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Mutation and Polymorphism of the Prion Protein Gene in Libyan Jews with Creutzfeldt-Jakob Disease (CJD)

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Summary

The inherited prion diseases are neurodegenerative disorders which are not only genetic but also transmissible. More than a dozen mutations in the prion protein gene that result in nonconservative amino acid substitutions segregate with the inherited prion diseases including familial Creutzfeldt-Jakob disease (CJD). In Israel, the incidence of CJD is about 1 case/10^4 Libyan Jews. A Lys_{200} substitution segregates with CJD and is reported here to be genetically linked to CJD with a lod score of >4.8. Some healthy elderly Lys_{200} carriers >age 65 years were identified, suggesting the possibility of incomplete penetrance. In contrast, no linkage was found between the development of familial CJD and a polymorphism encoding either Met_{129} or Val_{129}. All Libyan Jewish CJD patients with the Lys_{200} mutation encode a Met_{129} on the mutant allele. Homozygosity for Met_{129} did not correlate with age at disease onset or the duration of illness. The frequency of the Met_{129} allele was higher in the affected pedigrees than in a control population of Libyan Jews. The frequency of the Met_{129} and Val_{129} alleles in the control Libyan population was similar to that found in the general Caucasian population. The identification of three Libyan Jews homozygous for the Lys_{200} mutation suggests frequent intrafamilial marriages, a custom documented by genealogical investigations.

Introduction

The dementing form of the human prion diseases is usually referred to as Creutzfeldt-Jakob disease (CJD) (Jakob 1921, 1977). All of the prion diseases of humans and animals are fatal neurodegenerative diseases which can be manifest as sporadic, infectious, or inherited disorders (Prusiner 1991). CJD presents as a rapidly progressive dementia with pyramidal and cerebellar signs, generalized myoclonus, and periodic discharges on the electroencephalogram (Roos et al. 1973; Brown et al. 1986). Patients with CJD generally develop neurological dysfunction between the ages of 50 years and 70 years and die within 1 year. Transmission of CJD to apes was accomplished by intracerebral inoculation of extracts prepared from the brains of patients dying of CJD (Gibbs et al. 1968; Gibbs and Gajdusek 1969).

In the brains of patients dying of CJD, an abnormal isoform of the prion protein (PrP), designated "PrP^{Sc}" or "PrP^{CJD}," was found (Bockman et al. 1985, 1987). Clusters of CJD, once ascribed to common-source exposure to infectious prions (Neugut et al. 1979), are now known to be due to mutations in the PrP gene (Hsiao et al. 1991*b*). The human PrP gene is encoded by a gene on the short arm of chromosome 20 (Sparkes et al. 1986).

The largest focus of CJD in the world was identified among Libyan Jews who were initially thought to contract the disease by eating lightly cooked sheep brain from scrapie-infected sheep (Kahana et al. 1974; Neugut et al. 1979). CJD in this community is a familial disorder with an incidence about 100 times higher than that found worldwide (Zilber et al. 1991). A mutation at codon 200 of the PrP gene which results in the substitution of Lys for Glu was identified in this cluster (Goldfarb et al. 1990*b;* Hsiao et al. 1991*b*). The same mutation has been detected in patients dying of familial

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CJD in Czechoslovakia (Goldfarb et al. 1990*a*), Chile (Goldfarb et al. 1991), the United States (Bertoni et al. 1992), and Great Britain (Collinge et al. 1993).

Considerable attention has been focused on the role of a polymorphism at PrP codon 129 in the pathogenesis of, and susceptibility to, prion diseases. Homozygosity at codon 129 for Met or Val appears to predispose people to sporadic CJD (Palmer et al. 1991). Sporadic CJD patients who are heterozygous at codon 129 (Met_{129}/Val_{129}) appear to have a more protracted course than do those who are homozygous (Met_{129}/Met_{129}) (Doh-ura et al. 1991). Although the Met_{129} allele is the most common in Caucasians and Asians, patients with iatrogenic CJD after pituitary growth hormone therapy were found to encode predominantly Val_{129} (Collinge et al. 1991). In the inherited prion diseases, a correlation between age at disease onset and homozygosity at codon 129 has been reported in patients with a 144-bp insertion in the PrP gene (Baker et al. 1991). Patients with a codon 178 mutation who encode a Met_{129} on the same allele appear to develop a disease called "fatal familial insomnia," while those who encode a Val_{129} present a dementing disorder more characteristic of CJD. While the age at onset was early for those homozygous for Val_{129}, there was no correlation of age at onset with homozygosity for Met_{129} (Goldfarb et al. 1992).

