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Genetic Analysis of Creutzfeldt-Jakob Disease and Related Disorders

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Genetic analysis of Creutzfeldt–Jakob disease and related disorders

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SUMMARY

Genetic studies of over 200 cases of Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker syndrome (GSS), fatal familial insomnia (FFI) and kuru have brought a reliable body of evidence that the familial forms of CJD and all known cases of GSS and FFI are linked to germline mutations in the coding region of the PRNP gene on chromosome 20, either point substitutions or expansion of the number of 24-nucleotide repeat units. Phenotypic expression of FFI and familial CJD, clinically and pathologically distinct syndromes linked to the 178Asp→Asn substitution, is dependent on a polymorphism at codon 129. Synthetic peptides homologous to several regions of PrP spontaneously form insoluble amyloid fibrils with unique morphological characteristics and polymerization tendencies. Peptides homologous to mutated regions of PrP exhibit enhanced fibrillogenic properties and, if mixed with the wild-type peptide, produce even more abundant and larger fibrous aggregates. A similar process *in vivo* may be the primary event leading to amyloid accumulation and disease.

1. INTRODUCTION

Creutzfeldt–Jakob disease (CJD) is a subacute mental and neurological disorder with a prominent dementia and movement abnormalities which typically affects middle-aged individuals and leads to death in 3–12 months after onset of symptoms. Spongiform degeneration in various parts of the brain is the characteristic neuropathological feature. The disease is randomly distributed around the world with an annual mortality rate of one per million people, and in 5–10% of cases shows an autosomal dominant pattern of inheritance. Gerstmann–Sträussler–Scheinker (GSS) syndrome is a rare familial disorder with cerebellar ataxia and massive degeneration in the cerebellum with characteristic multicentric plaques (Masters *et al.* 1981). Fatal familial insomnia (FFI) is a disease of intractable insomnia, degeneration of thalamic nuclei, with little or no spongiform change in the cortex (Medori *et al.* 1992, Manetto *et al.* 1992). Kuru, characterized by the presence of spongiform degeneration and amyloid plaques in the cerebellum and clinically expressed as ataxia, reached epidemic proportions in New Guinea in the 1950s due to transmission during cannibalistic rituals (Gajdusek 1977). Human-to-human transmission was also implicated in a series of CJD cases with transmission occurring in the setting of medical interventions (Brown *et al.* 1988, 1992*c*). Immunostaining of brain tissue sections or Western immunoblots of detergent-treated brain extracts reveal presence of PrP in spongiform encephalopathy, whether familial, sporadic or environmentally acquired. Brain suspensions of patients with spongiform encephalopathy, including the familial forms, transmit the disease to experimental primates through intracerebral inoculation, proving that these disorders are hereditary and at the same time transmissible (Brown *et al.* 1991, 1992*b*).

Major efforts have been undertaken in the last several years to elucidate the nature of these disorders. Novel molecular biology techniques have become important tools for analysis of the genetic mechanisms controlling these disorders and for studies of the infectious agent (Prusiner 1991). This review is based on molecular genetic studies of Creutzfeldt–Jakob disease and other spongiform encephalopathies conducted in this laboratory.

2. GERM-LINE MUTATIONS ASSOCIATED WITH INHERITED FORMS OF CREUTZFELDT–JAKOB DISEASE AND RELATED DISORDERS

Each patient with familial spongiform encephalopathy so far studied was shown to carry one of several mutations in the coding region of the PRNP gene. Familial CJD is associated with point substitutions at codons 178 and 200, possibly at codons 180 and 232, and an expanding 24-nucleotide repeat. GSS is associated with a different set of point mutations at codons 102, 105, 117, 198, 217, and an 8-repeat insert mutation.

