α-Syn likely is but one of several cofactors that induce the formation of pathological tau lesions, because α-syn inclusions are not found in every brain with tau pathology. Other than glycosaminoglycans that have been observed in neurofibrillary tangles of patients with Alzheimer's, most of these cofactors remain to be identified. However, α-syn may still play a role in brains with tau pathology but no obvious α-syn pathology. In this scenario, we speculate that a limited amount of amyloidogenic α-syn fibrils can serve as seeds to initiate tau fibrillization. This residual amount of α-syn may be undetectable or may be degraded after the initiation of tau polymerization. This is consistent with the notion that tau inclusions are more resistant to degradation than α-syn inclusions are, as suggested by the greater abundance of "ghost" or extracellular fibrillary tangles than of extracellular Lewy bodies.

We conclude that α-syn induces the formation of tau fibrils and that both tau and α-syn synergistically effect the polymerization of each other into fibrillar amyloid lesions. These findings provide insights into mechanisms that underlie the formation of pathological inclusions in neurodegenerative diseases, and they suggest that therapeutic agents that directly or indirectly inhibit the formation of one form of amyloid might be effective on several of these neurodegenerative disorders.

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18. K114 is an analog of Congo red, and interactions of K114 with amyloid fibrils results in quantitative increases in fluorescence (*32*). Five μl of each sample was added to 100 μl of 50-μM K114 in 100 mM glycine, pH 8.5, and fluorescence (excitation wavelength = 380 nm, emission wavelength = 550 nm, cutoff = 530 nm) was measured with a SpectraMax Gemini fluorometer and SoftMax Pro 4.0 software.
19. Alternative splicing of exons 2 and 3 in the *tau* gene leads to the absence (0N) or the presence of inserted sequences of 29 (1N) or 58 (2N) residues in the NH_2-terminus of tau, whereas alternative splicing of exon 10 results in the presence of three (3R) or four (4R) microtubule-binding repeats of 31 or 32 residues.
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Supporting Online Material

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Materials and Methods
Figs. S1 to S4

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Balancing Selection at the Prion Protein Gene Consistent with Prehistoric Kurulike Epidemics

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Kuru is an acquired prion disease largely restricted to the Fore linguistic group of the Papua New Guinea Highlands, which was transmitted during endocannibalistic feasts. Heterozygosity for a common polymorphism in the human prion protein gene (*PRNP*) confers relative resistance to prion diseases. Elderly survivors of the kuru epidemic, who had multiple exposures at mortuary feasts, are, in marked contrast to younger unexposed Fore, predominantly *PRNP* 129 heterozygotes. Kuru imposed strong balancing selection on the Fore, essentially eliminating *PRNP* 129 homozygotes. Worldwide *PRNP* haplotype diversity and coding allele frequencies suggest that strong balancing selection at this locus occurred during the evolution of modern humans.

Prion diseases are invariably fatal, transmissible neurodegenerative conditions, which include Creutzfeldt-Jakob disease (CJD) and kuru in humans and bovine spongiform encephalopathy (BSE) and scrapie in animals. According to the protein-only hypothesis, the central molecular event in prion replication is the posttranslational recruitment of a normal neuronal glycoprotein (PrP^C) into a self-propagating conformational isomer that accumulates as aggregated material (PrP^{Sc}) (*1*). Kuru came to the attention of Western medicine in the 1950s as the affected area of the Eastern Highlands of Papua New Guinea came under Australian administrative control. The Fore and neighboring linguistic groups occupied a remote highland area that had had no direct contact with the outside world before this. It was the practice in these communities for kinship groups to consume deceased relatives at mortuary feasts. From the evidence of local oral history, this practice was not ancient among the Fore and is thought to have started around the end of the 19th century. The first remembered case of kuru was around 1920, and the disease rapidly increased in incidence. Adult women and children of both sexes were primarily affected, reflecting their selective exposure—adult males participated little at feasts. At its peak, kuru killed around 1% of the population annually, and some villages were almost devoid of

