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A Novel Protective Prion Protein Variant that Colocalizes with Kuru Exposure

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ABSTRACT

BACKGROUND

Kuru is a devastating epidemic prion disease that affected a highly restricted geographic area of the Papua New Guinea highlands; at its peak, it predominantly affected adult women and children of both sexes. Its incidence has steadily declined since the cessation of its route of transmission, endocannibalism.

METHODS

We performed genetic and selected clinical and genealogic assessments of more than 3000 persons from Eastern Highland populations, including 709 who participated in cannibalistic mortuary feasts, 152 of whom subsequently died of kuru.

RESULTS

Persons who were exposed to kuru and survived the epidemic in Papua New Guinea are predominantly heterozygotes at the known resistance factor at codon 129 of the prion protein gene (PRNP). We now report a novel PRNP variant — G127V — that was found exclusively in people who lived in the region in which kuru was prevalent and that was present in half of the otherwise susceptible women from the region of highest exposure who were homozygous for methionine at PRNP codon 129. Although this allele is common in the area with the highest incidence of kuru, it is not found in patients with kuru and in unexposed population groups worldwide. Genealogic analysis reveals a significantly lower incidence of kuru in pedigrees that harbor the protective allele than in geographically matched control families.

CONCLUSIONS

The 127V polymorphism is an acquired prion disease resistance factor selected during the kuru epidemic, rather than a pathogenic mutation that could have triggered the kuru epidemic. Variants at codons 127 and 129 of PRNP demonstrate the population genetic response to an epidemic of prion disease and represent a powerful episode of recent selection in humans.
Prion diseases are fatal, transmissible neurodegenerative conditions that include Creutzfeldt–Jakob disease in humans and bovine spongiform encephalopathy in animals. Kuru is a prion disease that had a dramatic impact on the Fore linguistic group of the Eastern Highlands Province of Papua New Guinea and that provides our major experience with epidemic prion disease in humans. It has assumed new relevance because of the occurrence of variant Creutzfeldt–Jakob disease, which is linked to epizootic bovine spongiform encephalopathy, to which there has been a wide population exposure through diet in the United Kingdom and other countries. It was the practice in the Fore society for kinship groups to consume deceased relatives at mortuary feasts, a practice that resulted in human-to-human prion transmission; male members of the Fore group who were older than 6 to 8 years of age participated little, if at all, in these feasts, with the result that kuru at its peak predominantly affected women and children. Kuru is associated with prolonged incubation periods that may exceed five decades.

Kuru was restricted to the Fore linguistic groups and their immediate neighbors with whom they intermarried. As recorded in oral history, the first cases appeared in the early 20th century, and thereafter the number of cases increased in incidence. A peak annual mortality of more than 2% was recorded in some Fore villages. Some villages became largely devoid of young women. Kuru has progressively disappeared from the younger Fore population, consistent with the cessation of endocannibalism in the late 1950s.

Prion diseases are associated with an accumulation of a disease-related isoform of host-encoded prion protein (PrP) through a posttranslational process involving conformational change and aggregation. According to the “protein-only” hypothesis, an abnormal PrP isoform is the principal, and possibly the sole, constituent of the transmissible agent or prion. A common coding polymorphism at codon 129 of the prion protein gene (PRNP), where either methionine (M) or valine (V) may be encoded, is a strong susceptibility factor for human prion diseases. Codon 129 heterozygosity is protective against the development of iatrogenic and sporadic Creutzfeldt–Jakob disease and kuru; all clinical cases of variant Creutzfeldt–Jakob disease to date have been in MM homozygotes. Heterozygosity at a different PRNP polymorphism, E219K, is also associated with resistance to sporadic Creutzfeldt–Jakob disease among persons in Japan but is not known to modulate acquired prion disease. Heterozygosity is thought to confer resistance to prion disease by inhibiting homologous protein–protein interactions, although PrP residue 129 also restricts the propagation of particular prion strains through conformational selection.

STUDY DESIGN
We obtained genealogic information and venous blood samples from patients with kuru and, when possible, from healthy family and community members, some of whom had participated in multiple mortuary feasts. The investigators who obtained genealogic information were unaware of the participants’ genetic data. For each village community, we calculated an exposure index, which was defined as the total number of deaths from kuru per village divided by the estimated village population in 1958 times 1000. Elderly women exposed to kuru were defined as women older than 50 years of age in 2000 who lived in a kuru-exposed region.

