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Mutation in codon 200 and polymorphism in codon 129 of the prion protein gene in Libyan Jews with Creutzfeldt–Jakob disease

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SUMMARY

Various mutations in the prion protein (PrP) gene are associated with Creutzfeldt–Jakob disease (CJD), a transmissible fatal neurodegenerative disorder. Among Libyan Jews, CJD is a familial disease with an incidence about 100 times higher than the worldwide population. CJD in this community segregates with a point mutation at codon 200 of the PrP gene which causes the substitution of lysine for glutamate. This mutation was found in all definitely affected individuals and yields a maximum lod score of 4.85. Some healthy elderly mutation carriers above 65 years of age were identified, suggesting partial penetrance. Homozygous patients have the same disease pattern and age of onset as heterozygous patients, which argues that CJD associated with the codon 200 lysine mutation is a true dominant disorder. In the caucasian population, Palmer *et al.* (1991) reported an association between homozygosity in a polymorphic site at codon 129 of the PrP gene, coding for either valine or methionine, with a tendency to acquire the sporadic or iatrogenic forms of CJD, as well as with disease age of appearance in the genetic type. The incidence of the polymorphism at codon 129 in the control Libyan population is similar to the one found in the caucasian population. In the Libyan CJD patients, the codon 200 mutation is within a Met_{129}-encoding allele. The incidence of the Met allele is significantly higher in the affected pedigrees than in the control Libyan population; however, no difference was detected between CJD patients, codon 200 healthy carriers, and their normal family members. Homozygosity at codon 129 did not correlate with age of onset or the clinical course in the Libyan Jewish patients. Our finding suggests that the codon 200 mutation causing CJD in Libyan Jews occurred in an isolated pedigree, and has not propagated since to the general Libyan Jewish community.

1. INTRODUCTION

Creutzfeld–Jakob disease (CJD), a human transmissible spongiform encephalopathy, can have a sporadic, iatrogenic or genetic etiology (Hsiao & Prusiner 1991). The disease is characterized clinically by rapidly progressive dementia, pyramidal and cerebellar symptoms, generalized myoclonus and periodic EEG pattern (Brown *et al.* 1986). Disease onset generally occurs between the ages of 50 and 70 years, and mean duration of disease is one year. CJD is associated with the accumulation in the brain of a protein called prion protein PrP^{Sc}, which is the abnormal isoform of PrP^{C} (Bolton *et al.* 1982; Oesch *et al.* 1985). Recently, mutations in the PrP gene have been reported in genetic clusters of human prion diseases (Doh-ura *et al.* 1989; Goldgaber *et al.* 1989; Hsiao *et al.* 1989, 1991*a*,*b*; Owen *et al.* 1989; Goldfarb *et al.* 1990*a*,*b*, 1991).

The largest focus of CJD in the world was identified among Libyan Jews (Kahana *et al.* 1974). CJD in this community is a familial disorder with an incidence about 100 times higher than the worldwide population (Zilber *et al.* 1991). A missense mutation at codon 200 of the PrP gene which results in the substitution of lysine for glutamate was identified in this cluster (Goldfarb *et al.* 1990*a*; Hsiao *et al.* 1991*b*). The same mutation was detected in another cluster of CJD in Czechoslovakia (Goldfarb *et al.* 1990*b*).

Recent publications suggest a correlation between homozygosity at codon 129 and susceptibility to sporadic CJD (Palmer *et al.* 1991). Most of the patients tested were shown to be homozygous for either methionine or valine at codon 129. Collinge *et al.* reported an excess of valine 129 homozygotes in iatrogenic CJD patients (Collinge *et al.* 1991). Baker *et al.* (1991) found a correlation between early age of disease onset and homozygosity at codon 129 in a

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large pedigree with CJD linked to a 144 base pair insertion within the PrP gene. Doh-ura *et al.* (1991) reported a different disease course among sporadic CJD patients, suggesting that heterozygous patients at codon 129 have a protracted course similar to GSS.