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young adult women. Kuru was the first human prion disease shown to be transmissible, by inoculation of chimpanzees with autopsy-derived brain tissue (*2*). It is hypothesized that kuru originated from consumption of an individual with sporadic CJD (*3*), a disease with a remarkably uniform worldwide incidence of around 1 per million and a lifetime risk of around 1 in 50,000. The ban on cannibalism imposed by the Australian authorities in the mid-1950s led to a decline in kuru incidence, and although rare cases still occur, these are all in older individuals and reflect the long incubation periods possible in human prion disease—kuru has not been recorded in any individual born after the late 1950s (*4*).

A coding polymorphism at codon 129 of *PRNP* is a strong susceptibility factor for human prion diseases. Methionine homozygotes make up 37% of the UK population, whereas valine homozygotes make up 12% (*5*). Homozygosity at *PRNP* codon 129 predisposes to iatrogenic and sporadic CJD (*5*, *6*). In iatrogenic CJD caused by exposure to contaminated pituitary hormones, heterozygotes have a longer mean incubation period (*7*). All cases of variant CJD to date have been methionine homozygotes (*8*), and homozygotes of either allele have an earlier age of onset for kuru (*9*). Heterozygosity at a different *PRNP* polymorphism, Glu^{219} replaced by Lys (E219K) (*10*), is also associated with resistance to sporadic CJD in Japan (*11*). Heterozygosity is thought to confer resistance to prion disease by inhibiting homologous protein-protein interactions (*5*).

Blood was obtained from Fore women aged 50 years or above. All had a history of multiple exposure to mortuary feasts. Twenty-three out of 30 women over the age of 50 were heterozygotes at codon 129, a finding that is significant (Fisher's exact test, $P = 0.01$) compared with the genotypes of the unexposed Fore population, which are in Hardy-Weinberg equilibrium ($n = 140$). Two large samples of elderly Europeans have also displayed Hardy-Weinberg equilibrium, which suggests that this age effect is local to the Fore (*12*, *13*). The age of onset of kuru in homozygotes of either allele at *PRNP* codon 129 has been estimated around 19 years, but over 30 years for heterozygotes (*9*). Thus the marked survival advantage for codon 129 heterozygotes provides a powerful basis for selection pressure in the Fore. This finding led us to examine for other evidence of balancing selection at *PRNP* in Papua New Guinea (PNG) and worldwide.

The primary sequence of the prion protein is highly conserved between primates (*14*). Methionine is the ancestral amino acid at codon 129 (*14*). Sequencing and genotyping of more than 2000 chromosomes in populations selected to represent worldwide genetic diversity were used to determine which *PRNP* nucleotide changes were intermediate polymorphisms (defined as a frequency >0.05 in at least one population studied). M129V was polymorphic in all world populations with a reducing cline toward East Asia; the lowest frequency was found in Japan (0.01). The E219K polymorphism was found in Japan and other populations in the Indian subcontinent and East Asia. One of these two established prion disease–resistance polymorphisms was found in every population studied. It is unusual that, considering the reducing cline of 129V in East Asia, the Fore population have the highest polymorphic frequency of 129V in the world (0.55), the only population for which 129V is

