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https://hdl.handle.net/2324/5542

出版情報：American Journal of Human Genetics. 64 (4), pp.1063-1070, 1999-04. American Society of Human Genetics
バージョン：
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Am. J. Hum. Genet. 64:1063–1070, 1999

Ancestral Origins and Worldwide Distribution of the *PRNP* 200K Mutation Causing Familial Creutzfeldt-Jakob Disease

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Summary

Creutzfeldt-Jakob disease (CJD) belongs to a group of prion diseases that may be infectious, sporadic, or hereditary. The 200K point mutation in the *PRNP* gene is the most frequent cause of hereditary CJD, accounting for >70% of families with CJD worldwide. Prevalence of the 200K variant of familial CJD is especially high in Slovakia, Chile, and Italy, and among populations of Libyan and Tunisian Jews. To study ancestral origins of the 200K mutation–associated chromosomes, we selected microsatellite markers flanking the *PRNP* gene on chromosome 20p12-pter and an intragenic single-nucleotide polymorphism at the *PRNP* codon 129. Haplotypes were constructed for 62 CJD families originating from 11 world populations. The results show that Libyan, Tunisian, Italian, Chilean, and Spanish families share a major haplotype, suggesting that the 200K mutation may have originated from a single mutational event, perhaps in Spain, and spread to all these populations with Sephardic migrants expelled from Spain in the Middle Ages. Slovakian families and a family of Polish origin show another unique haplotype. The haplotypes in families from Germany, Sicily, Austria, and Japan are different from the Mediterranean or eastern European haplotypes. On the bais of this study, we conclude that founder effect and independent mutational events are responsible for the current geographic distribution of hereditary CJD associated with the 200K mutation.

Received November 13, 1998; accepted for publication February 10, 1999; electronically published March 12, 1999.

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0002-9297/99/6404-0018$02.00

Introduction

Creutzfeldt-Jakob disease (CJD; MIM 123400) is a rapidly progressive neurodegenerative disorder characterized by prominent dementia, cerebellar ataxia, rigidity, pyramidal signs, myoclonus, and paroxysmal bursts of high-voltage waves on electroencephalogram (Brown et al. 1994). Spongiform degeneration, neuronal loss, and astrocytic gliosis are the major neuropathologic features (Beck and Daniel 1987). The disease typically affects middle-aged individuals and leads to death within 6–24 mo after onset. CJD is randomly distributed worldwide with a yearly incidence rate of ~1/1,000,000 (Brown et al. 1986). From 5% to 10% of CJD cases show a familial pattern corresponding to autosomal dominant inheritance (Masters et al. 1979; Brown et al. 1994). Brain suspensions of patients with CJD, including patients with familial CJD, transmit the disease to experimental animals through intracerebral inoculation (Gibbs et al. 1968), confirming that this disorder is both hereditary and transmissible.

The *PRNP* gene coding for the prion protein has been mapped to chromosome 20p12-pter (Sparkes et al. 1986), and a number of mutations in this gene have been associated with hereditary forms of spongiform encephalopathy, familial CJD, Gerstmann-Sträussler-Scheinker disease, and familial fatal insomnia (Goldfarb and Brown 1995).

An A→G transition in codon 200 of the *PRNP* coding region changes the codon sequence from GAG to AAG and results in a predicted substitution of lysine (200K) for glutamate (200E) (Goldgaber et al. 1989). The 200K mutation accounts for >70% of cases of familial CJD. This mutation was first identified in a CJD family from Poland (Goldgaber et al. 1989) and later in clusters of this disease in Slovakia (Goldfarb et al. 1990*c*), Libyan Jews (Goldfarb et al. 1990*b*; Hsiao et al. 1991), Chile