We therefore investigated the correlation between the genotype at codon 129 in the Libyan Jewish community suffering from CJD associated with the codon 200 mutation and the phenotype of the disease in this community.

2. MATERIALS AND METHODS

(*a*) *Patient population*

Patients (28) were diagnosed as dying from CJD based on clinical criteria and the presence of the Lys substitution at the codon 200. There were 46 codon 200 mutation carriers who were individuals belonging to CJD pedigrees; 38 non-carriers of the codon 200 mutation were siblings, cousins and other close relatives of the carriers; 90 unrelated Libyan individuals were examined as controls.

(*b*) *PCR amplification and allele-specific hybridization*

DNA samples from patients, mutation carriers and controls were amplified by PCR with primers AAG-GATCCCTCAAGCTGGAAAAAGA (sense) and AAGAATTCTCTGACATTCTCCTCTTCA (antisense) to generate an 864 base pair (b.p.) fragment including the PrP open-reading frame (ORF). Samples of the PCR-amplified DNA were dot blotted into Gene Screen+after denaturation and hybridized with oligonucleotide-specific probes for K or E at codon 200 (GGTCTCGGTGAAGTT for E, and GGTCTTGGTGAAGTT for K) or M or V at codon 129 (CGGCTACATGCTGGG for M, and CGGCTACGTGCTGGG for V) using conditions previously described (Hsiao *et al.* 1991).

(*c*) *Allele-specific amplification*

DNA from patients and mutation carriers was amplified by PCR to generate a 500 b.p. fragment of the PrP ORF by using primers that include the M/V polymorphic site at their 3′ end as sense (GCCTTGGCGGCTACA for M, and GCCTTGG-CGGCTACG for V) and AAGAATTCTCTGACAT-TCTCCTCTTCA as antisense. The samples were electrophoresed on a 1% agarose gel, blotted into Gene Screen +, and hybridized with probes K and E as described above.

(*d*) *Linkage analyses*

Eight families were used in this analysis. The disease was assumed to follow a dominant mode of inheritance with age-dependent penetrance, where penetrance was assumed to rise linearly from 0 at age 35 to 70% at age 75. We estimated the disease gene frequency (not available in the literature) by the following approximate procedure. With the assumption made above, age of onset, A, has a uniform distribution with a density of 1/40 in the range from 35 through to 75, given an individual is in the age range. About half the population falls into this age category, so the unconditional density is equal to $P(A) = 1/80$. The incidence, $P(N)$, is known to be about 1/7000 new cases per year, and can be expressed as

$$P(N) = P(A)P(N/A), \quad (1)$$

where $R(N/A)$ is the probability of developing the disease in the following year given that an individual is currently unaffected, and summation is over ages 35 through to 75. The disease can occur only in genetically predisposed individuals whose proportion is equal to $1-(1-p)^2$, where p is the disease gene frequency. Given that one has the disease genotype, the probability of becoming affected within the next year increases linearly as given above by the penetrance function, that is, $P(N/A) = (1/80)\ [1-(1/80)(A\text{-}35)][1-(1-p)^2]$, where p is the disease gene frequency. Inserting the expression in equation (1), and varying p such that equation (1) is satisfied, yields a disease gene frequency for CJD of $p = 0.0128$. As this is an approximate result, other values of p were also tried in the linkage analysis.

One of the objects of this study was to see whether there was genetic linkage, given that one allows for linkage disequilibrium (allelic association). Therefore we evaluated four different likelihoods of the data, assuming presence $(\Theta = 0)$ and absence $(\Theta = \frac{1}{2})$ of linkage, and presence δ_{max}) and absence $(\delta = 0)$ of disequilibrium, were Θ is the recombinant fraction and δ is the disequilibrium parameter. Because the prion protein gene is a candidate gene, no intermediate values between $\Theta = 0$ and $\Theta = \frac{1}{2}$ were tested. Meaningful estimation of δ from the data was difficult; therefore, and because disequilibrium was evidently very strong, only the maximum achievable disequilibrium and no disequilibrium were tested.

