Molecular Neurobiology

ISSN0893-7648/94/8(1): 1–13/$3.60

Spontaneous Generation of Infectious Nucleating Amyloids in the Transmissible and Nontransmissible Cerebral Amyloidoses

D. Carleton Gajdusek

National Institutes of Health, Bethesda, MD

Abstract

The unconventional viruses of the transmissible subacute spongiform encephalopathies (kuru-CJD-GSS-FFI-scrapie-BSE) are nucleants spontaneously generated from host precursor proteins altered to β-pleated sheet configuration that polymerize into insoluble infectious amyloid fibrils. The *de novo* conversion to infectious amyloids is facilitated or accelerated by many different point mutations causing amino acid changes, a stop codon, or octapeptide inserts that increase the likelihood of spontaneous conversion to infectious configuration by many orders of magnitude. Similar nucleating induction of configurational change to amyloid probably occurs in other amyloidoses of brain and in systemic amyloidoses. Thus, all amyloids, particularly so-called fibrillar amyloid enhancing factors, may be considered to be infectious scrapie-like agents. These events probably occur extracellularly, thus we are attempting to reproduce them in vitro, even from synthetic polypeptides.

Index Entries: Nucleation; infectious amyloids; spontaneous generation of infectious proteins; amyloidoses, of brain, nontransmissible; amyloidoses, of brain, transmissible; Creutzfeldt-Jacob disease; Gerstmann-Sträussler-Scheinker disease; familial fatal insomnia; scrapie; bovine spongiform encephalopathy; amyloidoses; familial amyloidotic polyneuropathy; Alzheimer's disease; protein configuration.

Introduction

In recent years, we have become aware that the unconventional or atypical slow virus diseases of kuru-Creutzfeldt-Jacob disease (CJD), Gerstmann Sträussler-Scheinker disease (GSS), or scrapie-bovine spongiform encephalopathy (BSE) are cerebral amyloidoses *(1–11)*. As with most amyloidoses, the spontaneous *de novo* generation of amyloid fibrils is under genetic control, although in many susceptible hosts, all individuals are susceptible to even a minimal intracerebral infective dose; thus, for such inoculation, there is no genetic control other than the species barrier.

Transmissible and infectious amyloidoses of brain, as with all amyloidoses, require three stages of fibrillar polymerization of the amyloid subunit into insoluble semisolid arrays or microfibrils, which coalesce or agglutinate (aggregate) into structures of diverse morphology: SAFs and kuru plaques. The first is preliminary processing of the precursor into the amyloid subunit or monomer (C-terminal truncation, N-terminal removal of the 22 amino acid signal peptide, and release from the

All correspondence and reprint requests should be addressed to: D. C. Gajdusek, Laboratory of Central Nervous System Studies, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.

inositol-stearic acid membrane anchor); the second is nucleation-induced configurational change in the subunit into a cross β-pleated configuration; and the third is the formation of oligomers or polymers—usually dimers, tetramers, octamers, or hexadecamers—which may polymerize to produce electron microscopically visible fibrils (SAFs) and coalesce into kuru plaques.

All β-pleated proteins can also form vitreous-like solid arrays that display extensive periodicity, but are not true three-dimensional crystals. They are, rather, semisolid, amorphous, vitreous, or mucilaginous pseudocrystals, thick emulsions, or gels that are insoluble precipitates in the hydrophilic media of the *milieu interieur*. On heating, drying, annealing, or aging, these may increase their hydrogen bonding and decrease their hydration to assume increasingly a β-pleated structure, even in the case of some proteins with α-helical structure *(12)*. This is the heart of amyloidology, and the basis of much of the biochemical alteration of proteins of cuisine and of aging of leather, parchment, rubber, natural fabrics, and even cartilage and the lens of the eye.

Genetic Control of Generation of Infectious Amyloids in Creutzfeldt-Jakob Disease Syndromes

Eight point mutations, each changing an amino acid, have been found to cause familial CJD or its GSS and familial fatal insomnia (FFI) variants. Most GSS families display an amino acid replacement of proline by leucine at codon 102 *(13–16)*. Two Japanese GSS families have the same proline to leucine change, but at codon 105 *(17)*. In two families with atypical GSS, there is instead a replacement of alanine by valine at codon 117 *(18,19)*. One GSS family has a codon 198 mutation replacing phenylalanine by serine *(20,21)*; and one other GSS family has a codon 217 mutation replacing glutamine by arginine *(14,22)*. The more common type of familial CJD has a codon 200 mutation that replaces glutamic acid with lysine *(13,23–26)*. This has now been found in over 60 families with over 100 cases of CJD *(27,28)*. However, in a large Finnish kindred with CJD *(29)*, there is a replacement of aspartic acid by asparagine at codon 178 *(30,31)*, and there are Dutch, French, Hungarian, English, and American CJD families also with a codon 178 point mutation: eight families in all with 97 CJD cases *(28,32–39)* (Fig. 1).

There is a Japanese family with a mutation in codon 145 that changes the codon to a stop codon. This so-called amber mutation produces a disease with a clinical course resembling Alzheimer's disease *(38)*.

Seven different insert mutations, which are insertions of additional copies of an octapeptide repeat in the region normally containing five repeating octapeptide-coding sequences between codons 51 and 90 of the precursor gene, also cause CJD in various modified clinical expressions. There are twofold and four- to ninefold octapeptide repeats in different families *(12,39–45)*. Two families with five repeats have been identified. All others have been found in only one family. Thus, we know seven different insertional mutations in eight families that are responsible for the alteration of the normal precursor protein into the infectious amyloid form (Fig. 1).

At codon 129, we have a nonpathogenic point mutation with substitution of valine for methionine, which is a silent polymorphism in the general population *(23)*. Another silent polymorphism is a point mutation at codon 117 of GCA to GCG that causes no amino acid change. Finally, we find some normal subjects carry four instead of five copies of the octapeptide normally at codon 41 to 91 *(40)*. Two amino acid changing point mutations have been found in sporadic cases of CJD in Japan *(17)*. Several other sporadic CJD cases and several kuru cases have been fully sequenced, and no mutations have been found.

Familial forms of CJD account for only about 5% of all cases. More than 90% are truly sporadic, and only about 1% have been shown to be iatrogenic from direct inoculation of the patient with infected material from a CJD patient. For all other cases, a chain of infection cannot be established, nor do they appear to be familial. At present, the best explanation for the regular incidence of sporadic nonfamilial CJD around the world is the *de novo* creation of the CJD amyloid infectious agent by a rare, spontaneous event occurring at a frequency of one per million population per annum, the surprisingly uniform worldwide incidence of CJD *(6)*. If one of the point mutations of familial CJD is present, this configurational change occurs with about a millionfold higher likelihood. One corollary of this paradigm is that the replication of the infectious amyloid caused by inoculations of a different species does not "breed true." The point mutation is not copied in the amyloid formed in the new host,

although it, in turn, is also infectious. The new CJD amyloid has the amino acid sequence of the precursor protein in the newly inoculated host. CJD from patients with the 102, 117, 178, 200, and 210 codon mutations has been transmitted to monkeys or chimpanzees, which do not carry these point mutations, nor do the infectious proteins made in these experimentally infected hosts contain those point mutations. The process of conformational change may well be an induced nucleation and homotaxic pattern-setting for crystalline or fibril growth. The further elucidation of this transformation to β-pleated insoluble, protease-resistant, and infectious configuration will require the full structural comparison of infectious and noninfectious forms of the molecule, using NMR, the synchrocyclotron, circular dichroism spectroscopy, infrared spectroscopy, and high-resolution electron microscopy *(12,46–49)*.