(Brown et al. 1992), Italy (D'Alessandro et al. 1998), and Japan (Miyakawa et al. 1998). The annual incidence rate of CJD in some of these populations is much higher than the world average: ~200/1,000,000 in a rural population of Slovakia (Mitrova 1991), ~75/1,000,000 among the Libyan Jewish migrants living in Israel (Hsiao et al. 1991), and nearly 18/1,000,000 in central Chile (Galvez et al. 1980). Familial CJD associated with the 200K mutation has also been described in Tunisian and Greek Jews (Goldfarb et al. 1990*a*; Brown et al. 1991) and in non-Jewish groups in Spain (Coria et al. 1995), Britain (Collinge et al. 1993; Windl et al. 1996), and Austria (Hainfellner et al. 1996). Genetic linkage between the 200K mutation and familial CJD was established in genetic studies of this disease in Libyan Jews (Gabizon et al. 1993).

Analysis of worldwide distribution of the 200K CJD variant (Goldfarb et al. 1991) led to an early hypothesis that the 200K mutation originated in Spain and spread to Mediterranean countries and South America with a massive migration that resulted from the expulsion of Sephardic Jews. This hypothesis was supported by the finding that in all these populations the 200K mutation is coupled with 129M at the *PRNP* polymorphic codon 129, which has a variable M/V ratio in different populations. However, this hypothesis was disputed on the basis of other aspects of history (Gabizon et al. 1993; Korczyn 1994). A considerable number of Sephardic Jews emigrated to Turkey and the Netherlands. Those who came to the Netherlands soon intermarried with Ashkenazi Jews, but the 200K mutation has not been found in Sephardic Jews of Turkey or among Ashkenazi Jews in the Netherlands, England, or other western European countries to which Dutch Jews have emigrated. In addition, there has been a close interconnection between the Jewish communities of Libya, Tunisia, Greece, and France (Meiner et al. 1997), but it seems unlikely that Jewish people would intermarry with Slovaks or Chileans (Korczyn 1994). With the purpose of establishing ancestral origins of the 200K mutation, we selected highly polymorphic microsatellite markers flanking the *PRNP* gene on chromosome 20p12-pter and an intragenic single-nucleotide polymorphism to analyze the 200K mutation–associated haplotypes in 62 families representing 11 world populations.

Families and Methods

CJD Families and Controls

Sixty-two families segregating CJD with the 200K mutation, including 15 Libyan Jewish, 6 Tunisian Jewish, 6 Italian, 6 Chilean, 23 Slovakian, 1 Spanish, 1 Polish, 1 Japanese, 1 German, 1 Sicilian, and 1 Austrian, were selected for this study. These families represent all major countries/populations in which the 200K variant of familial CJD has been reported. Two or more family members were available for testing in 35 studied families. One of the Libyan subjects was homozygous for the 200K mutation. Unaffected healthy controls included 41 individuals of Libyan Jewish and 92 of Slovakian origin. Genotypes of French reference samples were also analyzed (CEPH database).

Screening for the 200K Mutation and the 129M/V Polymorphism

The GAG→AAG change at *PRNP* codon 200 abolishes a *Bsm*A1 restriction site ([N]5GAGAC), providing an easy technique to screen for this mutation (Goldfarb et al. 1990*a*). The ATG/GTG polymorphism at codon 129, coding for methionine or valine (M/V), is also conveniently determined by restriction analysis with endonuclease *Mae*II (Goldfarb et al. 1989). In all studied subjects, the coding region of the *PRNP* gene was amplified in a total volume of 10 μl with 50 ng genomic DNA; 1.75 mM $MgCl_2$; Perkin-Elmer PCR buffer (10 mM Tris-HCl [pH 8.3] and 50 mM KCl); 0.8 μM each primer, foward (5′-ATGCTGGTTCTCTTTGTGGCC-3′) and reverse (5′-GAAAGAGATCAGGAGGATCAC-3′); 250 mM each dNTP; and 0.3 U AmpliTaq DNA polymerase (Perkin-Elmer). PCR was performed in a 9600 GeneAmp thermocycler (Perkin-Elmer) under the following conditions: 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 120 s, with a final extension step of 72°C for 10 min. The amplicons subsequently were digested with *Mae*II or *Bsm*A1 (New England Biolabs) and resolved in a 2% agarose gel (GIBCO BRL). We cloned the PCR product using a TA cloning kit (Invitrogen) and performed restriction analysis when the 200 E/K 129 M/V phase could not be determined unambiguously from the pedigree.

