclear palsy. This phenotype shows variability both within and between different families.[132-137] The variability of clinical symptoms suggests that tau pathology may play a role in a large number of neurodegenerative diseases, including ALS, where neurofilamentous deposits mainly consisting of hyperphosphorylated tau protein occur. *Tau* mutations were first recognized in a syndrome consisting of frontal lobe dementia, parkinsonism and amyotrophy (FTDP).[132, 138] FTDP is characterized by autosomal dominant inheritance of behavioral disturbances, reduced speech and memory impairment, whereas parkinsonism and amyotrophy may occur late in the course of the disease The prevalence of FTD is estimated to be 2.8 per 100.000 in the 60-70 year-olds.[139] The proportion of families with FTD having a mutation in the *tau* gene varies from 13% to approximately 40%.[140-142]
In FTDP the mutations of the *tau* gene affect the alternative splicing of exon 10 of the *tau* gene, resulting in an excess of four-repeat tau isoforms. The effect of four-

repeat protein tau- isoforms on neurodegeneration could consist of a reduced binding of tau to microtubules in axons. That might explain the accumulation of tau protein as hyperphosphorylated neurofilaments in the cytoplasma, leading to central and peripheral axonopathy that results in neuronal death and amyotrophy.[143-146]
Guadeloupean parkinsonism, associated with frontolimbic dementia and ALS is also considered a tauopathy because of the accumulation of tau proteins, predominantly in the midbrain. Although no mutations in the *tau* gene were observed in this group of patients, all cases were homozygous for the H1 *tau* haplotype.[147] The *tau* polymorphism CA3663 is associated with the ALS, dementia-parkinsonism complex of Guam, indicating that *tau* is not the cause of this disease but probably acts as a susceptibility gene.[148, 149]

ALS with dementia and parkinsonism

The clinical phenotype of FALS can consist of pure ALS, multisystem degeneration in the tauopathies, and ALS in association with dementia and parkinsonism. Some mutations in *SOD1* may cause ALS associated with dementia, as observed in a family with the A76T mutation.[80] Parkinsonism is seen less often, suggesting reduced toxicity of *SOD1* mutations to nigrostriatal dopaminergic neurons.[150]The Guamanian type ALS is characterized by the familial co-occurrence of dementia, parkinsonism and motor neuron signs. Analysis of the *SOD1* gene in this disease found an I113T mutation in (2 of 23 unrelated) ALS patients from the Kii Peninsula of Japan.[83, 98, 151, 152]
Also other genes predisposing to age-related multisystem neurodegeneration represent a genetic contribution to ALS. Mutations in the *tau* gene cause varying symptomatology as was recently demonstrated in the frontotemporal dementia and parkinsonism complex (FTDP)[132, 137, 138, 153, 154] and in familial atypical progressive supranuclear palsy.[133] Whereas the ALS-frontotemporal dementia-complex (FTD) is linked to chromosome 9q (Table 1).[155] However, these genes do not account for all of the familial aggregation of ALS, dementia and parkinsonism.[156, 157]
Epidemiologic studies have revealed familial aggregation of classic ALS, dementia and parkinsonism suggesting that common genetic factors may contribute to all three disorders. A significant increase in the risk for dementia as well as for parkinsonism in first- and second- degree relatives of ALS patients has been found in several case-control studies.[12, 158-160] The cumulative incidence of dementia to age 90 was significantly higher (15%) in relatives of ALS patients than in relatives of controls. Furthermore the lifetime risk for parkinsonism is 7% for relatives of ALS patients, a twofold increase compared to relatives of controls.[158] Epidemiologic data also indicated an excess of familial co-occurrence of dementia and parkinsonism in families of patients with ALS: patients with sporadic ALS were 12 times more likely to have relatives with parkinsonism, if a relative had dementia than if there was no family history of dementia.[161] This excess aggregation probably reflects

a genetic susceptibility to widespread neurodegeneration.
Apart from the 4 loci described above, many more genes are involved in syndromes with ALS, dementia and/or parkinsonism. Machado-Joseph disease shows familial amyotrophy associated with parkinsonism and spinocerebellar symptoms, and is caused by expansion of trinucleotide repeats in the *SCA3* gene on chromosome 14. Other examples of genetic disorders presenting with both pyramidal and extra-pyramidal symptoms are familial aggregation of motor neuron disease, tic disorder and parkinsonism due to neuroacanthosis,[162-164] and the autosomal dominant inherited entity of parkinsonism and amyotrophy.[165-170] A familial ALS- dementia complex with autosomal recessive inheritance was reported in some families, including inbred Old Amish sib ships. The latter type of ALS presents with a juvenile form of ALS and an exceptional long survival (from 9 to 27 years) and is variably accompanied by dementia.[171, 172] Another familial ALS-dementia complex shows an autosomal dominant inheritance pattern.[173-177] The familial occurrence of ALS with symptoms of dementia, parkinsonism, or other CNS symptoms is also observed in a growing number of other genetic multisystem neurodegenerative disorders including Huntington disease, or neuroaxonal dystrophy.[178, 179] Therefore family histories of persons with ALS might be carefully analysed not only for ALS, but also for the often-related disorders of dementia and parkinsonism. .

Susceptibility genes

Considering that mutations in ALS genes occur in familial as well as sporadic ALS patients, probably more than 10% of ALS can be explained by the multiple autosomal dominant and recessive forms. Since there is still no evidence for any specific environmental cause for sporadic or familial ALS, most remaining cases of the disease are thought to result from interaction of several genes and environmental factors.
We describe here some of the genes potentially contributing to the development of ALS. In this broader complex genetic context, these genes are referred to as ‘susceptibility genes’ as mutations in these genes may only lead to ALS in the presence of other genetic or environmental risk factors.

Neurofilaments

Abnormal accumulation of intermediate filaments (IF) in the perikarya and proximal axons of motor neurons is a common pathological hallmark of ALS, suggesting that abnormalities in neurofilament organization may be involved in the pathogenesis of ALS.[180] Neurofilaments are the principal intermediate filament type expressed by motor neurons. They are formed by the co-assembly of three sub-units: NF-L (light subunit), NF-M

(medium subunit), and NF-H (heavy subunit). NF-L is necessary for filament assembly, whereas NF-M and NF-H form links with other neurofilaments in axons.[181] Peripherin, another intermediate filament, is associated with axonal spheroids in the degenerating motor neurons of ALS patients.[182] Transgenic mice overexpressing peripherin in motor neurons develop late onset motor neuron disease.[183, 184]
It is unclear whether the IF accumulations in ALS occur as a secondary feature of ALS-associated neurodegeneration or represent the primary cause of disease.
Several lines of evidence suggest that neurofilament genes play a causal role. First, variant alleles of the neurofilament heavy-subunit gene (*NF-H*) have been found in ~1% of more than 1300 sporadic and in 0.3% of 295 familial ALS patients, but not in control DNA.[185-190] However in transgenic mice overexpression of wild type NF-H proteins leads to atrophy of motor neurons.[3, 191, 192] Second, in situ hybridization studies revealed considerable reduction in NF-L mRNA levels in degenerating spinal motor neurons of ALS patients.[193] A role of NF-L dysfunction in neurons is further suggested by the observation that NF-L deficiency accelerates motor neuron signs in mice overexpressing peripherin.[183, 194] Also a *NF-L* mutation in a family with neuronal Marie-Charcot-Tooth (type 2) disease was detected. [195] Furthermore, mice transgenic for a mutant *NF-L* gene develop severe perikaryal and axonal degeneration of motor neurons.[191, 196]
The presence of abnormal neurofilament accumulations in FALS associated with *SOD1* mutations,[95, 118, 121] and in mice expressing mutant *SOD1* endorses involvement of neurofilamentous pathology also in ALS1. Surprisingly, experiments with *SOD1* transgenic mice, show that both the absence of NF-L neurofilaments (causing a depletion of axonal IF and a decrease in axonal conductivity)[197] and overexpression of the *NF-H* gene (causing perikaryal increase of IF) can cause a decrease in vulnerability and increase in survival of the motor neurons.[110, 111, 181, 198, 199] These studies also showed that perikaryal and axonal aggregates contain peripherin.[200]
In conclusion, IF variants are likely modifying risk factors in sporadic ALS and probably modulate disease expression in SOD1- related ALS.[201]

Excitotoxicity genes

The term excitotoxicity refers to a phenomenon in which the excessive or prolonged activation of excitatory amino acid receptors results in damage and eventually death of the involved neurons. The activation of these receptors leads to depolarization and neuronal excitation and is normally transitory. Excitatory damage of neuronal cells appears to be mediated by sustained elevations of intracellular calcium levels. In line with this, high levels of the calcium-binding protein paralbumin were found to delay motor neuron degeneration in double transgenic *SOD1*/ overexpressing paralbumin mice.[202-204] That excitotoxic mechanisms are implicated in the pathogenesis of ALS is further based

upon several observations. Levels of glutamate, the major excitatory transmitter in the motor neuron system, are increased in the cerebrospinal fluid of ALS patients,[205] whereas glutamate transport is reduced in the brain and spinal cord of ALS patients.[206] Motor neurons are vulnerable to excitotoxicity mediated by the glutamate receptor α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA),[114,207,208] and ingestion of excitotoxins may contribute to the pathogenesis of the ALS-parkinson-dementia complex of Guam.[209,210] Riluzole, an antiglutamate drug, is yet the sole drug shown to provide some therapeutical effect in ALS.

Depolarization of the neuronal membranes after activation of neuronal glutamate receptors activates voltage-dependent calcium channels, allowing calcium to entry the cell. The excess activation of neuronal glutamate receptors causing cell death via increased cellular calcium may be one mechanism involved in motor neuron disease. Glutamate can also produce neuronal degeneration by increasing *tau* mRNA expression and phosphorylated tau protein, as observed in neuronal cultures.[211-214] Decrease of glutamate transport may provoke elevated synaptic glutamate concentration. One of the four glutamate transporter proteins, the EAAT2 isoform, is involved in keeping extracellular glutamate levels below excitotoxic levels in the nervous system.

In 60% of sporadic ALS, a 30%-95% loss of EAAT2 activity is observed in the motor cortex and spinal cord but not in other brain regions.[204, 215, 216] The presence of variant mRNA transcripts of EAAT2 in these ALS cases could not be explained by mutations in the *EAAT2* gene [216-218], and other mechanisms must therefore be responsible for these low levels of EAAT2. Since *SOD1* gene mutations were found to inactivate EAAT2, *SOD1* mutations may partially, explain the decreased levels of EEAT2 in ALS.[112, 113]

Apolipoprotein E

The frequencies of apolipoprotein E (*ApoE*) ε2, ε3 and ε4 genotypes in familial and sporadic ALS are not different from those in the general population,[219] thereby excluding *ApoE* mutations as a primary cause of ALS. However, the *ApoE* genotype was reported in some studies to have an effect on the age of onset and clinical presentation of sporadic ALS. The ε3/ε3 genotype may prolong survival in ALS patients, whereas the ε4 allele may predict an earlier age at onset.[220] A significantly higher proportion of ALS patients with the ε3/ε4 genotype had bulbar symptoms, while the ε2/ε3 genotype seemed to predispose to limb onset of the disease. Therefore, as in Alzheimer disease, a protective role in ALS of ε2 and ε3 alleles, and a deleterious role of the ε4 allele have been suggested.[221-223]

The exact mechanism of the deleterious effect of the ε4 allele is unknown, but it may simply result from the absence of the protective alleles ε2 and ε3. The ε2 and ε3 gene products of ApoE have cysteine residues that are important in the detoxification of a lipid peroxidation product, 4-hydroxynonenal (HNE), which causes apoptosis and which

is increased in the spinal cord of ALS patients. The ε4 gene product has no cysteine residues, and therefore lacks the ability to protect against HNE-mediated apoptosis. This might explain the relationship between these *ApoE* genotypes and susceptibility to neurodegeneration.[224] However, other studies do not confirm the association of the ε4 allele with early onset, shortened survival or the presence of bulbar symptoms at onset in ALS.[219, 225, 226] Also in patients with the ALS -parkinsonism-dementia complex of Guam no association was found with any of the ApoE alleles.[29, 227]

Ciliary neurotrophic factor (CNTF)

Diminished levels of ciliary neurotrophic factor (CNTF), a survival factor in spinal motor neurons, might contribute to the development of ALS. This is based upon several arguments. First, a decrease in CNTF levels was found in the corticospinal tract neurons of ALS patients.[228] Second, abolition of *CNTF* gene expression in knockout mice causes progressive motor neuron degeneration.[229] Third, the frequency of the homozygous state for a mutation in the *CNTF* gene leading to absent CNTF is slightly increased in sporadic ALS patients.[230] As heterozygosity for *CNTF* mutations, is not increased in ALS patients, the role of CNTF in ALS seems modest.[230, 231]

Cytochrome P450 debrisoquine hydroxylase (CYP2D6)

Protein polymorphisms in the cytochrome P450 debrisoquine hydroxylase gene (*CYP2D6*) are responsible for diminished metabolism of exogenous toxins, hereby forming one of the risk factors for Parkinson disease.[232] Three studies have addressed the role of the CYP2D6 (b) allele as a risk factor for ALS. One study found no association between this allele and ALS among 57 ALS patients and controls.[233] The second study found a significant increase in the frequency of the CYP2D6(b) allele in 50 ALS patients.[234] In the Chamorro individuals the frequency of this mutant allele was higher than in Caucasians, but no differences were found between healthy Chamorro people and patients with Guamanian ALS- dementia-parkinsonism complex.[29]

Apurinic apyrimidimic endonuclease (APEX)

The DNA repair enzyme apurinic/apyrimidinic endonuclease (APEX nuclease) might be a risk factor for ALS as reduced levels of APEX nuclease were found in the frontal cortex of ALS patients.[235] There are two reports of *APEX* gene mutations resulting in a decreased level and activity of the enzyme in ALS: mutations were found in 4/117 patients with sporadic ALS and in 4/9 twins with ALS, but not in controls.[236-238] Apex nuclease

may also play a role in the Guamanian ALS-dementia-parkisnonism complex, since a metabolite of cycasin, methylazoxymethanol (MAM), a potential environmental genotoxin in Guamanian ALS, was found to cause significantly reduced neuronal APEX levels and activity.[48] Although *APEX* mutations do not seem to account for a large proportion of ALS, the role of DNA damage in the etiology of ALS may be important.

Mitochondrial metabolism

Evidence that either inherited or acquired mitochondrial dysfunction plays a role in the pathogenesis of ALS is accumulating.[239] Mitochondrial dysfunction has been detected in muscle biopsies of sporadic ALS patients,[240] and increased oxidative damage has been found in the central nervous system tissue from autopsied familial and sporadic ALS patients.[241-243] Mitochondrial vacuolisation is an early pathological feature in *SOD1*-mutated transgenic mice, suggesting that the leakage or translocation of mutant *SOD1* in mitochondria may be the primary event triggering further degeneration.[105, 115, 244, 245] Additionally, mutations in mt DNA, including multiple mtDNA deletions or depletion of mtDNA,[246] and a mutation of the mitochondrial DNA-encoded subunit 1 of cytochrome-c oxidase have been reported in ALS.[247] Mitochondrial dysfunction in ALS may also be due to decreased levels of mitochondrial manganese superoxide dismutase (SOD2). This mitochondrial enzyme is encoded for by a nuclear gene on chromosome 6. An Ala9Val mutation in *SOD2* has been found in some patients with sporadic ALS,[248] whereas degeneration of neurons in basal ganglia and brainstem, and progressive motor disturbances with paresis occur in *SOD2* knockout mice.[249, 250] Furthermore, cultured murine cortical neurons with reduced levels of SOD2 are more sensitive to glutamate neurotoxity than neurons with intact SOD2 activity.[251] Dysfunction of SOD2 exacerbates disease expression in *SOD1* transgenic mice.[252]

Additional candidate genes

Various additional 'candidate genes' for ALS as a monogenic entity or a multifactorial disease have been proposed. Despite the fact that SOD1 is involved in ALS through mechanisms probably not related to antioxidant activity, candidate genes for familial ALS include genes involved in free radical removal. A cytosolic protein termed the copper chaperone for superoxide dismutase (CCS) mediates the delivery of copper to SOD1. Experiments with CCS knockout mice show a marked reduction in SOD1 activity,[251] suggesting that CCS might play a role in ALS.[253]
The association of the B allele of monoamine oxidase (MAO-B), a generator of free radicals, with a later age-at-onset of ALS suggests a role for oxidative damage in ALS.[254]

The *Bcl-2* gene, encoding a protein that inhibits apoptosis, might be involved in ALS, as it attenuates neurodegeneration in SOD1-mediated ALS models.[255] The spinal cords of patients with sporadic ALS may display increased expression of the transcription factor c-jun mRNA, suggesting that c-jun is involved in the neurodegenerative processes of ALS.[256, 257]

Another set of candidate genes is derived from the association of ALS and lymphoma.[258-261] A region on the long arm of chromosome 22 near the neurofilament gene may contain several genes that may modulate susceptibility to ALS: the blood group phenotype P2 is overrepresented in ALS patients (43% of U.S. veterans with ALS, more than twice as frequent as expected),[262] and appears extremely frequent (66%) in the indigenous Chamorros of the islands Guam and Saipan.[263] The P2 blood group is equally frequent in the population in the northern part of Sweden, where the recessively inherited D90A *SOD1* mutation prevails.[93, 264] Close structural similarity of P2 to GM_1 ganglioside may explain the characteristic immunologic features of ALS patients, such as the increase in IgM antibodies against GM_1 ganglioside by cross-reactivity against the P-system blood group antigens.[265] Other genes that might involve predisposition to ALS are the interleukin-2 receptor beta chain gene, localized to 22q11.2-q12, which is a locus for many neodysplasias of the lymphoid system, and the leukemia inhibitory factor gene (*LIF*) on 22q12.1-q12.2.[266] Recently, knockout mice for the hypoxia-response element (HRE) of the vascular endothelial growth factor (*VEGF*) gene were shown to develop an ALS-like condition, but *VEGF* mutations have not yet been identified in human ALS.[267]

ALS: a multifactorial disease

In view of the abundance of genetic and environmental factors that contribute to the pathogenesis of ALS, this disease is a paradigm for multifactorial diseases. These are also referred to as "complex diseases" because of the complex interaction between genetic and/or environmental factors.

Gene- gene interaction

There are strong indications of gene-gene interaction and/or interaction of gene products. The selective detrimental effect of mutated ALS genes suggests an interaction with genes that are specifically expressed in neurons. Alternatively a neuron-specific lack of protection against the detrimental effect of ALS genes may exist.

Recently, experiments with double transgenic mice demonstrated mutual effect-modification of ALS genes (*SOD1*, neurofilament, excititoxicity and mitochondrial genes). The genetic effect-modification may result from a novel interaction of altered

gene-products (protein-protein interaction). Alternatively, it is conceivable that abnormal proteins modulate gene expression (protein-gene interaction).[268]
Experiments with transgenic mice have effectively demonstrated that mutations in the neurofilament gene slow down SOD1- mediated ALS. The interactive effect of genes may also enhance neurodegeneration, like the increase in excitotoxic vulnerability observed in *SOD1* mutations, *tau* gene upregulation by glutamate toxicity,[212] and the coincidence of a high prevalence of the rare P2 blood group in the native inhabitants of Guam and in the Scandinavian population, where *SOD1* mutations cause a rare recessive form of ALS1.

Gene- environment interaction

Gene-environment interaction is suspected when a specific environmental risk factor modulates the risk for disease in persons with a susceptibility genotype. Exogenous control of gene expression should be considered as a possible cause of neurodegeneration for instance in the Guamanian ALS. The observation that intraneuronal neurofilamentous aggregations (a neuropathological hallmark for guamanian ALS/ PDC) are frequent in the indigenous Gamorro population of Guam, where consumption of cycad nuts is high, may lead to the hypothesis that for the specific subgroup of this population with a genetic predisposition to neurofilamentous accumulation, ingestion of cycad nuts may be more harmful.[269, 270] The prevalence of a specific polymorphism in the *tau* gene in the Guamaninan population, distinct from the splice site mutations in FTDP-17,[149] and the observation that cycasin may cause persistent upregulation of *tau* mRNA expression,[48] suggests that *tau* polymorphisms may increase susceptibility to cycasin-modulated *tau* expression. Also specific polymorphisms of the *CYP2D6* gene, occurring more frequently in the Chamorro population may increase susceptibility to locally prevalent environmental toxins.[29]
Detection of environmental risk factors involved in gene-environment interaction in ALS is difficult because ALS is genetically heterogeneous and a specific exposure may convey only a small increase in risk. Furthermore the pathogenic mechanism of mutated ALS genes may vary per gene or even per mutation, some acting through neurofilament accumulation others by an excitotoxic pathway. Each of the ALS genes or each mutation in those genes may respond to a different environmental trigger. Probably, the most feasible approach to study gene-environment interaction in ALS is to evaluate the additional effect of environmental factors on specific mutations in ALS genes (because variable expression in monogenic ALS may depend on exogenous exposure) or on allelic variance of susceptibility genes, as was previously done for Alzheimer's dementia.[271] Identification of gene-environment interaction is of great importance because identification of exogenous influences on the course of the disease will help to find ways to prevent disease in at risk individuals. Furthermore, it might shed light on the pathogenesis of ALS.

ALS: an unifying hypothesis?

Neuropathologically, the different types of familial and sporadic ALS are characterized by similar neurofilamentous neuronal inclusions. These findings suggest a common pathogenesis of motor neuron degeneration in all types of ALS. The major challenge of current research is to find out the role of mutations in the major ALS genes and of 'susceptibility' genes, in terms of pathogenic mechanism.
Many of the neurodegenerative diseases, including ALS, Alzheimer's dementia, Pick disease, Parkinson disease, prion diseases and trinucleotide repeat diseases are characterized by senile plaques, Lewy bodies, neurofibrillary tangles, glial cytoplasmatic inclusions, spheroids, etc. All these lesions consist of proteins such as β- amyloid, tau, neurofilament, α synuclein, prions or expanded polyglutamine-containing proteins. It is, therefore, possible that all these neurodegenerative diseases result from an abnormal interaction between proteins, that not only leads to a loss-of-function of those proteins, but more importantly to a gain-of-function e.g. toxic effect on neurons eventually resulting in neuronal death.[272] Novel therapeutic approaches aiming to prevent or reverse abnormal protein-protein interactions may show beneficial in the group of neurodegenerative disorders characterized by abnormal aggregation of proteins.

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CHAPTER 4

A shared genetic susceptibility for amyotrophic lateral sclerosis, dementia, and Parkinson's disease

Charcot faisant sa leçon.
Dessin à l'encre de Paul Richter. Extrait d'un cahier de note illustré pendant les leçons de Charcot à la Salpêtriè.
Provenance: La leçon de Charcot, voyage dans une toile. Exposition organisée au Musée de l'Asistance Publique de Paris 1986

CHAPTER 4.1

Familial aggregation of amyotrophic lateral sclerosis, dementia, and Parkinson's disease.

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Abstract

Clinicians have long suspected an association of classical amyotrophic lateral sclerosis (ALS) with Parkinson's disease (PD), dementia, or both. If proven, this would raise the possibility of a shared genetic susceptibility to the three disorders. To investigate this hypothesis, we compared 151 newly-diagnosed ALS patients (7 familial) with 140 controls, in terms of cumulative incidence of ALS, PD, and dementia in parents, siblings, and grandparents. We used Cox proportional hazards analysis to compute rate ratios (RRs) for ALS, dementia, and PD in relatives of ALS patients versus relatives of controls. The risk for dementia was significantly higher in relatives of ALS patients than in those of controls (RR=1.9, 95% CI 1.1-3.1), and was similar for relatives of patients with sporadic and familial ALS. The risk of PD was higher in relatives of patients with familial ALS (RR=5.6, 95% CI 0.6-50.3) than in relatives of patients with sporadic ALS (RR=1.8, 95% CI 0.5-6.0), but these differences were not statistically significant, probably due to insufficient statistical power with the available sample size. These findings indicate that ALS and dementia, and perhaps also PD, co-occur within families more often than expected by chance, suggesting there may be a shared genetic susceptibility to these disorders.