Although we and others (Goldfarb et al. 1990*a*; Hsiao et al. 1991*b*) have reported the presence of the codon 200 Lys substitution in Libyan Jews dying of CJD, to our knowledge no genetic linkage study has been published. We report here significant linkage between this PrP gene mutation and development of disease. This result is of particular importance, since the penetrance of familial CJD in these Libyan Jewish patients may be incomplete. Since the codon 129 polymorphism is thought to influence the phenotypic expression of the genetic, sporadic, and even the infectious forms of the prion diseases, we determined the codon 129 genotype in affected and nonaffected members of the Libyan Jewish community. Our studies indicate that the mutant allele encodes a Met_{129} in all Libyan Jews examined. No correlation of age at onset with homozygosity at codon 129 could be found.

Material and Methods

Patient Population

Twenty-eight patients were diagnosed as dying of CJD on the basis of (*a*) clinical signs and symptoms and (*b*) the presence of the Lys substitution at codon 200. Forty-six codon 200 mutation carriers were individuals belonging to CJD pedigrees. Thirty-eight noncarriers of the codon 200 mutation were siblings, cousins, and other close relatives of the carriers. Ninety unrelated Libyan Jews were examined as controls.

PCR Amplification and Allele-specific Hybridization

DNA samples from patients, mutation carriers, and controls were amplified by PCR with primers AAGGATCCCTCAAGCTGGAAAAAGAC (sense) and AAGAATTCTCTGACATTCTCCTCTTCA (antisense) to generate an 864-bp fragment including the PrP open reading frame (ORF). Samples of the PCR-amplified DNA were dot blotted onto GeneScreen + after denaturation and hybridized with oligonucleotide-specific probe K (GGTCTTGGTGAAGTT for Lys) or probe E (GGTCTCGGTGAAGTT for Glu) at codon 200 or with oligonucleotide-specific probe M (CGGCTACATGCTGGG for Met) or probe V (CGGCTACGTGCTGGG for Val) at codon 129, under conditions described elsewhere (Hsiao et al. 1991*a*).

Allele-specific Amplification

DNA from patients and mutation carriers was amplified by PCR to generate a 500-bp fragment of the PrP ORF by using primers that include the Met/Val polymorphic site at their 3′ end as sense (GCCTTGGCGGCTACA for Met and GCCTTGGCGGCTACG for Val) and antisense (AAGAATTCTCTGACATTCTCCTCTTCA). The samples were electrophoresed in a 1% agarose gel, blotted onto GeneScreen +, and hybridized with probes K and E as described above.

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 years to 70% at age 75 years. We estimated the disease gene frequency (unavailable in the literature) by the following approximate procedure: under the assumption made above, age at onset, A, has a uniform distribution with a density of 1/40 in the range from 35 years through 75 years, given that an individual is in the age range. About one-half the population falls into this age category, so that the unconditional density is equal to $P(A) = 1/80$. The incidence, $P(N)$, is known to be about 1/7,000 new cases/year and can be expressed as

$$P(N) = \Sigma_A P(A)P(N \mid A)\,, \qquad (1)$$

where $P(N \mid A)$ is the probability of developing the disease in the following year given that an individual is

Table 1

Number of Individuals Examined in the Present Study—Libyan Jewish CJD Patients, Mutation Carriers, and Unrelated Controls

	No. Verified	No. Suspected
CJD patients	28	17[a]
Healthy mutation carriers	46[b]	...
Mutation carriers age 65–70 years	3	5[c]
Unrelated Libyan controls	90	...

[a] Offspring are positive, but spouse is untested.
[b] All healthy mutation carriers were found within families of CJD patients.
[c] Obligate carriers.

currently unaffected, and summation is over ages 35–75 years. 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, $P(N/A) = (1/80)[1 - (1/80)(A - 35)][1 - (1 - p)^2]$, 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 $p = .0128$. 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, by assuming (*a*) presence ($\theta = 0$) and absence ($\theta = 1/2$) of linkage and (*b*) presence (δ_{max}) and absence ($\delta = 0$) of disequilibrium, where θ is the recombination fraction, and δ is the disequilibrium parameter. Because the PrP gene is a candidate gene, no intermediate values between $\theta = 0$ and $\theta = ½$ were tested. Meaningful estimation of δ from the data was difficult; therefore, and because disequilibrium was evidently very strong, only the maximum achievable δ and $\delta = 0$ were tested.