De novo spontaneous generation of the infectious form from the full-length CJD amyloid precursor may account for most sporadic CJD. It occurs to cause sporadic CJD as a rare stochastic event in one individual per million population base per annum (the worldwide incidence rate of CJD). In familial CJD, GSS, and FFI, these many point mutations (amino acid substitutions, a new stop codon, or octapeptide inserts) have increased the likelihood of this spontaneous configurational change about 10^6-fold.

It appears likely that a coordinate-covalent or covalent alteration in the precursor may be induced that endows the infectious nucleant with great stability *(46–49)*. We thus have reason to anticipate that the infectious form of the scrapie amyloid precursor may eventually be induced in vitro, even from synthetic polypeptides. These studies are under way, and monkeys and other animals inoculated with fibrils formed in vitro from synthetic peptides homologous to CJD pathogenic mutation-containing regions of the CJD amyloid precursor gene are under observation.

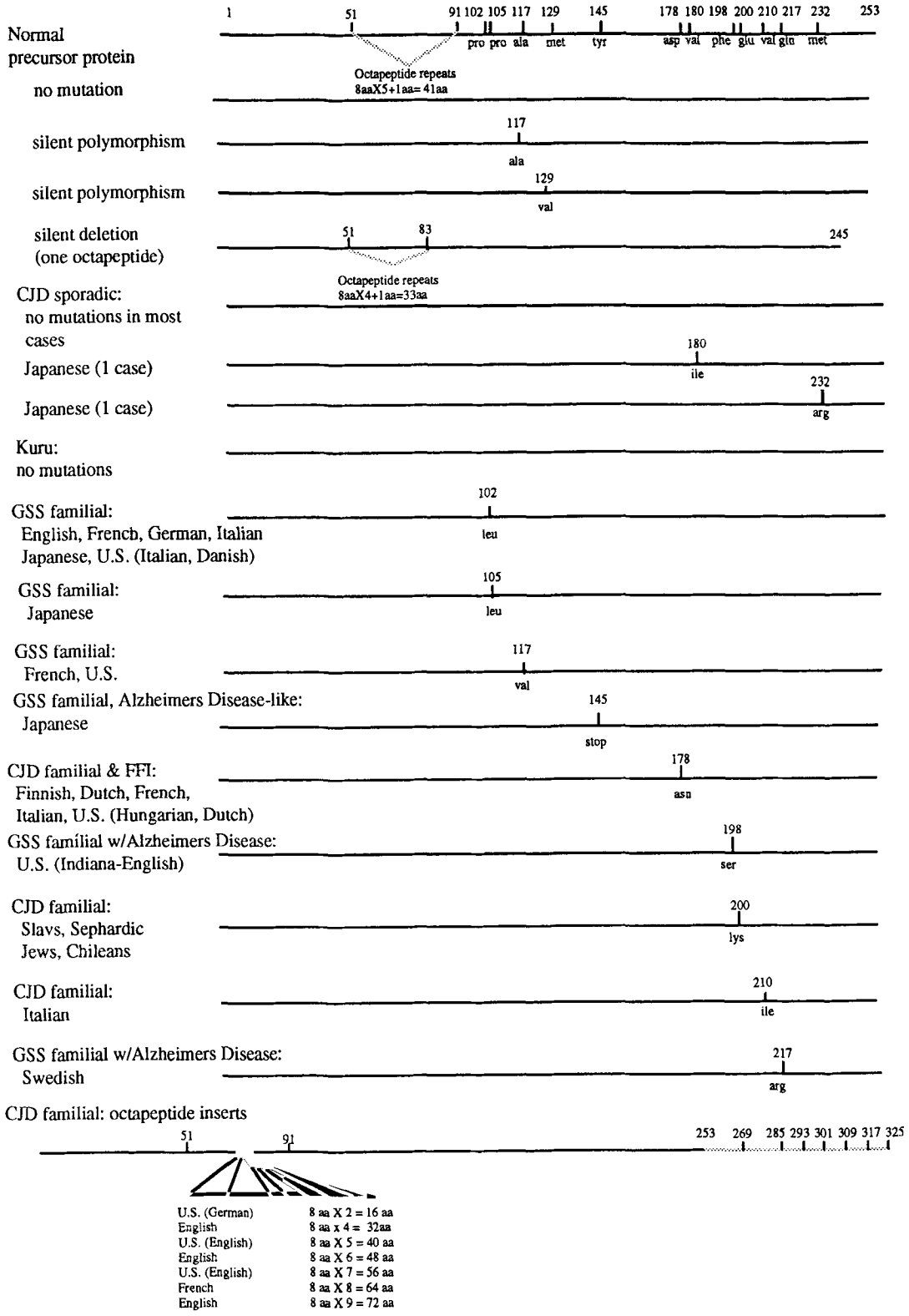
Autonucleation with induction of configurational change in the full-length infectious amyloid precursor protein is the basic process of scrapie replication. We have suggested that fibril amyloid-enhancing factor is a scrapie-like infectious agent *(5)*, operating by an analogous self-induction of configurational change, much as small fibrils of tropocollagen nucleate and pattern-set the polymerization of collagen monomers into specific fibril networks *(50)*.

Oravske Kuru

Mitrová *(52)*, who has been following the high incidence of CJD throughout Slovakia for more than two decades, has identified a new focus of CJD in high incidences in several villages of Orava in the western foothills of the High Tatra mountains near the Polish border. In 1980, she identified an unusually high incidence of CJD in the rural Lucenec area of south central Slovakia with many cases also across the border in Hungary *(51)*. During the past decade, cases have been found in increasing frequency from the most sparsely populated rural sheep-raising area of Slovakia *(52,53)*. Here an epidemic of CJD developed during the 1980s with some 30 cases occurring in patients born and reared in a dozen small villages with a total population of under 15,000. This yields an incidence over 1000 per million population per year in contrast to the worldwide incidence of one per million per year. The most intensely involved villages of Zuberec and Habovka with a total population of under 2000 have had over 20 cases of CJD in the past three years. The incidence in these villages has thus reached over 3000 times higher than that in such cities as Paris, London, New York, Sydney, Santiago, or Shanghai, or any other large city, and it is 100 times higher than that among the Sephardic Jews in Israel. Members of the same family who were 20 to 30 yr different in age became sick at nearly the same time. This suggested a common source of infection, rather than genetically determined etiology, as did the new "epidemic" of appearance of CJD in the 1980s. We suspected an accident of massive contamination of the population with sheep scrapie, which the Orava farmers have long recognized in their sheep as *klusavka*. For these reasons, at first we believed that this outbreak might not be explained genetically.