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive, incurable neurodegenerative disorder. Clinically, ALS is distinguished from two other forms of motor neuron disease, progressive spinal muscular atrophy and progressive bulbar palsy, by the presence of both upper and lower motor neuron signs. In some areas of the Pacific (Guam, the Kii Peninsula of Japan, and Western New Guinea), ALS occurs endemically; there, ALS has been associated with a parkinsonism-dementia complex. [1] In the United States and Europe, ALS is rare (incidence 2 to 7 per 100,000 per year). [2]

The pathogenesis of ALS is unclear. Like Alzheimer's disease (AD) [3-5] and Parkinson's disease (PD) [6, 7] ALS appears to be a complex disorder, in which the clinical manifestations used for diagnosis may be caused by more than one genetic defect, or may have other, nongenetic causes. Further, within some of the genetic forms, unidentified environmental exposures could be required for expression of the disease.

In 5 to 10% of cases, there is a family history of ALS, usually in a pattern consistent with autosomal dominant inheritance. In some, but not all, families with autosomal dominant ALS, there is close linkage with markers on the long arm of chromosome 21,[8] and associations have been found with several different defects in the superoxide dismutase (*SOD1*) gene on chromosome 21. [9]

ALS may be associated with clinical or pathological signs of AD or PD. All three are idiopathic degenerative diseases of aging, and all are characterized by the selective disappearance of specific sets of nerve cells followed by gliosis; hence, some have proposed a common etiopathology for the three disorders. [10-13] Some ALS patients also have signs of dementia, [14-24] parkinsonism, [25, 26] or both. [27, 28] In pedigrees with apparent autosomal dominant inheritance of ALS, dementia and ALS have co-occurred in the same individual [29, 30], or in different individuals in the family. [31-33] Similarly, there may be co-occurrence of ALS and parkinsonism, [34, 35] or of all three disorders. [36, 37]

We used epidemiologic methods to investigate the hypothesis of shared genetic susceptibility to ALS, PD, and dementia. We assessed familial aggregation of these three disorders by comparing ALS patients and controls in terms of cumulative incidence of ALS, dementia, and PD in their relatives.

Methods

We ascertained all clinically non-demented patients who were newly-diagnosed with ALS in 1989 to 1991 at the Neurological Institute, Columbia-Presbyterian Medical Center. We did not perform systematic neuropsychological testing on these patients, but none had symptoms of functional impairment suggestive of dementia. All ALS patients had electromyography (EMG) and were diagnosed by L.P.R. The clinical diagnosis of ALS

was restricted to patients with both upper (Babinski sign or clonus) and lower (weakness, wasting, fasciculation) motor neuron signs. We excluded patients with primary lateral sclerosis (N=5). [38]

For each ALS patient, we selected another patient admitted to the Department of Neurology for stroke or scheduled for EMG on the nearest day who matched the ALS case by age (±5 years), gender, and payment status (Medicaid or Medicare vesus private) to serve as a control. If the first selected control refused or was unable to participate, we selected the next patient who matched the case. This procedure was repeated until a suitable control was found. We excluded candidate control subjects if their discharge diagnoses indicated any kind of dementia, PD, hereditary neuropathy, spinal muscular atrophy, or motor neuron disease. The diagnoses of the controls included stroke (23%), radiculopathy or spinal cord compression (36%), peripheral (sensory or motor) neuropathy (23%), multiple sclerosis (4%), and myopathy (9%). In the remaining 5%, no neurological abnormality was detected.

To prevent bias in participation, we invited each subject to participate in a study of "the causes of neurological disorders", without emphasizing genetic factors. Subsequently, we administered a semistructured interview that asked for demographic and medical information on the patient and his/her siblings, parents and grandparents. Offspring were excluded from the study because we assumed they would be younger than the age periods at greatest risk for AD, PD, and ALS. D.M.-K. administered half of the interviews in person during the hospital admission or outpatient consultation, and half were administered by another interviewer by telephone within three weeks after discharge or outpatient consultation. There was no difference between the ALS patients and controls in terms of the proportion interviewed in person (ALS patients, 50%; controls, 40%). The interview included, for each relative, specific questions about diagnosed ALS, AD or any other kind of dementia, and PD and, independently of whether the relative was reported to be affected, additional information about decline in memory and functional ability, signs of PD (extrapyramidal signs including resting tremor, bradykinesia, postural instability, rigidity), and use of L-dopa or similar medication.

The following questions were used to ascertain decline of memory and function in each relative:

Did [relative] have any of the following problems for some time but not all of his/her life: (1)...trouble remembering things like a short shopping list, or things to do, or events that happened recently? (2)...trouble finding his/her way even in a familiar surrounding? (3)...needing help to dress or eat, not due to obvious physical disability? (4)...trouble remembering the exact date and/or year?

We classified relatives as affected with dementia if they were reported to have had AD or dementia, or had ≥ 2 affirmative answers to the four questions above. We did not attempt to differentiate among different types of dementia, because most affected relatives had died long before the introduction of clinical and pathologic criteria for subclassification

of dementia. [39] Eighty-two percent of relatives reported to have had AD also had ≥ 2 affirmative answers to the questions above. We classified relatives as affected with PD if they were reported to have had PD.

We also collected further data on other conditions in relatives, especially on syndromes presenting with dementia or parkinsonism (intracranial neoplasm, stroke, meningitis, alcoholism, multiple sclerosis, poliomyelitis, and arthritis). We classified relatives as unaffected if they were reported to have had a preexisting condition that might have led to dementia or parkinsonism.

We requested medical records and autopsy reports for 53 relatives reported to have had PD, dementia, or ≥ 2 signs of dementia. For the remaining relatives reported to have been affected with PD or dementia, data were insufficient to locate the medical record. Forty-three (81%) of the records requested were received, but only 23 of these (53%) contained useful information. (The remaining records contained information only on unrelated conditions.) All of the records with useful information confirmed the reported diagnoses. We did not request records for relatives reported to have had ALS because these family histories had already been well documented for the clinical records.

We used survival analysis methods to control for differences in the years-at-risk of each disorder between relatives of ALS patients and relatives of controls. This method is appropriate for genetic studies of disorders of late onset, where some family members are too young to express the disease and some have died of other causes before the age at risk. [40] We assumed that each relative was at risk of ALS, PD, or dementia from birth until current age (if alive and unaffected), age at death (if deceased and unaffected), or age at onset of symptoms. Then we used actuarial life table analysis [41] to calculate cumulative incidence of each disorder by age in relatives of ALS patients and relatives of controls, where cumulative incidence estimates the probability that a relative would be affected by the time he or she reached a specific age. We used Cox proportional hazards analysis [42] to calculate rate ratios (RRs) for each of the three disorders in relatives of ALS patients vs. relatives of controls. These RRs estimate the ratio of risk in relatives of ALS patients versus relatives of controls, assuming this ratio is constant with age. ALS patients who reported having one or more relatives (parent, sibling or grandparent) with ALS were classified as "familial ALS". Analyses of risk of each disorder were then stratified by relationship to the patient or control (sibling, parent, or grandparent), and by familial versus sporadic ALS.

Results

The participation rate was 96% for ALS patients and 89% for controls. The study population comprised 1,156 relatives (390 siblings, 302 parents, and 464 grandparents) of 151 ALS patients and 1,102 relatives (336 siblings, 280 parents, and 486 grandparents)

of 140 controls. Relatives with missing information on dementia were excluded from analyses of dementia (relatives of ALS patients: 5% of siblings, 9% of parents, and 68% of grandparents; relatives of controls: 2% of siblings, 7% of parents, and 59% of grandparents), and those with missing information on PD were excluded from the analyses of PD (relatives of ALS patients: 5% of siblings, 11% of parents, and 68% of grandparents; relatives of controls: 2% of siblings, 7% of parents, and 60% of grandparents). Analyses of dementia included the remaining 793 relatives of ALS patients and 790 relatives of controls, while analyses of PD included 787 relatives of ALS patients and 782 relatives of controls.

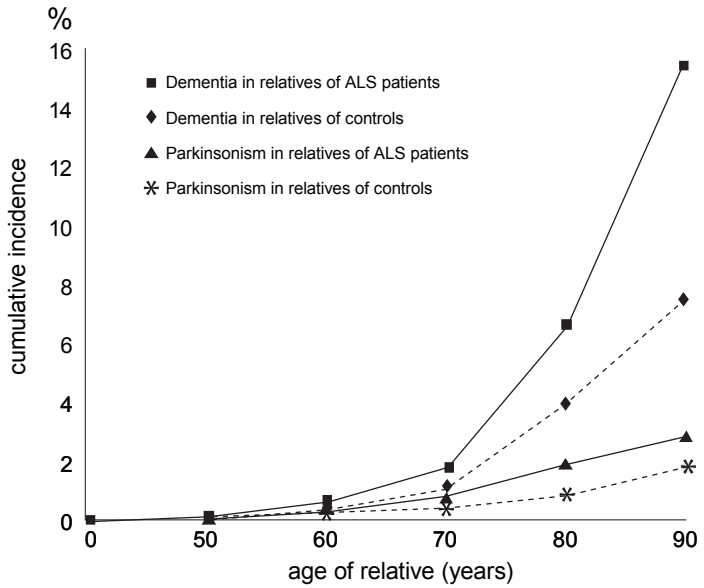

Figure. Cumulative incidence of reported dementia and PD in relatives (parents, siblings, and grandparents) of ALS patients and controls by age of relatives. Solid line, squares = dementia in relatives of ALS patients; dashed line, diamonds = dementia in relatives of controls; solid line, triangles = PD in relatives of ALS patients; dashed line, stars = PD in relatives of controls.

A family history of ALS was reported in 7 (4.6%) of the ALS patients and in none of the controls. Age at onset was similar in patients with familial ALS (mean ± SD:60 ± 17; range, 45 to 79 years) and sporadic ALS (mean ± SD: 62 ± 14; range, 23 to 88 years). For all cases, the male:female ratio was 3:2. In ALS patients with onset ≤ 50 years, there were equal numbers of men and women; in those with onset > 50 years, there were more men (male: female ratio, 2:1).

Dementia was reported twice as often in relatives of ALS patients (38/793) as in relatives

of controls (22/790). The cumulative incidence of dementia was significantly higher in relatives of ALS patients (risk to age 90: 15.4%, SE 2.9%) than in relatives of controls (risk to age 90: 7.5%, SE 2.1%) (figure) Among relatives of ALS patients, the risk of dementia to age 90 was 21.7% (SE 4.6%) in parents, 0.4% (SE 0.4%) in siblings, and 12.6% (SE 4.5%) in grandparents. Among relatives of controls, the risk of dementia to age 90 was 9.8% (SE 3.1%) in parents, 0.5% (SE 0.5%) in siblings, and 7.4% (SE 3.4%) in grandparents. The RR for dementia in relatives of cases compared with relatives of controls was 1.9 (95% CI 1.1-3.1), and was similar in relatives of familial and sporadic cases (table 1).

Table 1. **Cumulative incidence to age 90 and rate ratio (RR) for dementia in relatives (parents, siblings, grandparents) of sporadic and familial ALS patients and controls**

	No. of patients or controls	No. of relatives		Cumulative Incidence % (SE)	RR (95% CI)
		Total*	with dementia		
ALS patients					
Familial	7	37	2	10.5 (7.0)	1.6 (0.4-7.0)
Sporadic	144	756	36	15.7 (2.1)	1.9 (1.1-3.2)
Total	151	793	38	15.4 (2.9)	1.9 (1.1-3.1)
Controls	140	790	22	7.5 (2.1)	1.0 (reference)

*Relatives with missing information on dementia were excluded from the analyses

Table 2. **Cumulative incidence to age 90 and rate ratio (RR) for Parkinson's disease in relatives (parents, siblings, grandparents) of sporadic and familial ALS patients and controls**

	No. of patients or controls	No. of relatives		Cumulative Incidence % (SE)	RR (95% CI)
		Total*	with Parkinson's disease		
ALS patients					
Familial	7	37	1	3.9 (3.7)	5.6 (0.6-50.3)
Sporadic	144	750	7	2.7 (1.2)	1.8 (0.5-6.0)
Total	151	787	8	2.7 (1.2)	1.9 (0.6-6.4)
Controls	140	782	4	1.7 (1.0)	1.0 (reference)

*Relatives with missing information on PD were excluded from the analyses

The risk of PD was twice as high in relatives of ALS patients as in relatives of controls, but this difference was not statistically significant (figure and table 2). Among relatives of ALS

patients, the cumulative incidence of PD to age 90 was 3.0% (SE 1.4%) in parents, 1.7% (SE 1.7%) in siblings, and 3.1% (SE 2.4%) in grandparents. Among relatives of controls, the risk of PD to age 90 was 3.5% (SE 2.3%) in parents, zero in siblings, and 0.6% (SE 0.6%) in grandparents. The risk of PD was increased 5.6-fold in relatives of familial ALS patients, and 1.8-fold in relatives of sporadic ALS patients, but neither RR was statistically significant (table 2). The risk of PD was three times as high in relatives of familial versus sporadic ALS cases, but this difference was not statistically significant either (RR=3.1; 95% CI 0.4-24.9).

Discussion

Our finding of a two-fold increase in the risk of dementia in relatives of ALS patients compared with relatives of controls (table 1) implies that ALS and dementia co-occur in families more often than expected by chance. This familial aggregation of ALS and dementia is consistent with a previous report of an increased risk of ALS in relatives of dementia patients. [43] The 1.9-fold increased risk of PD in relatives of ALS patients compared with relatives of controls was not statistically significant. However, we cannot rule out a modest familial association of ALS and PD, because statistical power to detect the association with PD was insufficient with our available sample size.

Although previous reports have described patients and families with coexisting ALS, dementia, or PD, two previous epidemiologic studies failed to demonstrate significantly increased risk of neurodegenerative diseases in relatives of ALS patients. [44, 45] The findings of these two studies are difficult to interpret because of lack of specificity in the diseases examined in the relatives. The first study [44] compared ALS patients and controls in terms of their family histories in first-degree relatives of a diverse group of neurological disorders, and found an odds ratio of 2.2 that was not statistically significant. The second study [45] found no significant difference between ALS patients (12%) and controls (8%) in terms of the proportion of their first- and second-degree relatives over age 60 (parents, siblings, grandparents, aunts and uncles) affected with neurodegenerative diseases. It is unclear whether or not an association would have been observed if dementia and PD had been examined separately, and if the analysis had been restricted to first-degree relatives, in whom a shared genetic susceptibility would be expected to have the greatest effect (provided they are old enough to express late onset neurogenerative disorders).

Three aspects of our study design were intended to reduce bias. First, we avoided *selection bias* by describing our study objectives very generally when inviting subjects to participate, without mentioning genetic hypotheses. This should have eliminated the possibility of selective participation of ALS patients with a positive family history. Second, we attempted to reduce "family information bias" (ie, more complete recall of the family history by ALS patients than controls) [46] by choosing as controls patients who were hospitalized because

of severe neurological conditions, in whom the severity of their disorders might have evoked a level of concern comparable to that of the cases about the possible causes of their neurological problems. This should have minimized differences between cases and controls in recall of the family history, especially of neurological disorders. Third, to reduce reporting bias further, we used a very structured approach to obtaining the family history. Our family history questionnaire consisted of 30 questions on the family composition and an additional 17 questions for each relative separately. Except for two subquestions, these questionnaires were completely structured.

We also evaluated the possibility of differential recall of the family history, by comparing ALS patients and controls in terms of the proportion of their relatives reported to have conditions that were unlikely to be associated with ALS. We found no difference between relatives of ALS patients and relatives of controls in the prevalence of a history of cancer (13% versus 14%), multiple sclerosis (0.4% versus 0.5%), poliomyelitis (0.4% versus 0.4%), or stroke (6% versus 7%), suggesting that recall bias is unlikely to account for our findings.

We included grandparents in our study in order to increase the number of relatives who were old enough to be at risk of neurodegenerative disorders. (We excluded other second-degree relatives [aunts and uncles] because we found that few patients or controls were able to provide information about them.) For both ALS patients and controls, the validity of the family history data is expected to be inversely associated with distance in relationship, generally resulting in more underreporting in second-degree than in first-degree relatives. [47] Since approximately 20% of the relatives included in the analysis were grandparents, underreporting is likely. Underreporting may be a bigger problem for PD than for dementia, because there is probably less general awareness of parkinsonism; some extrapyramidal signs may go unnoticed. [48] This would be expected to be especially problematic in grandparents, who would have been diagnosed with PD between 1910 and 1930, when awareness of PD was even probably even lower than it is now. Underreporting may have lowered our estimates of cumulative incidence of dementia in relatives of ALS patients and controls and, if nondifferential between ALS patients and controls, may have biased our estimates of RR toward unity.

In addition, the effect of a shared genetic susceptibility is expected to be greater in first-degree relatives (such as parents and siblings), who share 50% of the proband's genes on average, than in second-degree relatives (such as grandparents), who share 25% of the proband's genes on average. The risk of dementia (cumulative incidence to age 90) was nearly twice as high in the parents of ALS patients as in their grandparents (21.7% versus 12.6%), whereas in controls the difference in risk of dementia between parents and grandparents was smaller (9.8% versus 7.4%). These results are compatible with the hypothesis of a shared genetic susceptibility to ALS and dementia.

We found very low risks of both dementia and PD in the siblings of ALS patients and controls. The average age of the siblings was only 60 years; thus, many very too young to

be affected by dementia or PD. These results are similar to those in the study of van Duijn et al., [49] where cumulative incidence of siblings of controls was 0.5% at age 65 (personal communication).

ALS is likely to be etiologically heterogeneous; the shared genetic susceptibility to ALS, dementia, and PD probably applies to only a subset of the families of patients with either familial or sporadic ALS. The modest RRs for dementia and PD that we observed may mask a greater increase in risk in this subset of families. As noted by Weiss et al., [50] the existence of nongenetic phenocopies (ie, clinical symptoms expressed in persons *without* the high risk genotype) and reduced penetrance (ie, clinical symptoms *not* expressed, or expressed to a lesser degree, in some persons *with* the high risk genotype) can lead to small relative risks in family studies even when genetic factors are important. These problems may be especially important for PD, where most cases are believed to be nongenetic. [51] Further, there is evidence for genetic heterogeneity in both AD [3-5] and PD. [6, 7]

There are several alternative explanations for disease familial aggregation: culturally inherited risk factors, common environmental exposures, and shared susceptibility genes. Khoury et al. [52] showed that the effect of a familial environmental exposure on disease risk would have to be very strong to produce familial aggregation of the disease, and no environmental exposures with such strong effects have been identified for the diseases we studied. Further, most of the ALS patients and controls in our study were either immigrants to the United States or offspring of immigrants; their lifestyles and histories of environmental exposure probably differed from those of their parents and grandparents. This suggests that genetic susceptibility probably played a more important role than environmental factors in familial aggregation of these diseases.

In some families a change in the activity of the *SOD1* gene on chromosome 21 is believed to play a role both AD and PD. [53, 54] Since different mutations in this gene are linked to ALS, [9] this raises the question of how the mutations affect neuronal degeneration, and which factors influence the preferential expression of the gene in a specific type of neurons. It also makes this gene a candidate for testing the mechanism of a shared genetic susceptibility to ALS, PD, and AD.

Familial co-occurrence of classical ALS and dementia (and possibly PD) may reflect pleiotropic expression of the same genetic predisposition, perhaps triggered by different environmental exposures. Families in which these disorders co-occur may form a distinct genetic subtype. It would be interesting to investigate whether, in some families with ALS and dementia or PD, all three disorders may be linked to the same genetic markers and, if so, to determine the factors responsible for variability in expression of clinical symptoms, whether genetic, environmental, or a combination. Our data suggest that further research into the common etiology of ALS, dementia, and PD may be fruitful, especially from a genetic point of view.

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CHAPTER 4.2

Increased co-occurrence of dementia and parkinsonism in the families of amyotrophic lateral sclerosis patients

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Abstract

Genetic causes for amyotrophic lateral sclerosis (ALS) include disorders with familial co-occurrence of ALS, dementia and parkinsonism. We investigated co-occurrence of dementia and parkinsonism in the families of 140 pairs of ALS patients and controls. Familial aggregation of dementia and parkinsonism was doubled in families of ALS patients (odds ratio 2.5; p=0.096). Dementia in families of sporadic ALS patients increased risk for parkinsonism, suggesting a familial association between the three disorders.

Introduction

Amyotrophic lateral sclerosis is a neurodegenerative disorder characterized by a progressive deterioration of motor neuron functions. Mendelian inherited ALS, observed in approximately 10% of the patients is clinically and neuropathologically indistinguishable from sporadic ALS. During the last decade various gene defects have been identified in hereditary forms of ALS. In ALS without a clear-cut inheritance pattern various 'susceptibility' genes have been identified. The hypothesis is that these 'susceptibility' genes have been reported which are thought to activate a cascade of neurodegenerative mechanisms by interaction with other low penetrant gene sequences or with environmental risk factors.[1]

Our understanding of the molecular pathogenesis of ALS is further complicated by the existence of genetic disorders with overlapping clinical and pathological features of ALS, dementia and parkinsonism. Examples are the tauopathies, frontotemporal dementia with parkinsonism and amyotrophy linked to chromosome 17 (FTDP-17) [2] and the Guamianan type of ALS. [3] Other examples of familial aggregation of ALS, dementia of the frontotemporal type are characterized by ubiquinated, tau-negative intraneuronal inclusions. [4-6] The indications that ALS and extrapyramidal features are part of a multi-

system disease process underlying motor neuron disease are accumulating. [7, 8]
In a previous study the occurrence of *either* dementia or parkinsonism was investigated in a large group of first- and second degree relatives of ALS patients and controls. The cumulative incidence of dementia to age 90 was two times higher in relatives of ALS patients than in relatives of controls. And the same was true for parkinsonism. [9] The current study aimed to determine whether occurrence of *both* dementia and parkinsonism is more frequent in the families of ALS patients**.**

Methods

A case control study using the family histories of ALS, dementia and parkinsonism was carried out in 1156 siblings, parents and grandparents of 151 newly diagnosed ALS patients and 140 age and gender matched controls with 1102 relatives. Sixty percent of the participants were men, with a mean age of 60 years (range 21-86). (table 1) ALS patients and controls had the same mean number of siblings. Ascertainment of patients and controls, and data collection were published earlier. [9] All newly diagnosed patients had the diagnosis definite or probable ALS according to the El Escorial criteria of both upper and lower motor neuron signs.

Table 1. **Characteristics of familial and sporadic amyotrophic lateral sclerosis (ALS) patients and controls.**

	Familial ALS	Sporadic ALS	Total ALS	Controls
Total	7	144	151	140
Men	4 (57%)	86 (60%)	91 (60%)	83 (60%)
Mean Age (range)	59 (43-77)	60 (21-86)	60 (21-86)	59 (24-87)
Mean no. of siblings (range)	3 (1-8)	3 (0-13)	3 (0-13)	3 (0-11)

To reduce bias in participation the study objective was indicated as addressing the “causes of neurological diseases” without emphasizing genetics. Information about symptoms of ALS, dementia and parkinsonism was sought for each parent, sibling, and grandparent individually using a semi-structured interview. About one third of the medical records of relatives who were reported to be affected could be obtained for confirmation of the diagnosis. For the remaining relatives reported to be affected no medical records were available for review. The familial association between dementia and parkinsonism was examined by calculating the odds ratios for a family history of parkinsonism in families with dementia. This analysis was carried out separately within the families of ALS patients and controls.

To compare the co-occurrence of dementia and parkinsonism in the family we used a matched-pairs univariate logistic regression analysis of 140 pairs of ALS cases and matched controls, each case-control pair forming a cluster.

Results

We evaluated associations between parkinsonism and dementia within the families of ALS patients and controls. To do this, we estimated the odds ratios for a family history of parkinsonism in subjects with vs. without a family history of dementia. (table 2) In five families of ALS patients and two families of controls dementia was reported in several relatives. Whereas multiple cases of parkinsonism occurred in two families of sporadic ALS patients, none of the families of the controls had more than one case of parkinsonism.