3. RESULTS

(*a*) *Allele and genotype analysis*

PCR amplification followed by dot-blot allele-specific hybridization analyses were used to screen known CJD patients, their family members, and normal Libyan controls (Hsiao *et al.* 1991). Libyan Jews (28) diagnosed with CJD tested positive for the codon 200 lysine substitution, either via direct testing or a positive result in offspring and a negative test in the spouse. Another 17 historical CJD patients were identified as suspected mutation carriers, when the spouse was unavailable for testing and offspring tested positive (table 1). One patient, a 42-year-old woman, was shown to be homozygous for the codon 200 mutation. We have identified two other probable homozygous patients among the historical cases, as all their offspring are carriers but their spouses are not. Unfortunately, tissue for direct probing from these patients was unavailable (table 2). Healthy Libyan

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Table 1. *Current data on codon 200 mutation in Libyan Jews*

	verified	suspected
CJD patients	28	17[a]
healthy mutation carriers	46	—
mutation carriers age 65–70	3	5[b]
unrelated Libyan controls	90	—

[a] Positive offspring but untested spouse.
[b] Obligated carriers.

Table 2. *Homozygous patients for the codon 200 mutation*

	verified	suspected
number of cases	1	2[a]
age of disease onset	42	52, 60
clinical course	typical	typical

[a] All offspring positive with negative spouse.

controls (120) without a history of CJD were shown to be negative for the mutation, but 46 healthy Libyan Jews tested positive for the mutation, three of them aged above 65.

We tested the codon 129 polymorphism among 23 Libyan Jewish CJD patients carrying the mutation at codon 200, 39 healthy codon 200 mutation carriers, their 38 unaffected family members, and 65 healthy Libyan controls. M/V polymorphism at codon 129 was tested by allele-specific oligonucleotide hybridization (figure 1). Among the normal Libyan Jewish population, the genotype frequency at codon 129 was

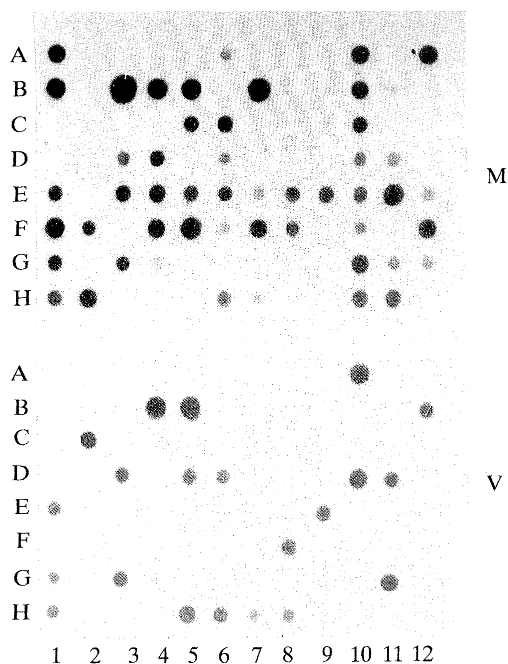

Figure 1. Allele-specific oligonucleotide hybridization for Met or Val at codon 129. The PrP ORF was amplified by PCR, blotted into Gene Screen +, and hybridized with specific labelled oligonucleotides for either Met (upper panel, designated 'M') or Val (lower panel, designated 'V') at codon 129. Samples reacting with both probes are heterozygous for Met/Val at codon 129, whereas samples reacting with one of the probes are homozygous for the respective amino acid.