However, we have now sequenced DNA from nine of the Orava and six of the Lucenec CJD brains, and all have shown the substitution of lysine for glutamic acid at codon 200. Four of the 11 studied healthy adult first-order relatives have the same mutation *(13)*. CJD had not been known in Orava before the 1970s *(52)*. The epidemic started with a few cases in the late 1970s and developed into an escalating epidemic in the late 1980s. We have found some family members with the mutation, although they are healthy and over 70 yr of age. We are now looking for the cofactor that turns on the expression of the mutation or a factor that in the

Infectious Amyloidoses of Brain



past inhibited the post-translational configuration change of the precursor to amyloid. Thus, the new question is not what has caused the Orava outbreak—it is the codon 200 glutamic acid to lysine point mutation—but rather what has prevented its expression in previous generations so that it has accumulated as a frequent silent nonpathogenic polymorphism, only expressing itself as a pathogenic mutation in these people in the past 15 yr.

The CJD Genetic Marker for the Wandering Jews of the Diaspora

On discovering the codon 200 glutamine to lysine point mutation responsible for the high-incidence foci of CJD in both the Lucenec and Orava regions of Slovakia and widely disseminated in Slavic peoples of Eastern Europe, we screened a large number of sporadic and familial CJD brain specimens from our archive of frozen brain accumulated over the past 30 yr *(25)*. This led us to discover the mutation in Greek CJD patients who were Sephardic Jews, and we quickly found the mutation in Sephardic Jews who had some diagnosis of CJD in France from Tunisia and in Sephardic Jews with CJD in Israel, both Libyan-born and Israeli-born. Ashkenazic Jewish CJD patients did not have the codon 200 glutamic to lysine point mutation *(24)*.

We are thus now investigating other Circum-Mediterranean Sephardic Jews with CJD, with particular attention being given to the Iberian Peninsula, especially Spain, where in 1492 the Catholic monarchs, Ferdinand and Isabella, forced the quick conversion of large numbers of Sephardic Jews to Catholicism. Many of the remainder fled and gave rise to the large Sephardic Jewish group in Greece where we have found the mutation. Of those remaining in Spain and converted to Catholicism, many emigrated in the 15th century to the New World. We have now found that in Chile, the proportion of familial cases among the CJD patients is severalfold higher than elsewhere, and these patients and their family members have the 200 codon glutamine to lysine substitution *(27,28,36)*.

The CJD Point Mutation for the Large Finnish Pedigree of Familial CJD

One of the largest familial CJD pedigrees is that published by Haltia et al. in Finland *(29)*, which we have now investigated and found therein none of the point mutations previously known in familial CJD or GSS, but instead a codon 178 replacement of aspartic acid by asparagine *(30)*. We have now found this codon 178 mutation in Dutch, French, Hungarian, and American cases of familial CJD *(32,37)*. Furthermore, in Italian, French, and US families it causes a clinical variant of CJD, familial fatal insomnia (FFI).

Thus, the paradigm from the amyloidosis literature of any one of several amino acid substitutions in the precursor molecule causing an enormously increased likelihood of its post-translational conversion to an amyloid configuration and its poly-

Fig. 1. *(previous page)* Twenty-one different mutations in the gene specifying the host precursor molecule of CJD amyloid: 11 cause amino acid (aa) changes, one produces a stop codon, one a base change in the codon with no amino acid change, seven are octapeptide inserts, and one an octapeptide deletion. Eight causing an amino acid change (102, 105, 117, 178, 198, 200, 210, 211) and one a stop codon (145) are found in families of diverse ethnic origins with familial CJD and its GSS and FFI variants. Three are silent polymorphisms: the codon 129 substitution of valine for methionine (which is found in about 20% of the normal population); a base mutation in codon 117 causing no amino acid change; and an octapeptide deletion (in about 20% of the population). Seven additional mutations in families with CJD are insertions of octapeptide repeats into a region where there are already five copies of the same repeat. In a US family, there are two copies of the octapeptide (8aa × 2 = 16aa) inserted, bringing the total to seven copies; in an English family, there are four copies of the octapeptide (8aa × 4 = 36aa) inserted, bringing the total to nine copies; in a second US family, there are five copies of the octapeptide (8aa × 5 = 40aa) inserted, bringing the total to 10 copies; in another English family, there are six copies (8aa × 6 = 48aa) inserted, bringing the total to 11 copies; in a US family from England, there are seven copies (8aa × 7 = 56aa) inserted, bringing the total to 12 copies; in a French family, there are eight copies (8aa × 8 = 64aa) inserted, bringing the total to 13 copies; in another English family, there are nine copies (8aa × 9 = 72aa) inserted, bringing the total to 14 copies.

merization and deposition in the form of amyloid fibrils in various tissues has proved amazingly predictive in unraveling the pathogenesis of familial CJD and GSS, and also of β-amyloid deposition in normal aging, Alzheimer's disease, and Down's syndrome.

Varieties of Phenotypic Expression Determined by Different Mutations in the CJD Precursor Gene

The pattern of clinical disease produced by the different mutations in different families is extremely uniform in some and quite variable in other families. It ranges from classical GSS in most families with codons 102, 105, and 117 mutations to classical CJD with codon 178 and codon 200 mutations and the various insertions of 2, 4, 5, 6, 7, 8, or 9 octapeptide repeats. However, FFI is the clinical form of the disease in several families with codon 178 mutations, but the clinical from of CJD without insomnia occurs in other families with the same mutation. However, we have reported that the presence of the valine polymorphism at codon 129 together with the 178 mutation on the same chromosome causes FFI, whereas the classical CJD-type disease appears in families with the more common methionine pleomorphism at codon 129 on the codon 178 mutated chromosome.

The age of onset is earlier and the duration of clinical disease is longer in codon 178 CJD than in codon 200 CJD, and in codon 200 disease, the EEG usually shows the characteristic CJD spike and slow-wave periodicity. No such EEG change is found in the codon 178 CJD patients. Even the incubation period for clinical disease in intracerebrally inoculated squirrel, spider, and capuchin monkeys differs for codon 178 and codon 200 cases of CJD, being considerably longer in codon 200 than in codon 178 cases.

Many other distinguishing phenotypical expressions of the pathogenic mutations have now been recorded. These include an Alzheimer's disease-type clinical course in the 145 stop codon mutation with no β(A4) amyloid deposits in the amyloid plaques. On the other hand, the codons 198 and 217 have produced an Alzheimer's disease-like clinical course and also a combined CJD and Alzheimer's disease-type neuropathology with both the CJD amyloid and the β(A4) amyloid plaques.