Results

Genotypic Determinations

PCR amplification followed by allele-specific oligonucleotide hybridization was used to screen known CJD patients, their family members, and normal, unrelated Libyan Jews (Hsiao et al. 1991*a*). Twenty-eight Libyan Jews diagnosed with CJD were found to carry the codon 200 Lys substitution, as determined by either direct testing or inference, when the living spouse was negative but an offspring was positive. Another 17 historical CJD patients were identified as suspected mutation carriers, when the spouse was unavailable for testing but some of the offspring were positive (table 1). One patient, a 42-year-old woman, was shown to be homozygous for the codon 200 mutation, and two other probable homozygous patients among the historical cases have been identified. While the spouses of these probable homozygotes were negative, all of their eight offspring (three from the first patient and five from the second patient) are carriers. Unfortunately, no tissue for genotyping from these probable homozygotes was available (table 2). Forty-six healthy Libyan Jews tested positive for the mutation, three of whom were older than 65 years of age. One hundred twenty healthy Libyan Jewish controls without a family history of CJD were found to be negative for the mutation.

We examined the codon 129 polymorphism among 23 Libyan Jewish CJD patients carrying the mutation at codon 200, 39 healthy codon 200 mutation carriers, 38 of their family members not carrying the mutation, and 66 healthy unrelated Libyan Jewish controls. The polymorphism frequency encoding either Met_{129} or Val_{129} was determined by allele-specific oligonucleotide hybridization (fig. 1). Among the normal Libyan Jewish population, the genotype frequency at codon 129 was 28% for Met_{129}/Met_{129} homozygotes, 56% for Met_{129}/Val_{129} heterozygotes, and 16% for Val_{129}/Val_{129} homozygotes. Tabulating the frequency of Met_{129}, we found that 63% of the total codon 129 alleles in this population encoded Met (table 3). This frequency is not significantly different from that reported for the Caucasian British population (Owen et al. 1990). Among the tested CJD patients, there were 24% heterozygous and 76% Met_{129}/Met_{129}, including a

Table 2

Patients Homozygous for the PrP Gene Codon 200 Mutation

	Verified	Suspected
No. of cases	1	2[a]
Age (in years) at disease onset	42	52 and 60
Clinical course	Typical	Typical

[a] All eight offspring (three from the first patient and five from the second patient) were positive for the mutation, while the spouses of these two patients were negative for the mutation.

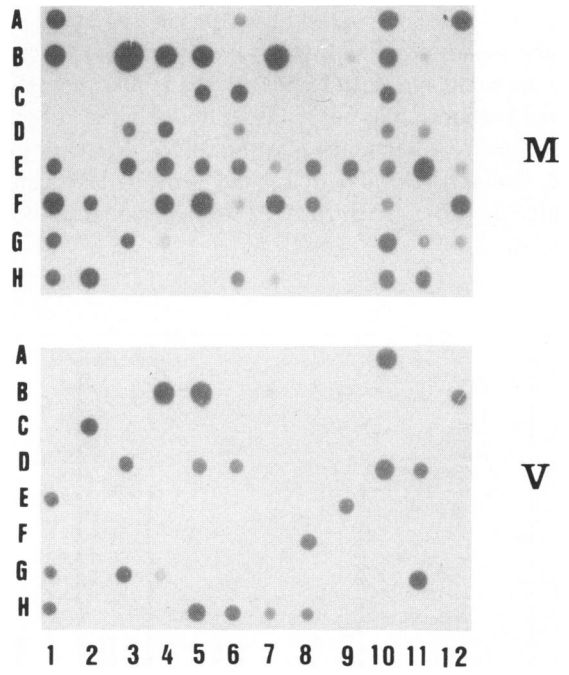

Figure 1 Allele-specific oligonucleotide hybridization for Met or Val at codon 129. The PrP ORF was amplified by PCR, blotted onto GeneScreen +, and hybridized with specific labeled oligonucleotides for either Met (*top;* designated "M") or Val (*bottom;* designated "V") at codon 129. Samples reacting with both probes are heterozygous for Met/Val at codon 129, while samples reacting with one of the probes are homozygous for the respective amino acid.

patient homozygous for the codon 200 Lys mutation. This results in a 90% Met_{129} frequency when both alleles are counted and in 78% Met_{129} frequency for the nonaffected allele. Similar results were obtained for the unaffected mutation carriers. Among family members of CJD patients without the codon 200 mutation, there were 61% Met_{129}/Met_{129}, 34% Met_{129}/Val_{129}, and 5% Val_{129}/Val_{129}, resulting in 78% Met_{129} (table 3). The difference in the frequency of Met_{129} in the general Libyan Jewish population compared with that in the members of Libyan Jewish families with CJD was statistically significant ($\chi^2 = 6.6$; $P < .025$). No differences in the frequency of Met_{129} in the normal allele were apparent among CJD patients, healthy codon 200 carriers, and normal family members without the mutation.