The clinical and laboratory studies were approved by the Medical Research Advisory Committee of the Government of Papua New Guinea and by the local research ethics committee of the Institute of Neurology and National Hospital for Neurology and Neurosurgery in London. Full participation of the communities involved was established and maintained through discussions with village leaders, communities, families, and individual participants. The field studies followed the principles and practices of the Papua New Guinea Institute of Medical Research, which included obtaining individual oral consent from all participants before any samples were obtained.

GENOTYPING AND GENETIC ANALYSIS
DNA from degraded archival serum samples of persons with kuru was isolated with the use of the QIAGEN QIAamp DNA Blood Mini Kit; whole-genome amplification was then performed. We sequenced the PRNP open reading frame and genotyped unlinked microsatellite markers in persons from the Eastern Highlands Province, in-
Figure 1. A Cline in PRNP 129V Allele Frequency in Papua New Guinea.

An increasing cline in 129V frequency centers on the kuru region. A total of 282 samples from young persons from the kuru region were matched by village to elderly women from midlevel-exposure and high-exposure zones (green) (see the Supplementary Appendix). The 546 persons in low-exposure zones comprised linguistic groups that border the kuru region among which there are at least some documented cases of kuru: Gimi (87 persons), Yate (157), Keiagana (221), Kanite (35), and Awa (46) (blue and gray). The codon 129 allele count did not differ significantly from that in the kuru region. Highland populations with no recorded kuru (red) comprised Agarabi (90 persons), Asaro (26), Bena Bena (46), Gadsup (42), Gahuku (91), Labogai (65), Morae (an Anga linguistic group, 43), Siane (68), Tairora (68), and Yakuyufa (92) (P = 0.04 for the comparison of allele frequency with the frequency in the kuru-exposed region, by a two-tailed chi-square test). More distant populations (313 persons, yellow and gray) in included Vanimo–Wewak (5), islands neighboring Papua New Guinea (44), Port Moresby (11), Western Highlands (4), Madang and its neighboring island area (239), and Lae (10) (P < 0.001 for the comparison of allele frequency with the frequency in the kuru-exposed region, by a two-tailed chi-square test). Error bars represent ±1.96 binomial standard errors. EHP denotes Eastern Highlands Province, El exposure index, NS not significant, and PNG Papua New Guinea.

**Results**

**Study Sample and Exposure Index**

We previously reported that there was Hardy-Weinberg disequilibrium of the codon 129 genotype in a small cohort of older Fore women who had participated in multiple mortuary feasts. We have now expanded these data to include 10 recently obtained samples and 142 archived samples from patients with kuru, findings from a nearly completed study of elderly survivors of mortuary feasts (557 persons from the kuru region who were born before 1960, including 30 women whose data were previously reported), and results of extensive sampling of healthy current populations in exposed and unexposed areas of the Eastern Highlands Province (2053 persons) and more distant regions of Papua New Guinea (313). We stratified these samples according to the critical determinants of exposure to kuru on the basis of participation in mortuary feasts: sex, date of birth, and village of residence.

By plotting exposure-index contours (see the Methods section), we defined three zones in the kuru region: high exposure (exposure index >200), midlevel exposure (exposure index of >30 to 200), and low exposure (exposure index ≤30), and two additional unexposed zones: areas of Eastern Highlands Province that are close to the kuru region but have no oral history of kuru and distant regions of Papua New Guinea (see the Methods section).

**PRNP 129V and Geographic Distribution of 129V**

In addition to these important extensions of our previous results with respect to the codon 129 genotype, we now report a novel PRNP coding change, G127V (c.380G→T, CCDS13080.1), which is located in a highly conserved and structured region of PrP. We examined whether this variant was a neutral polymorphism, a novel pathogenic mutation causing inherited prion disease (that might have constituted an infectious origin for the kuru epidemic), or a polymorphism conferring resistance to prion disease that might have been selected by the kuru epidemic.
Initially, we sequenced the PRNP open reading frame in 112 patients with kuru, 142 elderly women from the kuru region who were born before 1950, and 282 young persons from mid-exposure and high-exposure regions (exposure index >30) (Table 1 in the Supplementary Appendix). We went on to genotype codons 127 and 129 in the entire patient and control population (Table 2 in the Supplementary Appendix). The PRNP codon 129 polymorphism is common across the Eastern Highlands Province. The young population in kuru-exposed areas has a higher frequency of the 129V allele than do adjacent unexposed populations of the Eastern Highlands Province (0.57 vs. 0.52) (Fig. 1, and Table 2 in the Supplementary Appendix). Areas outside the Eastern Highlands Province, including coastal and inland regions of Papua New Guinea and neighboring islands, have a low frequency of the 129V allele, similar to the frequency in the rest of southeast Asia and the Pacific Islands (Fig. 1) and in marked contrast to the frequency in the Eastern Highlands Province (P<0.001 by a two-tailed chi-square test) (Table 1). An increasing cline in the frequency of the 129V allele thus centers on the kuru-exposed region (Fig. 1). The 127V polymorphism was invariably linked to a 129M allele and was geographically restricted to the Purosa valley and neighboring villages (Fig. 2). In this part of the south Fore region, 127GV is a common genotype (frequency, 0.08). We did not detect deletions of the entire gene or PRNP promoter polymorphisms.

