ABSTRACT The PRNP polymorphic (methionine/valine) codon 129 genotype influences the phenotypic features of transmissible spongiform encephalopathy. All tested cases of new variant Creutzfeldt–Jakob disease (nvCJD) have been homozygous for methionine, and it is conjectural whether different genotypes, if they appear, might have distinctive phenotypes and implications for the future “epidemic” curve of nvCJD. Genotype-phenotype studies of kuru, the only other orally transmitted transmissible spongiform encephalopathy, might be instructive in predicting the answers to these questions. We therefore extracted DNA from blood clots or sera from 92 kuru patients, and analyzed their codon 129 PRNP genotypes with respect to the age at onset and duration of illness and, in nine cases, to detailed clinical and neuropathology data. Homozygosity at codon 129 (particularly for methionine) was associated with an earlier age at onset and a shorter duration of illness than was heterozygosity, but other clinical characteristics were similar for all genotypes. In the nine neuropathologically examined cases, the presence of histologically recognizable plaques was limited to cases carrying at least one methionine allele (three homozygotes and one heterozygote). If nvCJD behaves like kuru, future cases (with longer incubation periods) may begin to occur in older individuals with heterozygous codon 129 genotypes and signal a maturing evolution of the nvCJD “epidemic.” The clinical phenotype of such cases should be similar to that of homozygous cases, but may have less (or at least less readily identified) amyloid plaque formation.

Kuru, the prototype human transmissible spongiform encephalopathy (TSE), was spread by exposure to contaminated human tissues through the practice of ritual cannibalism, and is by virtue of its oral and/or mucocutaneous route of infection the most appropriate comparison for nvCJD. Here, we compare codon 129 genotypes to age at onset and duration of illness for 92 kuru patients dying in the late 1950s, at the height of the kuru epidemic, of which nine cases had been the subject of extensive neuropathological examination (4).

MATERIALS AND METHODS

We retrieved a total of 92 frozen blood clots and sera from our tissue bank archives, stored at −20°C since collection in Papua, New Guinea in 1957–1958. Genomic DNA was extracted from blood clots after treatment with proteinase K using a standard phenol-chloroform extraction method and from sera using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN), with the following slight modification: 500 μl of plasma was centrifuged for 30 sec at 13,000 rpm in a microfuge (Beckman, Palo Alto, CA) and pelleted cells were treated with 300 μl of cell lysis solution. Further steps were performed according to the manufacturer’s instructions.

The 169-bp fragment of the PRNP gene spanning codons 94 through 150 was amplified by PCR using TaKaRa Ex Taq polymerase (TaKaRa Shuzo Co., Shiga, Japan) and oligonucleotide primers (GenSet Corp., La Jolla, CA) 5'-caccacgactagtgaa-3' and 5'-ataagtagctctcatgtca-3' under the following conditions: an initial denaturation (95°C, 3 min) followed by 35 cycles of denaturation (94°C, 30 sec), annealing (60°C, 30 sec), extension (72°C, 30 sec), and a final elongation (72°C, 5 min). The ATG/GTG polymorphism at codon 129 was screened in the amplified samples using endonuclease MaeI (Boehringer Mannheim) or TaqI (MBI Fermentas, Vilnius, Lithuania) (5). Digested fragments were resolved in a 3% MetaPhor agarose gel (FMC BioProducts, Rockland, ME) stained with ethidium bromide. Codon 129 genotypes (MM, VV, or MV) were determined on the basis of the distinct restriction patterns seen for each genotype.

Comparisons of genotype frequencies between different age groups were made by using the two-tailed Fisher’s exact test.

RESULTS

The distribution of homozygous methionine, homozygous valine, and heterozygous genotypes according to age at onset of kuru symptoms is shown in Fig. 1. It is evident that compared with the series as a whole, homozygous methionine patients are overrepresented in the younger age groups, and

Abbreviations: nvCJD, new variant Creutzfeldt–Jakob disease; TSE, transmissible spongiform encephalopathy.

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heterozygous patients are overrepresented in the older age
groups. When the genotype frequencies of the group of
children and young adolescents (<15 yr of age) were compared
with adults >30 yr of age, the difference between homozygotes
and heterozygotes was statistically significant (P = 0.0001),
Table 1. A less impressive but still statistically significant
correspondence also exists between the codon 129 genotype
and the duration of illness, shorter illnesses being associated
with homozygosity and longer illnesses being associated with
heterozygosity (P = 0.006).

Nine specimens in the series were matched with patients
whose brains had earlier been the subject of extensive neuro-
pathological examinations (4). An extract of these data is
presented in Table 2, along with clinical summaries and codon
129 genotypes. Irrespective of the age at onset or duration of
illness, or of the codon 129 genotype, all nine patients had a
clinical picture dominated by progressive locomotor ataxia
associated with the shivering tremor characteristic of kuru.
The occurrence of extrapyramidal signs, strabismus, dyspha-
gia, and mutism was also unrelated to age at onset or duration
of illness, or to the codon 129 genotype.

In regard to neuropathology, widespread gliosis occurred in
cases, associated with a variable pattern of neuronal ab-
normalities and vacuolation that was unrelated to genotype.
However, amyloid plaques were noted only in the four patients
with at least one methionine allele, and not in the five patients
with only valine alleles. Except for the presence of plaques, the
lone heterozygote was neuropathologically indistinguishable
from either the methionine or valine homozygotes.