Lys_{200} Mutant Allele Contains a Met_{129}

No Libyan Jewish CJD patients or healthy mutation carriers were found to be homozygous for the Val allele at codon 129. A patient homozygous for the Lys_{200} mutation was also homozygous for Met_{129}. These findings suggested that the Lys_{200} mutation was present on an allele encoding Met_{129}. We used allele-specific PCR amplification in order to test this hypothesis (fig. 2). The sense primer included on its 3′ end the nucleotides encoding either Met_{129} or Val_{129}, while the antisense primer was the same one used for amplification of the entire PrP ORF. This strategy resulted in the allele-specific amplification of a 464-bp fragment of DNA band stretching from codon 129 to codon 753 at the 3′ end of the PrP ORF. No amplification occurred either in Met_{129}/Met_{129} individuals with the Val_{129} primer or in Val_{129}/Val_{129} individuals with the Met_{129} primer. No difference between the results obtained by this procedure and those using allele-specific oligonucleotide hybridization was observed. In Met_{129}/Val_{129} individuals with the Lys_{200} mutation, 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 allele encoding Met_{129}.

Correlations between Genotype and Disease Phenotype

The ages of the CJD patients with the codon 200 mutation at the time at onset of clinically detectable

Table 3

PrP Gene Codon 129 Polymorphism in Libyan Jews

	Met/Met	Met/Val	Val/Val[a]	Met (%)
CJD patients	18	5	NF	78[b]
Healthy codon 200 carriers	32	7	NF	82[b]
Related controls	23	13	2	78
Unrelated controls	25	33	8	63

NOTE.—At codon 129 of the human PrP gene, a Met or a Val is encoded.

[a] NF = not found.

[b] Calculated only for the allele not carrying the codon 200 mutation.

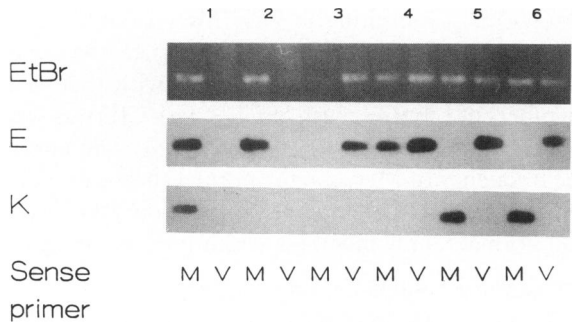

Figure 2 Allele-specific PCR amplification of the PrP gene. Primers specific for either Met (designated "M") or Val (designated "V") at codon 129 were used for allele-specific amplification. In each case, the 3′ nucleotide of the sense primer codes for the respective polymorphic site. The amplified fragments were then Southern blotted and hybridized with specific labeled 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).

neurological dysfunction are plotted in a histogram in figure 3 (*top*); the mean age is 56 years. The ages of the healthy, unaffected carriers with the Lys_{200} mutation are plotted in figure 3 (*bottom*). When the frequency of the Met_{129} polymorphism in the wild-type (wt) allele was compared for the two groups in figure 3, no difference was found. Next, we compared those CJD patients below and above the mean age at onset for the frequency of Met_{129} in the wt allele; again, no significant difference was found. The few Met_{129}/Val_{129} heterozygotes were dispersed among all age groups; furthermore, all healthy mutation carriers >56 years of age were homozygous for Met_{129} (table 4). The duration of clinical illness in CJD patients was also not different when those patients homozygous for Met_{129} were compared to heterozygotes (table 5). In addition, no substantial differences in the clinical course and age at disease onset were observed when patients homozygous for the Lys_{200} mutation were compared to heterozygotes.