28% Met_{129}/Met_{129}, 56% heterozygous, and 16% Val_{129}/Val_{129}, which results in a total of 56% Met in this population (table 3). This frequency is not significantly different from the one reported for the caucasian population (Owen *et al.* 1990). Among the tested CJD patients there were 24% heterozygous and 76% Met_{129}/Met_{129}, including a homozygous patient for the codon 200 lysine mutation; this results in a 90% frequency of Met_{129} when counting both alleles, and in 79% Met frequency for the non-affected allele. Similar results were obtained for the unaffected mutation carriers. Among family members of CJD patients without the codon 200 mutation there were 65% Met_{129}/Met_{129}, 31% heterozygous, and 2% Val_{129}/Val_{129}, resulting in 80% Met (table 3). The different incidence in Met between the general Libyan Jewish population and the CJD-affected families (including affected and unaffected individuals) is statistically significant ($\chi^2=6.6$, $p<0.025$). No difference in the Met_{129} incidence in the normal allele is apparent between CJD patients, healthy codon 200 carriers, and their normal family members.

(*b*) *The codon 200 lysine mutation is within a MET₁₂₉-encoding allele*

Among CJD patients and healthy mutation carriers, not a single individual was found homozygous for valine at codon 129. A homozygous patient for codon 200 lysine mutation was also homozygous for the codon 129 methionine genotype. This strongly suggests that the codon 200 lysine mutation is always present in an allele encoding methionine at codon 129. We used allele-specific PCR amplification to prove this hypothesis (figure 2). The sense primer included on its 3′ end the nucleotides encoding either methionine or valine; the antisense primer was the same used for the full PrP open-reading frame amplification. This strategy resulted in the allele-specific amplification of 464 base pairs DNA encoding from codon 129 to the 3′ prime end of the PrP ORF. No amplification occurred in Met_{129}/Met_{129} individuals with the Val_{129} primer, or in the Val_{129}/Val_{129} individuals with the Met_{129} primer. The correlation between this test and the allele-specific oligonucleotide hybridization is 100%. In Met_{129}/Val_{129} individuals with the codon 200 lysine substitution, hybridization of a Southern blot of allele-specific amplified samples with either codon 200 E or K oligonucleotide probes showed that the Lys_{200} mutation is present on the Met_{129}-encoding allele.

(*c*) *Genotype–phenotype correlation*

We divided the codon 200 mutation associated CJD patients in two age groups, below and above the average age for disease onset in this community (which is 56 years old), and calculated the incidence of Met_{129} in the unaffected allele in these groups as compared with healthy mutation carriers in the same age frames. No significant difference in Met_{129} incidence was found between younger and older patients on mutation carriers. The few patients heterozygous for the M/V polymorphism are dispersed between all

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Table 3. *PrP gene codon 129 polymorphism in Libyan Jews*[a]

	M/M	M/V	V/V	M (%)
CJD patients	15	5	not found	78[b]
healthy codon 200 carriers	32	7	not found	82[b]
related controls	23	13	2	78
unrelated controls	25	33	8	63

[a] At codon 129 of the human PrP gene (PRNP), a methionine (M) or valine (V) is encoded.
[b] Calculated only for the allele not carrying the codon 200 mutation.

age groups. Furthermore, all healthy mutation carriers over 60 years of age were homozygous for Met_{129} (table 4). Clinical course was not different between homozygous and heterozygous CJD patients at codon 129 (table 5). It is also significant that no differences in the clinical course and age of disease onset were observed in homozygous codon 200 patients, as compared with the heterozygous CJD patients.

(*d*) *Linkage analysis*

Linkage analyses were done with the MLINK program of the LINKAGE package (Lathrop *et al.* 1984). Because almost all affected individuals were homozygous at codon 129 (see below), the data were essentially uninformative for linkage between CJD and this locus. Thus the alleles at codon 200 were used for linkage analysis with or without disequilibrium, and for analysing disequilibrium with or without linkage. As the results in table 6 show, there is no significant evidence for linkage without disequilibrium (we obtained a maximum lod score of 2.63 at the estimated gene frequency for CJD). However, because of the apparent strong disequilibrium, a linkage analysis under equilibrium is not realistic; under disequilibrium, the lod score was equal to 4.85, which is significant evidence for linkage. The third and fourth columns of table 6 verify overwhelming evidence for disequilibrium, whether or not linkage is assumed.