Selection of Microsatellite Markers and Microsatellite Genotyping

Polymorphic markers flanking the *PRNP* gene in the 20p12-pter region were selected from the comprehensive linkage map of chromosome 20 (Center for Medical Genetics). To refine the location of the microsatellite markers in relation to the *PRNP* gene, we analyzed seven YAC clones (761-D-12, 894-D-11, 856-D-9, 753-G-9, 763-E-2, 938-C-2, and 898-D-9) from contig WC 20.0 (MIT Center for Genome Research). The YAC clones were screened by PCR for the presence and location of candidate markers (fig. 1).

The markers selected for genotyping were as follows: telomere-*D20S867* (*AFMb026xh5*)-2.1 cM-*D20S889* (*AFM234tf10*), *D20S116*-2.7 cM-*D20S482* (*GATA-51D03*), *PRNP* (*WI7784*), *D20S895* (*AFMb352xd9*), *D20S849* (*AFMa217zh9*)-centromere, spanning 4.8 cM. PCR amplification of the microsatellite markers was car-

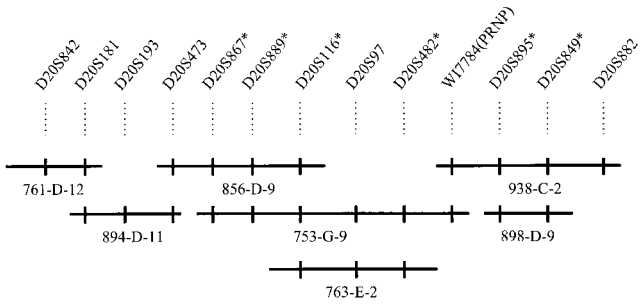

Figure 1 Integrated genetic and physical map of the *PRNP* region. Location of the microsatellite markers was determined by PCR analysis of a contig constructed of seven human YAC clones. WI7784 is the EST of the *PRNP* gene. Asterisk (*) denotes markers selected for haplotyping.

ried out with 80 ng genomic DNA in a 10-μl total reaction volume containing 2.5 mM $MgCl_2$, Perkin-Elmer PCR buffer (10 mM Tris-HCl [pH 8.3] and 50 mM KCl), 0.8 μM each forward and reverse primer, 250 mM each dNTP, and 0.3 U AmpliTaq DNA polymerase (Perkin-Elmer). The forward primers were fluorescently labeled with one of three phosphoamidites, 6-FAM, HEX, or TET (Research Genetics). PCR was performed in a 9600 GeneAmp thermocycler (Perkin-Elmer) under the following conditions: 95°C for 12 min, followed by 30 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 30 s, with a final 10-min extension at 72°C. The PCR-amplified fragments were mixed with a size standard (ABI GS500 ROX) and formamide-loading buffer and were loaded onto a 6% denaturing acrylamyde gel. We performed electrophoresis at 30W for 7 h using an ABI 373A sequencer (Perkin-Elmer). The size of the amplified microsatellite alleles was measured by GS ANALYSIS (2.1). The number of CA or GATA repeats was determined in each tested allele and standardized by comparison to genotypes of the reference sample CEPH 1347-02.

Linkage Disequilibrium and Haplotype Analysis

To estimate linkage disequilibrium between marker loci and the *PRNP* 200K mutation, allele frequency distributions in the disease and control chromosomes were compared. Using SAS version 6.11 (SAS Institute, Inc.), we applied Fisher's exact test for a $2 \times n$ contingency table, in which n is the number of observed alleles at each locus, with two rows representing 200K mutation carriers and the control group. Haplotypes were constructed manually, on the basis of the assumption of minimum recombination between markers. We prefered to use unambiguous microsatellite haplotypes for analysis. To use information from the ambiguous haplotypes, we estimated possible allele combinations using 3LOCUS.PAS (Long et al. 1995), on the basis of the EM algorithm. In the sample from the individual homozygous for the disease allele, only one chromosome was included, since consanguinity was strongly suspected in the pedigree.