Table 2. **Familial aggregation of dementia and parkinsonism in families of ALS patients and controls**

	Number of Patients			
	Total	With a family history of parkinsonism	Odds Ratio (95% CI)	
Sporadic ALS Patients				
Family history of dementia	30	9 (30%)	11.8 (3.32-1.84)	p<0.001
No family history of dementia	114	4 (3.5%)	1.0 (reference)	
Controls				
Family history of dementia	20	4 (20%)	4.8 (1.21-8.68)	p=0.037
No family history of dementia	120	6 (5.0%)	1.0 (reference)	

Patients with sporadic ALS who had a family history of dementia were almost 12 times more likely to have a family history of parkinsonism than those who did not have a family history of dementia (odds ratio 11.8; p<0.001). There were too few patients with familial ALS (n=7) to evaluate co-occurrence of dementia and parkisnonism in the family. Parkinsonism was reported in one of three families with familial ALS and dementia (OR 1.5, 95% confidence interval 0.06-40.64). Controls with a family history of dementia were five times more likely to have a family history of parkinsonism than those without a family history of dementia (odds ratio 4.8; p=0.037). These data suggest a difference in familial aggregation of the diseases between 140 ALS patients and the matched control (odds ratio 2.5; 95% confidence interval 0.8-8.3; p=0.096), considering the sample size of the present study. (table 3)

Table 3. **Co-occurrence of dementia and parkinsonism in the families of 140 pairs of matched ALS patients and controls**

	Family history of dementia and parkinsonism	No family history of dementia or parkinsonism	Odds Ratio (95% CI)	
ALS patients*	10 (7%)	113	2.5 (0.8-8.3)	p=0.096
Controls	4 (3)	114		

*Sporadic and familial ALS

Discussion

Genetic analysis of patients with Mendelian inherited ALS has led so far to the identification of four mutant genes: *SOD1*, *alsin*, *senataxin* and *VAPB*. In addition linkage to loci on chromosome 16q12, 18q21, 20p13 and 20q13 has been found for different autosomal dominant forms, an autosomal recessive form on chromosome 15q12-21 of ALS, and linkage for hereditary ALS with frontal lobe dementia has been assigned to a locus on chromosome 9q21. In an autosomal dominant form of parkinsonism (PARK8), mutations in the *LRRK2* gene are associated with a wide spectrum of clinical features, including parkinonism with dementia or amyotrophy or both. [10]

In our present epidemiological study we found a familial association between ALS, dementia and parkinsonism in families with sporadic ALS. The significant, twelve-fold increase in risk for parkinsonism in families of ALS patients *with* dementia points to a common genetic susceptibility and variable expression in a specific group of ALS patients. It is unlikely that co-occurrence of dementia and parkinsonism in ALS patient is due to bias, since a positive family history for other neurological diseases (multiple sclerosis, poliomyelitis and stroke) in ALS patients was similar to that in controls, irrespective a family history of dementia or parkinsonism.

Intrafamilial variability is characteristic of hereditary disorders associated with dementia, parkinsonism and amyotrophy. Although the molecular pathogenesis is not yet fully understood, the familial aggregation of ALS, dementia and parkinsonism might be the result of an interaction of a common genetic defect with other, low penetrant genetic factors and/or environmental influences leading to variable neurodegenerative mechanisms.

On the other hand, since dementia and parkinsonism are highly prevalent in the elderly and the difference between the ALS patients and controls in familial co-occurrence of these disorders in this epidemiologic study was not statistically significant, we cannot rule out that our observation occurred by chance. The results of epidemiologic studies on the occurrence of dementia in relatives of patients with Parkinson's disease (PD) and that of PD

in relatives of patients with Alzheimer's disease are inconsistent. [11 12]
The evidence for overlapping molecular features between ALS, parkinsonism and dementia is growing. For instance, the presence of tau protein accumulation in FTDP-17 and Guamanian ALS or ubiquitin reactive, tau negative pathology in patients with overlapping features of ALS and frontotemporal dementia. [4-8] Frontal dysfunction was found in a significant proportion (50%) of ALS patients. [13]
Our data suggest the existence of gene sequences predisposing to age related multisystem neurodegeneration and thus warrants further research for specific genes in ALS with co-occurrence of dementia and parkinsonism. Exact clinical, genetic and neuropathological characterization is a prerequisite for a molecular identification to be meaningful.

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CHAPTER 5

Environmental risk factors for amyotrophic lateral sclerosis

Cycas circinales: developing female megasporophylls, first with ovules (above) and then with large seeds and new leaves (below), from Rheede's 1682 Hortus Indicus Malabaricus

CHAPTER 5.1
Amyotrophic lateral sclerosis on Guam

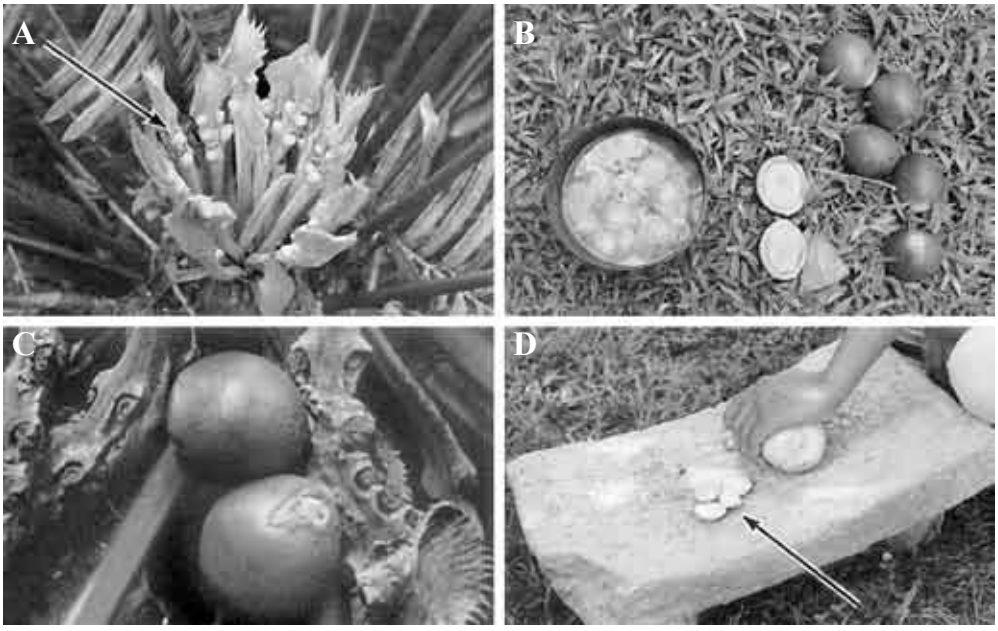
Toward the 1940s an extremely high prevalence of ALS in the Chamorro population of the western Pacific island Guam attracted attention and was associated with another neurodegenerative disorder, the parkinsonism-dementia complex (PDC). [1 2 3]
The co-occurrence of ALS and PDC in patients and families was observed in a few other areas in the Pacific as well. [4]
So far, no genetic causes for the ALS-PDC have been identified by analysis of the major ALS gene, copper –zinc superoxide dismutase (*SOD1*), or of apolipoprotein E and cytochrome debrisoquine hydroxylase genotypes. [5] Epidemiologic and genetic studies indicate that familial recurrence occurs more frequently (in 30-80%) in ALS/PDC compared to classical ALS, with a familial recurrence of 5-10%. [6-8]
The incidence rate of ALS worldwide is 2-7/100,000 persons per year. In the period between 1950 -1964 an exceptionally high incidence rate of 50-100 per 100,000 population per year was observed on Guam. On average the age at onset of ALS in the Chamorro population was 10 years earlier than was the case of classic ALS. [1] From the late 1960s on, the incidence of ALS declined steadily to 3-7/100,000 per year for both sexes. [9, 10] In the same time the incidence of PDC on Guam decreased from 55 to 25 per 100,000 for men, and 23 to 21 for women, persisting at about 18 per 100,000 per year. In the same period the age of onset of ALS and PDC increased to that elsewhere. [11]

Clinically the Guamanian type of ALS is indistinguishable from classic ALS. The neuropathology of ALS/PDC, however, differs from that in non-Chamorro cases of ALS. [12] The neuropathologic hallmark of ALS /PDC is abundant neurofibrillary tangles consisting of intracytoplasmic filamentous tau inclusions in widespread regions of the central nervous system, the brainstem and spinal cord. The neurofibrillary tangles exhibit very similar biochemical and ultrastructral properties of their phosphorylated tau protein, to those of Alzheimer's disease (AD), but the specific laminar and regional cortical distribution of the neurofibrillary tangles is different from that seen in AD. [13-15] In contrast to the frontotemporal dementia and parkinsonism complex (FTDP-17), a clinically similar tauopathy, where mutations in the *tau* gene generally result in overexpression of the four-repeat tau protein isoforms, mutations of the *tau* gene were not found in ALS/PDC. [16-18]
The endemic occurrence of ALS/PDC in Guam and its dramatic decline occurring within a couple of decades, cannot be explained by a change in genetic susceptibility because of the population composition.

Furthermore a high incidence of ALS/PDC in immigrants, from areas where risk for ALS is low, supports an environmental risk factor, possibly in interaction with a more prevalent genetic susceptiblility. [19-22]

Because the geographical incidence rates of ALS/PDC are correlated with the consumption of food containing the seed of the locally growing cycad palms, Cycas Circinalis, much effort was spend on studying the neurotoxic components of the sago fruit, to understand the underlying pathogenic mechanism. [23]

The traditional procedure used to prepare cycad seeds for consumption involves an extensive detoxifying soaking. The soaking diminishes the residue of toxic components like cycasin and the aminoacid BMMA [24] but not that of other, water insoluble toxins, like sterol beta-d-glucoside. The feeding of washed seeds containing this glucoside to mice inflicted neurologic deficits consistent with the clinical features of ALS/PDC as well as the formation of neurofibrillary tangles. [25, 26]



A. Immature seed (arrow) on seed-bearing megasporophylls of Cycas circinalis.
C. Two mature seeds of the type used to prepare flour.
B. Mature seed, split seed showing the starchy white endosperm and the latter removed and soaking in water to leach out water-soluble poisonous principle.
D. Traditional method of grinding the washed and sun-dried cycad seed endosperm (arrrow) to produce flower used in tortillas and other local foodstuffs.
From: Peter S. Spencer, Le Journal Canadien de Sciences Neurologiques, 1987;14:347-357
Guam ALS/Parkinsonism-Dementhia: A Long-Latency Neurotoxic Disorder Caused by "Slow Toxin(s)" in Food.

Also, recently additional insight in the mechanism behind exposure to toxic components of cycads on Guam was gained by the discovery that the Chamorro population of Guam probably ingested large quantities of cycad toxins indirectly by eating flying foxes. The indigenous flying foxes apparently fed on cycad seeds and were found to contain extraordinarily high concentrations of the toxic component BMMA of the cycad seeds. The decline in disease has been attributed to a change in consumption patterns. [27, 28]

Therefore the most important risk factors for ALS in Guam seem to be the predisposition in the Guamanian population to form neurofibrillary tangles and the ingestion of cycad. On one side experiments with transgenic mouse models suggest a dose-dependent association between overexpression of human tau protein in mice and an age-dependent phenotype similar to ALS/PDC. [29] On the other, there is evidence that at autopsy the characteristic neurofibrillary tangles occur in a large percentage of the adult, neurologically intact, native Guamanian population.[13, 30-32] The prevalence of the intraneuronal tau accumulations in the Chamorro population seems to remain high, despite the declining incidence of ALS/PDC. It seems that this tauopathy in the Chamorro population is insufficient by itself to cause symptoms and exposure to additional less prevalent risk factors, for instance the consumption of food containing neurotoxic components may trigger the neurodegenerative process.

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Preparing cycad flour and tortillas. From "Voyage autour du monde" by L. de Freycinet. in Stelle J.C. and Guzman T. Observations about amyotrophic lateral sclerosis and the parksinonism-dementia complex of Guam with regard to epidemiology and etiology. John C. Steele and Tomasa Guzman. The Canadian Journal of Neurological Sciences 1987;14(3 suppl) :358-362

A flying fox (Pteropus mariannus) eating the seeds of a cycad (Cycas rumpii) in Guam. Photograph by Merlin Tuttle, Bat Conservation International. From: Cox P.A. and Sacks O.W. Cycad neurotoxins, consumption of flying foxes, and ALS-PDC disease on Guam. Neurology, 2002;58:956-959.

A flying fox of the genus Pteropus prepared for consumption at a Chamoro feats in Guam. After boiling in coconut milk, the animal is consumed in its entirity. Photograph by Merlin Tuttle, Bat Conservation International. From: Cox P.A. and Sacks O.W. Cycad neurotoxins, consumption of flying foxes, and ALS-PDC disease on Guam. Neurology, 2002;58:956-959.

An advertisement for imported flying foxes in Guam. After the Guamanian species vanished, 18,000 were imported from Samoa during a 3-year period. Photograph by Merlin Tuttle, Bat Conservation International. From: Cox P.A. and Sacks O.W. Cycad neurotoxins, consumption of flying foxes, and ALS-PDC disease on Guam. Neurology, 2002;58:956-959.

CHAPTER 5.2

A link between amyotrophic lateral sclerosis and short residence on Guam

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Neurology in press

Introduction

Between 1940 and 1965 an unusually high incidence of amyotrophic lateral sclerosis (ALS), with co-occurrence in patients and families of a parkinsonism-dementia complex (PDC), was observed in the Chamorro population of Guam. So far, no genetic causes for ALS/PDC have been found. The increased risk of ALS in migrants to and from Guam suggests a combined effect of an environmental exposure and a genetic susceptibility. [1, 2]
The declining incidence of ALS on Guam has been attributed to changes in the consuming patterns of food containing the seed of the locally growing cycad palms. These seeds were either processed traditionally in cycad flour or ingested indirectly by the consumption of indigenous flying foxes. These foxes were foraging on the cycad seeds and are supposed to contain high concentrations of the neurotoxic components of the seeds. [3, 4]
We had information on residence on Guam in a previously conducted case-control study of ALS and we could therefore investigate the possibility that residence in Guam contributed to risk for ALS in citizens of the United States.

Methods

The study population consists of 140 pairs of ALS patients and neurologic controls, matched for age (± 5 years), gender (83 pairs of men and 57 pairs of women) and insurance status. Participants were patients newly diagnosed with ALS patients between 1989-1991

at the Neurological Institute Columbia-Presbyterian Medical Center in New York. [5] The family histories of ALS, dementia and parkinsonism, occupational and medical histories were ascertained in a semi-structured interview. Information on residence outside the continental United States for a period of at least one month (place, date and the duration of the stay) was recorded, without alluding to specific places or continents. Information on military service was obtained, including the date of conscription, a job description, whereabouts and serious health problems during military service. To investigate the association between Guam and ALS, a matched-pairs exact conditional logistic regression analysis was performed, each case-control pair forming a cluster. Multivariable analysis was performed to control for confounding.

Results and Discussion

The results show a significant association between short residence on Guam and ALS (OR 8.0, 95% CI 1.07-355). (Table) This association remained in multivariable analysis with age, gender, education, a family history of ALS, dementia or parkinsonism, enrolment and duration of military service, exposure to pesticides, and trauma (OR 9.40, 95% CI 1.08-81.91, p=0.04). The mean age at diagnosis of patients who were on Guam was not different (66 years, range 47-81) from patients who had not been on Guam (60 years, range 21-86)(p=0.6). ALS was not associated with residence in Central or South America, the Philippines, New Guinea or other areas in the South Pacific. Neither was ALS associated with military service. There was no difference in year of enrolment (OR 1.0, 95% CI 0.87-1.27) or the duration of military service (OR 1.0, 95% CI 0.93-1.23). None of the ALS patients were hospitalized, seriously injured or had a blood transfusion on Guam.Of the nine patients six were on Guam during their military service. One ALS patient was one month, seven were two months and one was fifteen months on Guam. The mean time between the end of the stay on Guam and the diagnosis of ALS was 43 years (range 27-57).
Before we can accept the association of short residence on Guam with ALS, methodological issues have to be addressed.The goal of the original study did not specify Guam as a putative risk factor. Therefore selection bias related to residence on Guam seems unlikely. For the same reason we did not collect further information e.g. on eating habits during the stay on Guam. It would have been intriguing to know if patients had traditional cuisine containing cycad seeds.
Because the Neurologic Institute was not known for an interest in the association between ALS and Guam it is unlikely that preferentially referral of patients who stayed on Guam biased the outcome of this study. However, given our study design we can not fully rule out selection or information bias have played a part.

Table. **Former residence on Pacific Islands among Amyotrophic Lateral Sclerosis (ALS) patients and controls.**

	ALS N=140	Controls N=140	Odds Ratio (95% CI)	p-value
Guam	9	1	8.0 (1.07-355)	0.04
New Guinea / South Pacific*	10	8	1.3 (0.26-7.47)	0.94
Military service:	54	53	0.8 (0.54-1.34)	0.49
Start* *Mean (range)	1942 (1939-1975)	1942 (1940-1970)	-	
Duration*** Mean (range) years	3 years (1-39)	4 years (1-18)	-	

Excluding Guam ** Forty (28%) ALS patients and thirty-three (23%) controls started military service before the end of World War II, twenty (14%) ALS patients and twenty (14%) controls after 1945 (p=0.85). ** *(p=0.53) Wilcoxon signed ranks test.

Despite the small sample size of this study we detected an association where two larger studies did not. The time from exposure to ascertainment in the two earlier studies was shorter, 16-25 years and 15-27 years, respectively, than in the present study (43 years, range 27-57). [6, 7] This could indicate that one of the key characteristics of the exposure occurring during the period of endemic ALS on Guam, is the long delay in clinical expression. In conclusion, our epidemiologic data bolster the vision that exposure to slow acting toxic agents are important in the pathogenesis of ALS, most likely in combination with a genetic predisposition.

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CHAPTER 5.3

Environmental risk factors and gene-environment interaction in amyotrophic lateral sclerosis

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Summary

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive loss of upper and lower motor neurons leading to severe disability and early death. The main etiologic hypothesis involves the interaction of specific environmental exposures with distinct genetic susceptibilities for the majority of ALS. The current case-control study of 140 pairs of ALS patients and controls, matched for age, gender and socioeconomic status, aims to re-investigate previously reported risk factors for ALS and to analyze the interactive effect of each of these proposed risks with a genetic predisposition to neurodegeneration, defined by a family history of amyotrophic lateral sclerosis, dementia or parkinsonism. We collected information on antecedent medical histories (specific infectious diseases, diabetes, autoimmune disorders, surgery and injuries), exposure to animals or to specific environmental toxins (pesticides, aluminum, lead and others), the occupational histories, lifelong smoking habits and the consumption of alcoholic beverages. In multivariable analysis ALS was associated with a history of tuberculosis (odds ratio 8.16, 95% CI 0.91-73,08, p=0.06), exposure to pesticides (odds ratio 2.21, 95% CI 0.92-5.36, p=0.08) and previous residence on Guam (odds ratio 9.5, 95% CI 1.08-81.91, p=0.04). Interactive effects of exposures with a family history of neurodegenerative disorders was not detected.

Introduction

Amyotrophic lateral sclerosis (ALS), or Lou Gehrig's disease, is characterized by a progressive loss of motor neurons in the cerebral cortex, brainstem, and the anterior horn cells of the spinal cord. In most patients onset occurs in middle life and death is attributed to respiratory failure 3-5 years after onset. Amyotrophic lateral sclerosis is predominantly (80%) a sporadic disorder. Established risk factors for ALS are age, male gender and a growing number of genetic defects involving different molecular pathways. The age at onset, the survival of ALS and the occasionally observed extrapyramidal pathology in some ALS patients, is variable both within families with specific genetic forms of ALS, and in patients with identical genetic defects from different families with ALS. [1, 2] These observations strongly suggest that the effect or expression of genetic defects can be modulated by epigenic or environmental factors.

Small clusters of ALS, related to shared professional or residential proximity, have been reported throughout the world. [3-7] Similarly, the observation of conjugal ALS strongly suggests etiologic factors in the environment or in lifestyles. [8, 9] The small number of affected individuals in these clusters complicates differentiating between a common exposure, transmission of a causal agent or pure coincidence. Nevertheless, the clusters keep the discussion alive about potential environmental causes for ALS.

Previous epidemiological studies investigated potential risk factors including environmental exposures related to residence, occupation or lifestyle, physical trauma, and medical histories. [10, 11] The current study was conducted to re-investigate alleged environmental risk factors for ALS and to analyze a possible interactive effect of each of these risk factors with a genetic predisposition to neurodegeneration.

Subjects and Methods

Study population

The study population consists of 140 pairs of ALS patients and controls, matched for age (± 5 years), gender (83 pairs of men and 57 pairs of women) and insurance status, as a proxy for socioeconomic status. Participants were newly- diagnosed patients with ALS, ascertained from the Neurological Institute, Columbia Univeristy Medical Center, New-York, in the years 1989-1991. The clinical diagnosis of ALS was restricted to patients with both upper (Babinski sign or clonus) and lower (weakness, wasting, fasciculation) motor neuron signs. The patients would therefore correspond to those with definite or probable ALS according to the El Escorial criteria (which were not introduced until 1994; after our patients had been selected).

For each ALS patient, a control was selected from patients admitted for stroke or scheduled for EMG on the nearest day to the ALS patient, who matched the ALS case

by age (±5 years), gender, and payment status (Medicaid or Medicare vs. private). If the first selected control refused or was unable to participate, we selected the next patient who matched the case. This procedure was repeated until a suitable control was found. We excluded candidate control subjects if their discharge diagnoses indicated any kind of dementia, parkinsonism, hereditary neuropathy, spinal muscular atrophy, or motor neuron disease. The diagnoses of the controls included: stroke (23%), radiculopathy or spinal cord compression (36%), peripheral (sensory or motor) neuropathy (23%), multiple sclerosis (4%), and myopathy (9%). In the remaining 5%, no neurological abnormality was detected.

Exposure assessment

Participants where invited to participate in an interview for a research study of neurological diseases. A semi-structured questionnaire was administered to ascertain detailed information on the family histories, and the medical, occupational, and residential histories. Questions were read to the participant and answers recorded by the interviewer on the standard interview form. Interviews were administered in person during the hospital admission or outpatient consultation (50%) or administered by telephone within three weeks after discharge or outpatient consultation. There was no difference between the ALS patients and controls in terms of the proportion interviewed in person (ALS patients 50%; controls 40%) or by telephone.

Family history

For each relative (sibling, parent and grandparent) a separate questionnaire was administered with questions in non-technical language addressing Alzheimer's disease, dementia or symptoms of irreversible cognitive decline in relatives. Similarly, the diagnosis of Parkinson disease or symptoms of parkinsonism and symptoms of ALS was sought for each sibling, parent and grandparent individually. Offspring were excluded from the study because we assumed that the offspring would not yet have reached the age periods at risk for ALS, dementia or parkinsonism. About one third of the medical records of relatives who were reported to be affected were obtained for confirmation of the diagnosis. The family history of ALS, dementia and parkinsonism was defined as positive if at least one relative was reported affected. The size of the family is determined by the number of siblings, and was similar for ALS patients and controls (mean number of siblings was 3). Familial ALS was reported in seven (5%) ALS patients; none of the controls reported a relative with ALS. In this study risk for ALS was significantly associated with a family history of neurodegenerative disorders (ALS, dementia and/or parksinonism).

Medical history

The interview included questions on the lifetime occurrence of the following medical conditions: diabetes, cancer, eczema, asthma, rheumatoid arthritis, ulcerative colitis and multiple sclerosis (table 1). We asked if the participants had suffered injuries such as fractures, muscle lacerations, burns or electrical shocks. Details were ascertained from the injuries and we subsequently classified the injuries according to the affected part of the body (head or spine, abdomen or chest and limbs). We recorded whether an injury necessitated medical attendance or hospitalization. We asked if trauma resulted in loss of consciousness and the number and duration of these events and the age at the time of each event was recorded. All surgical procedures or blood transfusions were recorded separately. The medical records were used to validate the medical histories.