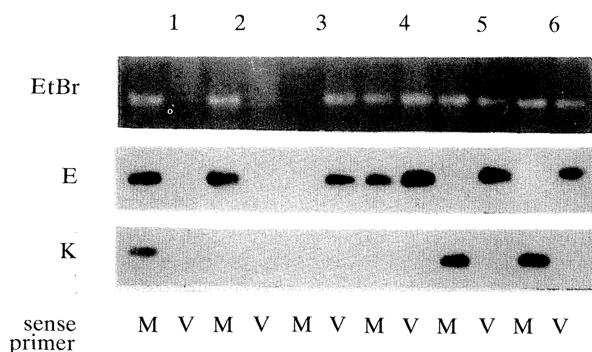

Figure 2. Allele-specific PCR amplification of the PrP gene. Primers specific for either Met (designated 'M') or Val (designated 'V') at codon 129 were used for allele-specific amplification. In each case, the 3′ nucleotide of the sense primer codes for the respective polymorphic site. The amplified fragments were than Southern blotted and hybridized with specific labelled oligonucleotides for Lys (designated 'K') or Glu (designated 'E') at codon 200. Lane 1, heterozygous codon 200 (MM); lane 2, control MM; lane 3, control VV; lane 4, control MV; lanes 5 and 6, heterozygous codon 200 (MV).

4. DISCUSSION

Our results show both strong evidence for disequilibrium (with or without linkage) and strong evidence for linkage (given disequilibrium) between CJD and PRNP in Libyan Jews. It is a common observation, also found in the present data, that tightly linked loci also show association between some alleles at the same loci. The two effects, disequilibrium and linkage, are somewhat confounded, but there is clearly a strong main effect of disequilibrium and a smaller, but still strong, effect of linkage. Pedigree exploration until now suggests partial penetrance, but only long-term follow-up will reveal the probability of a specific mutation carrier acquiring the disease.

Considerable attention has been focused on the PrP codon 129 polymorphism with respect to modifying the phenotypic characteristics of CJD. In the inherited prion diseases, a correlation between age of disease onset and homozygosity at codon 129 has been reported in patients with a 144 b.p. insertion in the PrP gene (Baker *et al.* 1991). Patients with a codon 178 mutation (Asp to Asn) who encode a Met_{129} on the same allele appear to develop a disease called fatal familial insomnia, whereas those who encode a Val_{129} present a dementing disorder more characteristic of CJD. Although the age of onset was early for those homozygous for Val_{129}, there was no correlation between age of onset and homozygosity for Met_{129} (Golfarb *et al.* 1992). In sporadic CJD, homozygosity at codon 129 was found to predispose patient to disease, whereas heterozygosity is thought to be protective

Table 4. *Correlation of codon 129 genotype with age of carriers*

	methionine at unaffected allele (%)	
age	CJD patients	codon 200 carriers
> 56	83	100
35–56	77	82

Table 5. *Correlation of codon 129 genotype and clinical course of CJD in Libyan Jews*

genotype	number of patients	age of onset/years	disease dutation/years
M/M	14	60.8 (43–73)	4.3 (2–10)
M/V	5	58.6 (35–70)	5.2 (3–13)

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Table 6. *Lod scores (test for linkage) and chi-square (1 d.f.) values (test for disequilibrium) for different disease gene frequencies*

(The relevant likelihood ratios for the four columns of the table are given as follows: column 1, $L(\Theta=0,\delta=0)$; column 2, $L(\Theta=0,\delta_{max})/L(\Theta=1/2,\delta_{max})$; column 3, $L(\Theta=0,\delta_{max})/L(\Theta=0,\delta=0)$; column 4, $L(\Theta=1/2, \delta_{max})/L(\Theta=1/2,\delta=0)$.)

	test for linkage (lods)		test for disequilibrium (χ^2)	
Gene frequency	$\delta=0$ (column 1)	$\delta=\max$ (column 2)	$\Theta=0$ (column 3)	$\Theta=\frac{1}{2}$ (column 4)
0.05	1.753	4.200	36.43	25.21
0.0128	2.630	4.850	64.87	45.51
0.001	3.010	5.160	95.59	85.61
0.0001	3.022	5.192	132.30	122.31

(Palmer *et al.* 1991). These findings suggest that homozygosity at codon 129 may enhance the formation of PrP^{C}/PrP^{Sc} complexes, which are thought to feature in catalysing the conversion of PrP^{C} to PrP^{Sc} based on transgenic mice (Prusiner 1991; Prusiner *et al.* 1990).