Results

The Mediterranean Haplotype

Genetic maps based on linkage analysis do not provide optimal resolution or precise ordering of markers located in close proximity to the *PRNP* gene. Since such a problem may complicate haplotype analysis, we determined the location of markers by testing seven human YAC clones spanning the *PRNP* gene region on chromosome 20p12-pter (761-D-12, 894-D-11, 856-D-9, 753-G-9, 763-E-2, 938-C-2, and 898-D-9). The resulting integrated map of the *PRNP* region (fig. 1) defines the individual location of each marker. This analysis proved useful in selection of microsatellite markers for this study.

The Libyan Jewish families with CJD and controls were studied most extensively. We analyzed frequencies of the 200K mutation–associated microsatellite alleles of three markers closest to the *PRNP* gene and compared them with allele frequencies in the background population (table 1). The mutation-associated allele frequency of each marker deviated significantly from the controls ($P < .021$), suggesting that the 200K mutation–associated chromosomes in this population are identical by descent. Next, three-marker haplotypes were constructed for the Libyan Jewish families with CJD and compared with control haplotypes (table 2). Two related haplotypes, 20-14-18 and 20-14-19, show an outstandingly high frequency among the 200K mutation–associated chromosomes (.47 and .28). Of eight unambiguous Libyan Jewish haplotypes, 20-14-18 and 20-14-19 were found four times each. Six of seven ambiguous haplotypes were in agreement with one of the above haplotypes. Since 20-14-18 and 20-14-19 haplotypes share alleles at two loci, it is likely that one of them is derived from the other, supporting the idea of a single historic mutational event.

All six Tunisian Jewish families show an unambiguous 20-14-18 disease-associated haplotype, indicating that patients of Tunisian Jewish descent share one of the Libyan Jewish haplotypes. The haplotypes in six Italian and six Chilean families were determined with use of a computer program developed by Long et al. (1995) that allows researchers to analyze ambiguous haplotypes. The results suggest that the 20-14-18 and 20-14-19 haplotypes combined (.33 in Italian and .43 in Chilean populations) are significantly more common than any of the other possible haplotypes (table 2). Although controls for Italian and Chilean populations were not available for this study, we assume that the frequency of the 20-

Table 1

Microsatellite Allele Frequencies in Unambiguous Libyan Jewish and Slovakian 200K Mutation–Associated and Control Chromosomes

Locus and Allele	Libyan 200K Chromosomes (n = 8)	Libyan Control Chromosomes (n = 82)	Slovakian 200K Chromosomes (n = 18)	Slovakian Control Chromosomes (n = 184)
D20S116:				
17	.00	.00	.00	.002
18	.00	.23	.11	.30
19	.00	.07	.00	.13
20	1.00	.36	.89	.20
21	.00	.25	.00	.17
22	.00	.02	.00	.16
23	.00	.01	.00	.01
24	.00	.06	.00	.01
		P<.001		P<.001
D20S482:				
12	.00	.00	.00	.05
13	.00	.14	.00	.25
14	1.00	.61	.17	.38
15	.00	.15	.83	.24
16	.00	.09	.00	.07
17	.00	.01	.00	.01
		P<.021		P<.001
D20S895:				
18	.50	.05	.00	.03
19	.50	.37	.94	.43
20	.00	.04	.00	.03
21	.00	.15	.06	.06
22	.00	.10	.00	.07
23	.00	.22	.00	.32
24	.00	.00	.00	.03
25	.00	.07	.00	.03
		P< .001		P<.001

14-18 and 20-14-19 haplotypes in the background populations could not be significantly different from Libyan Jewish or CEPH reference samples (table 2). The 20-14-18 haplotype was observed unambiguously in the large Spanish family. The consistent presence of a rare haplotype in the disease chromosomes of subjects with CJD from four Mediterranean countries and Chile strongly suggests a common ancestral origin.