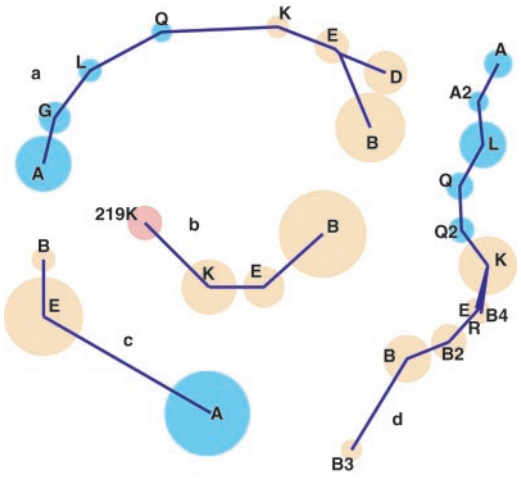

Fig. 1. Examples of predicted haplotype genealogy from four populations: (**a**) European, (**b**) Japanese, (**c**) Fore, and (**d**) African. Haplotypes are colored in orange (129M), blue (129V), or pink (129M, 219K) (*16*). Note the linear structure, loss of diversity in the Fore, and the location of 129V and 219K at extremes of all genealogies. Results are similar using maximum likelihood, parsimony, or distance methods. For method details, see SOM text, note 4.

Table 1. Frequency of human *PRNP* open reading frame polymorphisms in various world populations. *n* = number of individuals genotyped for M129V, A117A and the 1–octapeptide repeat deletion (1-OPRD) polymorphism by PCR and restriction digestion. UP, Uttar Pradesh; CEPH, Centre d'Etude du Polymorphisme Humain. Dash indicates no data. For sequencing details, see SOM text, note 3.

Population	*n*	Frequency of human *PRNP* polymorphisms (%)					
		M129V	E219K	N171S	G142S	1-OPRD	A117A
African							
Yemeni Hadramout	15	27	0	0	6	0	3
Yemeni Sena	22	32	0	0	0	2	5
Cameroon	39	35	0	8	0	-	9
Jamaican	100	32	0	5	3	30	0
South Asian							
Sri Lankans	35	23	13	0	0	0	1
Non-UP Indians	88	21	5	0	0	2	2
UP Indians	64	28	6	0	0	0	3
East Asian							
Japanese	36	1	7	0	0	-	0
Taiwan (5 populations)	70	3	3	0	0	0	0
Pacific							
PNG (Madang)	83	30	1	0	0	0	0
PNG (Fore)	48	55	0	0	0	0	0
Fiji (Taveuni)	10	5	-	-	-	5	0
Fiji (others)	18	17	-	-	-	3	0
Vanuatu (Port Olry)	33	5	13	0	0	8	0
Vanuatu (Maewo)	32	17	8	0	0	13	0
Tonga	22	14	7	0	0	0	0
European							
Turkish	61	48	0	0	0	3	4
CEPH parents	122	38	0	0	0	1	3
Georgian Jews	74	26	0	0	0	0	3
South American							
Columbian	148	41	0	0	0	3	3

Table 2. Calculated values for Tajima's statistic in four world populations. These are shown together with their *P* values under the assumption of a standard, neutral, constant size evolutionary model.

Statistic	CEPH	UK	Africa	Japan	PNG
Tajima *D*	3.799	3.433	2.200	1.148	2.982
P	$<10^{-3}$	0.004	0.03	0.168	0.01

the most frequent allele. In total, we found only six intermediate frequency polymorphisms in our screen: five coding changes, M129V, E219K, N171S, G142S, 1-octapeptide repeat deletion, but only one intermediate frequency silent change at Ala^{117}, A117A (Table 1) (*9*).

The McDonald and Kreitman (MK) test compares the number of silent and coding nucleotide changes between two species with polymorphisms within a species in a 2 by 2 contingency table (*15*). *PRNP* does not appear to be a rapidly evolving gene as there are only four noncoding and two coding changes between chimpanzee and human. When a comparison is made between humans and more divergent species, such as other great apes, all New and all Old World monkeys, the MK test suggests a significant departure from neutral evolution with an excess of human coding polymorphism. (The ratio of noncoding to coding polymorphisms in humans is 1:5; the ratios in other species and the associated *P* values for the comparison with the human ratio are gibbon 13:2, $P = 0.0055$; Rhesus monkey 23:9, $P = 0.0185$; spider monkey 27:7, $P = 0.006$; *P* values were calculated by using Fisher's exact test).