Transthyretin Amyloidoses of Familial Amyloidotic Polyneuropathy (FAP) as a Paradigm for the Genetic Control of Transmissible and Nontransmissible Brain Amyloidoses

Of most pertinence to our problem of the unconventional viruses, which are infectious amyloids, have been the transthyretin amyloidoses of familial amyloidotic polyneuropathies (FAP) *(7,8,10,54)*. Patients are members of several hundred families scattered around the world in which the disease is an autosomal dominant trait. The onset of the clinical disease may occur at different ages and leads to the destruction of peripheral nerves by progressive deposition of amyloid in the perineurium. The human transthyretin gene has been cloned, and its full sequence of 6.9 kb composed of four exons and three introns is known. Its encoding gene is located on chromosome 18 *(55,56)*. Transthyretin in its pure and crystalline form is a soluble prealbumin of 14-kDa mol wt with 127 amino acids. Its secondary, tertiary, and quaternary structures have been determined by X-ray crystallography. It is a symmetrical tetramer of 55 kDa made of four subunits showing extensive β-pleated sheet structure *(57–60)*. Thus, it is amyloidogenic by structural chemical considerations.

Members of different affected families have a mutation resulting in a one amino acid substitution in the precursor that increases the statistical mechanical likelihood of the molecule falling into the amyloid conformation by a factor of about 10^4–10^6. There is no one specific mutation causing the disease in all families. Thus, in over 100 investigated families, more than 30 different mutations have been detected (Fig. 2). The transthyretin amyloid may be deposited in the anterior chamber of the eye to cause familial amyloidotic blindness, in the heart to cause amyloidotic cardiopathy, or asymptomatically in the intestinal wall, as well as around peripheral nerves to cause FAP. Different amino acid changing mutations cause different clinical pictures ranging from disease in which several different organs are targeted by amyloid deposition to pure single organ-targeted amyloidosis. The FAP is

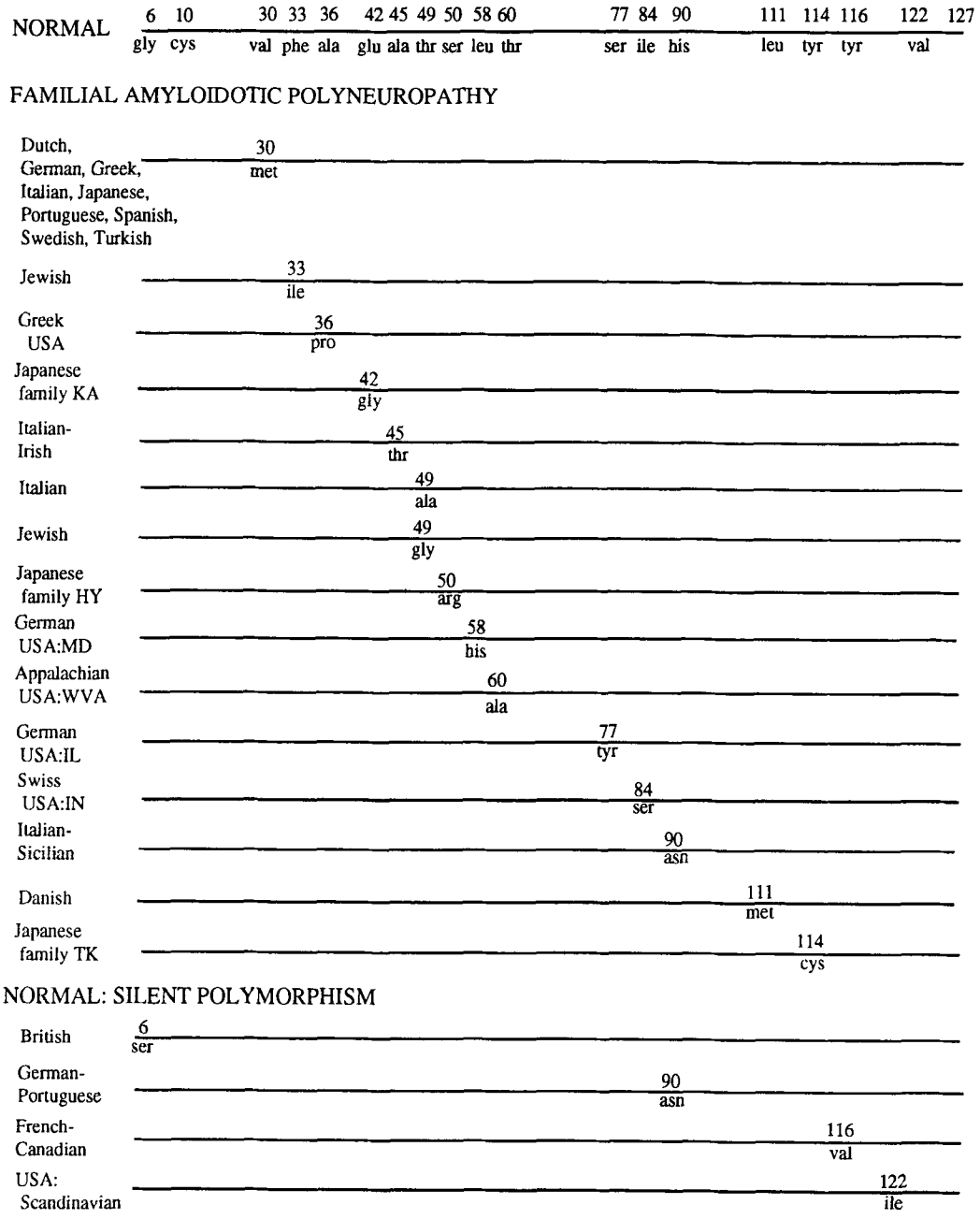


Fig. 2. Sixteen different amino acid substitutions caused by point mutations in the gene specifying the transthyretin prealbumin precursor molecule in over 20 families of various ethnic origins are shown. Four of these families are normal without FAP, and the mutation is a silent, nonpathogenic polymorphism in these. On codon 49 are two different amino acid substitutions, in a Jewish and Italian family, respectively. Codon 90 (asparagine replaces histamine) mutation has apparently caused FAP in the Italian Sicilian family, but not in the German, Portuguese families. There are over 20 additional such point mutations reported in the recent literature as responsible for different symphonies of pleomorphic clinical expression of familial amyloidotic polyneuropathy, familial amyloidotic blindness, and familial amyloidotic cardiopathy.

thus caused by precipitation of amyloid formed from the transthyretin precursor, with any of a set of point mutations each causing a single amino acid replacement that increases the likelihood of amyloid formation. This amyloid is not a replicating infectious molecule. Without one of these point mutations, it is difficult to change the transthyretin polypeptide by concentration and nucleation into the amyloid configuration. With these single amino acid substitutions, amyloid formation occurs spontaneously as a much more likely stochastic event, even extracellularly and in vitro.