Infections

To complete the medical histories questions were asked about the occurrence of specific infectious diseases (poliomyelitis, meningitis/encephalitis, mumps, shingles, herpes, tuberculosis, rubella, chicken pox, whooping cough and hepatitis) (table 2).

Occupational history

A lifetime occupational history was ascertained and for each occupation a job description, the period spent on each job were recorded separately (table 3). The occupations were then grouped into ten different categories: white collar (office workers, teachers, salespersons, clergymen, musicians, psychoanalyst); medical (physicians, nurses, dentists); food and meat handling (chefs, butchers); professions with high exposure to electromagnetic fields (electricians, powerplant operators, radiologists); jobs with exposure to chemical substances and solvents (dry cleaning, chemist, photographer, painter, tobacco industry, hairdressers); jobs requiring heavy physical work (construction workers, laborers, movers), mechanics, and jobs in textile industry. Furthermore, we asked whether the patient had ever worked with a jackhammer or any other kind of tool that uses compressed air. Information on military service was obtained, including the date of conscription, a job description, whereabouts and serious health problems during military service, as described in the previous section of this chapter. The interview also included questions about level of education. Physical activity in youth was assessed by participation in physical education classes.

Toxic exposures

We inquired about occupational or residential exposure to radioactivity, lead, mercury, selenium, copper, mercury, aluminum, manganese, nickel, plastic manufacturing, arsenic, leather, hides, pesticides, petroleum products, gas or oil (table 4).

Exposure to animals

The patients were asked if they had ever lived with a household pet or if they had ever worked with or close to farm animals or to other animals, and for how long (years) the exposure lasted. The animals were grouped into one category of domestic pets (dogs, cats, guinea pigs), one for birds or poultry, one for cow, horse, or sheep and a separate category was created for other household pets (fish, turtle, snake and lizards) that we will further refer to as 'fish' (table 5).

Lifestyle

Information was collected on lifelong smoking habits of cigarettes, cigars and pipe. For persons who reported smoking, the age at which they started smoking, the number of cigarettes a day smoked and the number of years they had been smoking was recorded. The average consumption per week of glasses of beer, wine or hard liquor was recorded. The patients were also asked whether they had used illicit drugs (marijuana, hashish,cocaine, heroin, morphine, LSD) more than 10 times during their entire life.
The interview included questions about level of education, rural residence and the nature of the farms (cattle breeding, dairy, poultry, agriculture). When cattle- breeding or dairy farming was reported, the information was registered as exposure to cattle (see above). Residence outside the United States for more than one month was recorded by country, duration (months) and date (year). The interview also addressed possible prenatal exposures, by requesting information on the health condition of both parents in the period around the birth of the participant, including information on smoking habit, alcohol use, and medical treatments.

Statistical analysis

We used univariable conditional logistic regression analysis to obtain odds ratios and their confidence intervals for each exposure. Although we had probed for information on the duration of the exposures, for many variables this information was missing for too many subjects to add any meaningful results. Nonparametric paired tests were used for

comparison of the exposure variables between cases and matched controls. The McNemars test was used for univariable analysis of categorical variables and the Wilcoxon signed rank test for univariable analysis of continuous variables. Variables missing data for greater than 10% of subjects, or with p-values greater than 0.3 in the univariable test were not included in further analysis. For each of the remaining variables we separately tested for interaction with a family history of neurodegenerative disease (ALS, dementia or parkinsonism).
For the remaining variables multiple conditional logistic regression analysis was performed with the case control pair as stratum and the exposures as independent variables. Family history of neurodegenerative disease was also included as a covariate. Further selection was made using backward stepwise methods, retaining variables with a p-value below 0.15 in the logistic regression model. Independent variables (exposures) that did not contribute to the variance were excluded from the analysis (and recalculation was performed).
Age and gender were included in the model as a continuous variable to control for any residual confounding not accounted for by the matching of cases and controls. Given the potential for confounding education was also included as a covariate in the regression analysis, although its inclusion had little effect on the relative risk estimates.

Results

Of the 140 matched pairs 84 (60%) were male. Familial recurrence of ALS was reported in seven ALS patients (5%) and not in controls. The mean age of men in this study was 60 years (range 22-82) and of women 62 years (range 24-88). In familial ALS the mean age of men was 55 years (range 44-74) and of women 64 years (range 53-78). A family history of ALS, dementia or parkinsonism was a significant risk factor for ALS (odds ratio 2.06, 95% confidence interval 1.12-3.83), independent of the age of the subject (p for interaction 0.78).

Medical history

From the medical conditions only diabetes was reported differently by ALS patients and controls. Diabetes was associated inversely with ALS. This association was not apparent when controls who were diagnosed with stroke were excluded from the analysis. (table 1)
No evidence for an association with autoimmune disorder or malignancies was found. ALS patients reported more injuries, in particular injuries of the head and spine and fractures of other parts of the body that required hospitalization.

Table 1. **Number of ALS cases and controls, and univariable odds ratios for medical histories**

	ALS	Controls	Odds ratio	CI (95%)	
Diabetes	10/140	22/140	0.36	0.15	0.87
Diabetes *	10/139	17/107	0.43	0.16	1.11
Rheumatoid arthritis	24/140	19/140	1.38	0.67	2.82
Eczema	20/140	20/140	1.0	0.51	1.05
Asthma	39/140	38/140	1.03	0.62	172
Ulcerative colitis	8/140	8/140	1.0	0.37	2.66
Carcinoma	21/140	17/140	1.33	0.63	2.81
Multiple sclerosis	1/140	5/140	0.20	0.02	1.71
Surgery	113/140	117/140	0.78	0.40	1.55
Blood transfusions	22/140	25/140	0.9	0.47	1.70
Injuries:	78/140	69/140	1.34	0.81	2.23
medical treatment	64/140	59/140	1.01	0.78	1.32
hospitalization	32/140	24/140	0.82	0.54	1.24
unconsciousness	7/140	7/140	1.0	0.35	2.85
head or spine	20/140	14/140	1.60	0.72	3.52
chest or abdomen	2/140	2/140	1.0	0.14	7.09
limb	73/140	65/140	1.27	0.78	2.07
muscle	28/140	27/140	1.05	0.55	2.01
fracture	50/140	39/140	1.16	0.83	1.60
burn	2/140	2/140	1.0	0.14	7.09
electrical shock	1/140	2/140	0.5	0.04	5.51

* excluding controls with stroke from the analysis

Infections

A history of tuberculosis was associated with an increase in risk for ALS (table 2). In multivariable analysis the association with tuberculosis remained. None of the patients or controls with a history of tuberculosis reported living or working on a dairy or cattlebreeding farm. A history of poliomyelitis was more frequent in ALS patients, but was not associated with ALS in the multivariable analysis. Neither did a history of any viral infection affect risk for ALS (odds ratio 0.61, 95% CI 0.31-1.23).

Table 2. **Number of ALS cases and controls, and univariable odds ratios for specific infectious diseases**

	ALS	Controls	Odds ratio	CI (95%)	
Bacterial					
Tuberculosis	7/138	1/139	7.00	0.86	56.89
Whooping cough	31/116	40/123	0.71	0.37	1.39
Viral					
Poliomyelitis	6/140	3/138	2.00	0.50	8.00
Shingles	12/140	15/138	0.77	0.34	1.75
Hepatitis	4/135	8/136	0.50	0.15	1.66
Rubella	43/116	41/125	1.53	0.80	2.94
Measles	71/113	78/126	0.95	0.51	1.78
Chicken pox	85/121	97/131	0.83	0.46	1.51

Table 3. **Number of ALS cases and controls, and univariable odds ratios for occupational histories**

	ALS	Controls	Odds ratio	CI (95%)	
White collar	114/140	92/140	3.20	1.57	6.50
Medical	7/140	12/140	0.50	0.17	1.46
Food handling	9/140	5/140	1.80	0.60	5.37
Electricity	3/140	7/140	0.42	0.11	1.65
Electromagnetic fields	4/140	7/140	0.57	0.17	1.95
Chemical	14/140	13/140	1.08	0.49	2.37
Textile	6/140	4/140	1.50	0.42	5.31
Mechanic	14/140	15/140	0.92	0.43	1.97
Plastic	3/140	3/140	1.0	0.20	4.95
Radioactivity	1/140	0/140			
Physical labor	21/140	25/140	0.78	0.40	1.55
Jackhammer	15/140	8/140	2.0	0.80	4.95
Farmer	31/140	32/140	0.84	0.56	1.70
Cattlebreeding	3/140	2/140	1.50	0.25	8.97
Dairy	8/140	9/140	0.87	0.31	2.41
Poultry	2/140	0	65.28	0.001	5658
Agriculture	8/140	11/140	0.72	0.29	1.80

Occupational history

We found an excess of ALS with occupations that were classified as 'white collar'. The professions that involve physical labor was less frequent in ALS patients. (table 3) None of the other occupations were associated with ALS; including professional exposure to electromagnetic field (to exposure to electricity or radioactivity and reported electrical shocks), or professions with exposures to chemicals and solvents, professions handling leather or hides or the processing of petroleum-, gas- or oil products. Farming was reported similarly in ALS patients and controls.

Toxic exposures

The use of pesticides was significantly associated with ALS. (table 4) The association with pesticides remained in the multivariable analysis. (table 7) Exposure to pesticides was not associated with agricultural farming (p=0.82) nor with exposure to horses, cow or sheep (p=0.18); exposure to pesticides was reported in 3/8 ALS patients involved in agriculture (Fisher exact p=0.21) and in 2/11 controls involved in agriculture (Fisher exact p=0.42). Exposure to pesticides was reported in 5/21 ALS patients with exposure to horses, cow or sheep (Fisher exact p=0.34) and 4/16 controls (Fisher exact p=0.02). Reported exposure to lead, copper, mercury, aluminum, manganese, nickel, magnesium, petroleum products, gas or oil and heavy metals were not associated with an increase in risk for ALS. (table 4)

Table 4. **Number of ALS cases and controls, and univariable odds ratios for toxic exposures**

	ALS	Controls	Odds ratio	CI (95%)	
Pesticides	23/139	11/137	2.33	1.06	5.09
Lead	20/135	18/136	1.18	0.61	2.30
Copper	17/137	17/137	1.0	0.47	2.09
Mercury	10/138	6/135	1.66	0.60	4.58
Heavy Metals	16/139	11/137	1.50	0.67	3.33
Aluminum	13/139	12/138	1.09	0.48	2.47
Manganese	8/139	7/138	1.14	0.41	3.15
Nickel	8/138	5/137	1.60	0.52	4.89
Magnesium	8/138	7/138	1.14	0.41	3.15
Arsenic	2/139	6/138	0.33	0.67	1.65
Leather/hides	4/140	4/139	1.0	0.25	3.99
Petroleum/gas/oil	15/139	12/138	1.3	0.57	2.96

Exposure to animals

The bivariate analyses of contacts at home or at work, including living on a farm, with horses, cow or sheep showed a significant association. (table 5) The association with exposure to horse, cow or sheep did not remain in the multivariable analysis.(table 7) There was no report of tuberculosis in any of the persons exposed to cattle (including the subjects who lived or worked on dairy or cattle breeding farms). The risk for ALS was not increased by exposure to common household pets, birds and/or poultry, or other pets (turtles, fish, snakes). (table 5)

Table 5. **Number of ALS cases and controls, and univariable odds ratios for exposure to animals**

	ALS	Controls	Odds ratio	CI (95%)	
Horses, cow or sheep	16/140	7/140	2.80	1.01	7.77
Birds	19/140	17/140	1.18	0.53	2.64
Fish	9/140	6/140	1.50	0.53	4.21
Pets	103/140	106/140	0.89	0.52	1.53

Lifestyle

Neither smoking nor alcohol consumption was associated with ALS. (table 6) The data concerning the health and medication of the parents, and a job-description of the parents during the pregnancy were insufficient for analysis. The results of the analysis of stay outside the US for a period of more than six months, the information on having been in military service and in particular the significant association with previous residence on Guam were presented in a separate report.

Table 6. **Number of ALS cases and controls, and univariable odds ratios for lifestyle**

	ALS	Controls	Odds ratio	CI (95%)	
Smoking*	92/140	89/140	1.13	0.64	2.01
Alcohol consumption (mean number of glasses a day)**	0.9 (0-15)	1.1 (0-14)	1.01	0.97	1.03
Soft drugs (marijuana, hashish)	6/140	10/140	0.5	0.15	1.66
Hard drugs+ (cocaine, heroin, LSD)	3/140	2/140	2.0	0.18	22.05

*ever smoked; ** beer, wine or hard liquor; + used hard drugs more than 10 times.

Interaction with family history of ALS, dementia or parkinsonism

We found a significant interaction between a family history of neurodegenerative disease and the exposure to copper (p=0.08), living on a dairy farm (p=0.07) and physical education in youth (p=0.04).

Table 7. **Multivariable Model.***

	Odds Ratio	CI (95%)		p-value
Tuberculosis	8.16	0.91	73.08	0.06
Pesticides	2.21	0.91	5.36	0.08
Jackhammer	2.23	0.84	5.92	0.11
Guam	9.40	1.08	81.91	0.04

* Adjusted for age, gender, education, family history of ALS, dementia or parkinsonism, exposure to cattle (horse, cow or sheep), dairy or agricultural farming, a history of fractures, injury to head or spine with or without unconsciousness, and physical labor.

Discussion

This case-control study was an exploratory approach to confirm previously reported risk factors for ALS. Our study supports the hypothesis that exposure to pesticides, a history of tuberculosis, previous residence on Guam and a family history of ALS, dementia or parkisnonism are associated with ALS.

Before we can accept these findings the following methodologic comments have to be made. Three aspects of our study design were intended to reduce bias. First, we avoided ascertainment or selection bias by describing our study objectives very generally when inviting subjects to participate. This should have reduced the possibility of selective participation of ALS with specific antecedent medical conditions or other presumed risk factors. Second we attempted to avoid a general problem of the case- control study, the recall or information bias, by selecting controls who were hospitalized because of severe neurologic illnesses, in whom the severity of their disorder might have evoked a level of concern comparable to that of the ALS patients about the possible causes of their neurologic problems. This should have minimized the differences between patients and controls in recall of antecedent events. Third, to reduce reporting bias further we use a very structured approach to obtain information on the exposures. The association of specific exposures with ALS depends on the method of exposure assessment. We have attempted to reduce information bias of occupational histories by using job titles and job descriptions to characterize occupations at increased risk for specific exposures. However

these procedures cannot prevent potential misclassification of true exposure. In absence of direct measurements of exposure in this study, self- reported information on exposures might have lead to misclassification bias. The absence of strong associations with ALS for instance with exposure to lead or aluminum, for which well known hypothesis exist, gives some assurance that over-reporting by cases was not a major source of bias in exposure assessment. Furthermore, considering a broad range of different risk factors in a multivariable analysis decreased risk for confounding in this study.

We did not find interactions with a family history of ALS, dementia and parkinsonism because the statistical power was probably diminished with the available sample size.

Our data suggest an association with a history of tuberculosis. Few preliminary data exist on the association of tuberculosis and ALS. Some studies suggested that ALS is associated with an increased milk ingestion, especially in the youth. [12, 13] In our study living on a dairy or cattle farm was not associated with the transmission of tuberculosis.

Several explanations could be considered for the association with tuberculosis: a long-term neurotoxic effect of a tuberculosis infection or, a neurotoxic effect of the antibacterial medication, a tuberculosis infection mediated immune respons involving antibodies against motorneurons, or a common (immunologic) increased susceptibility for tuberculosis and ALS.

This study confirmed an association with exposure to pesticides. [14, 15] This association was not explained by more ALS patients than controls involved in agricultural farming, or exposed to cattle, horses or sheep. The association of pesticides, agricultural chemical or insecticides and ALS has been investigated in several studies, some showing a modest association with ALS. [16-18] In addition, a cohort study found an increase of ALS in workers in a chemical industry involving exposure to pesticides. [19-20]

The association of occupational pesticide exposure with Parkinson disease suggests a neurodegenerative effect of pesticides, particularly in combination with a genetic predisposition to neurodegeneration. [21, 22, 23] Our results are not consistent with the idea that motor neuron disease is a delayed sequel to infection with poliomyelitis or other infections. [24-27] Similarly, reports of ALS after a poliomyelitis infection are not supported by epidemiological studies, [28-30] although one study reported that DNA polymorphisms of the poliovirus receptor in cell cultures allowed persistent viral infection, and in humans the same polymorphism were associated with persistent poliomyelitis infection in ALS and progressive spinal atrophy. [31]

Our dat do not support that cattle farmers and sheperds have a higher risk for ALS.[32]

Since the legendary baseball player Lou Gehrig ALS was diagnosed with ALS, sport-related vigorous premorbid physical activity has been hypothesized, but has never been proven, to be a risk factor for ALS. [33] In this study risk of ALS was not affected by participation in sports at school. On the other hand, many types of sports predispose to injuries and fractures and many studies found that these types of injuries might play a role.

[33-36] An increase of dementia in people suffering repeated head injuries suggested a dose dependent relation in neurologic trauma and neurodegeneration. [37] We could not detect a relation with injuries, although there was a discrete association with injuries particularly to head and spine, and in the number of fractures before the onset of neurologic symptoms in ALS patients. However, our data do not support the hypothesized association of working with a jackhammer or any other kind of tool that use compressed air. [7, 12] Our data also do not support an association with smoking. [38-40]

We found a protective effect of diabetes mellitus for ALS in the current study, partly because we included stroke patients as controls, for which diabetes is an acknowledged risk factor. When the stroke patients were excluded from the control group the protective effect of became smaller. On the other hand, the protective effect could correlate with a loss of the neuroprotective vascular endothelial growth factor protein (VEGF). [25-27, 41] Loss of these neuroprotective properties of the VEGF protein are associated with ALS, [42, 43] and in patients with diabetes mellitus the over-expression of the VEGF gene in the neuronal cell bodies and axons possibly enhance the normal neuroprotective functions of this protein.

The overrepresentation of 'white collar' professions in ALS patients in this study probably reflects a bias in ascertainment of ALS patients with easier access and particular knowledge of the ALS referral center at the Neurological Institute.

This referral bias makes it hard to draw conclusions about other occupational risk for ALS in this study.

Genetic causes play a major role in the etiology of ALS. During the last decade various gene defects have been identified in mendelian inherited ALS. Although the exact pathologic mechanism of these genetic defects are not yet fully understood, similar disruptions of various biochemical pathways, may play a role in sporadic ALS as well. Furthermore, various 'susceptibility' genes have been reported in sporadic ALS. [44] The hypothesis is that these 'susceptibility' genes may act by interaction with other low penetrant gene sequences or with environmental risk factors.

Addressing the genetic complexity of sporadic ALS in search of gene-environment interaction poses a major challenge. It is tempting to believe that large studies collecting data on genetic variation and exposure of potential environmental risks will eventually permit proper analysis of gene-environment interaction in ALS.

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CHAPTER 6

General discussion

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Introduction

During the last decade, genetic approaches to the study of ALS have resulted in new insights in the etiology of neurodegeneration. In a small fraction of patients with this most frequent paralytic disease in adults, defects in genes were identified, which cause intracellular protein accumulation, mitochondrial dysfunction, enhance excitotoxicity, or decrease axonal transport in motor neurons. [1]

However the loss of motor neurons in the most common sporadic form of ALS is still largely unexplained. The etiology is likely to be multifactorial and, in particular, to involve gene-environment interactions. For this reason, the identification of genes that predispose to the development of ALS could help to understand possible toxic events that may lead to the loss of motor neurons. Conversely, studies of the mechanisms of action of environmental factors might help to identify specific genes that modulate neurodegenerative processes.

The same holds for the other two major neurodegenerative disorders dementia and parkinsonism, which generally occur late in life. The identified genetic defects in each of these disorders account for only a small proportion of all cases. The etiology for the majority of sporadic cases probably involves multiple genes, which are common in the general population but have a relatively weak effect on their own, and are likely to interact with each other and with non-genetic factors to modulate susceptibility to neurodegeneration.

During the last three decades a considerable increase has been observed of neurodegenerative disorders like ALS, dementia and parkinsonism. This is paradoxical, because populations have overall become healthier and thus more individuals are living to the ages at which these diseases are clinically expressed.

Taken together, further clues about genetic susceptibility and environmental triggers for neurodegeneration are urgently needed and may have far- reaching implications, including the development of preventive strategies and policies that could identify individuals at risk and limit exposure to harmful agents.

In this thesis we have followed the leads provided initially by the observations on the pacific island Guam and occasional case reports from elsewhere of familial co-occurrence of ALS with dementia and parkinsonism.

Using an epidemiologic approach we have tested the hypothesis that in some families these three disorders occur more frequently than expected by chance. Apparently, in these families the genetic susceptibility to neurodegeneration is associated with a broad clinical phenotype of ALS, dementia and parkinsonism.
Subsequently, we explored the possibility of associations of putative environmental risk factors with ALS, and the possibility of an interactive effect with a family history suggestive for a genetic susceptibility to neurodegeneration i.e. a family history of ALS, dementia or parkinsonism.
In this chapter we will discuss methodologic issues concerning the design of this study and the analysis of the data. Furthermore, we discuss the main findings in the light of the current knowledge and ongoing research in the field of adult onset neurodegenerative disorders. We end with our expectations for future research and practical suggestions for attending patients with a genetic susceptibility to the late-onset neurodegenerative disorder ALS.

Methology

Two elements, the validity and the precision of the study design, will be discussed here.

Validity

Selection bias

We avoided ascertainment or selection bias by describing our study objectives very generally when inviting subjects to participate. This should have reduced the possibility of selective participation of ALS with specific antecedent medical conditions or other presumed risk factors.
The Neurological Institute of Columbia Univeristy Medical Center is known for an interest in the research for ALS. For that reason we cannot exclude that patients who were better informed about this were more likely to be referred. Because the Neurologic Institute was not known for research on specific environmental exposures, is seems unlikely that this Institute may have attracted disproportionately more ALS patients with specific exposures, such as those who lived in Guam.
The participation rate was 96% for ALS patients and 89% for controls. This argues against strong selection related to non-participation.
The validity of the data also depends on the recall of the requested information and on the willingness of the participant to share this information. To diminish the possibility of information bias, we selected controls that were hospitalized or seen as outpatients for severe neurologic conditions, because we expected that the severity of their disorder might have evoked a level of concern comparable to that of the ALS patients about the possible

causes of their neurologic problems. Nevertheless, since ALS is more severe than most of the diseases in the controls, some recall bias cannot be excluded. The diagnoses of the controls included stroke (23%), radiculopathy or spinal cord compression (36%), peripheral (sensory or motor) neuropathy (23%), multiple sclerosis (4%), and myopathy (9%);in the remaining 5%, no neurological abnormality was detected.

The selection of controls should have minimized the differences between patients and controls in recall of antecedent events and reduce 'family information bias' (ie, more complete recall of the family history by ALS patients than by controls).

For family history data the ascertainment of information on relatives depends on the knowledge of the participant regarding the health status of relatives. The validity of the information will thus not be equally reliable for all relatives. To determine the validity of the family data, when possible, medical record of relatives should be reviewed. Some investigators choose to interview more than one relative, to enhance the validity of the family data.

Generally, the sources of misclassification are difficult to avoid in these types of studies, especially when the research concerns fatal adult onset disorders, for whom most affected relatives are deceased. Underreporting and under-evaluation are bigger concerns than overreporting in family history studies. The validity of family history data is expected to be inversely associated with distance in relationship, generally resulting in more underreporting and under-evaluation of second-degree than in first-degree relatives. [2]

To reduce reporting bias of the family history we used a very structured approach to obtain the family histories. Our family history questionnaire consisted of 30 questions about the family composition and an additional 17 questions for each relative separately (see Appendix).

We also evaluated the possibility of differential recall of the family history by comparing ALS patients and controls in terms of the proportion of their relatives reported to have conditions that were unlikely to be associated with ALS.