The 178^{Asn} mutation was first identified by direct sequencing in two members of a Finnish CJD kindred (Goldfarb *et al.* 1991*b*) and a member of the U.S.–Dutch family (Nieto *et al.* 1991). The GAC-to-AAC

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Table 1. *Creutzfeldt–Jakob disease in families segregating the 178^{Asn} mutation*

country of origin	number of affected members	neuro-pathologically verified	experi-mentally transmitted
Finland	14	4	1
Hungary	9	5	2
Netherlands	10	4	2
Canada	6	4	0
France	14	6	1
France	3	1	0
total	56	24	6

substitution at codon 178 results in an amino acid change from aspartic acid to asparagine. By using the *Tth III* 1 restriction test for screening, we have initially identified seven CJD families with 64 affected individuals, of which 17 were shown to carry the 178^{Asn} mutation. However, a subsequent analysis demonstrated that one of the families had in fact fatal familial insomnia. Data concerning the six families with true dementing illness and spongiform encephalopathy characteristic for CJD are shown in table 1. Twenty-four patients were neuropathologically verified and six experimentally transmitted. All 15 patients available for genetic screening showed the mutation, as well as 10 of 26 first-degree relatives, but none of 83 unrelated controls. Analysis has confirmed strong linkage (lod score of 5.3) between the mutation and CJD in the Finnish and U.S.–Hungarian families. In the Finnish pedigree, of 20 individuals screened for this mutation, ten were positive and they all had CJD and died of this disease, whereas of the other ten family members who tested negative neither had CJD and five have survived beyond 60 years of age. This observation suggests that the disease penetrance is close to 100%.

The 200^{Lys} mutation was first found in siblings from a Polish family (Goldgaber *et al.* 1989), then in seemingly sporadic patients from Slovakia and Chile, and a Sephardic Jewish family from Greece (Goldfarb *et al.* 1990*a*). It was later established that patients from a CJD cluster in Slovakia (Goldfarb *et al.* 1990*c*), patients from a similar cluster in Libyan Jews living in Israel (Goldfarb *et al.* 1990*b*; Hsiao *et al.* 1991), CJD patients from families originating in Greece and Tunisia (Brown *et al.* 1991*c*), and familial CJD patients in Chile (Brown *et al.* 1992*a*), all carried the same 200^{Lys} mutation.

The codon 200 GAG to AAG change results in a substitution of glutamic acid to lysine in the encoded protein. Restriction endonuclease analysis with *Bsm* A1 and single nucleotide extension reaction were used for screening.

The current annual mortality rate of CJD in the Slovakian clusters is approximately 200 per million population per year (in some northern villages it approaches 2000 per million); the Libyan Jewish population in Israel is characterized by rates close to 100 per million per year, and the frequency of CJD in some populations in Chile is 18 per million per year. The codon 200^{Lys} mutation was detected in 54 families with 98 known CJD cases (table 2) by testing CJD patients and their first-degree relatives: 51 patients were neuropathologically verified and 16 experimentally transmitted to primates. All tested CJD patients, and 24 of 71 first-degree relatives, but none of 103 unrelated healthy control individuals coming from the same populations, had the mutation. A very strong association was demonstrated between the mutation and disease by comparing frequencies of the mutation in patients and control individuals.

Eleven mutation-carrying families emigrated from cluster areas to Western countries. From these families, 30 affected individuals belonged to the second, third, or the fourth generation after emigration, and have never revisited the country of origin. The fact that branches of these families migrating from cluster areas to other countries continue to have CJD over several generations argues against a role of local environmental factors, and supports the view that familial CJD is a primarily genetic disorder, in which the mutation is responsible for disease.

The codon 200^{Lys} mutation probably originated in Spain and was dispersed in the Middle Ages by mass migration of expelled Sephardic Jews to North African and European countries, and migration of Spanish people to Latin American countries, including Chile. Historical data suggest that Sephardic Jewish communities were established in the areas of Krakow, Vienna and Prague, surrounding Slovakia.

Unlike 178^{Asn}, the codon 200^{Lys} mutation often occurs in families with 'skipped' generations, with cases limited to a single generation, or even apparently sporadic cases. From calculations based on analysis of 65 mutation carriers the disease penetrance was found to be 0.56, i.e. presence of the 200^{Lys} mutation results in disease in approximately half of the carriers.