In spite of the amyloidogenicity of the normal transthyretin by structural and chemical considerations, spontaneous amyloid formation does not occur in the absence of a facilitating mutation causing an amino acid substitution until the ninth decade, with the rare sporadic appearance of senile cardiac decompensation with cardiac amyloidosis. In these patients in their 80s, the full-length molecule without any mutation is the amyloid subunit. There are also several silent polymorphisms in the population with point mutations causing nonpathogenic single amino acid substitutions (Fig. 2). The codon 30 point mutation with proline replaced by methionine has been expressed in transgenic mice that develop deposits of human amyloid containing the methionine-30 mutation similar to depositions in FAP, but also in the intestine and other tissue and they pass this trait to their offspring *(61,62)*.

Analogies with Transmissible Dementias (CJD, GSS) Suggest that Transthyretin Amyloidoses of FAP and Other Amyloidoses May Also Be Transmissible and Infectious

The close parallel between multiple amino acid changing mutations, each enormously facilitating the conversion to amyloid of the precursor protein, which usually fails to fall spontaneously into amyloid configuration, except as a rare event (one per million population per annum, the worldwide incidence of sporadic CJD) in the absence of any mutation in the precursor, and the transthyretin amyloidoses of FAP, determined by any one of many different point mutations, strongly suggests that FAP may also be transmissible. It will be difficult to demonstrate this in experimental animals since long-term observation will be essential and since subtle clinical signs, rather than flagrant, fatal disease should be expected. This will also have to be controlled by histopathological search for amyloid deposits in the perineurium and in other tissues. Amyloidoses based on other precursors may likewise be transmissible under the proper experimental conditions. This might be demonstrated using transgenic mice expressing the human precursors.

Nucleating Induction of Configurational Change in Host Precursors and Polymerization to Fibrils as a General Phenomenon in Amyloidogenesis

We have recently demonstrated spontaneous generation of congophilic amyloid fibrils using synthetic polypeptides corresponding to sequences encoded by normal and mutant familial CJD alleles in the regions of codon 178 and codon 200. The 178 mutant and normal peptides formed fibrils with distinct morphological characteristics, and differing aggregation tendencies from fibrils formed from the 200 mutant or normal peptides. The mutant peptides produce more filaments and denser masses of aggregate filaments than the unmutated peptides. Furthermore, mixtures of the normal with the mutant peptides for either codon region produce denser masses of fibrils than either peptide alone *(63)*. The amyloid deposits of FAP contain both the mutated and the unmutated molecules, the mutated having nucleated and induced the β-pleating and copolymerization of the unmutated molecules in the heterozygous patients (A. F. de Frietas, University of Oporto, personal communication).

Frangione has shown that the amyloid deposits in the vascular wall in hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA-D) contain both the codon 695 (glutamine substituted for glutamic acid) mutated amyloid β(A4) protein and the unmutated molecule with the normal β(A4) sequence. The heterozygous patients are obviously polymerizing both the amyloid β(A4) protein derived from the normal brain amyloid precursor protein

(APP) and the mutated β(A4) protein. He and his colleagues have synthesized polypeptides of the first 28 amino acids of the β(A4) protein with and without the codon 695 mutation of HCHWA-D, and studied the dynamics of fibril polymerization with each. The synthetic 28 amino acid polypeptide with the mutation forms amyloids much faster than the polypeptide without the mutation. However, if both synthetic polypeptides are placed together in solution, the mutated polypeptide accelerates amyloid fibril formation by the unmutated polypeptide. Frangione uses the phrase accelerated instructive fibrillogenesis for this nucleating phenomenon of facilitation or induction of amyloidogenesis by a different amyloid *(64)*.

We are probably seeing the same phenomenon in the conversion of insulin-associated proteins to an amyloid in insulin–amyloid deposits in the amyloidosis of late diabetes. The same amyloid induction may also be occurring in the simultaneous appearance of β(A4) amyloid and τ-amyloid in the neurofibrillary tangles of Alzheimer's disease, Down's syndrome, and normal aging brain.

Fibril Amyloid-Enhancing Factor in Experimental AA Amyloidosis May Be a Scrapie-Like Nucleating Infectious Protein

Amyloid-enhancing factor *(65–71)* is a low-molecular-weight glycoprotein found in tissues containing AA amyloid that accelerates the laying down of AA amyloid in tissues in experimentally induced AA amyloidosis in mice and hamsters, shortening the lag time to 2 d from several weeks. It apparently serves as a nucleus for fibril formation and deposition *(70)* in animals with high serum level of acute-phase reactant AA amyloid precursor produced by the nonspecific activation of inflammatory response with injections of casein, silver nitrate, or lipopolysaccharide *(72,73)*.

Fibril amyloid-enhancing factor *(70)* is an example of pattern-setting induction of amyloid formation and polymerization, which is basically a nucleation process such as is required in all fibril polymerization. Such configurational change in the host precursor protein to the β-pleated infectious amyloid form occurs in the case of the transmissible agents of kuru-CJD-GSS-scrapie-transmissible mink encephalopathy-BSE. In these infections with infectious amyloid proteins, a pattern-setting configurational change is induced in the host precursor. Thus, we suggest that the fibril amyloid-enhancing factor is a scrapie (or kuru-CJD-GSS)-like agent that induces a change in its own precursor to produce more of itself by copying its altered configuration. We have mistaken this pattern-setting nucleation for viral replication. It may be that we need to broaden our concept of a virus as have the computer virologists *(6,50)*.

Alzheimer's Disease

The universal brain amyloidoses, which everyone begins to get in old age and which is neuropathologically evident in all human brains from the ninth decade through the century, is caused by amyloid deposits formed from a 42 or 43 amino acid (aa) peptide (β[A4]) proteolytically cleaved from the 80-kDa brain amyloid precursor protein (APP). This precursor is a transmembrane protein the amyloid subunit from which extends from the center of the transmembrane region to the 15th extracellular amino acid. It is normally processed with a high rate of turnover with cleavage in the extracellular segment, thereby preventing the formation of the amyloidogenetic 42–43 aa β(A4) peptide. All metabolic interferences, environmental or genetic, with the high rate of turnover, lead to the possibility of cleavage resulting in this amyloidogenic peptide. This is the same process that, when accelerated from environmental factors or from point mutations on the precursor or on other chromosomes, specifying still unidentified proteins that must be either enzymes, chaperonins, or binding proteins, or other rate-influencing molecules, is the cause of Alzheimer's disease. In Down's syndrome, overproduction of the precursor appears to be enough to lead to formation of the β(A4) peptide.

Familial Alzheimer's disease (FAD) families with pathological mutants of the APP gene account for only 3–5% of the FAD families, whereas FAD accounts for 10–20% of all Alzheimer's disease, 80–90% of Alzheimer's disease being sporadic. The majority of FAD families with early onset appear to have a point mutation now localized on chromosome 14, whereas the late-onset FAD families appear to have a mutation less firmly localized on chromosome 19.