To reduce information bias further we use a very structured approach to obtain information on the exposures. The association of specific exposures with ALS depends on the method of exposure assessment. We attempted to reduce information bias of occupational histories by using job titles and job descriptions to characterize occupations at increased risk for specific exposures. However these procedures cannot prevent potential misclassification of true exposure. In absence of direct measurements of exposure self- reported exposure information may lead to misclassification bias. However, we found it reassuring that cases and controls did not differ in self-reported exposure to lead or aluminum, two exposures whose possible relation to ALS has been widely publicized. This provides some some assurance that overreporting by cases was not a major source of bias in exposure assessments.

Confounding

The study was designed to control for the possible confounding effects of age, gender and socioeconomic status. In the analysis we have further controlled for confounding by the use of a logistic conditional regression model.

Precision

The precision of the study depends on its statistical power and thereby on the sample size. In this study the precision was qualified with 95% confidence intervals.
Although this was one of the largest case-control studies of ALS, our sample size was limited. As a general rule we had sufficient sample size for the analysis of individual determinants (perhaps not for some of the determinants that were less frequent, i.e parkinsonism). The sample size was not sufficient to study the interaction of individual determinants with a family history of ALS, dementia or parksinonism, i.e. to study gene-environment interaction.

Genetic associations between amyotrophic lateral sclerosis, dementia and parkinsonism

Many neurodegenerative disorders, including ALS, Parkinson's disease, Alzheimer's disease, frontotemporal and Lewy body dementia are characterized by neuronal damage that may be caused by toxic or structurally abnormal, aggregation–prone proteins. These disorders are clinically separate disorders, yet they seem to share common neuropathologic features, which eventually may cause overlapping clinical symptoms. [3]
They are all characterized by the accumulation of proteinaceous inclusions in neuronal cells and the loss of neurons. When misfolded proteins, including tau, beta amyloid, or α-synuclein accumulate in sufficient quantity, they are prone to aggregation. Insoluble aggregates of disease-related proteins may be deposited in microscopically visible inclusions or plaques, the characteristics of which are often disease specific. These include ubiquitin positive intracellular inclusions in ALS and in some forms of frontotemporal dementia, tau containing intracellular neurofibrillar tangles in other forms of frontotemporal dementia and in Guamanian ALS, beta-amyloid containing extracellular plaques and tau containing intracellular neurofibrillary tangles in Alzheimer's disease, α-synuclein containing Lewy bodies in parkinsonims and Lewy Body dementia, and the intranuclair ubiquitin positive inclusions in Kennedy's disease (spino-bulbar muscular atrophy). [4, 5, 6]
The explanation for the formation of the pathological hallmarks of the disease and the selective vulnerability of specific groups of neurons to cell death is difficult. Different

regions of the brain may significantly vary in susceptibility to specific genetic defects, in expression of specific proteins and in interactions with environmental factors.
The recent cloning and subsequent functional analysis of several genes involved has begun to reveal the molecular pathogenesis of some of these disorders, and will eventually yield an answer for the controversial issue whether inclusion bodies are pathogenic, incidental or a beneficial coping response of the affected cells. [7]
Clearly, the genetic classification of disorders bearing overlapping features of ALS, dementia and parkinsonism will also be helpful in gaining insight in the pathways leading to neurodegeneration.
The number of genes implicated in the pathogenesis of ALS is constantly increasing, and now also includes genetic defects that are predominantly associated with clinical signs of only upper or only lower motor signs. Since motor neurons are amongst the longest cells in the body they require highly specialized adaptations for protein trafficking. Genetic vulnerability to defects in the processing or the axonal transport of proteins was identified in a number of genetic diseases of the motor neurons with overlapping features of ALS, hereditary spastic paraparesis, spinal muscular atrophies or peripheral motor neuronopathies. [8 9 10 11 12 13 14 15]Several highly penetrant genes have been identified for rare mendelian inherited forms of the major neurodegenerative disorders, including some disorders with overlapping features (see table).

The identification of genes increasing susceptibility to neurodegeneration, which are more and more associated with the majority of sporadic forms of ALS, dementia or parkinsonism is very difficult. The same is true for the estimation of the magnitude of their effect and their relationship to environmental exposures, principally for two reasons.
First, neurodegeneration is a heterogeneous concept, perhaps more so than any other multifactorial condition. Neurodegeneration occurs in various clinical syndromes with a varying decline of cognitive, motor and coordination skills of the neural system. Thus the exact delineation of the clinical phenotype, historically regarded as a fundamental prerequisite for any genetic analysis, can be clinically difficult.
Second, neurodegeneration is most often age-related and a rarely has sufficient affected relatives for genetic analysis let alone a large pedigree.
This picture has led investigators to change from classic linkage analysis to alternative strategies, usually association studies with polymorphisms in candidate genes and comparison of their frequencies between unrelated cases and controls.
Greater success has been achieved with the mendelian inherited conditions of ALS, dementia, and parkinsonism. Although these studies have helped our understanding of the pathophysiology, their public health implications have as yet remained limited.
We find ourselves in a curious position, because we are searching for one gene or a few that play a major role in at least three distinct pathological processes. Perhaps the answer

lies with a more fundamental understanding of polygenic disorders. We are dealing with probabilities not certainties. Carriage of an at risk haplotype does not guarantee disease. Maybe genetic predisposition to a neurodegenerative disease ought to be seen in terms of molecular processes rather than of specific clinical diagnoses. Identifying a susceptibility gene for a clinical syndrome should not be so dogmatically compartimentalised but must be interpreted in view of the pathophysiology.

The work presented in this thesis seems to support this view.
Using a genetic epidemiologic strategy we found evidence for a shared genetic susceptibility for degeneration of the motor system (ALS), the cortical areas with cognitive functions (dementia) and the subcortical nuclei involved in motor control (parkinsonism). In the families where we observed familial co-occurrence of ALS, dementia or parkinsonism, these diseases occurred randomly. This variability within families with ALS in age at onset, the rate of progression and the occurrence of extrapyramidal features is now well recognized. [4, 16-19] The phenotypic variability suggests that the selective vulnerability of specific groups of neurons to the formation of pathological hallmarks or cell death depends on a complex variation within different regions of the brain in the expression and interaction of genes as well as in the interactions with environmental factors.

Gender maybe one of the factors that influence the expression of disease.
All epidemiologic surveys of ALS report consistently a male preponderance. Although the extend of the excess of ALS in men varies across studies, the male preponderance is on average at least 1.5 to 1, and concerns all forms of sporadic ALS, both the Western type of ALS as the Guamanian ALS-PDC. Efforts to explain the male preponderance have addressed issues such as gender related job exposure to environmental risk factors (for example weldering, using a jackhammer, or being exposed to pesticides, solvents or electrical shock) and misdiagnosis of X-linked bulbar dystrophy (SMBA, Kennedy's disease).
Alternatively, evidence for protective influences in women is accumulating from observations in familial ALS. Specifically in the L126S, L84V and the A4T in the *SOD1*-gene mutations non-penetrance or reduced penetrance in female relatives has been observed. The L126S mutation of *SOD-1* presented with extremely mild symptoms in the legs in male patients and non-penetrance at age 80 in female carriers.[20] The L84V mutation showed rapid progression in males while the female patients began to be affected about 20 years later than the male patients. [21]

An other missense mutation of the *SOD1* gene, A4T, showed rapid progression in affected males and non-penetrance in the mother of the patient, who carried the same mutation. [22]

Estrogens are supposed to have a role as free radical scavengers [23] or anti-apoptotic agents [24, 25] and these functions may compete with the neurotoxic activity of mutant SOD-1 protein. It remains to be elucidated how these neuroprotective effects sustain neuronal survival even after menopause.

In one case control study menarche occurred later and menopause earlier in women affected with ALS, implicating a relatively short reproductive period. This inverse association supports the hypothesized protective effect of estrogens in the etiology of ALS. [26]

Several lines of evidence from human and animal studies suggest a protective role for estrogens in the predisposition to parkinsonism and dementia. [27] [28] The prevalence of parkinsonism is higher in men than in women by an approximately 3:2 ratio. [29]

While the exact mechanisms by which estrogen exerts its effect are still unclear, there is increasing evidence that estrogens may bind to intracellular receptors that enter the nucleus and act as transcription factors to regulate gene expression in the striatum. Estrogens may also act via nongenomic mechanisms such as membrane receptors, interaction with striatal dopamine receptors, and regulation of ion channels, protect against β-amyloid toxicity or excitoxicity. [27, 30, 31]

To date, the findings about efficacy of estrogens therapy in preventing cognitive decline and dementia in postmenopausal women are mixed. Some case-control studies, cross-sectional studies, and prospective studies have reported an association between lower risk for dementia and postmenopausal estrogen supplementation. Meta-analyses of the potential protective effect of estrogen against dementia have reported risk reductions. Clinical trials of estrogen therapy did not confirm a protective effect for dementia nor did women with Alzheimer's disease benefit on cognitive performance. [32]

In men, testosterone concentrations diminish with age, which suggests a link between testosterone depletion and the frequency of ageing-related neurodegenerative disorders. [33-35] The mechanisms by which testosterone could improve cognition and prevent cognitive impairment include modulation of neurotransmitters, stimulation of neuronal connectivity, decreased β-amyloid peptide production, and prevention of excitotoxicity. Alternatively the conversion of testosterone to oestradiol (via aromatase) might influence cognition.

Androgen receptors that bind testosterone co-localize in the brain with estrogen receptors and are found mainly in regions involved in learning and memory, such as the thalamus, hippocampus, and the deep layers of the cerebral cortex. [36] The X-linked, *androgen receptor* (*AR*) gene associated with spino- bulbar muscular atrophy (Kennedy's disease) bears resemblance in clinical features with ALS. The *AR* gene contains a CAG repeat expansion within exon 1 that codes for a polyglutamine sequence of variable length. [37] Lengths of the CAG repeat appears to be inversely correlated with transcriptional activity, and long CAG repeats in the *AR* gene has been associated with androgen insensitivity. [38] These results suggest that the greater the impairment of AR function, the higher the risk for cognitive decline in elderly men. [39]

Neurodegenerative disorders such as ALS, diseases, Alzheimer's disease, frontotemporal dementia (FTD) and parkinsonism are increasingly being realized to have common cellular and molecular mechanisms including the toxic effect of misfolded protein, protein aggregation and inclusion body formation. [40] [41] These neurodegenerative diseases exhibit loss of nerve function in different ways, from memory lapses to uncontrollable muscular movements, but it is now believed that these diseases share many common molecular mechanisms.

The question is to find which mechanisms initiate the neurodegenerative process and to discover if aggregation is the cause or the effect of the diseased neuron. Although, the conspicuous nature of large aggregates in affected tissue makes them attractive candidates for causing disease, whether these aggregates are integrally involved in the disease process or are beneficial in sequestering toxic by-products is unknown. [42]

Initially misfolding of the protein can induce novel toxic properties and/or prevent normal protein functioning. There is evidence that in these diseases, disease-causing proteins severely interferes with the working of the ubiquitin-proteasome system (UPS), the cellular machine responsible for eliminating damaged proteins within the cell. [43] [40, 44] Another possibility is that misfolded or damaged proteins, common to all human neurodegenerative diseases, may clump together to form aggregates. The protein aggregates, bind to the UPS machine irreversibly and prevent the complete degradation of the proteins, explaining the disease process. [45]

The ubiquitin-proteasome system is responsible for cell homeostasis. In healthy cells, proteins perform their function and then, with the help of the proteasome, disappear. If idle and damaged proteins remain, their presence can affect cell behavior. Once bound, the toxic proteins do not release the proteasome. This interference with the normal clearance of proteins has a cumulative and amplifying negative effect. The proteins that are normally degraded build up. The toxic proteins and proteasome are bound together in a close and stable fashion, indicating that the proteins are trapped within the proteasome. This could explain the negative consequences on the health of the cell in which disease builds over decades before symptoms result.

Conversely, it has been suggested that failure to identify, detect abnormal and misfolded protein and transport them for degradation to the UPS system, causes aggregation.

When proteins accumulate in sufficient quantity, they are prone to aggregation. Insoluble aggregates of disease-related proteins may be deposited in microscopically visible inclusions and plaques, the characteristics which are often disease specific. [46] [47]

Most of our knowledge of UPS dysfunction has come from identification of genes linked to familial forms of parkinsonism. Two of these genes, *Parkin* (PARK2) and *UCHL1* (PARK5), are functional in the ubiquityl ligase pathway. The normal function of these ligases is to recognize target proteins for degradation, or transport to other part of the cell. [48-50]

Mutated, *Parkin* and *UCHL1* reduce the normal ability to degrade protein and decrease net UPS activity. [51, 52]

Pathogenic mechanisms in Alzheimer's disease are strongly related to the destabilisation of the structure of β amyloid, resulting in formation of insoluble, disease causing protein aggregates in the brain. [47] The concept that monomeric aberrant proteins are the initiating event pertains also to α-synuclein in parkinonism. In the tauopathies, the pathogenic effects of aberrant tau includes the inhibition of the UPS by the soluble protein and the potentially toxic effect of large aggregates of hyperphophorylated and misfolded tau as paired helical filaments, leading to neurofibrillary tangles. [46]
Novel toxic properties of misfolded proteins may also affect mitochondrial functioning. A major cause of ALS is a mutation in the ubiquitous expressed *SOD-1* gene, but the mechanisms for toxicity to motor neurons is unknown. Multiple disease causing mutants, not wild type *SOD-1*, are now demonstrated to be recruited to mitochondria, only in affected tissue. [53] Highly preferential association with spinal cord mitochondria is seen in human ALS for a mutant SOD1 that accumulates only to trace cytoplasmic levels, suggesting that damage from action of spinal cord-specific factors that recruit mutant SOD1 to spinal mitochondria as the basis for their selective toxicity in ALS. [54, 55]
Mitochondrial localization of *parkin, PINK1* and *DJ-1*, the proteins of respectively PARK2, PARK6 and PARK7, also suggest a role in mitochondrial dysfunction in parkinsonism. [5]
Interestingly, the most common cause of the axonal type of peripheral neuropathy, CMT2, involves mutations of the *MFN2* gene, a mitochondrial GTPase. [56]
Oxidative stress is another neurotoxic mechanism involved in the UPS inhibition. Further evidence for the involvement of oxidative stress in neurodegeneration is presented by the association of ALS with changes in the hypoxia induced expression of vascular endothial growth factor VEGF. [57] These data confirm that chronic vascular insufficiency plays an important role in the degeneration of motor neurons. In parkinsonism oxidative stress has a role in the pathogenic process, as the protective function against oxidative stress. [58]
The initiating events in the neurodegenerative processes briefly described here involve genetic defects and may involve additional interaction with environmentally induced toxicity in the more common sporadic forms of disease.

Table. **Major genes for amyotrophic lateral sclerosis and**

Disease	Genes	Locus	Inheritance	Main clinical features
ALS1	*SOD1*	21q22	AD AR	adult onset, short survival adult onset, slowly progressive
ALS2	*Alsin*	2q33-34	AR	juvenile ALS or PLS, infantile ascending HSP with progressive spasticity of limb and facial muscles, bulbar or pseudobulbar symptoms, distal muscular weakness and atrophy
ALS3		18q21	AD	Adult onet
ALS4	*Senataxin*	9q34	AD	slowly progressive, distal spinal muscular atrophy with normal life expectancy with and without pyramidal signs; spastic paraplegia with amyotrophy (Silver syndrome); absence of bulbar involvement
ALS5		15q12-21	AR	
ALS6		16q12.1-2	AD	adult onset ALS; ALS/FTD survival 3-20 years , reduced penetrance
ALS7		20p13	AD	
ALS 8	*VAPB*	20q13	AD	intrafamilial varability of adult onset slowly progressive SMA, adult onset slowly progressive ALS with tremor and long survival, rapidly progressive ALS
ALS/FTD		9q21-22	AD	ALS-dementia/FTD
ALS/FTD		t(18;21) (q23;q22.1)	AD	ALS-FTD
FTDP-17	*Tau*	17q21	AD	FTD-parkinsonisme-amyotrophy
Guam ALS/PDC				ALS, dementia, parksinonism
FTDP		17q21-22	AD	FTD-parkinsonism
FTD		3	AD	FTD
PARK8	*LRRK2* *Dardarin*	12p11.2- q13.1	AD	parkinsonism with dementia, amyotrophy or both

associated dementia and parkinsonism

Suggested molecular mechanism	Neuropathology	Ref.
toxic gain of function; proteasomal inhibition, aggregate formation; oxidative stress; axonal transport impairment; mitochondrial dysfunction	ubiquitin containing cytoplasmatic and axonal inclusions	84-86
premature truncated alsin is rapidly degradated, impairing endosomal trafficking		87-92
		93
dysfunction of helicase activity, impairing mRNA processing		13, 15, 94
		950
		96-98
		98
impairment of intracellular membrane trafficking		83
	ubiquitin containing inclusions without tauopathy	99
		100
assembly of microtubules	tauopathy	101, 102
	tauopathy	103, 104
	tauopathy	105
	tauopathy	106, 107
	pure nigral degeneration with or without neurofibrillary tangles, brainstem Lewy bodies, or widespread brainstem and cortical Lewy bodies	108-112

Environmental risk factors for amyotrophic lateral sclerosis

During the past decade, genetic approaches to the study of ALS have been paralleled only partly by an increase in our understanding of the role of environmental risk factors and the mechanisms of gene-environment interaction.
The finding that initiated the intensive search for environmental toxins in neurodegenerative disorders was the discovery in the late 1970's that the drug containing l-methyl-4phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) induces in intravenous drug users neurological features resembling Parkinson's disease. [59] This observation could be reproduced in various animal models.

Also investigators have been trying to identify the etiologic trigger of a prototypical neurological disorder found in the Chamorro people in the western Pacific, with features of ALS, parkinsonism and Alzheimer-like dementia (ALS/PDC) [60]. Viral agents have been virtually been ruled out [61] and the disease incidence obviously declined in all three affected western Pacific areas. Since then the search for the etiology of this disorder has focused on genetic susceptibility and non-transmissible environmental factors that are vanishing as the susceptible population adapts to a modern lifestyle.
Epidemiologic studies made the link between preference for Chamorro food and the high incidence of Guamanian ALS/PDC. [62] Data were consistent with the hypothesis that the seeds of the neurotoxic cycad palm, a traditional but declining source of food for the Chamorro, plays a role in the etiology of western Pacific ALS/PDC. The cycad seeds were either processed traditionally in cycad flour or ingested indirectly by the consumption of indigenous flying foxes. These foxes were foraging on the cycad seeds and are supposed to contain high concentrations of the neurotoxic components of the seeds. [63] [64] The cycad hypothesis has been strengthened by recent studies that demonstrate a direct correlation between the presence of ALS/PDC in populations and the exposure to the toxin.
The results of this study bolster the vision that exposure to slow acting toxic agents are important in the pathogenesis of ALS, most likely in combination with a genetic predisposition. In studies described in this thesis we found that for citizens of the United States even a short stay on Guam during the time of endemic ALS was associated many years later (mean 43 years, range 27-57) with an increased risk for ALS.

The study of environmental risk factors for age-related diseases as ALS is difficult for several reasons. Important environmental exposure and gene-environment interactions may occur well before the onset of clinical manifestations and remain undetected years later. Moreover, the severe neurodegenerative changes that underlie the symptoms of ALS may

be the result of additive or synergistic effects of multiple exposures and, over the years, these effects could be compounded by increased vulnerability of the ageing motor neuron system to toxic injury. Hence, repeated insults, chemical interaction, age-related effects and genetic susceptibility should also be considered when investigating the role of neurotoxins in the pathogenesis of ALS.

The results of our study on environmental effects (chapter 5.3) suggests an association between ALS and exposure to pesticides, a history of tuberculosis and short residence on Guam.
Pesticides are a broad range of substances most commonly used to control insects, weeds, and fungi (plant diseases). They are frequently classified by target organism or mode of use as insecticides, herbicides, fungicides, or fumigants. Individuals are frequently exposed to many different pesticides or mixtures of pesticides, either simultaneously or serially, making it difficult to identify effects of particular agents. Poisoning by acute high-level exposure to pesticides has well-known neurotoxic effects, but whether chronic exposure to moderate levels of pesticides is also neurotoxic is more controversial. It is possible that the most sensitive manifestation of pesticide neurotoxicity is a general malaise lacking in specificity and related to mild cognitive dysfunction, similar to that described for Gulf War syndrome. [65]
Pesticide exposure may also be associated with increased risk of neurodegenerative disease, particularly Parkinson's disease. [66] [67]
The association with pesticides, agricultural chemicals or insecticides and ALS was investigated in several studies, some of which show a modest association with ALS. [68] [69] [70] [71] [72] In addition, a cohort study found an increase of ALS in workers in a chemical industry involving exposure to pesticides. [73, 74]

We also found that ALS was associated with a history of tuberculosis. To our knowledge this association has not been reported before and we cannot rule out that this observation was due to chance. The outcome of some studies suggested that ALS is associated with an increased milk ingestion, especially in the youth, which might be linked with bovine tuberculosis infections. [69, 75] However, in our study we did not find a link between a history of tuberculosis and living on a dairy or cattle farm. Neither was ALS associated with neurologic sequelae of meningitis tuberculosa or spinal tuberculosis in our study.
Several explanations could be considered for the association with tuberculosis: a long-term neurotoxic effect of a tuberculosis infection or, a neurotoxic effect of the antibacterial medication, a tuberculosis infection mediated immune respons involving antibodies against motorneurons, or a common (immunologic) increased susceptibility for tuberculosis and ALS. [76]
Further stratification of the analysis of environmental risk factors did not show interactions

with a family history of ALS, dementia and parkinsonism, probably because the statistical power was not sufficient with the available sample size.

Suggestions for genetic counselling

In bioethical considerations about illnesses such as ALS there is increased recognition that the patient and family must be allowed to share crucial decision making. This refers both to questions regarding treatment as to genetic issues. However, the genetic counselling of incurable neurodegenerative disorders is not a straightforward matter and requests to be tailored to the needs and the psychological makeup of the patient and his family. [77, 78]
The severity of the disorder is likely to evoke concern about possible causes and about risk for the family. In our point of view, we (the physicians) owe the patients and their families appropriate information on the genetic complexity of the disorder and possible recurrence risks. This will help preventing the feeling of to be left with unanswered questions and unaccounted issues, especially in cases where the disease is familial. Moreover, the reliable assessment of possible genetic risks, will allow reassurance in the majority of cases.
Essentially, following the model of genetic testing for Huntington's disease, consensus guidelines for ALS genetic counselling and testing should be developed. [79, 80]
In view of the accumulating evidence for complex genetic causes in ALS, we urge to ascertain the family history of neurodegenerative disorders in all patients.
The phenotypic variability of the major ALS genes shows that clinical diagnosis of distal spinal muscular atrophy (dSMA type V), distal Charcot Marie Tooth disease type 2D (dCMT2D), primary lateral sclerosis and spastic paraplegia can belong to the broad spectrum of familial ALS. Therefore we propose that ascertainment of the family history of ALS patients include questions about diagnosed ALS, distal spinal muscular atrophy, spasticity, Alzheimers's disease, Pick's disease or frontotemporal dementia, or any other kind of dementia, or parkinsonism in relatives. Probing for the specific features (personality changes, extreme suspiciousness, or loss of decorum preceding the onset of decline in memory) may help differentiate the frontotemporal type of dementia. Additional information about functional ability, signs of parkinsonism (resting tremor, bradykinesia, postural instability, rigidity), and use of L-dopa or similar medication may help to identify extrapyramidal involvement. Furthermore information should be collected on preexisting conditions, causing symptoms of dementia or parkinsonism (intracranial neoplasm, stroke, alcoholism). When appropriate a molecular analysis should be performed to confirm the clinical diagnosis.