**ASSOCIATION STUDY**

A total of 36 of 48 patients with kuru who were younger than 20 years of age were homozygous for the PRNP open reading frame (127GG–129MM...
Figure 2. The Kuru-Exposed Region in Detail, Showing Areas of High Exposure and Persons with the 127V Allele.

We divided the kuru region into three zones of increasing exposure: villages with at least one recorded case of kuru but an exposure index of 30 or less (low-exposure group); a zone with an exposure index of more than 30 to 200; and a high-exposure zone, with an exposure index of more than 200. Red dots show the locations of persons with the 127V allele. The Purosa valley includes the villages of Purosa-Takai, Ketabi, Ai, and Mugaiamuti. The figure is adapted from a figure in Collinge et al.,26 which shows the location of all villages with a history of kuru.
or 127GG–129VV), as compared with 36 of 125 elderly women (P = 3.4×10^{-8} by the two-tailed chi-square test) and 27 of 104 patients with kuru who were older than 20 years of age at the onset of the disease (P = 1.2×10^{-8} by the two-tailed chi-square test). Heterozygosity is thus associated with resistance to kuru (since there is an excess of 129MV in elderly women who were resistant to kuru) and older age at onset of kuru (since there is an excess of 129MV in older patients with kuru). Both 129MM and 129VV were associated with young persons with kuru, as compared with 129MV, which was associated with elderly women in midlevel-exposure and high-exposure zones (P = 3.5×10^{-8} and P = 0.001 for the comparison of the frequency of 129MM and 129VV, respectively, with the frequency of 129MV, by the chi-square test with 1 df). Samples were available from 51 patients with kuru and 51 elderly women from the Puroso valley and neighboring villages where the 127V polymorphism was found. The 127V polymorphism was not found in any of the patients with kuru (P = 0.006 by Fisher’s exact test for the comparison with elderly women in mid-exposure and high-exposure zones). Heterozygosity at codon 127 provides strong, and possibly complete, resistance to kuru.

**Population Stratification**

Although we matched young controls to elderly women according to village of residence, and although the patients with kuru from whom we obtained samples were from a geographic area that was very similar to that in which the controls and elderly women lived (Fig. 2 in the Supplementary Appendix), we looked for genetic evidence that these samples were derived from the same homogeneous population. Principal component analysis of 1039 neutral single-nucleotide polymorphisms (SNPs) in 143 patients with kuru, 125 elderly women in midlevel-exposure and high-exposure villages, and 282 young controls from the kuru region showed no evidence of population stratification in the exposed region (P = 0.42 by EIGENSTRAT analysis for the comparison of patients with kuru with elderly women in mid-exposure and high-exposure villages), but persons in the kuru region could be readily distinguished from adjacent unexposed populations (282 persons, P(0.001) (Fig. 3 in the Supplementary Appendix). In a further investigation, we applied the genomic control method to the same set of 1039 neutral SNPs (in a comparison of patients with kuru with elderly women). Again the results are not compatible with the presence of population stratification (inflation factor, 1.09; 95% confidence interval [CI], 0.98 to 1.20).