Owen *et al.* (1989, 1990) first reported and described six extra 24-nucleotide repeat sequences in the PRNP gene in a British family with CJD and atypical variant dementias. Goldfarb *et al.* (1991*a*) confirmed that the area of tandem 24-base pair (b.p.) repeats between codons 51 and 91 is variable. Of a total of 532 individuals screened for the number of repeat units, members of five families with CJD, a family with GSS, and a non-neurological control patient were identified

Table 2. *Creutzfeldt–Jakob disease families with the 200^{Lys} mutation*

country of origin	number of families	number of affected members	neuro-patho-logically confirmed	experi-mentally trans-mitted
Slovakia	25	41	29	4
Poland, Germany	3	10	4	2
Libya	13	16	6	1
Tunisia	2	9	3	2
Greece	2	7	3	2
Chile	6	11	4	5
US	3	4	2	0
total	54	98	51	16

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Table 3. *24-nucleotide repeats in the PRNP gene in Creutzfeldt–Jakob disease, Gerstmann–Sträussler syndrome and non-neurological control patients*

number of repeats	medical condition	order of repeat units
5	normal	R1, R2, R2, R3, R4
7	CJD	R1, R2, R2, R2a, R2a, R3, R4
9	cirrhosis	R1, R2, R2, R3, R2, R3, R2, R3, R4
10	CJD	R1, R2, R2, R3, R2, R3g, R2, R2, R3, R4
11	CJD	R1, R2, R2, R2, R3, R2, R3g, R2, R2, R3, R4
12	CJD	R1, R2, R2c, R3, R2, R3, R2, R3, R2, R3g, R3, R4
13	GSS	R1, R2, R2, R3, R2, R2, R2, R2, R2, R2, R2, R2a, R4
14	dementing illness	R1, R2, R2, R3, R2, R3g, R2a, R2, R2, R2, R3g, R2, R3, R4

regular repeats									
CCT	CAG	GGC	GGT	GGT	GGC	TGG	GGG	CAG	R1
pro	his	gly	gly	gly	gly	trp	gly	gln	
CCT	CAT		GGT	GGT	GGC	TGG	GGG	CAG	R2
pro	his		gly	gly	gly	trp	gly	gln	
CCC	CAT		GGT	GGT	GGC	TGG	GGA	CAG	R3
pro	his		gly	gly	gly	trp	gly	gln	
CCT	CAT		GGT	GGT	GGC	TGG	GGT	CAA	R4
pro	his		gly	gly	gly	trp	gly	gln	
irregular repeats									
CCT	CAT		GGT	GGT	GGC	TGG	GGA	CAG	R2a
pro	his		gly	gly	gly	trp	gln		
CCT	CAT		GGC	GGT	GGC	TGG	GGG	CAG	R2c
pro	his		gly	gly	gly	trp	gly	gln	
CCC	CAT		GGT	GGT	GGC	TGG	GGG	CAG	R3g
pro	his		gly	gly	gly	trp	gly	gln	

as having an expanded number of repeat units in this region (table 3). The non-neurological patient, who died at age 63 of advanced micronodular cirrhosis, had no family history of neurological disease, no clinical or pathological signs of spongiform encephalopathy, and brain tissue did not transmit disease to experimental primates. The PRNP coding region was completely sequenced, and nine repeats were detected. No irregular nucleotide substitutions were seen. In contrast, CJD patients from American families with 7, 10, 10 and 12 repeats, and a French GSS family with 13 repeats, all had irregular nucleotide substitutions (table 3). The reported families with 11 and 14 repeats also had irregular substitutions (Owen *et al.* 1990, 1992). Members of the same family (affected or unaffected) always had an identical number of repeats and the same irregular substitutions. The occurrence of extra coding repeats in the PRNP gene is associated with neuropathologically verified (without spongiform change in some cases) and experimentally transmitted familial CJD with an unusually early age of onset and prolonged disease.

Kitamoto *et al.* (1993) have recently identified two new mutations in four cases of apparently sporadic CJD with clinically and pathologically typical disease. One case had a valine-to-isoleucine change at codon 180, two cases had a methionine-to-arginine substitution at codon 232, and the fourth case had both mutations.

3. PHENOTYPIC EXPRESSION OF DIFFERENT MUTATIONS

The age of onset and disease duration in familial CJD varies with the type of mutation. At the time of disease onset, the patients carrying the 200^{Lys} mutation were in their fifties, patients from the 178^{Asn} families were in their midforties, and most CJD patients with an expanding number of 24-nucleotide tandem repeats were in their thirties (table 4). The disease duration had a different distribution: it was the longest in the repeat expansion patients, and increasingly shorter in the 178^{Asn} and 200^{Lys} cases (Brown 1992).

The 178^{Asn} mutation patients were distinctive in that the initial sign of disease was invariably an insidious memory loss and, during the course of illness, the triphasic periodic slow waves characteristic of other familial and sporadic CJD patients were never seen (Brown *et al.* 1992*b*).