In some instances, we have to consider the psychological burden of a genetic diagnosis for the patient and the family. Based upon the pattern of inheritance in the family the

possibility of genetic counselling must be discussed with individuals at risk and the possible importance for healthy relatives must be mentioned. [80, 81]
Furthermore, families should be allowed to have an option to store DNA for future genotyping of genetic susceptibility to neurodegeneration. Specific genetic changes leading to susceptibilities, might in the future lead to preventive and therapeutic measures.
The same considerations pertain to the neuropathologic classification of ALS and of the relation with dementia and parkinsonism. Discussion of these issues might help make preliminary arrangements for a post-mortem examination that may simultaneously be instrumental for the counselling of the family and for medical research of this complex of neurodegenerative diseases.
In our own experience patients generally welcome the opportunity to share their family history and/or participate in this family history study. Most patients hope that their families will eventually profit from the family history of neurological disorders.

Suggestions for future research

The approach of studying potential familial aggregation through self-reported family history is useful as a starting point. The results of this thesis illustrate that the application of epidemiologic methods might be helpful to test a hypothesis of a shared genetic susceptibility. The follow-up is likely to be of a molecular genetic approach.
The recent identification of gene mutations responsible for mendelian inherited ALS and susceptibility genes in sporadic cases gives new opportunities to study the interaction between the expression of a predisposing gene and exposure to specific neurotoxic chemicals. The effect of selected chemicals can be compared in clonal cell lines, primary cultered neurones and transgenic animals expressing normal endogenous levels of normal protein, overexpressing normal protein and expressing mutant protein. The selective vulnerability of individual populations of neurons must be considered. This approach will offer an opportunity to study gene-gene and gene-environment interaction and help to figure out which environments turn up a gene and which turn it down, which environments interact with which proteins and which environments interfere in normal processing and functioning of which proteins. [82 83] This hopefully will lead to understanding of the pathogenic mechanisms that cause neurodegeneration and to develop effective means for cure and prevention.

Overall rates of the related neurodegenerative disorders, ALS, dementia and parkinsonism are much the same in developed countries, and the incidence rises exponentially until the eight decade of life. Advances in medical technology have prolonged survival up to high ages which has led to a rapid increase in the proportion of patients. The causes of the disorders are complex. Workers in epidemiology will have to develop risk models in

parallel with changing knowledge of biological risk factors.
Many promising research avenues are yet to be explored: properly powered genetic studies, neuroimaging and other biomarker techniques for diagnosis and disease monitoring. Improved diagnosis, wider recognition, and the prospect of disease modification make future management of neurodegenerative disorders an exciting specialty.

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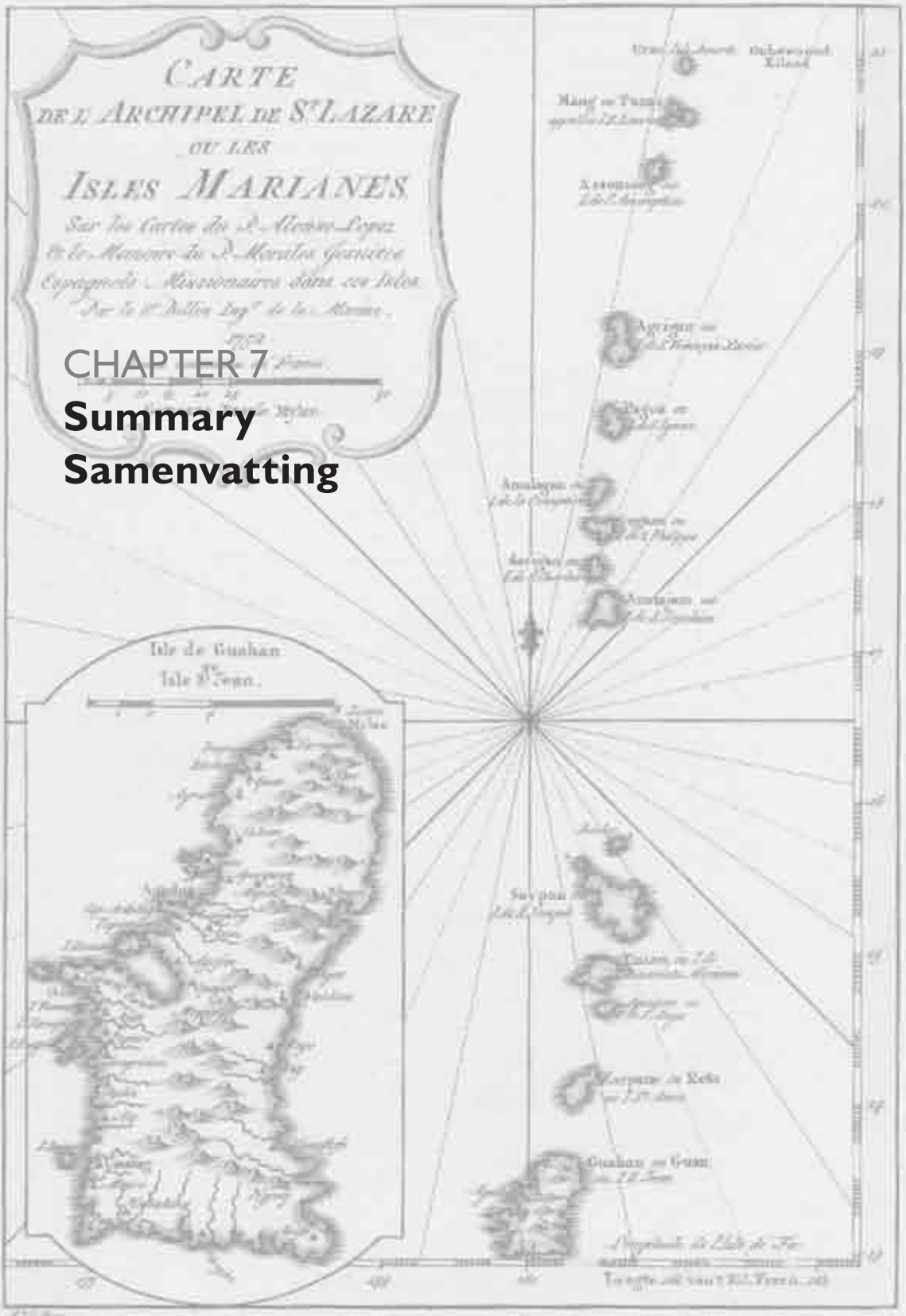

ARCHIPELAGUS St LAZARI OF DE MARIANES EILANDEN.
Volgens de Kaarten en Schriften der Spaanſche Zendelingen Jesuiten Door den Hr Bellin.

CHAPTER 7

Summary
Samenvatting

J. N. BELLIN “Carte De L’Archipel De St.Lazare ou Les Isles Marianes Sur les Cartes du P. Alonso Lopez Et Le Memoire di P.Morales Jesuites Espagnols Missionaires dans ces Isles. Par le Sr. Bellin Ingr. de la Marine 1752.” Paris 1762. Colored. 9X6. Attractive chart shows the Mariana Islands with a fine depiction in an inset of Guam with its harbors, rivers, elevations. Title is in a decorative cartouche with scroll motif, Large compass rose in center.

CHAPTER 7
Summary

This thesis aims to use epidemiologic methods to investigate genetic and environmental factors playing a role in ALS. We conducted a case control study between 1989-1991 at the Neurological Institute Columbia University Medical Center in New York. The study population consisted of 151 newly diagnosed ALS patients and 140 neurologic controls, matched for age (± 5 years), gender (83 pairs of men and 57 pairs of women) and insurance status.
In **chapter 2** the current insights in the relation of ALS, dementia and parkinsonism is summarized.

As described in the review (**chapter 3**) until the 1960s, ALS was considered to be a non-hereditary neurologic disease. In a land-mark report in 1955, Kurland and Mulder described 18 families with hereditary ALS from a 100-year review of the world literature. Only in the past decade, genetic analysis of familial forms of ALS led to the identification of several mendelian inherited forms of ALS. These familial forms comprise 5-10% of all cases. Thus far six autosomal dominant and three autosomal recessive forms were found to be associated with familial ALS. In addition, a growing number of genetic defects are being associated with syndromes featuring familial recurrence of ALS, frontotemporal dementia and parkinsonism. In the 90% of cases were ALS is sporadic, changes in various genes have been linked to an increased risk for ALS, so called susceptibility genes. Susceptibility genes are thought to interact with other low penetrant genes and/or with environmental risk factors.

In **chapter 4** of this thesis we used epidemiologic methods to find evidence for a shared genetic susceptibility for ALS, dementia and parkinsonism. To do so, we investigated familial aggregation of these three disorders in families of ALS patients. In the case-control study we compared the family histories of ALS patients and controls. A semi-structured questionnaire was used to ascertain valid information on neurologic conditions for each sibling, parent and grandparent, individually.

The data showed familial recurrence of ALS in five percent of the patients and not in controls. We found that the risk (cumulative incidence) of dementia was twofold increased for relatives of ALS patients. This association between ALS and dementia was apparent in

familial as well as in sporadic ALS. A similar association between ALS and parkinsonism was detected. We found that the association between ALS and dementia was stronger in first- degree relatives (parents and siblings) than in second- degree relatives (grandparents). These findings endorse a genetic effect in familial aggregation of ALS and dementia. We then evaluated the associations between dementia and parkinsonism within and between the families of ALS patients and controls. In families of ALS patients the risk for parkinsonism was significantly higher when dementia occurred in the family than if there were no known relatives with dementia. Co-occurrence of dementia and parkinsonism was observed twice more frequently in families of sporadic ALS, compared to controls. The results of this study show a familial association between ALS, dementia and parkinsonism. This suggests shared genetic susceptibilities for the neurodegenerative features in the families where these disorders co-occur. An important observation is, that the supposed genetic susceptibility to neurodegeneration also occurs in sporadic ALS.

In **chapter 5** we describe that between 1940 and 1965 an unusually high incidence of amyotrophic lateral sclerosis (ALS), with co-occurrence in patients and families of a parkinsonism-dementia complex (PDC), was observed in the Chamorro population of the pacific island of Guam. So far, no genetic causes for ALS/PDC have been found. The increased risk of ALS in migrants to and from Guam suggests a combined effect of an environmental exposure and a genetic susceptibility. The declining incidence of ALS on Guam has been attributed to changes in the consuming patterns of food containing the seed of the locally growing cycad palms. These seeds were either processed traditionally in cycad flour or ingested indirectly by the consumption of indigenous flying foxes. These foxes were foraging on the cycad seeds and are supposed to contain high concentrations of the neurotoxic components of the seeds.

In the second part of chapter 5 we have examined if residence on Guam during the period of endemic ALS, contributed to the risk for ALS in citizens returned to the United States. We have compared places, dates and the duration of residence outside the continental United States for more than one month. We found that a short stay (2-15 months) on Guam is associated with ALS in citizens of the United States. The link between ALS and temporary stay in Guam remained even after accounting for other putative risk factors such as age, gender, education, traumas, exposure to pesticides, history of tuberculosis and family history of ALS, dementia or parkinsonism, and military service. Despite the small sample size of this study we detected an association where two larger studies did not. The follow-up period in the two earlier studies was shorter, 16-25 years and 15-27 years, respectively, than in the present study (43 years, range 27-57). This might indicate that one of the key characteristics following the exposure during the period of endemic ALS on the pacific island of Guam is the long delay in clinical expression. The toxic exogenous factor

in ALS pathogenesis seems to act after quite a short exposition (maybe on genetically susceptible people) during the period of endemic ALS on Guam and has a long delay in clinical expression.

In the third part of chapter 5 we further aimed to identify environmental factors which may act against a background of increased genetic susceptibility. In the case control study described before we investigated putative environmental risk factors for ALS and analyzed the interactive effect of each of these proposed risks with a family history of ALS, dementia or parkinsonism, which we used as a marker for a genetic susceptibility to neurodegeneration. We collected information on antecedent medical histories (specific infectious diseases, diabetes, autoimmune disorders, surgery and injuries), exposure to animals or to specific environmental toxins (pesticides, aluminum, lead and others), occupational histories, smoking habits and the consumption of alcoholic beverages. In multivariable analysis, the association with exposure to pesticides and a history of tuberculosis remained.
The epidemiologic analysis of gene-environment interaction in ALS indicates that studies with larger sample sizes are needed to allow further stratification for interactions between infrequent exposures and the large number of rare genetic susceptibilities to neurodegeneration in sporadic forms of ALS.

This thesis illustrates that a genetic predisposition to ALS might be considered in terms of susceptibility to neurodegenerative disease rather than of specific clinical diagnosis. Consequently, patients and their families should be given genetic counselling, tailored to specific circumstances. When appropriate a molecular genetic analysis should be performed to confirm and specify the clinical diagnosis. Also, patients should be offered the option to store DNA for future genotyping of susceptibility to neurodegeneration. Relatives might profit from future preventive or therapeutic measures for specific genetic changes leading to susceptibility for neurodegeneration.
Clearly, the genetic classification of disorders bearing overlapping features of ALS, dementia and parkinsonism will be helpful in gaining insight in the molecular pathways leading to neurodegeneration. The identification of gene mutations responsible for familial aggregation of ALS, dementia and parkinsonism will open new opportunities to understand at the molecular level the influence of predisposing genes and the exposure to specific environmental factors. This interaction might explain the selective vulnerability of specific groups of neurons to the pathologic hallmark of disease and cell death.

CHAPTER 7
Samenvatting

Het doel van dit proefschrift was met epidemiologische onderzoeks methoden een gezamelijke genetische predispositie voor amyotrofische lateraal sclerose (ALS), dementie en parkinsonisme, en de invloed van omgevingsfactoren te bestuderen. Hiervoor werd een patient-controle studie verricht van 1989 to 1991 op het Neurological Institute van het Columbia University Medical Center in New York. De onderzoekspopulatie bestond uit 151 patienten bij wie recent de diagnose ALS was gesteld en 140 controle patienten, met andere neurologische aandoeningen, die dezelfde leeftijd (± 5 jaar) en geslacht hadden als de ALS patienten, en die in dezelfde klasse medisch verzekerd waren.

Tot ver in de twintigste eeuw was men zich nauwelijks bewust van de erfelijke vormen van amyotrofische lateral sclerose (ALS). Hierin kwam geleidelijk verandering na de publicatie van Kurland en Mulder in 1955, waarin zij een overzicht gaven van achtien families met ALS, die in de afgelopen 100 jaar in de literatuur werden beschreven.
In **hoofdstuk 2** wordt de huidige kennis over de relatie tussen ALS, dementie en parkinsonisme samengevat.

Zoals in het overzichtsartikel (**hoofdstuk 3**) wordt beschreven heeft de groeiende belangstelling voor en onderzoek naar familiaire ALS er inmiddels toe geleid dat nu bekend is dat bij ongeveer 5 tot 10 procent van de ALS patienten een erfelijke vorm van de ziekte met een mendeliaans overervingspatroon voorkomt. Pas in het afgelopen decennium kon met de toepassing van moderne moleculaire onderzoekstechnieken het gen of de locatie van verschillende - maar lang nog niet alle- mendeliaans overervende vormen van ALS worden geidentificeerd. Inmiddels kennen we zes autosomaal dominant overervende en drie autosomaal recessief overervende erfelijke vormen van ALS. Bovendien worden in rap tempo steeds meer genen ontdekt, die het voorkomen van combinaties van ALS, frontaalkwab dementie en/of parkinsonisme in families verklaren.
Ook worden, ondanks dat bij het merendeel (90%) van de ALS patienten de ziekte niet in de familie voorkomt, nu ook bij sporadische patienten veschillende genmutaties of -polymorphismen waargenomen. Vermoedelijk bestaat er ook bij sporadische patienten een wisselwerking tussen predispositie genen en schadelijke omgevingsfactoren, die ieder opzich onvoldoende penetrant zijn om ALS te veroorzaken.

H**oofdstuk 4** van dit proefschrift bevat het epidemiologische onderzoek naar een gemeenschappelijke genetische aanleg voor ALS, dementie en parkinsonisme. Hiertoe werd onderzocht of deze drie aandoeningen vaker in de families van ALS patienten voorkomen dan in families van controle personen. Dit werd onderzocht door de familiegeschiedenis van ALS patienten met die van controle personen te vergelijken. Om zo betrouwbaar mogelijk informatie te krijgen over neurologische aandoeningen in de familie werd van alle deelnemers voor ieder familielid afzonderlijk (broers, zusters, ouders en grootouders) een gestructureerde vragenlijst afgenomen.
Uit deze gegevens bleek dat in vijf procent van de families van de ALS patienten er familieleden waren met ALS terwijl in de families van de controles geen ALS voorkwam. Ook kon worden vastgesteld dat de familieleden van ALS patienten twee keer meer kans (cumulatieve incidentie) hadden op dementie dan de familieleden van de controle personen. De associatie tussen ALS en dementie werd zowel waargenomen in familiaire ALS als in families van sporadische ALS patienten. Een soortgelijk verband werd vastgesteld tussen ALS en parkinsonisme. De associatie tussen ALS en dementie was duidelijker in eerstegraads familieleden (de ouders, broers en zusters) van de ALS patienten dan in hun tweedegraads bloedverwanten (grootouders).
Vervolgens werd onderzocht of er in de families van ALS patienten een verband is tussen het voorkomen van dementie en het voorkomen van parkinsonisme. We vonden dat de kans op parkinsonisme statistisch significant hoger was in families van ALS patienten wanneer bij tenminste één van de familieleden dementie was vastgesteld dan wanneer dit niet het geval was. Tevens bleek dat bij sporadische ALS patienten de kans op het samen voorkomen van dementie en parkinsonisme in de familie twee keer groter was dan bij controle personen.
De resultaten van dit onderzoek bevestigden dientengevolge het vermoeden dat er een genetisch verband is tusssen ALS, dementie en parkinsonisme. Het veronderstelde familiaire verband tussen ALS, dementie en parkinsonisme zou verklaard kunnen worden door een gemeenschappelijke erfelijke aanleg voor neurodegeneratie in de families waarin deze drie neurologische aandoeningen voorkomen. Opvallend was dat de veronderstelde genetische aanleg voor neurodegeneratie ook in families van de sporadische ALS patienten werd waargenomen.

In **Hoofdstuk 5** worden omgevingsfactoren onderzocht.
In de periode tussen 1940 en 1965 werd een ongewoon hoge incidentie van ALS waargenomen in the inheemse Chamorro bevolking van het eiland Guam in de Stille Oceaan. Opmerkelijk was dat bij veel van de Chamorro patienten evenals in hun familie een parkinsonisme-dementie complex (PDC) voorkwam. Tot nu toe is de genetische oorzaak voor ALS/PDC op Guam nog niet opgehelderd. Wel zijn er recent aanwijzingen gevonden voor schadelijke omgevingsfactoren.

Omdat migranten van en naar Guam een verhoogd risico op ALS hadden werd een wisselwerking tussen blootstelling aan schadelijke omgevingsfactor(en) en een erfelijke aanleg voor neurodegeneratie verondersteld. De recent waargenomen dalende incidentie van ALS op Guam werd toegeschreven aan een verandering in het consumptiepatroon van voedsel dat zaden bevat van de inheemse cycade palm. Deze zaden werden hetzij op traditionele wijze tot meel verwerkt of indirect genuttigd via het eten van inheemse vleermuisjes. Omdat deze dieren zich voedden met de cycade zaden veronderstelde men dat zij hoge concentraties bevatten van het neurotoxische bestanddeel van de zaden. Inmiddels zijn de betreffende vleermuizen van het eiland verdwenen.
In het eerste deel van het vijfde hoofdstuk werd onderzocht of een verblijf op Guam gedurende de periode waarin ALS endemisch was op het eiland, ook voor inwoners van de Verenigde Staten na hun terugkeer nog van invloed was op de kans op ALS.
In de vragenlijst werden de naam van de plaatsen en de periode van verblijf buiten de Verenigde Staten, mits langer dan een maand, genoteerd. Bij het vergelijken van deze gegevens bleek dat de ALS patienten vaker op Guam waren geweest (duur verblijf van 2 tot 15 maanden) dan de controles. Verblijf op Guam bleef een significante risicofactor voor ALS te blijven, ook in de multivariabele analyse met andere mogelijke risicofactoren. Deze observatie is in tegenstelling met resultaten van twee eerdere studies, met grotere onderzoekspopulaties. In onze studie was de periode tussen het verblijf op Guam en het stellen van de diagnose ALS veel langer dan in de twee eerdere studies nl. gemiddeld 43 jaar (spreiding 27 tot 57 jaar).
Hieruit zou de conclusie getrokken kunnen worden dat een van de karakteristieken van de endemische ALS op Guam de lange periode (‘long latency’) was tussen het tijdstip waarop de blootstelling heeft plaatsgevonden en het optreden van klinische symptomen. Dit zou betekenen dat zelfs een kortdurende verblijf ten tijde van endemische ALS op Guam van invloed zou kunnen zijn op de pathogenese van ALS. Wellicht bij personen met een specifieke genetische predispositie. Voorts dat het kennelijk heel lang duurde (decennia) voor bij deze patienten de klinische symptomen optraden.

Het tweede deel van hoofdstuk 5 beschrijft onderzoek naar andere omgevings factoren die mogelijk, samen met erfelijke factoren, ALS zouden kunnen veroorzaken. In het eerder beschreven patient-controle onderzoek werden gegevens over een aantal mogelijk schadelijke omgevingsfactoren separaat geanalyseerd. Vervolgens werd voor ieder van deze factoren onderzocht of er een interactie was met het effect van een familiegeschiedenis voor ALS, dementie of parkinsonisme. Er werden anamnestische gegevens verkregen over specifieke infectieziekten, auto-immuun ziekten, operaties en verwondingen, blootstelling aan dieren of aan specifieke toxinen (onder anderen: pesticiden, aluminium, lood). Tevens werd informatie verzameld over de achtereenvolgende werkzaamheden en beroepen, over rookgewoonten en over de consumptie van alcoholische drank.

In de multivariabele analyse werd alleen een verband tussen ALS en het gebruik van pesticiden en tussen ALS en tuberculose waargenomen.
De genetisch epidemiologisch analyse in deze studie maakte duidelijk dat onderzoek naar een interactie tussen genen en omgevingsfactoren nadere studies vereist met veel grotere onderzoekspopulaties om laagsgewijs de invloed van weinig voorkomende omgevingsfactoren en de verschillende erfelijke predisposities voor neurodegeneratie in sporadische ALS te kunnen analyseren.

De resultaten van dit proefschrift bevestigen de hypothese dat een erfelijke aanleg voor ALS als een erfelijke aanleg voor een spectrum van neurodegeneratie kan worden beschouwd, in plaats van louter als aanleg voor één specifieke neurologische ziekte. Het is van belang hiermee ook rekening te houden bij het erfelijkheidsonderzoek en -advies aan de patienten en hun familieleden. In toenemende mate wordt DNA onderzoek mogelijk ter bevestiging van de klinische diagnose van neurodegeneratieve ziekten. Ook wanneer DNA diagnostiek nog niet mogelijk is, lijkt het zinvol DNA op te laten slaan voor genetische diagnostiek in de toekomst. Van deze kennis kunnen familieleden van de patient later voordeel hebben, bijvoorbeeld wanneer er geneesmiddelen of methoden worden ontwikkeld om, bij een specifieke aanleg voor neurodegeneratie, het ziekteproces af te remmen of te voorkomen.
Het is duidelijk dat een genetische classificatie van ziekten met klinisch overlappende kenmerken van ALS, dementie en parkinsonisme het inzicht in de moleculaire pathologie van neurodegeneratie zal verdiepen. Er zullen nieuwe genen worden gevonden in families waarin ALS, dementie en parkinsonisme samen voorkomen, en dit zal de mogelijkheid geven om op moleculair niveau de wisselwerking tussen erfelijke aanleg en bepaalde omgevingsfactoren te onderzoeken. Met behulp van die kennis zal er hoepelijk een verklaring worden gevonden voor de gevoeligheid van bepaalde groepen neuronen voor een degeneratief proces en voor het tijdstip van optreden en de daaropvolgend verloop van de pathologie.

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Curriculum Vitae

Danielle F. Majoor-Krakauer, date of birth December 8th 1954, married to Frank Majoor and mother of Emilie and Nina.