In contrast to familial CJD, caused by an insert encoding six additional octarepeats or a point mutation at codon 178, no correlation was found between age of onset of disease and homozygosity at codon 129 in Libyan Jews developing CJD. Homozygous patients for the codon 200 mutation, which have an identical protein on both alleles, do not show a different disease pattern to heterozygous patients (Hsiao *et al.* 1991). Older individuals with the codon 200 mutation who are homozygous at codon 129 are not uncommon in this community. Furthermore, the youngest patient tested (35 years of age) was of M/V genotype. In contrast to the Japanese group report (Doh-Ura *et al.* 1991), in our patients no difference can be appreciated in the disease pattern between the patients homozygous or heterozygous at codon 129.

Clinically, the patients with a mutation at codon 200, as compared with other mutations in the PrP gene, are the ones that most resemble sporadic CJD (Kahana *et al.* 1991). The discrepancy between Palmer *et al.*'s (1991) results, suggesting requirement for homozygosity at codon 129 in sporadic patients, and our results in the Libyan Jew genetic patients can be explained as follows. In all mechanisms invoked to explain the sporadic form of prion diseases – direct toxicity of PrP^{Sc}, somatic mutation, or spontaneous transformation of PrP^{C} to PrP^{Sc} – only a small number of pathological molecules are formed initially. Homozygosity may favour the transformation of PrP^{C} to PrP^{Sc} by dimer formation, resulting in disease spreading. However, in the genetic diseases, mutant molecules are produced from birth and may transform into PrP^{Sc} by an age-dependent spontaneous mechanism. It is therefore possible that the load of internal aberrant molecules is enough without the need to transform the protein from the normal allele to induce the disease. Indeed, the mutant protein, at least in codon 200 CJD, may have different properties from wild-type PrP (Meiner *et al.* 1992).

The difference in Met_{129} incidence in affected and unaffected families suggests that the codon 200 mutation occurred in one individual, probably homozygous for Met_{129}, and propagated in one pedigree that did not significantly intermingle with the general Libyan Jewish population for the generations since the mutation occurred. Indeed, the Lys_{200} mutation is found in a limited number of pedigrees and is completely absent in other Israeli Libyan families (R. Gabizon, unpublished data). This conclusion is favoured by historical facts which suggest that Jews living in the areas of Tripoli and Djerba were isolated from other Jewish communities inside and outside Libya. In the affected families, interfamily marriages were a common practice for generations, a fact reinforced by the presence of homozygous individuals for the codon 200 mutation. If this is the case, it is possible that other genes will also have more prevalence in the CJD-affected pedigrees than in the general Libyan Jewish population, and may play a role in the predisposition of specific individuals with the codon 200 mutation to acquire the disease. In Czechoslovakia and Chile, CJD associated with the codon 200 mutation is also present in isolated communities (Goldfarb *et al.* 1990*b*). In the next generation, when young Libyan Jews from CJD-affected families marry into the general Israeli population, we will be able to appreciate whether changes will occur in the phenotype of CJD caused by the codon 200 mutation.