**Hardy–Weinberg Equilibrium**

Further evidence of the genetic effect of kuru on PRNP is provided by deviation from Hardy–Weinberg equilibrium. The entire population of both sexes born before 1960 (480 persons in midlevel-exposure and high-exposure villages) shows marked loss of Hardy–Weinberg equilibrium at codon 129 (P = 4.6×10^{-4} by an exact method with the use of the PLINK software program [http://pngu.mgh.harvard.edu/~purcell/plink/], but not at codon 127, although since there are expected to be extremely few persons who are homozygous for 127VV, we have insufficient power to show loss of Hardy–Weinberg equilibrium at codon 127. When these data are stratified according to age, sex, and exposure, the most striking deficit of codon 129 homozygosity is found in elderly women from midlevel-exposure and high-exposure zones, in whom there is a marked loss of Hardy–Weinberg equilibrium at codon 129 — in contrast to persons in low-exposure or unexposed zones (P = 3.1×10^{-5} by an exact method with the use of PLINK) (Table 1 and Fig. 3). The loss of Hardy–Weinberg equilibrium at codon 129 is not found in a stratum of slightly younger women born between 1950 and 1960, which was the last period in which the dead were consumed at mortuary feasts, and was not detected in elderly men and unexposed elderly women. A stratum of men born between 1950 and 1960, who would have been young children in the final decade of the practice of mortuary feasts, do show distortion in Hardy–Weinberg equilibrium (P = 0.02 by an exact method with the use of PLINK). In traditional Fore society, boys older than approximately 7 years of age would no longer participate in the consumption of highly infectious material from mortuary feasts. The finding that Hardy–Weinberg equilibrium is maintained in the cohort of men born in the 1940s or earlier might thus be explained if they had had low exposure to kuru when they participated in feasts as children. This is consistent with the increase in the incidence of kuru through the early 20th century that was reported by elderly members of the Fore society.
Figure 3. Distribution of Heterozygosity at PRNP 127V and 129V in Elderly Female Survivors of Kuru.

The size of each pie chart is proportional to the number of elderly women from each village from whom samples were obtained. Heterozygosity at 127 or 129, or both, is highest in the zone of high exposure, resulting in Hardy–Weinberg disequilibrium. At equilibrium frequency, 48% of elderly women would be expected to be homozygous for PRNP. The figure is adapted from a figure in Collinge et al., which shows the location of all villages with a history of kuru.
KURU IN 127V PEDIGREES

No persons with the 127V polymorphism (51 persons from 32 families; mean age, 35 years; range, 16 to 78 years; 11 persons 50 years of age or older) had clinical evidence of neurodegenerative disease. Genealogies had been obtained as part of our ongoing studies, before the detection of the variant, from 18 probands with the 127V polymorphism. Parents of the living 127V probands would have lived through the peak kuru epidemic. Since one parent would be expected to be a 127V carrier, an increased or reduced history of kuru in this generation would provide further evidence to determine whether the 127V polymorphism was acting as a resistance factor or as a cause of inherited prion disease. The 127V pedigrees were compared with all 127G pedigrees obtained from villages in the Purosa valley in which more than one person with the 127V polymorphism had been detected (see Table 3 in the Supplementary Appendix). With the exception of Agakamatasa and Ilesa with moderate exposure (exposure index >100), all pedigrees, including those among Ai, Ivaki, Kalu, Kamira, Ketabi, Mugiamutu, Purosa-Takai, Takai, Waisa, and Wanatabi, were found in the region of highest kuru exposure (exposure index >200). Despite this, only 1 of 36 parents from 127V genealogies was recorded as having died from kuru, whereas 33 of 1 of 36 parents from 127V genealogies was recorded as having died from kuru, whereas 33 of 1 of 36 parents from 127V genealogies was recorded as having died from kuru, whereas 33 of 1 of 36 parents from 127V genealogies was recorded as having died from kuru, whereas 33 of 127G pedigrees were recorded as having died from kuru in the matched 127G pedigrees (P = 0.04 by a two-tailed chi-square test).

Given that the 127V polymorphism is highly geographically restricted, we suspected that there was a very recent common ancestor. We genotyped 13 microsatellite markers over 3 megabases linked to PRNP in order to investigate this possibility; 8 of them were informative. As assessed with the use of the PHASE software for haplotyping, 25 of 51 127V chromosomes share an identical microsatellite haplotype across the region (Fig. 4 in the Supplementary Appendix); the same haplotype was found in only 1 of 69 127G chromosomes. A point estimate for the time to the most recent common ancestor of 127V was 10 generations (95% CI, 7 to 15) (see Methods in the Supplementary Appendix for similar estimates with the use of an alternative method).