The Eastern European Haplotype

Microsatellite allele frequencies in the Slovakian unambiguous 200K chromosomes were significantly different from the Slovakian population controls ($P <$ .001) for all three microsatellite markers tested (table 1), suggesting that a single ancestral chromosome is responsible for familial CJD in the Slovakian cluster. Consensus haplotype 20-15-19 is seen much more frequently (.70) than other possible haplotypes among the Slovakian 200K chromosomes or in Slovakian controls (table 2). This haplotype was found in 14 of 18 unambiguously determined 200K chromosomes. The single family of Polish origin shares the Slovakian consensus haplotype, confirming that the 200K mutation in this family has a common origin with the Slovakian cluster.

In an attempt to clarify the relationship between the Mediterranean and eastern European 200K haplotypes, we constructed and compared six-marker consensus haplotypes (table 3). The unambiguous Libyan Jewish chromosomes are divided between 18-28-20-14-18-13 (Libyan-1) and 21-28-20-14-19-19 (Libyan-2) haplotypes that most likely drifted apart as a result of a double recombination between *D20S867* and *D20S889*, at the telomeric end, and between *D20S482* and *D20S895*, at the centromeric end. The Tunisian Jewish mutated chromosomes are also represented by two haplotypes, 18-28-20-14-18-13 (Tunisian-1), which is identical to Libyan-1, and 23-25-20-14-18-17 (Tunisian-2), which is original. The Spanish haplotype is identical to Libyan-1 and Tunisian-1. The Slovakian consensus haplotype 19-27-20-15-19-13 is different from any of the Mediterranean haplotypes in three of six alleles, suggesting an independent origin. A disease haplotype distinct from any of the preceding was found in a Japanese family (tables 2 and 3).

The Western European Haplotypes

The unambiguous 200K mutation–associated microsatellite haplotype 19-13-23 in a family of German or-

Table 2

Microsatellite Three-Marker Haplotypes and *PRNP* 129M/V Polymorphism in the 200K-Associated and Control Chromosomes