A detailed linkage disequilibrium single-nucleotide polymorphism (SNP) map of the *PRNP* locus was generated in the European population (*16*). The region is block-like in structure, and the *PRNP* gene is found within a single haplotype block extending over 30 kb. Haplotype diversity was characterized in four world populations (European, African, Japanese, and Fore). The most parsimonious explanation for the haplotype diversity worldwide requires only a single methionine-valine mutation. In terms of haplotype genealogy, the effect of balancing selection is to produce a gene tree with two long deep branches. The European, African, and Fore-speaking PNG population are characterized by highly distinct divergent clades associated with different alleles at codon 129 (Fig. 1), which suggests that codon 129 is an ancient polymorphism. Within the *PRNP* haplotype block, 4.3 kb was sequenced in a chimpanzee to compare nucleotide divergences between human and chimpanzee: 53 divergent nucleotides were found (1.2%). For the same 4.3 kb in human, there were five divergent nucleotides between methionine and valine clades (0.12%), which was similar to the methionine-valine clade divergence across 13 kb of the *PRNP* intron (0.10%).

Old balancing selection is characterized by a deep split in the genealogy, which leaves a particular signature on the nucleotide diversity of tightly linked polymorphisms: an excess of highly polymorphic sites and a deficit of low-frequency polymorphisms when compared with neutrally evolving sites (*17*). Tajima's *D* statistic allows us to assess this quantitatively. An excess of old polymorphisms will result in large positive values of the statistic and is indicative of population structure and/or balancing selection. The *D* values calculated in five different population samples and the corresponding *P* values (Table 2) show that apart from Japan, all populations have large positive *D* values that are significantly different from the neutral null-hypothesis. The *P* values were calculated through