Linkage Analyses

Linkage analyses were carried out 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 δ and for analyzing δ with or without linkage. As the results in table 6 show, there is no significant evidence for linkage without δ (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 δ, the lod score was equal to 4.85, which is significant evidence for linkage. Columns 3 and 4 of table 6 verify overwhelming evidence for δ, regardless of whether linkage is assumed.

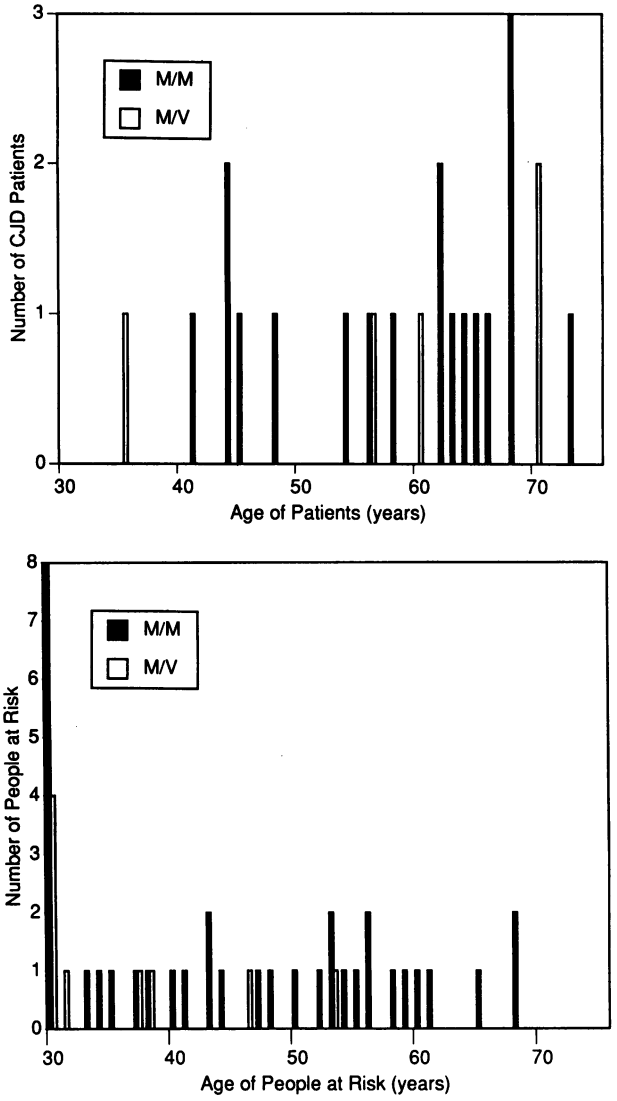

Figure 3 Codon 129 polymorphism as a function of the age at onset of CJD and the current age of codon 200 mutation carriers. *Top*, Histogram of the number of patients with the codon 200 mutation as a function of age at onset of CJD. *Bottom*, Histogram of the number of at-risk people with the codon 200 mutation as a function of their current age. Blackened bars denote Met/Met; unblackened bars denote Met/Val.

Table 4

PrP Gene Codon 129 Polymorphism in CJD Patients and Carriers, as a Function of Age

Age (years)	Met at Unaffected Allele (%)	
	CJD Patients	Codon 200 Carriers
>56	83	100
35–56	77	82

Discussion

Although all Libyan Jewish patients with CJD have been found to carry the Lys_{200} substitution, no reported linkage between the development of CJD and the Lys_{200} mutation has been published. Our results show both strong evidence for δ (with or without linkage) and strong evidence for linkage (given δ) between CJD and the PrP gene 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, δ and linkage, are somewhat confounded, but there is clearly a major effect of δ and a smaller but still substantial 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 at disease onset and homozygosity at codon 129 has been reported in patients with a 144-bp insertion in the PrP gene (Baker et al. 1991). Patients with a codon 178 mutation who encode a Met_{129} on the same allele appear to develop a disease called "fatal familial insomnia," while those who encode a Val_{129} present a dementing disorder more characteristic of CJD. While the age at onset was early for those homozygous for Val_{129}, there was no correlation of age at onset with homozygosity for Met_{129} (Goldfarb et al. 1992). In sporadic CJD, homozygosity at codon 129 was found to predispose patients to disease, whereas heterozygosity is thought to be protective (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 catalyzing the conversion of PrP^C to PrP^{Sc} on the basis of studies with transgenic mice (Prusiner et al. 1990; Prusiner 1991).