Table 4. *Phenotypic expression of mutations associated with familial Creutzfeldt–Jakob disease*

		familial CJD		
	sporadic CJD	200^{Lys}	178^{Asn}	24-b.p. repeat expansion
age at onset/years (mean ± s.d.)	62 ± 9	55 ± 8	46 ± 7	34 ± 10
duration of illness/months (mean ± s.d.)	6 ± 8	8 ± 18	22 ± 13	84 ± 5

Table 5. *Disease transmission rate in experimental primates*

condition		number tested	number transmitted	percentage
familial	CJD	47	36	77
	GSS	9	4	44
	FFI	3	0	0
sporadic	CJD	249	225	90
iatrogenic	CJD	8	8	100
	kuru	19	18	95

The mutation at codon 178 is responsible for both, FFI and familial CJD, clinically and pathologically distinct syndromes. Very recently, it has been shown that the genotype at polymorphic codon 129 determines the phenotype: the CJD phenotype was observed with allele $178^{Asn}129^{Val}$; however, the FFI phenotype occurs with allele $178^{Asn}129^{Met}$ (Goldfarb *et al.* 1992).

Data on experimental transmission of the disease to squirrel monkeys (table 5) show that the transmission rate of familial CJD and GSS is lower than that of sporadic CJD cases (Brown *et al.* 1994).

4. MUTATIONS INCREASE AMYLOIDOGENICITY OF PrP

Amyloid of the spongiform encephalopathies is composed primarily of PrP, a host-encoded protease resistant protein (Prusiner 1991). Synthetic polypeptides corresponding to several sequences of PrP were shown to produce insoluble fibrils (Gasset *et al.* 1992; Tagliavini *et al.* 1992). Our experiments (Goldfarb *et al.* 1993) confirmed that peptides analogous to regions of PrP encoded by normal and mutant alleles at the regions of codons 178 and 200 spontaneously formed amyloid fibrils with different morphological characteristics and aggregation tendencies, and demonstrated that the mutated version of peptide is more amyloidogenic than the normal sequence. Two peptides from the area of codons 195–213 were prepared, namely the normal 200Glu GENFTETDVKMMERVVEQM and the mutant 200Lys GENFTKTDVKMMERVVEQM; two peptides from the area of codons 169–185 were normal 178Asp YSNQNNFVHDCVNITIK and mutant 178 Asn YSNQNNFVHNCVNITIK; and two peptides from the area of codons 119–137 were 129Met GAVVGGLGGYVLGSAMSRPI and 129Val GAVVGGLGGYMLGSAMSRPI.

Electron microscopic study revealed the presence of fibrils in 10 mg ml^{-1} solutions of 200Glu, 200Lys, 178Asp and 178Asn peptides. Peptide 200Glu produced long rod-like fibrils showing moderate aggregation. The number of fibrils was approximately between two and seven per grid square. The mutant 200Lys peptide produced similar fibrils, but these had a stronger tendency to aggregate and covered noticeably more grid area. A mixture of both peptides produced very abundant and large fibrillar aggregates. The normal 178Asp peptide produced shorter and narrower fibrils than the codon-200 peptides. The fibrils in the mutant (178Asn) peptide solution were morphologically similar to 178Asp, except that the