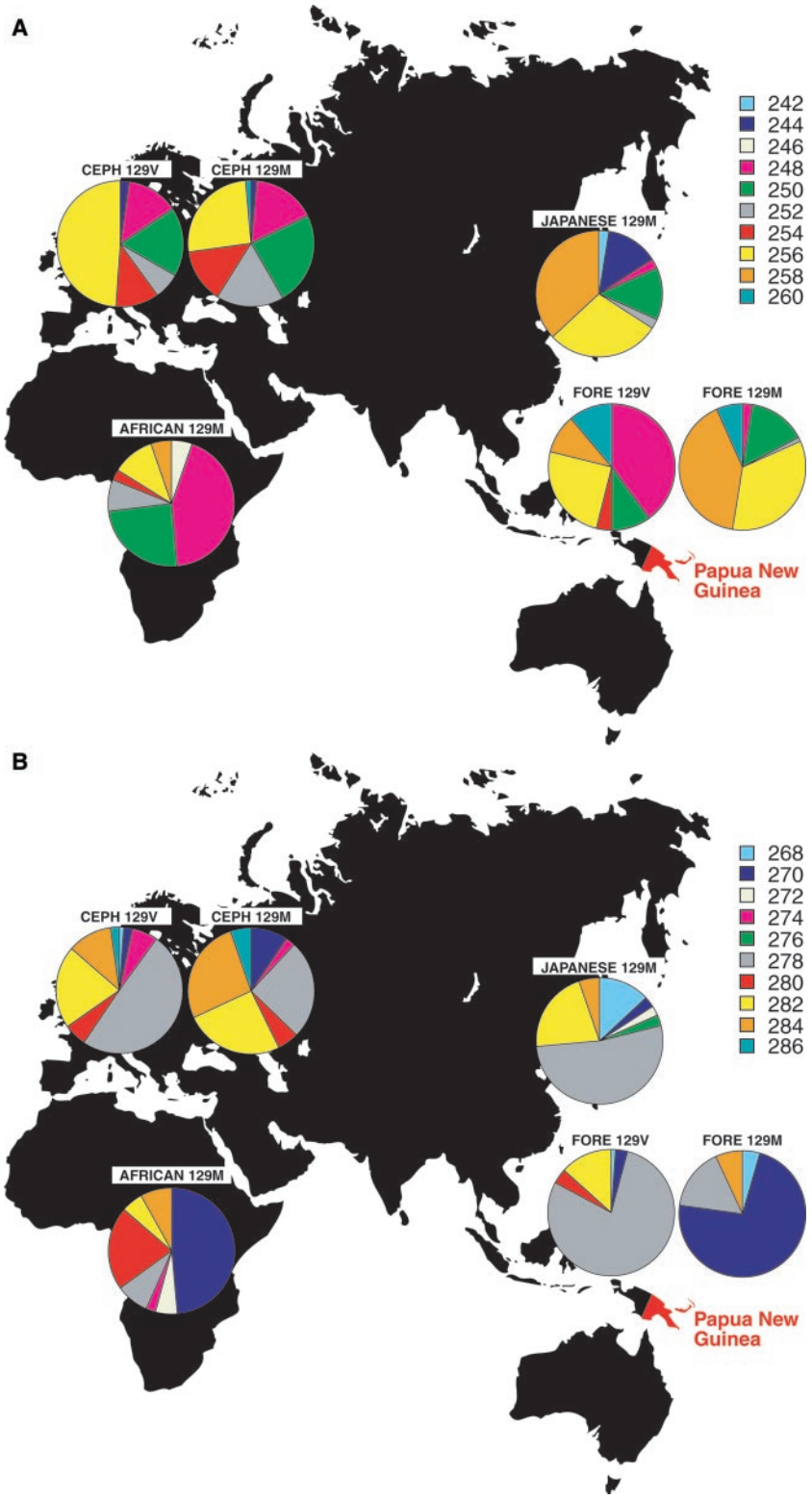

Fig. 2. Allele frequencies at microsatellites tightly linked to *PRNP*. Allele frequency at microsatellites in four populations: (**A**) microsatellite 53, which is 24 kb downstream of codon 129, and (**B**) microsatellite 108, which is 30 kb upstream of codon 129. Note the marked difference between PNG 129M and 129V when compared with CEPH 129M and 129V, although this is less striking for 53 than for 108. Note also the reduced diversity of alleles in PNG compared with other populations. See SOM text, note 5.

simulations assuming a neutrally evolving constant size and unstructured population. The real demography of the human population is, of course, very different from these assumptions. Empirical distributions of Tajima's *D* statistic are now available from a study of 313 genes (*18*). The empirical distribution was determined by using a mixed panel and differs markedly from the theoretical values with most *D* values being negative [mean, –0.973; confidence interval (95% CI): (–2.00, 0.955); 99% CI: (–2.12, 1.68); minimum, –2.32; maximum, 1.64). All populations are significant at the empirical 99% level. We note that the population sample used by Stephens (*18*) is geographically more inclusive than the populations considered here; because population structure will generally lead to more positive values of Tajima's *D* statistic, we expect the comparison with the empirical values to be conservative. Thus, on the level of background population expansion (corresponding to large negative values of *D*) that seems to have shaped much of human genetic variation, *PRNP* appears exceptional, and our results are in agreement with what would be expected under balancing selection.

If we plot the distribution of pairwise differences between M and V lineages and within M and V lineages we observe that, in European, African, and PNG populations, the major contributions to the overall pairwise difference comes from the between-lineage contribution. The distribution within the M and V lineages considered on their own is centered at low values, comparisons between M and V lineages