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 at onset of disease and homozygosity at codon 129 in Libyan Jews developing CJD. Older individuals with the Lys_{200} mutation who are homozygous for Met_{129} are not uncommon in the Libyan Jewish community, indicating that homozygosity at codon 129 does not necessarily result in disease at a relatively young age (table 4). Furthermore, the youngest CJD patient (35 years of age) tested was heterozygous at codon 129. In Libyan Jewish CJD patients, no difference could be appreciated in the duration of disease between patients homozygous or heterozygous at codon 129 (table 5), in contrast to one report describing shorter disease duration in Met_{129}/Met_{129} patients with sporadic prion diseases (Doh-ura et al. 1991).

Although our findings establish genetic linkage between the development of CJD and the Lys_{200} mutation, our observations also argue that factors other than the PrP gene sequence contribute to the disease pheno-

Table 5

Age at Onset of Symptoms and Duration of Disease in Libyan Jews with CJD, as a Function of the Codon 129 Polymorphism

Codon 129[a]	No. of Patients	Age at Onset (years)	Disease Duration (years)
Met/Met	14	60.8 (range 43–73)	4.3 (range 2–10)
Met/Val	5	58.6 (range 35–70)	5.2 (range 3–13)

[a] At codon 129 of the human PrP gene, a Met or a Val is encoded.

Table 6

Lod Scores (Test for Linkage) and χ^2 Values (Test for δ) for Different Disease Gene Frequencies

Gene Frequency	Lod Score		χ^2 Value	
	$\delta = 0$[a]	$\delta = max$[b]	$\theta = 0$[c]	$\theta = 1/2$[d]
.05	1.753	4.200	36.43	25.21
.0128	2.630	4.850	55.78	45.51
.001	3.010	5.160	95.59	85.61
.0001	3.022	5.192	132.30	122.31

[a] Relevant likelihood ratio = $L(\theta = 0, \delta = 0)$.
[b] Relevant likelihood ratio = $L(\theta = 0, \delta_{max})/L(\theta = {}^1/_2, \delta_{max})$.
[c] Relevant likelihood ratio = $L(\theta = 0, \delta_{max})/L(\theta = 0, \delta = 0)$.
[d] Relevant likelihood ratio = $L(\theta = {}^1/_2, \delta_{max})/L(\theta = {}^1/_2, \delta = 0)$.

type. In mice with experimental scrapie, genes other than that encoding PrP contribute significantly to control of the incubation time (Carlson et al. 1988). Since mice with ablated PrP genes (Prn-$p^{0/0}$) develop normally, the neurological dysfunction observed in the prion diseases cannot be due to an inhibition of PrP^{C} function but rather is due to an accumulation of PrP^{Sc} (Büeler et al. 1992). While the prominent role of PrP^{Sc} in the pathogenesis of the prion diseases is well established, gene products other than PrP and the aberrant metabolism of mutant PrP^{C} (Meiner et al. 1992) may also modify the course of the disease. It will be important to establish whether Libyan Jewish CJD patients produce only mutant PrP^{Sc} or whether they synthesize both mutant and wt PrP^{Sc}. Such studies should also clarify the influence of the codon 129 polymorphism, by comparing the PrP^{Sc} molecules produced in homozygous (Met_{129}/Met_{129}) and heterozygous (Met_{129}/Val_{129}) patients with the Lys_{200} mutation.

The increased frequency of the Met_{129} allele in members of affected families compared with members of unaffected pedigrees (table 3) suggests that the codon 200 mutation occurred in a single founder, probably homozygous for Met at codon 129, and was propagated in a limited number of families, the members of which did not significantly intermingle with the general Libyan Jewish population for many generations. Indeed, the mutation at codon 200 is restricted to a limited number of pedigrees and is completely absent in other Israeli Libyan families. This conclusion is supported by historical records indicating that Jews living in the areas of Tripoli and Djerba were isolated from other Jews inside and outside Libya and Tunisia. Intrafamily marriages were a common practice, a fact reinforced by the presence of individuals homozygous for the codon 200 mutation (table 2). Intrafamily marriages would favor the inheritance of a particular genotype in addition to PRNP, which could influence the phenotypic expression of CJD. In both Slovakia and Chile, CJD associated with the codon 200 mutation is also present in isolated communities (Goldfarb et al. 1990*a*). In the next generation, when young Libyan Jews from CJD-affected families marry into the general Israeli population, we shall be better able to appreciate whether changes in the phenotype of CJD caused by Lys_{200} will occur.

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