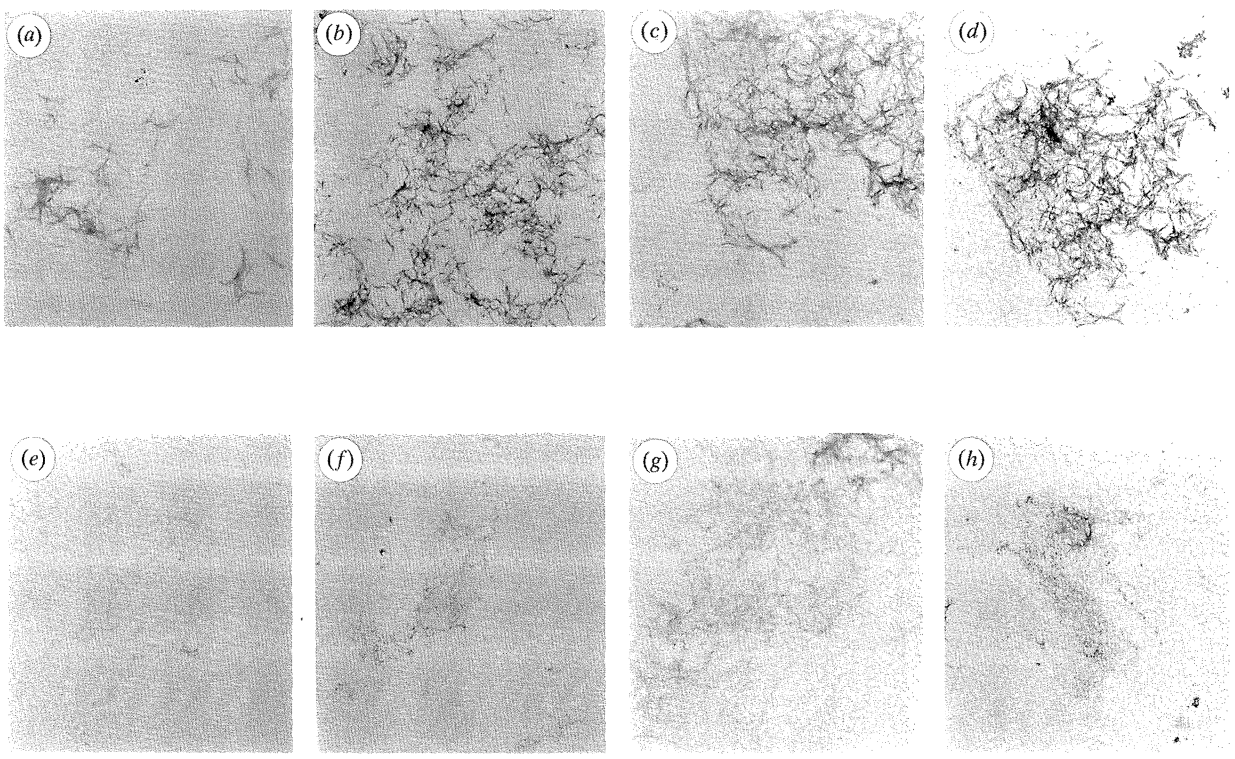

Figure 1. Electron micrographs of aggregates of distinct rod-like fibrils 8–12 nm in width, and several hundred nanometers in length, formed by normal and mutant peptides and their mixtures: (*a*) normal 200Glu, (*b*) mutant 200Lys, (*c*) and (*d*) mixture of 200Glu with 200Lys, (*e*) normal 178Asp, (*f*) mutant 178Asn, (*g*) and (*h*) mixture of 178Asp with 178Asn. Magnification ×52 500 in (*a–c*) and (*e–g*); ×26 250 in (*d*) and (*h*); 2% uranyl acetate stain.

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number of aggregates and the overall amount of material was noticeably greater. These aggregates were structurally different from those seen in the peptides of the codon 200 mutation area: the fibrils were much thinner, and the smaller and thinner aggregates could be seen on the background of many needle-like separate fibrils (figure 1). The mixed solution of 178Asp and 178Asn peptides showed a pattern of fibrils and aggregates similar to 178Asn, with larger aggregates and the amount of fibrillar material greater than in either 178Asn or 178Asp peptide solutions. Staining with Congo-red demonstrated characteristic green–yellow birefrigence confirming presence of amyloid. Neither of the peptides, 129Met or 129Val, or both mixed together, produced any fibrils. The 129 peptides mixed with the other peptides from the mutations 200 or 178 areas did not change the patterns characteristic for the 200 and 178 peptides.

The results of this study suggest that synthetic peptides homologous to several regions of PrP spontaneously form insoluble amyloid fibrils with unique morphological characteristics and polymerization tendencies. Peptides homologous to mutated regions of PrP exhibit enhanced fibrillogenic properties and, if mixed with the wild-type peptide, produced even more abundant and larger fibrous aggregates. Fibril production (an intrinsic feature of PrP, apparently independent of the function of its normal precursor protein) enhanced by mutations is viewed as the primary event leading to amyloid accumulation and disease. Inoculation of 'baby fibrils' to an intact animal may initiate the same process.