Figure 3 presents the mutations thus far identified on the β(A4) amyloid precursor protein (APP)

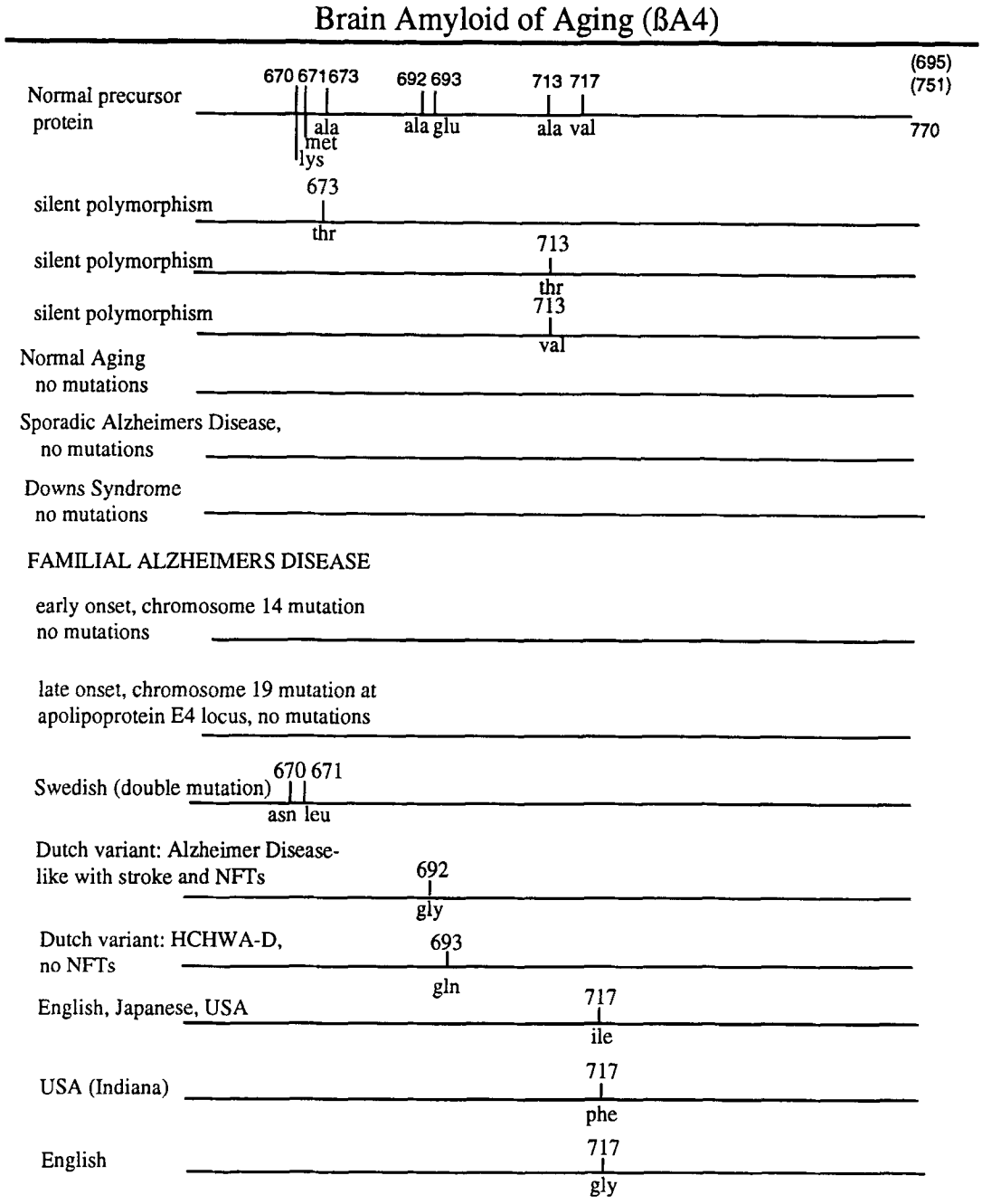


Fig. 3. Eleven point mutations on the β(A4) amyloid precursor protein of normal aging and Alzheimer's disease: three different amino acid substitutions at the same codon 717 all cause familial Alzheimer's disease (FAD) in rare British, Japanese, and US families. A double mutation at codons 670 and 671 causes FAD in a Swedish family. Two adjacent mutations on codons 692 and 693 each cause hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D) in a different Dutch family. A mutation on codon 673 and two different amino acid changes on codon 713 are silent polymorphisms. Sporadic Alzheimer's disease and Down's syndrome patients show no mutations on the β(A4) amyloid precursor protein. Only 3% of FAD have one of the five mutations causing the disease in rare families. Most of the early onset FAD families have a mutation on chromosome 14 and late-onset FAD families appear to have a mutation on chromosome 19, but neither of these are associated with any mutation on the β(A4) amyloid precursor protein gene.

and shows clearly the mounting parallel with the infectious brain amyloids of the spongiform encephalopathies (Fig. 1). Both these transmissible and nontransmissible brain amyloidoses now demonstrate close similarities to the paradigm of the transthyretin amyloidoses of familial amyloidotic polyneuropathy (Fig. 2) in which some 40 pathogenic point mutations have now been found.

The Semantic Word Wars of Slow Virologists and Amyloidologists

The amyloidologists assiduously avoid the terminology of microbiology in their discipline. We, from microbiology, have entered their field through the infectious amyloids of the subacute spongiform viral encephalopathies (SSVEs) of kuru-CJD-GSS-scrapie-BSE. Thus, we have used the term replication, and the concepts of virulence, host range, and incubation period, in describing phenomena for which they use other words and phrases. Various authors have exhausted the thesaurus to find terminology different from that used by their competing colleagues to describe the production of configurational change of host precursor proteins to β-pleated structure and the polymerization of amyloid fibrillogenesis. Such terminology includes:

Nucleation;
Induction;
Augmentation;
Enhancement;
Facilitation;
Acceleration;
Instruction; and
Heterodimer formation.

Since the unconventional and atypical viruses of SSVE (kuru-CJD-GSS-scrapie-BSE) have been identified as infectious amyloid molecules, our laboratory has slowly switched to designating them to infectious amyloids instead of unconventional viruses. Others have accepted the term prions for these agents. We prefer to draw on the important and informative paradigms of amyloidology that have directed much of our thinking over the past decade. I have facetiously pointed out that the founders of virology define a virus as an obligate parasite of submicroscopic size requiring the informational and energy systems of the host for replication: this embraces viroids, virules, virettes, virinos, nucleating agents of industrial infections, and computer viruses.