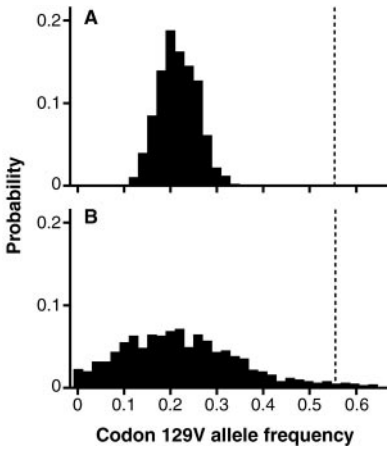

Fig. 3. Probability distribution resulting from a simulation of the effect of a population bottleneck on the frequency of the M129V allele. The model assumes a very severe recent bottleneck that reduced the population size to 1/300th of the initial size. The starting 129V frequency used was the Centre d'Etude du Polymorphisme Humain (CEPH) population frequency. (**A**) Bottleneck for one generation, (**B**) bottleneck for 10 generations. The use of the African or Japanese 129V frequency makes the probability of attaining the present day PNG (Fore) 129V frequency, shown by the dashed vertical line, even smaller. See SOM text, note 2.

are significantly different from zero and are centered around moderate to high values (fig. S1). Thus the M and V lineages are highly divergent within the *PRNP* genealogy, consistent with their being under old balancing selection, as indicated by Tajima's *D*. Age estimation of the most recent common ancestor of the M and V lineages suggests a genealogical depth of about 500,000 years [supporting online material text (SOM, note 1)].

Microsatellite and SNP diversity was examined at sites outside the *PRNP* haplotype block, ~30 kb upstream and downstream of codon 129, in the Fore and European populations. In the European population, there are significant differences between allele frequencies at two microsatellites and two SNPs on M and V chromosomes. The findings in the Fore are more dramatic with extensive linkage disequilibrium (LD) between codon 129 alleles and the same microsatellites; allele diversity is also reduced (Fig. 2, A and B). More generally, average gene diversity as measured by the heterozygosity is also reduced significantly compared with other populations from around the world (and including PNG coastal populations). All of this points to a major bottleneck in the recent history of the Fore. However, simulations suggest that a simple demographic event is highly unlikely to be able to lead to the observed distribution of M/V allele distribution at codon 129 (Fig. 3, A and B; SOM text, note 2).

Thus, kuru in the Fore imposed an exceptionally strong balancing selection on the prion locus, and global patterns of diversity in the same gene indicate historical balancing selection. What might have provided the selection pressure at *PRNP* around the world? The well-established examples of balancing selection are the major histocompatibility complex–human leukocyte antigen (MHC-HLA) locus (*19*), the β-hemoglobin locus, and more recently, the glucose-6-phosphate dehydrogenase (G6PD) locus (*20*), related to resistance to common infectious disease. Although a role for the prion protein in the defense against common conventional infectious agents cannot be ruled out, transgenic animals that have the prion protein gene ablated do not obviously have impaired immunity (*21*). Alternatively, we propose that acquired prion disease may have provided the necessary selective pressure worldwide, as it apparently has in PNG. The source of an acquired prion disease that might provide widespread and significant selection pressure is open to speculation. A prehistoric endemic animal prion disease that was able to cross the transmission barrier to carnivorous humans is a possibility. However, there is now strong evidence for widespread cannibalistic practices in many prehistoric populations, for example, telltale scratches and burn marks on Neanderthal bones (*22*) and biochemical analysis of fossilized human feces (*23*). For kuru, it has been supposed that a rare individual with sporadic CJD was consumed and that, subsequently, prion disease was amplified by the systematic and habitual practice of consumption of the dead at mortuary feasts, leading to recycling of prions in the population. A similar argument has been put forward for the origins of the BSE epidemic in the UK. With the recognition that prion incubation times can exceed a normal lifetime (*24*) and with increased awareness of subclinical prion disease (*25*), it may not be necessary to postulate the consumption of a rare sporadic CJD patient; more prevalent pre- or subclinically infected individuals may be responsible. Although the prion gene could have been subject to other unknown forms of selection, available evidence appears consistent with the explanation that repeated episodes of endocannibalism-related prion disease epidemics in ancient human populations made coding heterozygosity at *PRNP* a significant selective advantage leading to the signature of balancing selection observed today.

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