RELATIVE FITNESS

The viability of persons with different PRNP genotypes in the kuru region may be expressed as relative fitness, denoted by the Greek letter omega (ω) and defined as the number of surviving persons of a particular genotype standardized to the most viable genotype. This was calculated in two ways: for both 129 and 127, with all heterozygous combinations (127GV–129MM, 127GV–129MV, and 127GG–129MV) considered as equally the most viable genotypes, or for codon 129 alone, with the 129MV genotype considered as the most viable. In the entire sample of 480 persons born before 1960 in midlevel-exposure and high-exposure villages, ω = 0.64 for 127GG–129MM and ω = 0.74 for 127GG–129VV relative to the three heterozygous genotypic combinations; for codon 129 alone, ω = 0.69 for 129MM and ω = 0.74 for 129VV relative to the 129MV genotype. Subgroups that were more highly exposed to kuru have an even lower estimated viability relative to the three heterozygous genotypic combinations: among elderly exposed women from high-exposure villages (exposure index >200), ω = 0.23 for 127GG–129MM and ω = 0.42 for 127GG–129VV. For codon 129 alone, ω = 0.36 for 129MM and ω = 0.41 for 129VV relative to the 129MV genotype.

Although our failure to observe any 127VV homozygotes in the elderly population is consistent with Hardy–Weinberg equilibrium, 127V alleles were not in equilibrium with codon 129 genotypes. Among persons born before 1960, 127V alleles were more likely to be found in otherwise susceptible 129MM homozygotes than in 129MV heterozygotes (P = 0.02), whereas 127V and 129M were in equilibrium in persons who were born after 1960. These findings are consistent with the notion that 127V confers resistance to kuru in otherwise susceptible 129MM homozygotes (see the Supplementary Appendix for more detail).

DISCUSSION

These data support the conclusion that G127V in the heterozygous state confers resistance to kuru. In the folded PrPC conformation, which is the normal form of the protein, this residue lies in a small β-sheet, so the higher β-propensity of valine might increase PrPC stability. However, prion propagation is favored by homologous PrP interactions. A crystal structure of human PrP shows that interactions between neighboring PrP molecules are mediated by homotypic contacts between residues around position 129, leading to a four-strand intermolecular β-sheet. The impor-
tance of this region of the protein in mediating protein-to-protein contact could explain the susceptibility determined by the polymorphic residues 129 and 127.

Since the mean life expectancy in the kuru region was thought to be between 40 and 45 years, we cannot exclude the possibility that 127V is associated with a late-onset, low-penetrance, or recessive inherited prion disease that might indeed have triggered the kuru epidemic. However, there are few examples of mutations that are causative of autosomal dominant neurodegenerative disease achieving the polymorphic frequency observed in the Purosa valley. The inherited prion diseases caused by point mutations are generally poorly transmissible, and in some cases not transmissible, to experimental animals and are associated with a molecular type of the disease-associated prion protein (PrPSc) that is distinct from that of sporadic Creutzfeldt–Jakob disease or kuru. Finally, if 127V had triggered kuru, the localization to the southeastern part of the Fore linguistic group would be inconsistent with oral history that recorded that the first kuru patient was observed in the Keiagana linguistic group, which is located to the northwest of the Fore region.

In an earlier work, we proposed the theory that kuru imposed balancing selection on the Fore, acting at codon 129 of PRNP, although at the time we had sampled only 30 elderly exposed women. Our more recent genetic data confirm this effect in an additional 450 persons from the exposed population born before 1960. In addition, we have discovered at the epicenter of kuru exposure a novel PRNP allele — codon 127V — that is associated with resistance to kuru in patients and pedigrees and is found at the highest frequency in otherwise susceptible 129MM elderly women. The 127V-associated haplotype on chromosome 20 and the highly restricted geographic distribution of the variant suggest that there is a very recent common ancestor that slightly predates the estimates of the onset of the kuru epidemic that are based on recollections of the elderly members of the Fore group. This supports the inference that selection pressure imposed by kuru in the 20th century inflated the frequency of the 127V allele in the Purosa valley from a very small number of carriers. Our new data thus provide evidence of a complex selection event in the Fore population at PRNP during the kuru epidemic, with balancing selection acting to maximize heterozygosity at codon 129, coincident with positive selection acting to increase 127V alleles on a 129M background. Whether putative 127VV homozygotes would have had higher susceptibility to kuru than 127GV heterozygotes, in an analogous way to the situation at codon 129, remains a matter of speculation. The relative viability of combined codon 127–129 genotypes of PRNP in elderly persons from the kuru region suggests that there was stronger selection, in this specific situation, than that in the classically quoted examples of endemic malaria and hemoglobin S or C alleles.

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