Haplotype			200K-Associated Chromosomes											Control Chromosomes		
D20S116	D20S482	D20S895	Libyan Jews ($n = 15$)	Tunisian Jews ($n = 6$)	Italy ($n = 6$)	Chile ($n = 6$)	Spain ($n = 1$)	Slovakia ($n = 23$)	Poland ($n = 1$)	Japan ($n = 1$)	Germany ($n = 1$)	Sicily ($n = 1$)	Austria ($n = 1$)	Libyan ($n = 43$)	Slovakian ($n = 92$)	CEPH ($n = 28$)
18	13	18	…	…	…	…	…	…	…	…	…	…	…	…	…	…
18	13	19	.03	…	.00	…	…	.02	…	…	…	…	…	.03	.03	.06
18	13	21	…	…	…	.08	…	…	…	…	…	…	…	.02	…	…
18	13	22	…	…	…	…	…	…	…	.50	…	…	…	…	.01	…
18	13	23	…	…	…	…	…	…	…	.50	…	…	…	…	…	.04
18	14	18	…	…	.25	…	…	…	…	…	…	…	…	.04	…	…
18	14	19	…	…	…	.08	…	.09	…	…	…	…	…	.01	…	…
18	14	23	.06	…	…	…	…	…	…	…	…	.50	…	.01	.08	…
18	15	23	…	…	…	…	…	…	…	…	…	.50	…	.02	.03	…
19	12	25	…	…	…	…	…	.02	…	…	…	…	…	…	…	…
19	13	23	…	…	…	…	…	…	…	…	1.00	…	…	.04	…	…
19	15	19	…	…	…	…	…	.02	…	…	…	…	…	…	…	…
20	13	18	…	…	…	…	…	…	…	…	…	…	…	…	.01	…
20	13	19	.03	…	.08	…	…	…	…	…	…	…	…	…	.03	.02
20	14	18	.47	1.00	.25	.42	1.00	…	…	…	…	…	…	…	.01	.02
20	14	19	.28	…	.08	…	…	.07	…	…	…	…	…	.14	.04	.03
20	14	21	.03	…	…	…	…	…	…	…	…	…	.13	.04	.04	.02
20	14	22	…	…	…	.08	…	…	…	…	…	…	…	.05	…	…
20	14	23	…	…	…	.08	…	…	…	…	…	…	.13	…	…	.08
20	14	27	…	…	.08	…	…	…	…	…	…	…	…	.04	…	…
20	15	19	…	…	…	…	…	.70	1.00	…	…	…	…	…	.05	.10
20	15	21	…	…	…	…	…	.04	…	…	…	…	.13	…	…	…
20	15	23	…	…	…	…	…	.02	…	…	…	…	.13	.02	…	.02
21	13	19	…	…	.08	…	…	…	…	…	…	…	…	…	…	…
21	13	23	…	…	…	.17	…	…	…	…	…	…	…	…	.05	.02
21	14	19	.03	…	…	…	…	.02	…	…	…	…	…	.13	.05	.05
21	14	21	…	…	…	…	…	…	…	…	…	…	.13	…	…	…
21	14	23	…	…	…	…	…	…	…	…	…	…	.13	.09	.01	…
21	15	21	…	…	…	…	…	…	…	…	…	…	.13	…	…	…
21	15	22	…	…	…	.08	…	…	…	…	…	…	…	…	…	…
21	15	23	…	…	.08	…	…	…	…	…	…	…	.13	…	…	…
22	14	19	.03	…	…	…	…	…	…	…	…	…	…	…	…	…
22	14	21	…	…	.08	…	…	…	…	…	…	…	…	.01	…	…
24	14	21	.03	…	…	…	…	…	…	…	…	…	…	.03	…	…
Other	…	…	…	…	…	…	…	…	…	…	…	…	…	.28	.57	.53
PRNP 129M/V	…	…	M	M	M	M	M	M	M	M	V	V	V	M/V	M/V	M/V

Table 3

Six-Marker Haplotypes in 200K Mutation–Associated Chromosomes from Mediterranean, Eastern European, and Japanese Populations

		Microsatellite Marker					
Haplotype	No. of Families	D20S867	D20S889	D20S116	D20S482	D20S895	D20S849
Libyan -1	5	18	28	20	14	18	13
Libyan-2	2	21	28	20	14	19	19
Tunisian-1	3	18	28	20	14	18	13
Tunisian-2	2	23	25	20	14	18	17
Spanish	1	18	28	20	14	18	16
Slovakian	11	19	27	20	15	19	13
Japanese	1	18/22	22/24	18	13	22/23	16/20

igin is unique (table 2). The disease haplotypes in the Sicilian and Austrian families are ambiguous, and it is not clear whether they are related to the German haplotype. In all three western European families, the 200K mutation is coupled with GTG sequence coding for valine at the *PRNP* polymorphic codon 129 (200K/129V haplotype), in contrast to the Mediterranean, eastern European, and Japanese CJD 200K chromosomes, with 129 ATG sequence coding for methionine (200K/129M haplotype). Since the distance between the 200K mutation and the polymorphic codon 129 is only 213 bp, no recombination in this short fragment could be expected. Thus, the 200K chromosomes in patients from Germany, Sicily, and Austria has an ancestral origin independent of the Mediterranean, eastern European, or Japanese disease-associated chromosomes.