References

1. Gajdusek D. C. (1986) in *Biological Substrates of Alzheimer's Disease* (Scheibel A. B., Wechsler, A. F., and Brazier M. A. B., eds.), Academic, New York, pp. 33–54.
2. Gajdusek D. C. (1986) in *Unconventional Virus Diseases of the Central Nervous System* (Court L. A., Dormont D., and Brown P., eds.) (Proceedings of Conference, Paris, December 2–6, 1986). Commissariat a l'Energie Atomique (CEA), Service de Documentation, Fontenay-aux-Roses, France, pp. 641–659.
3. Gajdusek D. C. (1988) *J. Neuroimmunol.* **20,** 95–110.
4. Gajdusek D. C. (1988) in *Genetics and Alzheimer's Disease* (Sinet P. M., Lamour Y., and Christen Y., eds.) Proceedings of a meeting held by the Foundation IPSEN pour la Recherche Thérapeutique, Paris, March 25. Springer-Verlag, Berlin, pp. 174–176.
5. Gajdusek D. C. (1989) in *Modern Trends in Human Leukemia VIII* (Neth R., Gallo R. C., Greaves M., Gaedicke G., Gohla S., Mannweiller K., and Ritter J., eds.), Springer-Verlag, New York, pp. 481–499.
6. Gajdusek D. C. (1990) in *Virology,* 2nd ed. (Fields B. N., Knipe D. M., Chanock R. M., Hirsch M. S., Melnick J. L., Monath T. P., and Roizman B., eds.), Raven, New York, pp. 2289–2324.
7. Gajdusek D. C. (1991) in *Proceeding of Paul Ehrlich Institute Scientific Conference: Concepts in Biomedical Research.* Springer-Verlag.
8. Gajdusek D. C. (1991) in *Sub-Acute Spongiform Encephalopathies, Current Topics of Veterinary and Animal Science,* vol. 55 (Bradley R., Savey M., Marchant B. A., eds.), Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 91–114.
9. Gajdusek D. C. (1991) *Eur. J. Epidemiol.* **7,** 567–577.
10. Gajdusek D. C., Beyreuther K., Brown P., Cork L. C., Cunningham D. D., Frangione B., Gibbs C. J., Jr., Goldfarb L. G., Goldgaber D., Hsiao K. K., Koo E. H., Martin L. J., Masters C. L., Odenwald W. F., Price D. L., Prusiner S. B., Ruddle F. H., Safar J., Scangos G., Schmechel D. E., Shashikant C. S., Shlichta P. J., Sisodia S. S., Trapp B. D., Unterbeck A., Van Nostrand W. E., Violette S. M., Walker L. C., and Wirak D. (1991) *Brain Res. Rev.* **16,** 83–114.
11. Gajdusek D. C. and Gibbs C. J., Jr. (1990) in *Biomedical Advances in Aging* (Goldstein AL, ed.), Plenum, New York, pp. 3–24.
12. Safar J., Roller P. P., Ruben G. C., Gajdusek D. C., and Gibbs C. J., Jr. (1993) *Biopolymers* **33,** 1461–1476.
13. Goldgaber D., Goldfarb L. G., Brown P., Asher D. M., Brown W. T., Lin S., Teener J. W., Feinstone S. M., Rubenstein B., Kascsak R., Boellaard J. W., and Gajdusek D. C. (1989) *Exp. Neurol.* **106,** 204–206.

14. Hsiao K., Baker H. F., Crow T. J., Poulter M., Owen E., Tenwilliger J. D., Westaway D., Ott J., and Prusiner S. B. (1989) *Nature* **338,** 342–345.
15. Hsiao K. K., Doh-ura K., Kitamoto T., Tateishi J., and Prusiner S. B. (1990) *Ann. Neurol.* **26,** 137.
16. Kretzschmar H. A., Kufer P., Riethmüller G., DeArmond S., Prusiner S. B., and Schiffer D. (1992) *Neurology* **42,** 809–810.
17. Kitamoto T., Ohta M., Doh-ura K., Hitash S., Tarao Y., and Tateishi J. (1993) *Biochem. Biophys. Res. Commun.* **192,** 525–531.
18. Doh-ura K., Tateishi J., Sasaki H., Kitamoto T., and Sakaki Y. (1989) *Biochem. Biophys. Res. Commun.* **163,** 974–979.
19. Hsiao K., Cass C., Schellenberg G., Bird T., Devine-Gage E., Wisniewski M., and Prusiner S. B. (1990) *Neurology* **41,** 681–684.
20. Hsiao K., Cass C., Conneally P. M., Dloughy S. R., Hodes M. E., Farlow M. R., Ghetti B., and Prusiner S. B. (1990) *Neurobiol. Aging* **11,** 3, 302.
21. Hsiao K., Dloughy S. R., Farlow M. R., Cass C., Da Costa M., Conneally P. M., Hodes M. E., Ghetti B., and Prusiner S. B. (1992) *Nature Genetics* **1,** 68–71.
22. Dloughy S. I., Hsiao K., Farlow M. R., Foroud T., Conneally P. M., Johnson P., Prusiner S. B., Hodes M. E., and Ghetti B. (1992) *Nature Genetics* **1,** 64–67.
23. Goldfarb L. G., Brown P., Goldgaber D., Asher D., Rubenstein R., Brown W. T., Piccardo P., Kasasak J., Boellaard J. W., and Gajdusek D. C. (1990) *Exp. Neurol.* **108,** 247–250.
24. Goldfarb L. G., Brown P., Goldgaber D., Garruto R. M., Yanagihara R., Asher D. M., and Gajdusek D. C. (1990) *Lancet* **336,** 174–175.
25. Goldfarb L. G., Korczyn A. O., Brown P., Chapman J., and Gajdusek D. C. (1990) *Lancet* **336,** 637.
26. Goldfarb L. G., Mitrová E., Brown P., Toh B. H., and Gajdusek D. C. (1990) *Lancet* **336,** 514–515.
27. Brown P., Gálvez S., Goldfarb L. G., Nieto A., Cartier L., Gibbs C. J., Jr., and Gajdusek D. C. (1992) *J. Neurol. Sci.* **112,** 65–67.
28. Goldfarb L. G., Brown P., Mitrová E., Cervenáková L., Goldin L., Korczyn A. D., Chapman I., Gálvez S., Cartier L., Rubenstein R., and Gajdusek D. C. (1991) *Eur. J. Epidemiol.* **7,** 477–486.
29. Haltia M., Kovanen J., van Crevel H., Bots G. T. A. M., and Stefanko S. (1979) *J. Neurol. Sci.* **42,** 381–389.
30. Goldfarb L. G., Haltia M., Brown P., Nieto A., Kovanen J., McCombie W. R., Trapp S., and Gajdusek D. C. (1991) *Lancet* **337,** 425.
31. Haltia M., Kovanen J., Goldfarb L. G., Brown P., and Gajdusek D. C. (1991) *Eur. J. Epidemiol.* **7(5),** 494–500.
32. Nieto A., Goldfarb L. G., Brown P., Wexler P., Chodosh H. L., McCombie W. R., and Trapp S. (1991) *Lancet* **337,** 622–623.
33. Goldfarb L. G., Brown P., Haltia M., Cathala F., McCombie W. R., Kovanen J., Cervenakova L., Goldin L., Nieto A., Godec M., Asher D. M., and Gajdusek, D. C. (1992) *Ann. Neurol.* **31,3,** 274–281.
34. Brown P., Goldfarb L. G., Cathala F., Vrbovská A., Sulima M., Nieto A., Gibbs C. J., Jr., and Gajdusek D. C. (1991) *J. Neurol. Sci.* **10,** 240–246.
35. Brown P., Goldfarb L. G., and Gajdusek D. C. (1991) *Lancet* **337,** 1019–1022.
36. Brown P., Goldfarb L. G., Gibbs C. J., Jr., and Gajdusek D. C. (1991) *Eur. J. Epidemiol.* **7(5),** 469–476.
37. Brown P., Goldfarb L. G., Kovanen J., Haltia M., Cathala F., Sulima M., Gibbs C. J., Jr., and Gajdusek D. C. (1992) *Ann. Neurol.* **31,** 282–285.
38. Kitamoto T., Iizuka R., and Tateishi J. (1993) *Biochem. Biophys. Res. Commun.* **191,** 706–714.
39. Brown P., Goldfarb L. G., McCombie W. R., Nieto A., Squillacote D., Sheremata W., Little B. W., Godec M. S., Gibbs C. J., Jr., and Gajdusek D. C. (1992) *Neurology* **42,** 422–427.
40. Goldfarb L. G., Brown P., McCombie W. R., Goldgaber D., Swergold G. D., Wills P. R., Cervenáková L., Baron H., Gibbs C. J., Jr., and Gajdusek D. C. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10,926–10,930.
41. Goldfarb L. G., Brown P., Vrbovska A., Baron H., McCombie W. R., Cathala F., Gibbs C. J., Jr., and Gajdusek D. C. (1992) *J. Neurol. Sci.* **111,** 189–194.
42. Goldfarb L. G., Brown P., Little B. W., Cervenáková L., Kenney K., Gibbs C. J., Jr., Gajdusek D. C. (1993) *Neurology* **43,** 2392–2394.
43. Owen F., Poulter M., Collinge J., and Crow, T. (1990) *Am. J. Hum. Genetics* **46,** 1215–1216.
44. Owen F., Poulter M., Lofthouse R., Collinge J., Crow T. J., Risby D., Baker H. F., Ridley R. M., Hsiao K., and Prusiner S. B. (1989) *Lancet* **i,** 51–52.
45. Owen F., Poulter M., Shah T., Collinge J., Lofthaus R., Baker H., Ridley R., McVey J., and Crow T. J. (1990) *Mol. Brain Res.* **7,** 273–276.
46. Safar J., Wang W., Padgett M. P., Ceroni M., Piccardo P., Zopf D., Gajdusek D. C., and Gibbs C. J., Jr. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6373–6377.
47. Safar J. (1993) *Neurobiol. Aging,* in press.
48. Safar J., Roller P. P., Gajdusek D. C., and Gibbs C. J., Jr. (1993) *J. Bio. Chem.* 268, 20,276–20,289.
49. Safar J., Roller P. P., Gajdusek D. C., and Gibbs C. J., Jr. (1993) *Prot. Sci.* **2,** 2206–2216.
50. Guiroy D. C. and Gajdusek D. C. (1988) in *Molecular Genetic Mechanisms in Neurodegenerative Disorders* (Brown P., Bolis L., and Gajdusek D. C., eds.), *Discuss. Neurosci.* **5,** 69–73.
51. Mitrová E. (1980) in *Search for the Cause of Multiple Sclerosis and Other Chronic Disease of the Central Nervous System* (Boese, A., ed.), Weinheim Verlag Chemie, Weinheim, Germany, pp. 356–366.
52. Mitrová E. (1990) in *Unconventional Virus Diseases of the Central Nervous System* (Court L. A., Dormont D., Brown P., and Kingsbury D. T., eds.) Commissanat á l'Energie Atomique, Service de Documentation, Paris.
53. Mitrová E., Brown P. Hroncová D., Tatara M., and Zilák, J. (1991) *Eur. J. Epidemiol.* **7,** 487–489.