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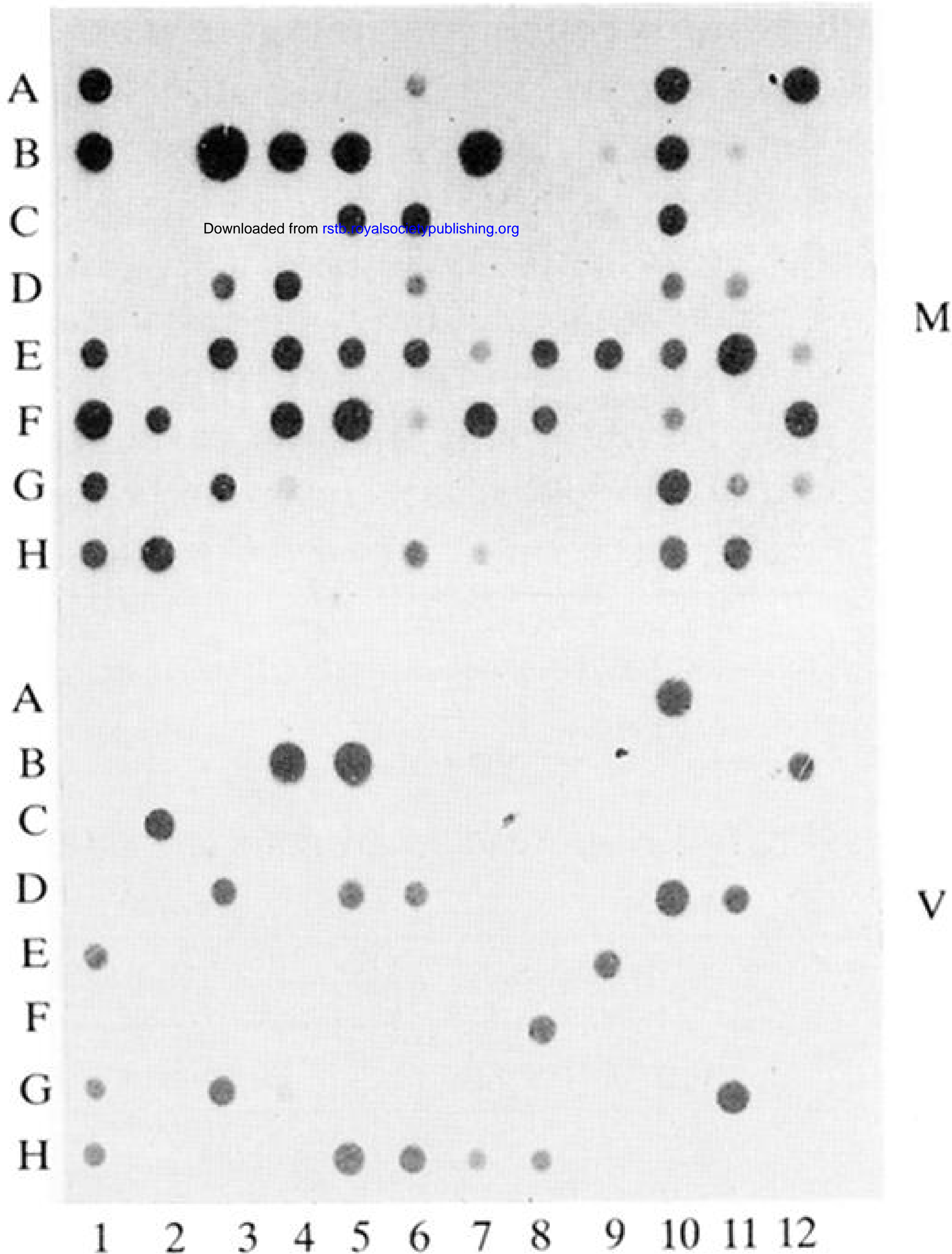