Discussion

Because of an unusually high prevalence in several geographically distant clusters, the origin of the 200K mutation has been a subject of interest. Our results show that patients with CJD who originate from Libyan Jewish and Tunisian Jewish populations, and from Spanish, Italian, and Chilean non-Jewish populations all share the 200K mutation–associated haplotype. The fact that families affected by CJD, from five different geographic locations, share a rare disease-associated haplotype suggests that this haplotype has a common origin. We concur with the first part of the hypothesis, presented elsewhere, that the 200K mutation originated in the Iberian Peninsula and spread to Mediterranean and South American countries, on the basis of the following historic records. In 1492, the monarchs of Spain issued a decree of expulsion of every Spanish Jew who refused to accept Christianity. Two hundred thousand Jews moved to North Africa, Turkey, France, Greece, Yugoslavia, Syria, Palestine, Italy, and later to the Netherlands (Alpher 1986). Sephardim (the Spanish Jews) joined Jewish communities that had existed in these areas for>1,000 years. For many of the converted new Christians who remained in Spain and Portugal, life was still insecure. Some of them migrated to South American countries, including Chile and Argentina, where their descendants are still being remembered among the old Spanish families, as, for example, the Medinas of Chile (Coon 1965). A Chilean family affected with the 200K variant of familial CJD was identified as progeny of 17th century Spanish immigrants (Salvatore et al. 1996).

The presence of several 200K mutation–associated Mediterranean haplotypes may correspond to a different scenario. The Libyan-2 haplotype 20-14-19 is present in the Libyan Jewish control population with a slightly higher frequency than in other control populations, suggesting that this may have been the ancient chromosome on which the 200K mutation originally occurred. This finding supports the previously expressed view that CJD has been endemic in Libya longer than in other populations (Gabizon et al. 1993). The original Libyan-2 haplotype may have been brought to Spain with the ancient Jewish migrants and changed into the modern 20-14-18 haplotype as a result of a double recombination. This newer haplotype has subsequently spread with the Middle Ages Sephardic migration to South America and the Mediterranean countries including Libya. The Italian population affected with the 200K variant of familial CJD is not known to have Jewish roots, but the presence of Sephardic communities in Italy in the Middle Ages has been reported (Alpher 1986).

The eastern European 200K mutation–associated consensus haplotype shows major differences from the Mediterranean haplotypes. The Slovakian families affected with CJD living in a mountainous area of central Slovakia are descendants of 13th–16th century immigrants from Romania and Russia (Mitrova et al. 1991). On the basis of haplotype analysis and historic data, the 200K mutation in the Slovakian cluster had an origin different from the 200K mutation that was spreading in the Mediterranean countries and South America. However, some caution is needed, since the intragenic M/V polymor-

phism at codon 129 of the *PRNP* gene used in this study shows the presence of the same 129M allele in the Slovakian and Mediterranean 200K mutation–associated chromosomes. The origin of the German, Sicilian, and Austrian 200K/129V haplotypes is definitely different from either the Mediterranean or Slovakian haplotypes, since they contain the alternative amino acid at position 129. The haplotype in the Japanese family is also unique.

A change from CG to TG is one of the most common human mutations. Cytosine in the CpG dinucleotide sequence is known to be methylated frequently to produce a 5-methylcytosine and these spontaneously deaminate to thymine, resulting in a C→T transition (Bird 1980; Cooper and Krawczak 1993).

We conclude from these data that at least four independent mutational events are responsible for the current geographic distribution of the 200K variant of hereditary CJD. The 200K mutation of Spanish origin drifted around the world with the migrating Sephardic population. A similar historic and genetic analysis of the world distribution of Machado-Joseph disease showed a Portuguese founder haplotype that had spread to France (Stevanin et al. 1995) and Japan (Takiyama et al. 1995), whereas independent mutations have occurred in the Algerian, Belgian, Guianese (Stevanin et al. 1995), and Brazilian (Iughetti et al. 1996) populations.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

CEPH database, http://www.cephb.fr/cephdb/ (for genotypes of French reference samples)

Center for Medical Genetics, http://www.marshmed.org/genetics (for chromosome 20 markers)

MIT Center for Genome Research, Whitehead Institute for Biomedical Research, http://www-genome.wi.mit.edu (for microsatellite markers)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for CJD [MIM 123400])

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