DISCUSSION

One of the more interesting features of the chromosome 20
PRNP gene is the phenotypic influence of polymorphic codon
129. Although not in itself pathogenic, it has been shown to
influence susceptibility to iatrogenic and sporadic forms of
TSE and to affect age at onset and duration of illness in familial
TSE; in association with a pathogenic mutation in codon 178,
the entire disease phenotype is altered (5–11).

Because all cases of nvCJD recognized to date have tested
homozygous for methionine at codon 129, it is not known
whether other genotypes (should they occur) will change the
nvCJD phenotype, possibly even to the point of obscuring its
distinctive clinical and neuropathological features.

One clue that this may not happen comes from experience
with iatrogenic Creutzfeldt–Jakob disease, resulting from pe-
ripheral injection by contaminated human growth hormone,
which shows that, other than a longer period of latency
between infection and the onset of symptoms, the clinicopath-
ological picture of Creutzfeldt–Jakob disease in patients with
a heterozygous codon 129 genotype does not differ from that
in patients with either methionine or valine homozygosity (ref.
12; D. Dormont, personal communication; M. Preece, per-
sonal communication).

An even more appropriate comparison comes from pheno-
type-genotype studies in kuru patients, in whom infection
occurred, at least in part, by the oral route. Three recent
studies comparing the neuropathological features of kuru and
nvCJD include kuru cases in which the genotype of codon 129

![FIG. 1. Distribution of PRNP codon 129 genotypes according to age at onset of illness in 92 kuru patients.](image-url)
was determined. In one study, the brains from two valine homozygotes showed a tendency for “proper plaque formation” in PrP-stained immunohistochemical sections (13). In another study, the brain from a valine homozygote showed numerous plaques by both histological and immunohistochemical staining (14). In the third study, brains from three valine and two methionine homozygotes had plaques visible by both histological and immunohistochemical staining, and no genotypic correlations were noticed (15).

In contrast to these studies, our series of nine cases showed a distinct correlation between the presence of the methionine allele and the presence of histologically recognizable amyloid plaques. It is possible that these differing results are a consequence of chance or of undefined differences in tissue fixation and staining methods. It is also possible that as a group, kuru cases with a valine allele at codon 129 have a comparatively reduced potential for plaque formation, and, by analogy, nvCJD cases with a codon 129 valine allele might not always show the prominent daisy plaque formation observed in methionine homozygotes.

Our finding that homozygotes have an earlier age at onset (and thus probably a shorter incubation period) than heterozygotes, but show a generally similar clinical phenotype, is in keeping with the effect of codon 129 homozygosity in other forms of TSE, and, if applicable to nvCJD, would predict (i) that codon 129 nvCJD heterozygotes will not pose a problem in recognition or diagnosis and (ii) that the appearance of an increasing proportion of heterozygotes may signal that the nvCJD epidemic is already evolving beyond its “leading edge,” and thus provide a more solid foundation for predictive modeling studies of its overall extent and duration.


<table>
<thead>
<tr>
<th>Case</th>
<th>Sex/age at onset</th>
<th>Duration of illness, mo</th>
<th>Abnormal affect</th>
<th>Extra-pyramidal</th>
<th>Other signs</th>
<th>Clinical signs (all cases had progressive ataxia associated with tremor)</th>
<th>Pathology (all cases had severe gliosis in all brain regions)</th>
</tr>
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<tbody>
<tr>
<td>6</td>
<td>F/6</td>
<td>8</td>
<td>+</td>
<td>Rigidity</td>
<td>Dysphagia</td>
<td>N</td>
<td>NV</td>
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<tr>
<td>7</td>
<td>F/6</td>
<td>5</td>
<td>–</td>
<td>–</td>
<td>Dysphagia, mutism</td>
<td>N</td>
<td>NV</td>
</tr>
<tr>
<td>16</td>
<td>M/7</td>
<td>8</td>
<td>–</td>
<td>–</td>
<td>Strabismus, mutism</td>
<td>NP</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>M/7</td>
<td>8</td>
<td>+</td>
<td>ChAth</td>
<td>Myoclonus</td>
<td>N</td>
<td>NV</td>
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<tr>
<td>24</td>
<td>F/11</td>
<td>12</td>
<td>+</td>
<td>–</td>
<td>Strabismus</td>
<td>P</td>
<td>NV</td>
</tr>
<tr>
<td>11</td>
<td>F/13</td>
<td>5</td>
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<td>Strabismus</td>
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<td>N</td>
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<tr>
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<td>M/17</td>
<td>10</td>
<td>–</td>
<td>–</td>
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<td>NV</td>
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<tr>
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<td>F/45</td>
<td>9</td>
<td>–</td>
<td>ChAth</td>
<td>Mutism</td>
<td>P</td>
<td>NV</td>
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<tr>
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<td>–</td>
<td>Dysphagia, mutism</td>
<td>N</td>
<td>PV</td>
</tr>
</tbody>
</table>

ChAth, choreo-athetosis; N, neuronal changes; V, vacuolation; P, plaques.