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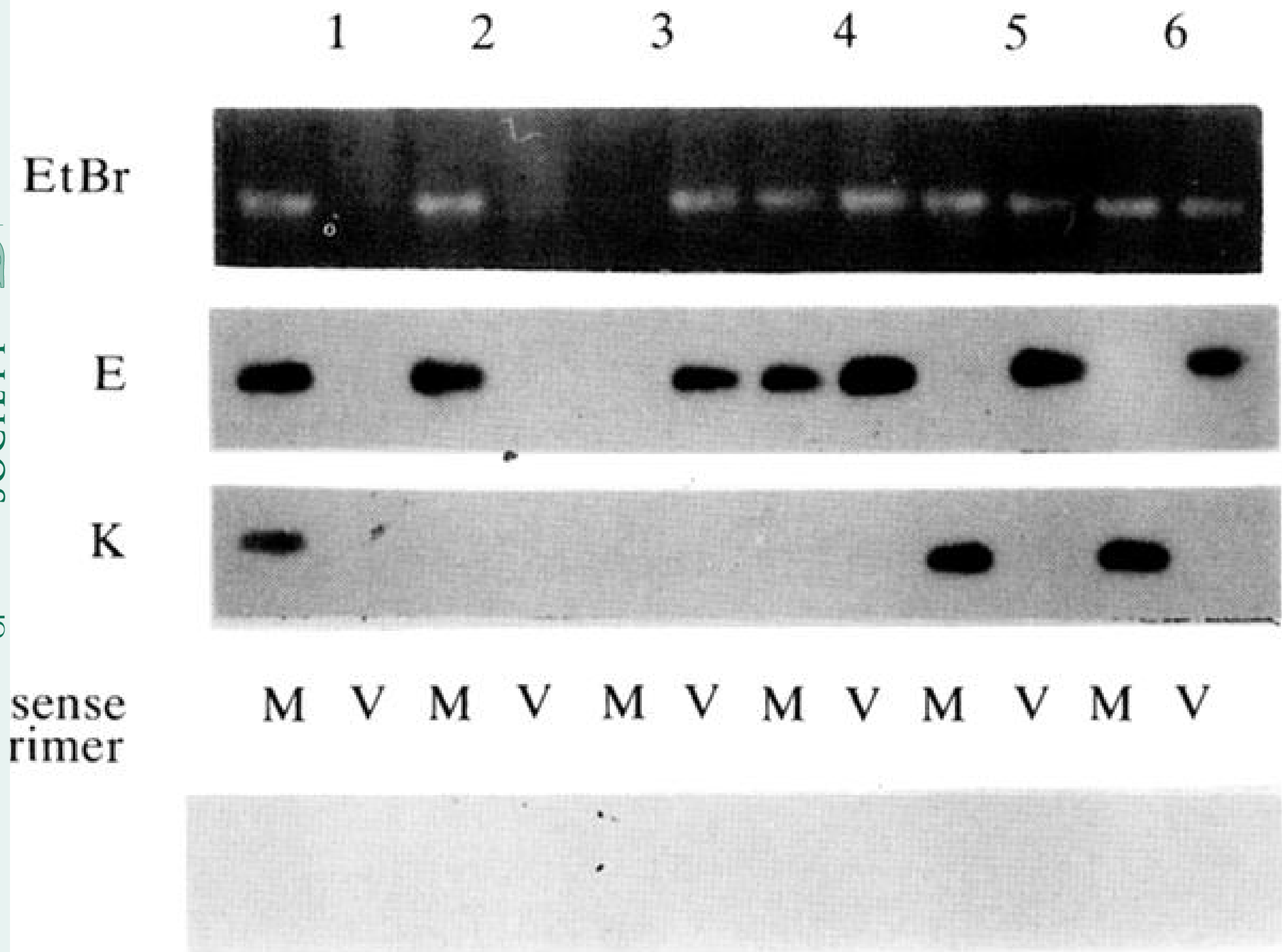

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gure 2. Allele-specific PCR amplification of the PrP gene. imers specific for either Met (designated 'M') or Val esignated 'V') at codon 129 were used for allele-specific nplification. In each case, the 3′ nucleotide of the sense imer codes for the respective polymorphic site. The nplified fragments were than Southern blotted and hybrized with specific labelled oligonucleotides for Lys (desigted 'K') or Glu (designated 'E') at codon 200. Lane 1, terozygous codon 200 (MM); lane 2, control MM; lane 3, ntrol VV; lane 4, control MV; lanes 5 and 6, heterozygous don 200 (MV).

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