Educational Background

1972-1979	Medical studies at Leiden University Medical School.
1975	Elective in Clinical Genetics, Department of Human Genetics, Tel Hashomer Medical School, Tel Aviv, Israel.
1978	Elective in Pediatrics and Clinical Genetics, Hôpital des Enfants Malades Necker, Paris, France.
1980-1981	Internship in Clinical Genetics and Cytogenetics, Department of Human Genetics, Friedrich Wilhelm University, Bonn, Germany (Prof. Dr.H.Weicker, and Prof. Dr. G.Schwanitz, cytogeneticist). Eligible for board certification as Medical Geneticist by the Human Genetics Society of Germany.
1987-1987	Internship in Clinical Genetics (Prof. Dr. M.F.Niermeijer), Department of Clinical Genetics, Erasmus University Medical School.
1992-1992	Master of Science program in Epidemiology, Mailman School of Public Health, (Prof. Dr. Z.Stein and Prof.Dr. R.Ottman, genetic epidemiology) Columbia University, New York, NY, USA. Registration as Master of Science in Epidemiology in 1992.
1995	Board certified registration as Clinical Geneticist in the Netherlands.

Employment's

1979-1981	Internship in Clinical Genetics and Cytogenetics, Department of Human Genetics, Friedrich Wilhelm University, Bonn, Germany.
1981-1987	Appointment as Clinical Geneticist, Department of Clinical Genetics, Erasmus University, Rotterdam (head: Prof. Dr. H. Galjaard).
1987-1992	Graduate Research Assistant at the Department of Neurology, Columbia University Medical Center (Prof. Dr..L.P.Rowland, M.D.), New York, USA and the Gertrude H. Sergievsky Center and Epidemiology Division,

Mailman School of Public Health, Columbia University, New York, USA (Prof.Dr.M. Susser, Prof.Dr.Z.Stein).

1992 Staff member of the Department of Clinical Genetics, Erasmus Medical Center Rotterdam (head Prof.Dr. H. Galjaard, since 2001 Prof.Dr. J.W.Wladimiroff).

APPENDIX

Genetic epidemiology of ALS: questionaire

SECTION I

ALS ID NUMBER: ___ ___ ___

STATUS: Case.....1
Control.....0

DATE OF INTERVIEW: ___ ___ / ___ ___ / ___ ___
Month Day Year

TIME STARTED: _____ AM PM
(Circle one)

GO TO PERSONAL IDENTIFICATION FORM FOR Q1 - Q3.

Q4. **CODE WITHOUT ASKING**

SEX:

male.....1
female.....2

Q5. What is your exact date of birth?

___ ___ / ___ ___ / ___ ___
Month Day Year

Q6. A. Do you consider yourself Hispanic?

YES.....1
NO.....0

B. Are you white, black, Asian, or another ethnicity?

White.....1
Black.....2
Asian.....3
Other (specify)_____..9

I'd like to begin now by asking some questions about your medical background. Most of them can be answered either by "yes", "no", or "don't know". Please feel free to tell me if you don't know the answer to a question because it is important for us to be able to tell the difference between a "no" answer and a "don't know" answer. OK, let's begin.

Now I'd like to ask some questions about your parents' health at the time of your birth. Let's begin with your mother.

Q7. How old was your mother when you were born? (DK=99) ___ ___

Q8. Did your mother have any serious health problems when she was pregnant with you?

YES.....1
NO...[GO TO Q9]....0
DK...[GO TO Q9]....9

A. What was the problem? **(RECORD VERBATIM)**

_____

_____

Q9. Did she receive any medical treatment during this pregnancy such as prescription mediation, an operation, radiation treatment, or any other type of medical treatment?

YES.....1
NO...[GO TO Q10]...0
DK...[GO TO Q10]...9

A. What kind of medication, operation or other kind of treatment did she receive? **(RECORD VERBATIM)**

_____

_____

Q10. Did your mother <u>smoke</u> during her pregnancy with you?

YES.....1
NO.....0
DK.....9

Q11. A. Did your mother drink any alcoholic beverages while she was pregnant with you?

YES.....1
NO..[GO TO Q12 - NEXT PG].0
DK..[GO TO Q12 - NEXT PG].9

B. Consider a "drink" to be about one 6 ounce glass of wine, one 12 ounce beer, or one cocktail. How many "drinks" of alcohol each week would you say your mother had while she was pregnant with you?...

one to seven.[GO TO Q12]..1
more than seven.....2
DK.....[GO TO Q12]..9

C. Was she an alcoholic?

YES.....1
NO.....0
DK.....9

Q12. Did your mother have any kind of a job when she was pregnant with you, including an unpaid job such as working on a farm or helping in a family business?

YES.....1
NO....[GO TO Q13].....0
DK....[GO TO Q13].....9

1. What kind of business or industry did she work in?

_____

2. What kind of work did she do?

_____

3. What was her job title?

_____

FOR OFFICE USE: ___ ___ ___

Now, I'd like to ask some questions about your <u>father's</u> health before you were born.

Q13. How old was your <u>father</u> when you were born? (DK=99) ___ ___

Q14. Did your father have any serious health problems when your mother was pregnant with you?

YES.....1
NO..[GO TO Q15 - NEXT PG].0
DK..[GO TO Q15 - NEXT PG].9

What was the problem? **(RECORD VERBATIM)**

_____

_____

Q15. Did your father receive any special medical treatment for this problem, such as prescription medication, an operation, radiation treatment, or any other kind of medical treatment?

YES.....1
NO...[GO TO Q16]...0
DK...[GO TO Q16]...9

A. What kind of treatment did he receive? (RECORD VERBATIM)

_____

_____

Q16. Did he smoke when your mother was pregnant with you?

YES.....1
NO.....0
DK.....9

Q17. A. Did he drink any alcoholic beverages when she was pregnant with you?

YES.....1
NO...[GO TO Q18]...0
DK...[GO TO Q18]...9

B. Again, consider a "drink" to be about one 6 ounce glass of wine, one 12 ounce beer, or one cocktail. How many "drinks" of alcohol each week would you say your father had around the time your mother was pregnant with you?...

one to seven.....[GO TO Q18]...1
more than seven.....2
DK.....[GO TO Q18]...9

C. Was he an alcoholic?

YES.....1
NO.....0
DK.....9

Q18. A. Did he have a paying job when your mother was pregnant with you?

YES.....1
NO..[GO TO B - NEXT PG]...0
DK..[GO TO B - NEXT PG]...9

1. What kind of business or industry did he work in?

_____

2. What kind of work did he do?

_____

3. What was his job title?

_____

FOR OFFICE USE: ___ ___ ___155

B. Was he retired at that time?

YES.....1
NO...[GO TO Q19]...0
DK...[GO TO Q19]...9

1. What kind of business did he work in before he retired?

_____

2. What kind of work did he do before he retired?

_____

3. What was his job title?

_____

Q19.1 Have you ever lived on a farm?

YES.....1
NO..[GO TO Q20 - NEXT PG].0
DK..[GO TO Q20 - NEXT PG].9

A. Where was the farm? **(PROBE FOR CITY, STATE, AND COUNTRY)**

_____
city

_____
state

_____
country

B. What kind of a farm was it? For example:
cattlebreeding/dairy/poultry/agriculture **(PROBE TO FIND OUT CROP(S))**

_____

C. How long did you live on the farm? **(PROBE FOR YEARS; UP TO ONE-01 DK-99)**

__ __

Q19.2 Have you ever lived or worked outside of the continental United States for 6 months or longer?

YES.....1
NO.....0
DK.....9

A.1 Where did you live?
(RECORD VERBATIM; PROBE FOR EACH NON-USA RESIDENCE)

_____

_____

B.1 How old were you when you first lived there? __ __

C.1 How long did you live there?
(PROBE FOR MONTHS; RECORD VERBATIM) __ __

_____

A.2 Where did you live?
(RECORD VERBATIM; PROBE FOR EACH NON-USA RESIDENCE)

_____

_____

B.2 How old were you when you first lived there? ___ ___

C.2 How long did you live there?
(PROBE FOR MONTHS; RECORD VERBATIM) ___ ___

_____

Now I want to ask you about diseases you might or might not have had.

Q20. First, I am going to ask you about <u>infectious</u> diseases you may have had. Did you have any of the following?

		YES	NO	DK
1.	poliomyelitis or infantile paralysis	1	0	9
2.	meningitis	1	0	9
3.	encephalitis	1	0	9
4.	mumps	1	0	9
5.	shingles	1	0	9
6.	herpes	1	0	9
7.	tuberculosis	1	0	9
8.	German measles (rubella)	1	0	9
9.	other measles	1	0	9
10.	chicken pox	1	0	9
11.	whooping cough	1	0	9
12.	hepatitis - (Probe to find out which type)	1	0	9
13.	Any other serious infectious disease	1	0	9

Specify:_____

_____

_____

ASK QUESTIONS A - E FOR EACH DISEASE R HAD. IF NO TO ALL, GO TO Q21 ON PAGE 10.

A.1 If YES: Disease _____
(fill in one of the above mentioned diseases)

B.1 How old were you when you first got this infection?
(DK-99; UP TO AGE 1-01) years ___ ___

C.1 How long were you ill? **(PROBE FOR MONTHS; DK-99; IF LESS THAN ONE MONTH, CODE 01)** months___ ___

D.1 Were you hospitalized?

YES.....1
NO..[GO TO NEXT DISEASE]..0
DK..[GO TO NEXT DISEASE]..9

1. Can you tell us in which hospital?

_____
Name of hospital

_____
address

_____

2. Do you remember the name of the physician who treated you?

_____
Name of physician

A.2 If YES: Disease _____
(fill in one of the above mentioned diseases)

B.2 How old were you when you first got this infection?
(DK-99; UP TO AGE 1-01) years ___ ___

C.2 How long were you ill? **(PROBE FOR MONTHS; DK-99; IF LESS THAN ONE MONTH, CODE 01)** months___ ___

D.2 Were you hospitalized?

YES.....1
NO..[GO TO NEXT DISEASE]..0
DK..[GO TO NEXT DISEASE]..9

1. Can you tell us in which hospital?

_____
Name of hospital

_____
address

_____

2. Do you remember the name of the physician who treated you?

_____
Name of physician

A.3 If YES: Disease _____
(fill in one of the above mentioned diseases)

B.3 How old were you when you first got this infection?
(DK-99; UP TO AGE 1-01) years ___ ___

C.3 How long were you ill? **(PROBE FOR MONTHS; DK-99; IF LESS THAN ONE MONTH, CODE 01)** months___ ___

D.3 Were you hospitalized?
YES.....1
NO..[GO TO NEXT DISEASE]..0
DK..[GO TO NEXT DISEASE]..9

1. Can you tell us in which hospital?

_____
Name of hospital

_____
address
_____

2. Do you remember the name of the physician who treated you?

_____
Name of physician

Q21. Have you ever had any serious injuries, such as broken bones, burns, poisoning, a head injury, electrical shock, or any other type of injury?

YES.....1
NO..[GO TO Q22 - NEXT PG].0
DK..[GO TO Q22 - NEXT PG].9

A. How many serious injuries have you had? ___

B. Now I'd like to ask some questions about (each of) (this/these) injuries. Let's start with the earliest one.

		1st	2nd	3rd
1.	What part(s) of your body were injured in the injury? **(RECORD VERBATIM)**	_____	_____	_____
2.	What type of injury do you have?	_____	_____	_____
3.	How did it happen? **(RECORD VERBATIM)**	_____	_____	_____
		_____	_____	_____
		_____	_____	_____

		1st	2nd	3rd
4.	How old were you when it happened? (DK-99, UP TO AGE 1-01)	__ __	__ __	__ __
5.	Did you go to a doctor because of the injury? (YES-1, NO-0, DK-9)	__	__	__
6.	What kind of treatment did you receive? (RECORD VERBATIM	_____	_____	_____
		_____	_____	_____
		_____	_____	_____
7.	Were you admitted to a hospital because of the injury? (YES-1, NO-0, DK-9)	__	__	__

(ASK 8-9 ONLY FOR HEAD INJURIES, OTHERWISE GO TO NEXT INJURY)

8.	Did you lose consciousness or were you "knocked out" because of the injury? (YES-1, NO-0, DK-9)	__	__	__
	(IF NO, OR DK, GO TO NEXT INJURY)			
9.	How long were you unconscious? (Probe for minutes IF LONGER THAN 1 HOUR, RECORD VERBATIM AND LEAVE CODING BLANK; DK-999)	__ __ __	__ __ __	__ __ __
		_____	_____	_____

Q22. Have you ever had any operations or surgery?

YES.....1
NO..[GO TO Q23 - NEXT PG]..0
DK..[GO TO Q23 - NEXT PG]..9

A. How many different operations have you had? __ __

B.	What was the reason for the operation?	How old were you at that time?
1st.	_____	__ __
2nd.	_____	__ __
3rd.	_____	__ __

C. What was the name of the hospital where you had the (1st, 2nd, etc.) operation?

I.	II.	III.
_____ (name of hospital)	_____ (name of hospital)	_____ (name of hospital)
_____ (address)	_____ (address)	_____ (address)
_____	_____	_____

D. What was the name of the physician who told you you needed the operation?

1)_____

2)_____

3)_____

Q23. Have you ever had a blood transfusion?

YES.....1
NO...[GO TO Q24].....0
DK...[GO TO Q24].....9

A. How many times? **(CODE NUMBER DIRECTLY, DK=99)** ___ ___

B. How old were you (the ____ time)? **(DK=99, UP TO AGE 1=01))**

1st ___ ___

2nd ___ ___

C. What was the reason for the tranfusion?

1) _____

2) _____

D. What was the name of hospital?

(1) _____ (name of hospital)
_____ (address)
_____

(2) _____ (name of hospital)
_____ (address)
_____

Q24. When you were born, was there any problem in the formation of your body parts such as cleft lip or palate, club foot, extra or missing fingers or toes, a heart defect other than a heart murmur, or any other problem?

YES.....1
NO...[GO TO Q25]...0
DK...[GO TO Q25]...9

A. What was the problem?

_____

_____

Q25. Did anyone ever tell you that when you were born, you were much smaller than other babies?

YES.....1
NO.....0
DK.....9

Q26. A. Did anyone ever tell you that you started to walk much later than other children?

YES.....1
NO..[GO TO Q27 - NEXT PG]..0
DK..[GO TO Q27 - NEXT PG]..9

(1) About how old were you when you started to walk? **(CODE MONTHS, DK=99)** ___ ___

Q27. Did anyone ever tell you that you started talking much later than other children?

YES.....1
NO...[GO TO Q28]...0
DK...[GO TO Q28]...9

(1) About how old were you when you started to talk? **(CODE MONTHS, DK=99)** ___ ___

Q28. What is the highest grade of school you have completed? **(IF EDUCATED OUTSIDE U.S., ASK WHAT IT WOULD EQUAL IN U.S.)**

Less than High School graduate.....1
High School graduate.....2
Some college but not a graduate (or <4 years past High School).....3
College graduate.....4
Graduate school.....5
DK.....9

Q29. When you were under 12 years old, did you take physical education classes with the other children at school?

YES...[GO TO Q30]....1
NO.....0
DK....[GO TO Q30]....9

A. What were your reason(s) for not taking physical education classes? **(RECORD VERBATIM)**

_____

_____

162 _____

Q30. Have you ever had a seizure or a convulsion? YES.....1
NO..[GO TO Q33 - NEXT PG]..0
DK..[GO TO Q33 - NEXT PG]..9

A. How many times did it happen? Would you say... 1.....1
2.....2
3.....3
4 or more.....4
DK.....9

B. How old were you (the first time)? (DK-99) ___ ___

Q31. Have you ever been told that you had epilepsy?
YES.[GO TO Q32 - NEXT PG]..1
NO.....0
DK.....9

A. What was the cause of the seizure(s)? (RECORD VERBATIM)

_____

_____

Q32. Have you ever taken anticonvulsant medication for seizures? YES.....1
NO...[GO TO Q33]...0
DK...[GO TO Q33]...9

A. How old were you when you started? (DK-99, UP TO AGE 1-01) ___ ___

Q33. Have you ever had severe neck pain or back pain?

YES.....1
NO...[GO TO Q34]...0
DK...[GO TO Q34]...9

A. What type of pain was it? (RECORD VERBATIM)

_____

_____

B. How old were you when that started? (DK-99, UP TO AGE 1-01) ___ ___

Q34. Have you ever taken prescription medicines regularly for six months or longer?
YES.....1
NO...[GO TO Q35 - NEXT PG].....0
DK...[GO TO Q35 - NEXT PG].....9

A. What prescription drugs have you taken in this way? For each medication, please tell me more or less how long you took it. **(PROBE FOR MONTHS; IF LONGER THAN 5 YEARS, RECORD VERBATIM AND LEAVE CODING BLANK)**

	Medication	No. of months
(1)	_____	__ __
(2)	_____	__ __
(3)	_____	__ __
(4)	_____	__ __

Q35. Have you ever smoked tobacco?

YES.....1
NO...[GO TO Q36]...0

A. How old were you when you started smoking? **(DK-99)** __ __

B. Which of the following have you smoked?...

	YES	NO	DK
cigarettes	1	0	9
cigars	1	0	9
a pipe	1	0	9

C. Do you still smoke?

YES...[GO TO E]....1
NO.....0

D. How old were you when you stopped? **(DK-99)** __ __

E. Which of the following did you smoke during the last year, and how much of each?...

	YES	NO	DK	How many per day? (DK-99)
cigarettes	1	0	9	__ __
cigars	1	0	9	__ __
a pipe	1	0	9	__ __

Q36. Have you used any of the following drugs more than ten times?

	YES	NO	DK
marijuana	1	0	9
hashish	1	0	9
cocaine	1	0	9
LSD	1	0	9
heroin	1	0	9
morphine	1	0	9

Q37. Approximately how many of the following alcoholic drinks would you say you usually have each week?

A. 12 ounce beers.....___ ___

B. 6 ounce glass(es) of wine.....___ ___

C. 1 ounce cocktails made with whiskey, vodka, skotch, gin, or other hard liquor.....___ ___

Q38. Have you ever had any of the following diseases?

	YES	NO	DK
1. diabetes	1	0	9
2. asthma or allergies	1	0	9
3. colitis	1	0	9
4. recurrent infections (more than 3 infections a year, that needed antibiotic therapy)	1	0	9
5. rheumatoid arthritis	1	0	9
6. rashes or other skin diseases	1	0	9
7. any kind of cancer, including leukemia or lymphoma	1	0	9
8. cerebral stroke	1	0	9
9. Multiple Sclerosis	1	0	9
10. Parkinson's Disease	1	0	9

(IF NO TO ALL, GO TO PART II ON PAGE 17.)

A.1 IF YES, specify the disease mentioned above: _____

B.1 How old were you when you first started to have symptoms? **(DK=99)** ___ ___

C.1 What kind of treatment have you received for it? **(RECORD VERBATIM)**

_____

D.1 Can you tell me the name and address of the physician who (is/was) treating you for it?

_____
name of physician

_____
street

_____
city state zip

A.2 IF YES, specify the disease mentioned above.:_____

B.2 How old were you when you first started to have symptoms? (DK-99) __ __

C.2 What kind of treatment have you received for it? (RECORD VERBATIM)

_____

D.2 Can you tell me the name and address of the physician who (is/was) treating you for it?

_____
name of physician

_____
street

_____
city state zip

A.3 IF YES, specify the disease mentioned above.:_____

B.3 How old were you when you first started to have symptoms? (DK-99) __ __

C.3 What kind of treatment have you received for it? (RECORD VERBATIM)

_____

D.3 Can you tell me the name and address of the physician who (is/was) treating you for it?

_____
name of physician

_____
street

_____
city state zip

PART II

OCCUPATIONAL HISTORY

I am also interested in any jobs or employment you have had.

Q1. Have you ever been in military service?

YES.....1
NO...[GO TO Q2]....0
DK...[GO TO Q2]....9

A. Which branch were you in?

Army.....1
Navy.....2
Airforce.....3
Marines.....4
Coast Guard.....5
National Guard.....6

B. (1) When did you first enter the service?
(PROBE FOR MONTH AND YEAR, DK=01 FOR MONTH)
99 FOR YEAR)

__ __ / __ __
Month year

(2) When did you leave the service?
(PROBE FOR MONTH AND YEAR, DK=01 FOR MONTH
99 FOR YEAR)

__ __ / __ __
Month year

C. What kind of work did you do in military service? (RECORD VERBATIM)

_____

D. Where were you during that time? (PROBE FOR CITY, STATE, AND COUNTRY)

_____
city state

_____
country

E. [ASK ONLY IF R WAS IN VIETNAM -- OTHERWISE GO TO Q2]

Were you ever exposed to any defoliants such as Agent Orange?

YES.....1
NO...[GO TO Q2]...0
DK...[GO TO Q2]...9

(1) What were you exposed to? (RECORD VERBATIM)

_____

(2) How were you exposed - what were you doing at the time?
(RECORD VERBATIM)

_____

_____

Q2. What kind of jobs have you had, (besides military service?) Let's start with your earliest job and go in order until we reach your current or last job.

What kind of business or industry was that?	What did you actually do?	What was your job title?	What year did you start?	How many years did you have the job?
1.			— — — —	— —
2.			— — — —	— —
3.			— — — —	— —
4.			— — — —	— —
5.			— — — —	— —
6.			— — — —	— —
7.			— — — —	— —
8.			— — — —	— —
9.			— — — —	— —
10.			— — — —	— —

Q3. As far as you know, have you ever been exposed in any of your jobs to one or more of the following substances? Please feel free to ask me to explain if you don't know what one of these substances is. Also, if you don't know whether or not you were exposed to one of the substances, feel free to tell me.

		YES	NO	DK
a.	lead	1	0	9
b.	copper	1	0	9
c.	plastic manufacturing	1	0	9
d.	mercury	1	0	9
e.	heavy metals	1	0	9
f.	aluminium	1	0	9
g.	manganese	1	0	9
h.	nickel	1	0	9
i.	magnesium	1	0	9
j.	arsenic	1	0	9
k.	pesticides	1	0	9
l.	leather, hides or meats	1	0	9
m.	petroleum products, gas or oil	1	0	9

Q4. Have you ever worked with a jackhammer or any other kind of tool that uses compressed air?

YES.....1
NO...[GO TO Q5]....0
DK...[GO TO Q5]....9

A. What kind of tool have you used?

_____

Q5. Have you ever lived with a household pet such as a cat, dog, bird, fish or any other kind of pet?

YES.....1
NO...[GO TO Q6]....0
DK...[GO TO Q6]....9

A. For each pet you have had, can you tell me what kind of pet it was and how long you had it?

	What kind of pet did you have?...	How long did you have it? (CODE YEARS, UP TO ONE YEAR=01)
1st.	_____	__ __
2nd.	_____	__ __
3rd.	_____	__ __
4th.	_____	__ __

Q6. Have you ever worked with or close to animals, such as farm animals?

YES.....1
NO...[GO TO NEXT PG].....0
DK...[GO TO NEXT PG].....9

For each animal, please tell me what type of animal it was and how long you worked near it.

	What was the...type of animal?	How long did you work near it? (CODE YEARS, UP TO ONE YEAR=01)
1st.	_____	__ __
2nd.	_____	__ __
3rd.	_____	__ __

PART III

FAMILY HISTORY

Now I would like to ask you some questions about your family - and I will be drawing a pedigree.

In order to make sure that our information on your family is as accurate and complete as possible it might be important for us to contact one or more of your relatives. So, during this part of the interview, when I ask about your relatives, I may ask for your permission to contact a relative. If you give your permission, then I will ask you for his or her address and telephone number, and I will then send a letter or telephone this relative, describing the study and asking if he or she would like to help us.

We will not contact any of your relatives unless you give your permission for us to do so and none of the information you, or your relative gives us will be revealed to any other relative.

If the relative prefers not to cooperate, he or she is free to do so.

If you are in contact with a relative who we would like to contact, it might be helpful if you could tell the relative that we will be contacting him or her for the study.

Reproductive History of Proband

Q1. **IF RESPONDENT IS FEMALE ASK:** Have you ever been pregnant, including any miscarriages or abortions you may have had?

IF RESPONDENT IS MALE ASK: Have you ever made a woman pregnant, even if the pregnancies ended in miscarriage or abortion?