54. Costa P. P., de Freitas A. F., and Saraiva M. J. M., eds. (1990) *Familial Amyloidotic Poyneuropathy and Other Transthyretin Related Disorders.* Archives de Medicina, Porto, pp. 414 and xxvi.
55. Sasaki H., Yoshioka N., Takagi Y., and Sakaki Y. (1985) *Gene* **37,** 191–197.
56. Tsuzuki T., Mita S., Maede S., Araki S., and Shimada K. J. (1985) *Biol. Chem.* **260,** 12,224–12,227.
57. Blake C. C. F., Swan I. D. A., Rerat C., Berthou J., Laurent A., and Rerat B. (1971) *J. Mol. Biol.* **61,** 217–224.
58. Blake C. C. F., Geisow M. J., Swan I. D. A., Rerat C., and Rerat B. (1974) *J. Mol. Biol.* **88,** 1–12.
59. Blake C. C. F. and Oatley S. J. (1977) *Nature* **268,** 115–120.
60. Blake C. C. F., Geisow M. J., Oatley S. J., Rerat B., and Rerat C. (1978) *J. Mol. Biol.* **121,** 339–356.
61. Wakasugi S., Inomoto T., Yi S., Naito M., Ushira M., Iwanaga T., Maeda S., Araki K., Miyasaki J., Takahashi K., Shimada K., and Yamamura K. (1988) in *Amyloid and Amyloidoses* (Takashi I., Shakuro A., Fumike O., Shozo K., and Eiro T., eds.), Plenum, New York, London, pp. 393–398.
62. Yi S., Takahashi K., Tashiro F., Wakasugi S., Yamamura K., and Araki S. (1990) Abstract No. 07/5 in *Program and Abstracts of the VIth International Symposium on Amyloidosis,* Oslo, Norway, August 5–9, 1990, p. 58.
63. Goldfarb L. G., Brown P., Haltia M., Ghiso J., Frangione B., and Gajdusek D. C. (1993) *Proc. Natl. Acad. Sci. USA,* **90,** 4451–4454.
64. Frangione B., Wisniewski T., and Ghiso J. (1992) *J. Cell Biochem. Abstract Supplement* **16E,** 200.
65. Axelrad, M. A. and Kisilevsky R. (1980) in *Amyloid and Amyloidosis* (Glenner G. G., Costa P. P., and de Freitas A. F., eds.), Excerpta Medica, Amsterdam, pp. 527–533.
66. Axelrad M. A., Kisilevsky R., Wilmer J., Chen S. J., and Skinner M. (1982) *Lab. Invest.* **47,** 139–146.
67. Hol P. R., van Andel A. C. J., van Ederen A. M., Draäyer J., and Gruys E. (1985) *Br. J. Exp. Path.* **66,** 689–697.
68. Janigan D. T. (1969) *Am. J. Pathol.* **55,** 379–393.
69. Janigan D. T. and Druet R. L. (1968) *Am. J. Pathol.* **52,** 381–390.
70. Niewold T. A., Hol P. R., van Andel A. C. J., Lutz E. T. G., and Gruys E. (1987) *Lab. Invest.* **56,** 544–549.
71. Ranlov P. (1967) *Acta. Pathol. Microbiol. Scand.* **70,** 321–335.
72. Cohen A. S. (1965) *Int. Rev. Exp. Pathol.* **4,** 159–243.
73. Janigan D. T. (1965) *Am. J. Pathol.* **47,** 159–171.