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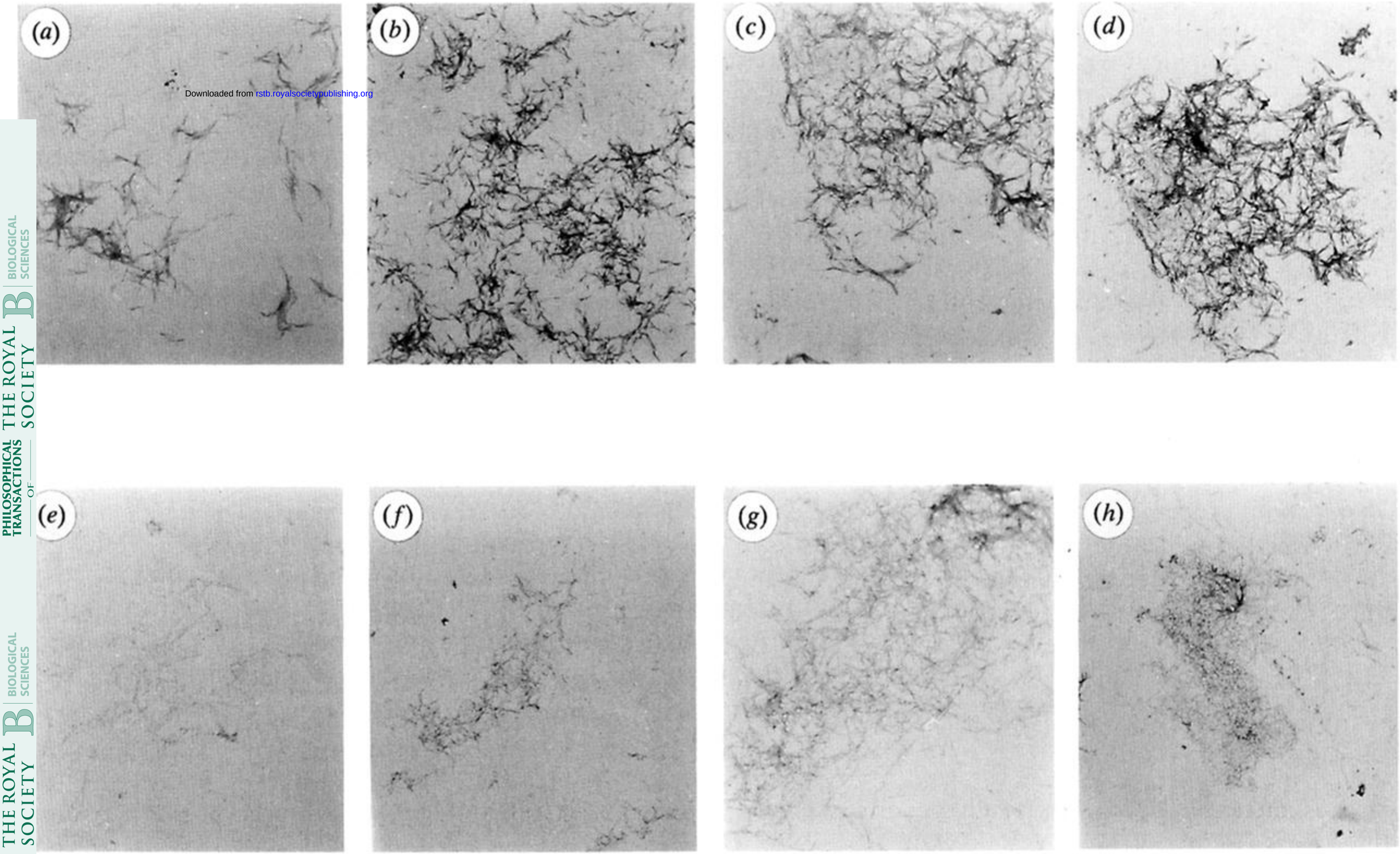

Figure 1. Electron micrographs of aggregates of distinct rod-like fibrils 8–12 nm in width, and several hundred nanometers in length, formed by normal and mutant peptides and their mixtures: (*a*) normal 200Glu, (*b*) mutant 200Lys, (*c*) and (*d*) mixture of 200Glu with 200Lys, (*e*) normal 178Asp, (*f*) mutant 178Asn, (*g*) and (*h*) mixture of 178Asp with 178Asn. Magnification ×52 500 in (*a–c*) and (*e–g*); ×26 250 in (*d*) and (*h*); 2% uranyl acetate stain.