Yes.....1
No...[GO TO Q9 - PG 26].....0
DK...[GO TO Q9 - PG 26].....9

(IF R IS FEMALE AGE 50 OR OVER, GO TO Q3 ON THE NEXT PAGE)

Q2. **IF FEMALE UNDER AGE 50 ASK:** Are you pregnant now?

IF MALE ASK: Are you the father of a pregnancy in progress now?

Yes.....1
No.....0
Not sure.....9

Q3. How many pregnancies have you (had/fathered) all together, including any that may have ended in miscarriages or abortions? (CODE NUMBER DIRECTLY; IF DON'T KNOW, ASK: How many are you sure of?)

__ __

(IF ONLY ONE PREGNANCY, GO TO Q5.)

Q4. Did all of the pregnancies have the same (father/mother)?

Yes.....1
No.....[GO TO B].....0
DK.....[GO TO B].....9

A. What was (his/her) first name? _____

(GO TO Q5)

B. How many different (fathers/mothers) were there? (DK=9) __

C. ASK (1) - (2) FOR EACH FATHER/MOTHER SEPARATELY

	(1) Could you tell me the first name of the (first, second, etc.) (man/woman) you (had/fathered) a pregnancy with?	(2) How many pregnancies did you (have/father) with (him/her)?
1st	_____	__ __
2nd	_____	__ __
3rd	_____	__ __
4th	_____	__ __

Q5. Did (any of) the pregnanc(y/ies) end in abortion, miscarriage, stillbirth, or ectopic pregnancy (pregnancy in tubes)?

Yes.....1
No.....[GO TO Q6 - NEXT PG].....0
DK.....[GO TO Q6 - NEXT PG].....9

A. How many pregnancies ended in these ways? __ __

Q6. How many liveborn children have you (had/fathers), including any who may have died after birth? __ __

ASK A-H FOR EACH LIVE BIRTH SEPARATELY.

	A. What was the first name of the child who was born...?	B. If obvious, code without asking. SEX: M =1 F =2	C. What year was (he/she) born? (RECORD AGE IF YEAR UNKNOWN: DK=9999)	D. Is (he/she) still living? YES.....1 NO.....0 DK.....9 If YES or DK go to H	E. How old was (he/she) when (he/she) died? (DK=00)	F. What was the cause of (his/her) death? (RECORD VERBATIM)	G. In which (town/city/state) did (he/she) die?	H. How accurately do you feel you could tell us about any medical problem (he/she) may have had? Would you say.... VERY ACCURATELY =1 SOMEWHAT =2 A LITTLE =3 NOT ACCURATELY AT ALL =4
1st								
2nd								
3rd								
4th								
5th								
6th								
7th								
8th								
9th								
10th								

Q7. (Has/Did) any of your (child/children) ever (gone/go) to a neurologist?

YES.....1
NO...[GO TO Q8 - NEXT PG]....0
DK...[GO TO Q8 - NEXT PG]....9

How many? ___

A.1 What is the name of this (son/daughter)? (CODE BIRTH ORDER)

_____ ___ ___

B.1 What was the reason for (him/her) to visit a neurologist? (RECORD VERBATIM)

_____

_____

C.1 How old was (he/she) the first time?
(CODE AGE DIRECTLY, DK=99) ___ ___

D.1 May I have your permission to contact the neurologist to review your (son's/daughter's) _____(name) records?

YES.....1
NO...[GO TO Q8 - NEXT PG]....0

E.1 What is the name, address, and telephone number of the neurologist?

_____
name of neurologist

_____
street

_____
city state zip

A.2 What is the name of this (son/daughter)? (CODE BIRTH ORDER)

_____ ___ ___

B.2 What was the reason for (him/her) to visit a neurologist? (RECORD VERBATIM)

_____

_____

C.2 How old was (he/she) the first time?
(CODE AGE DIRECTLY, DK-99) ___ ___

D.2 May I have your permission to contact the neurologist to review your (son's/daughter's) _____(name) records?

YES.....1
NO.....[GO TO Q8].....0

E.2 What is the name, address, and telephone number of the neurologist?

_____
name of neurologist

_____
street

_____
city state zip

Q8. (Has/Did) (any of) your (child/children) ever had one or more of the following diseases?

Disease	YES	NO	DK	What (is/are) the name(s) of your (son(s)/daughter(s)?)	Birth Order	How old was (he/she) when it started?
Multiple sclerosis	1	0	9	(1)_____	___ ___	___ ___
				(2)_____	___ ___	___ ___
ALS, or amyotrophic lateral sclerosis or Lou Gehrig's Disease	1	0	9	(1)_____	___ ___	___ ___
				(2)_____	___ ___	___ ___
any kind of cancer, including leukemia or lymphoma	1	0	9	(1)_____	___ ___	___ ___
				(2)_____	___ ___	___ ___
poliomyelitis	1	0	9	(1)_____	___ ___	___ ___
				(2)_____	___ ___	___ ___
(cerebral) stroke	1	0	9	(1)_____	___ ___	___ ___
				(2)_____	___ ___	___ ___
meningitis	1	0	9	(1)_____	___ ___	___ ___
				(2)_____	___ ___	___ ___

Disease	YES	NO	DK	What (is/are) the name(s) of your (son(s)/daughter(s)?)	Birth Order	How old was (he/she) when it started?
muscular dystrophy	1	0	9	(1)_____	__ __	__ __
				(2)_____	__ __	__ __
any diseases of the muscles or brain that we haven't mentioned (specify below)	1	0	9			
(1)_____				_____	__ __	__ __
(2)_____				_____	__ __	__ __

(IF NO OR DK TO ALL OF ABOVE, GO TO Q9)

Q9. How many full brothers and full sisters do you have, including any who may have died? (A full brother or full sister has both the same natural mother and the same natural father that you have.) __ __

ASK A-H SEPARATELY FOR EACH FULL SIBLING

Q10. (Are/were) your parents related by blood?

YES.....1
NO...[GO TO Q11]...0
DK...[GO TO Q11]...9

A. What (is/was) their relationship? _____

Q11. Were your mothers parents related by blood?

YES.....1
NO...[GO TO Q12]...0
DK...[GO TO Q12]...9

A. What was their relationship? _____

Q12. Were your father's parents related by blood?

YES.....1
NO...[GO TO Q13]...0
DK...[GO TO Q13]...9

A. What was their relationship? _____

Q13.	A. What was the first name of your.....?	B. If obvious, code without asking. SEX: M =1 F =2	C. What year was (he/she) born? (RECORD AGE IF YEAR UNKNOWN: DK=9999)	D. Is (he/she) still living? YES.....1 NO.....0 DK.....9 If YES or DK go to H	E. How old was (he/she) when (he/she) died? (DK=00)	F. What was the cause of (his/her) death? (RECORD VERBATIM)	G. In which (town/city/state) did (he/she) die?	H. How accurately do you feel you could tell us about any medical problem (he/she) may have had? Would you say.... VERY ACCURATELY =1 SOMEWHAT =2 A LITTLE =3 NOT ACCURATELY AT ALL =4
Father		1						
Mother		2						
Paternal Grand-father		1						
Paternal Grand-mother		2						
Maternal Grand-father		1						
Maternal Grand-mother		2						

	A. What was the name of your mother's brother or sister who was born...?	B. If obvious, code without asking. SEX: M =1 F =2	C. What year was (he/she) born? (RECORD AGE IF YEAR UNKNOWN: DK=9999)	D. Is (he/she) still living? YES.....1 NO.....0 DK.....9 If YES or DK go to H	E. How old was (he/she) when (he/she) died? (DK=00)	F. What was the cause of (his/her) death? (RECORD VERBATIM)	G. In which (town/city/state) did (he/she) die?	H. How accurately do you feel you could tell us about any medical problem (he/she) may have had? Would you say.... VERY ACCURATELY =1 SOMEWHAT =2 A LITTLE =3 NOT ACCURATELY AT ALL =4
1st								
2nd								
3rd								
4th								
5th								
6th								
7th								
8th								
9th								
10th								

	A. What was the name of your father's brother or sister who was born...?	B. If obvious, code without asking. SEX: M =1 F =2	C. What year was (he/she) born? (RECORD AGE IF YEAR UNKNOWN; DK=9999)	D. Is (he/she) still living? YES.....1 NO.....0 DK.....9 If YES or DK go to H	E. How old was (he/she) when (he/she) died? (DK=00)	F. What was the cause of (his/her) death? (RECORD VERBATIM)	G. In which (town/city/state) did (he/she) die?	H. How accurately do you feel you could tell us about any medical problem (he/she) may have had? Would you say.... VERY ACCURATELY =1 SOMEWHAT =2 A LITTLE =3 NOT ACCURATELY AT ALL =4
1st								
2nd								
3rd								
4th								
5th								
6th								
7th								
8th								
9th								
10th								

COMPLETE RELATIVE MEDICAL HISTORY QUESTIONNAIRE PROBAND I.D.:___ ___ ___
FIRST DEGREE RELATIVES

FILL OUT ONE QUESTIONNAIRE FOR EACH RELATIVE, IN ORDER LISTED BELOW.

Now I'd like to ask you about your...

	NAME	RELATIONSHIP (CIRCLE)	BIRTH ORDER
FATHER	_____	1	0 0
MOTHER	_____	2	0 0
BROTHER/SISTER	_____	3	___
MATERNAL AUNT/UNCLE	_____	4	___
PATERNAL AUNT/UNCLE	_____	5	___

Q1. (Has/Did) your _____(relative) ever (gone/go) to a neurologist?

YES.....1
NO...[GO TO Q2 - NEXT PG]....0
DK...[GO TO Q2 - NEXT PG]....9

A. What was the reason for (him/her) to visit a neurologist? (RECORD VERBATIM)

_____

_____

B. How old was (he/she) the first time?
(CODE AGE DIRECTLY, DK-99) ___

(DO NOT ADMINISTER Q2 FOR AUNTS/UNCLES)

Q2. Has your _____(relative) ever had one or more of the following diseases?

Disease	YES	NO	DK	How old was (he/she) when it started?
Alzheimer's disease or any other type of dementia.....	1	0	9	__ __
Parkinson's disease.....	1	0	9	__ __
Multiple sclerosis.....	1	0	9	__ __
ALS, or amyotrophic lateral sclerosis or Lou Gehrig's Disease.....	1	0	9	__ __
any kind of cancer, including leukemia or lymphoma.....	1	0	9	__ __
poliomyelitis.....	1	0	9	__ __
(cerebral) stroke.....	1	0	9	__ __
meningitis.....	1	0	9	__ __
muscular dystrophy.....	1	0	9	__ __
any disease of the muscles or brain that we haven't mentioned **(SPECIFY BELOW)**....	1	0	9	__ __

_____

Q3. (Has/Did) (relative) (had/have) any of the following problems for longer than 3 months but not all his/her life?

A. (Does/Did) (he/She) have trouble remembering things like a short shopping list, or things to do, or events that happened recently?

YES.....1
NO.....0
DK.....9

B. (Does/Did) (he/she) have trouble finding (his/her) way even in a familiar surrounding?

YES.....1
NO.....0
DK.....9

C. (Does/Did) (he/she) need help to dress or eat, not due to obvious physical diability?

YES.....1
NO.....0
DK.....9

D. (Does/Did) (he/she) either have trouble remembering the exact date (and/or) year?

YES.....1
NO.....0
DK.....9

E. How old was (he/she) when these problems began?
(CODE AGE DIRECTLY; DK=99) ___ ___

Q4. (Has/Did) your _____ ever (develop/developed) a muscle weakness of any part of the body such as (his/her) arms, hands, legs, feet, or neck?

YES.....1
NO...[GO TO Q5]....0
DK...[GO TO Q5]....9

A. Which part of the body? **(RECORD VERBATIM)**

_____

B. Was this problem only on one side of the body? YES.....1
NO...[GO TO Q5]....0
DK...[GO TO Q5]....9

1. Which side was it?

_____

Q5. (Are/Were) (his/her) muscles so stiff that (he/she) (could/couldn't move (his/her) limbs smoothly?

YES.....1
NO.....0
DK.....9

Q6. (Has/Did) (he/she) (had/have) any change in (his/her) voice such as slurring of (his/her) speech or gradually growing softer?

YES.....1
NO.....0
DK.....9

Q7. (Does/Did) (he/she) have muscle twitches in (his/her) arms or legs?

YES.....1
NO.....0
DK.....9

IF NO TO Q4 THROUGH Q7, SKIP TO Q9.

Q8. About how old was (he/she) when these problems with weakness, stiffness, muscle twitches and/or (his/her) voice started? ___ ___

A. How rapidly would you say the problems got bad? Would you say...

very rapidly (within 1 year).....1
somewhat rapidly (within 2-5 years).....2
not very rapidly (more than 5 years).....3
DK.....9

Q9. (Does/Did) (he/she) sometimes have trouble getting a movement started -- such as starting to walk or getting out of a chair -- because (his/her) body (won't/wouldn't) immediately obey?

YES.....1
NO.....0
DK.....9

Q10. (Does/Did) (he/she) have uncontrollable trembling or shaking of the legs, hands, arms or head?

YES.....1
NO.....[GO TO Q11].....0
DK.....[GO TO Q11].....9

A. Which part of the body? _____

B. (Is/Was) it worse when (he/she) (is/was) moving or when (he/she) (is/was) not moving?

not moving.....1
moving.....0
DK.....9

Q11. (Does/Did) (he/she) have a tendency to "shuffle" when (he/she) (walks/walked)?

YES.....1
NO.....0
DK.....9

Q12. (Has/Did) (his/her) handwriting (gotten/get) smaller over time?

YES.....1
NO.....0
DK.....9

Q13. (Does/Did) (he/she) have an unsteady or unbalanced way of walking?

YES.....1
NO.....0
DK.....9

IF NO TO Q9 - Q13, SKIP TO Q15.

Q14. About how old was (he/she) when these problems (with (his/her) walking, handwriting or shaking began?

__ __

Q15. Has any part of _____'s body become paralyzed without getting better?

YES.....1
NO...[GO TO Q16].....0
DK...[GO TO Q16].....9

A. What parts? (RECORD VERBATIM)

_____

_____

_____

B. What was the reason? (RECORD VERBATIM)

_____

_____

(ASK ONLY IF RELATIVE HAS PROBLEMS WITH LOWER BODY, UPPER BODY, OR PARALYSIS -- OTHERWISE GO TO NEXT RELATIVE)

Q16. Has your _____(relative) seen a doctor about these problems?

YES.....1
NO.....0
DK.....9

Q17. (Has/Did) (he/she) ever (taken/take) L-dopa, Sinemet, Artane or Cogentin?

YES.....1
NO.....0
DK.....9

Q18. (Does/Did) (he/she) have any other diseases or conditions that you think are important for us to know?

YES.....1
NO...[GO TO Q19]...0
DK...[GO TO Q19]...9

A. What (is/was) the condition?

_____

_____

Q19. May I have permission to contact _____ to ask (him/her) about his/her medical history?

YES.....1
NO.....0

(IF YES, WRITE NAME AND ADDRESS ON PERSONAL IDENTIFYING INFORMATION SHEET)

Thank you very much for your time and your patience. We appreciate your cooperation very much and we hope that with your contribution this study will help us to reveal some of the causes of neurological diseases.

RELATIVE MEDICAL HISTORY
GRANDPARENTS

PROBAND I.D.:___ ___ ___

RELATIONSHIP:

Maternal.....6
Paternal.....7

Now, I'm going to ask some questions about your (mother's/father's) mother and father -- your (maternal/paternal) grandparents.

		Grandfather	Grandmother
Q1.	(Has/Did) either your grandfather or grandmother ever (gone/go) to a neurologist?		
	YES.....1		1
	NO...**[GO TO Q2 - NEXT PG]**....0		0
	DK...**[GO TO Q2 - NEXT PG]**....9		9

A. What was the reason for (him/her) to visit a neurologist? **(RECORD VERBATIM)**

Grandfather	Grandmother
_____	_____
_____	_____

		Grandfather	Grandmother
B.	How old was (he/she) the first time? **(CODE AGE DIRECTLY, DK-99)**	___ ___	___ ___

Q2. (Has/Did) either your grandfather or your grandmother ever (had/have) one or more of the following diseases?

Disease	A. Grandfather YES	NO	DK	How old was he when it started?	B. Grandmother YES	NO	DK	How old was she when it started?
Alzheimer's disease or any other type of dementia	1	0	9	__ __	1	0	9	__ __
Parkinson's disease	1	0	9	__ __	1	0	9	__ __
Multiple sclerosis	1	0	9	__ __	1	0	9	__ __
ALS, or amyotrophic lateral sclerosis or Lou Gehrig's Disease	1	0	9	__ __	1	0	9	__ __
any kind of cancer	1	0	9	__ __	1	0	9	__ __
poliomyelitis	1	0	9	__ __	1	0	9	__ __
(cerebral) stroke	1	0	9	__ __	1	0	9	__ __
meningitis	1	0	9	__ __	1	0	9	__ __
muscular dystrophy	1	0	9	__ __	1	0	9	__ __
any diseases of the muscles or brain that we haven't mentioned (specify below)	1	0	9	__ __	1	0	9	__ __

(IF NO OR DK TO ALL OF ABOVE, GO TO Q6 ON THE NEXT PAGE.)

C. GRANDFATHER

Disease	What type of treatment has he received?
(1) _____	_____
(2) _____	_____
(3) _____	_____

D. GRANDMOTHER

Disease	What type of treatment has she received?
(1) _____	_____
(2) _____	_____
(3) _____	_____

Grandfather Grandmother

Q3. (Has/Did) either your grandfather your grandmother (had/have) any of the following problems for some time but not all his/her life?

A. (Does/Did) either of them have trouble remembering things like a short shopping list, or things to do, or events that happened recently?

	Grandfather	Grandmother
YES	1	1
NO	0	0
DK	9	9

B. (Does/Did) either of them have trouble finding their way even in a familiar surrounding?

	Grandfather	Grandmother
YES	1	1
NO	0	0
DK	9	9

C. (Does/Did) either of them need help to dress or eat, not due to obvious physical disability?

	Grandfather	Grandmother
YES	1	1
NO	0	0
DK	9	9

D. (Did/Does) either have trouble remembering the exact date (and/or) year?

	Grandfather	Grandmother
YES	1	1
NO	0	0
DK	9	9

E. How old was (he/she) when these problems began?
(CODE AGE DIRECTLY; DK-99) ___ ___ ___ ___

Grandfather Grandmother

Q4. (Has/Did) either your grandfather or your grandmother ever (develop/developed) a muscle weakness of any part of the body such as (his/her) arms, hands, legs, feet, or neck?

	Grandfather	Grandmother
YES	1	1
NO....[GO TO Q5]	0	0
DK....[GO TO Q5]	9	9

A. Which part of the body? (RECORD VERBATIM)

Grandfather	Grandmother
_____	_____
_____	_____

B. Was this problem only on one side of the body?

	Grandfather	Grandmother
YES	1	1
NO....[GO TO Q5]	0	0
DK....[GO TO Q5]	9	9

1. Which side was it?

Grandfather	Grandmother
_____	_____
_____	_____

Q5. (Are/Were) either your grandfather's or your grandmother's muscles so stiff that they (can't/couldn't) move their limbs smoothly?

YES.....1	1
NO.....0	0
DK.....9	9

Q6. (Has/Did) either of them (had/have) any change in their voice such as slurring or gradually growing softer?

YES.....1	1
NO.....0	0
DK.....9	9

Q7. (Does/Did) either your grandfather or your grandmother have muscle twitches in their arms or legs?

YES.....1	1
NO.....0	0
DK.....9	9

IF NO TO Q7 THROUGH Q10, SKIP TO Q12.

Q8. About how old was your (grandfather/grandmother) when these problems with weakness, stiffness, muscle twitches and/or (his/her) voice started? ___ ___ ___ ___

A. How rapidly would you say the problems got bad? Would you say...

very rapidly (within 1 year).....1	1
somewhat rapidly (within 2-5 years).....2	2
not very rapidly (more than 5 years).....3	3
DK.....9	9

Q9. (Does/Did) either your grandfather or your grandmother sometimes have trouble getting a movement started such as starting to walk or getting out of a chair because their body (won't/wouldn't) immediately obey?

YES.....1	1
NO.....0	0
DK.....9	9

Grandfather Grandmother

Q10. (Does/Did) either of them have uncontrollable trembling or shaking of the legs, hands, arms or head?

	Grandfather	Grandmother
YES.....1	1	
NO...[GO TO Q11]..0	0	
DK...[GO to Q11]..9	9	

A. Which part of the body?

Grandfather _____ Grandmother _____

B. (Is/Was) it worse when (he/she) is not moving or when (he/she) (is/was) moving?

	Grandfather	Grandmother
not moving.....1	1	
moving.....0	0	
DK.....9	9	

Q11. (Does/Did) either your grandfather or your grandmother have a tendency to "shuffle" when (he/she) (walks/walked)?

	Grandfather	Grandmother
YES.....1	1	
NO.....0	0	
DK.....9	9	

Q12. (Has/Did) your grandfather's or your grandmother's <u>handwriting</u> (gotten/get) smaller over time?

	Grandfather	Grandmother
YES.....1	1	
NO.....0	0	
DK.....9	9	

Q13. (Does/Did) either your grandfather or your grandmother have an unsteady or unbalanced way of walking?

	Grandfather	Grandmother
YES.....1	1	
NO.....0	0	
DK.....9	9	

Q14. About how old was (he/she) when these problems (with (his/her) walking, handwriting or shaking) began?

Grandfather __ __ Grandmother __ __

Q15. Has any part of your grandfather's or your grandmother's body become paralyzed without getting better?

	Grandfather	Grandmother
YES.....1	1	
NO.....0	0	
DK.....9	9	

A. What parts? **(RECORD VERBATIM)**

Grandfather	Grandmother
_____	_____
_____	_____
_____	_____

B. What was the reason? **(RECORD VERBATIM)**

Grandfather	Grandmother
_____	_____
_____	_____

(ASK ONLY IF RELATIVE HAS PROBLEMS WITH LOWER BODY, UPPER BODY, OR PARALYSIS -- OTHERWISE GO TO NEXT RELATIVE)

Q15. Has your (grandfather/grandmother) seen a doctor about these problems?

YES.....1	1
NO.....0	0
DK.....9	9

Q16. (Has/Did) (he/she) ever (taken/take) L-dopa, Sinemet, Artane or Cogentin?

YES.....1	1
NO.....0	0
DK.....9	9

Grandfather Grandmother

Q17. **(Does/Did) either your grandfather or your grandmother have any other diseases or conditions that you think are important for us to know?**

YES	1	1
NO	0	0
DK	9	9

A. What (is/was) the condition?

Grandfather	Grandmother
_____	_____
_____	_____

IF RELATIVE SCREENED NEGATIVE ON PRECEDING QUESTIONNAIRE GO TO NEXT RELATIVE.

IF RELATIVE SCREENED POSITIVE ON PRECEDING QUESTIONNAIRE GO TO Q18 AND/OR Q19.

IF RELATIVE IS ALIVE ASK Q18, OTHERWISE GO TO Q19.

Q18. In order to make sure that our information on your family is as accurate and complete as possible, we would like to contact your (grandfather/grandmother). We will not contact any of your relatives unless you give your permission for us to do so and none of the information you or your relative gives us will be revealed to any other relative.

May I have permission to contact your (grandfather/grandmother) to ask (him/her) about (his/her) medical history?

YES	1	1
NO	0	0
NOT APPLICABLE	8	8

(IF YES, WRITE NAME AND ADDRESS ON RELATIVE IDENTIFYING INFORMATION SHEET)

IF RELATIVE IS DECEASED ASK:

Grandfather Grandmother

Q19. **May I have permission to contact the physician and/or hospital (institution, nursing home, etc.) who would have the most information on your (grandfather's/grandmother's) medical history?**

YES	1	1
NO	0	0
NOT APPLICABLE	8	8

(IF YES, WRITE NAME AND ADDRESS ON CONSENT FORM FOR RELEASE OF MEDICAL RECORDS - DECEASED SUBJECTS.)