CREUTZFELDT-JAKOB DISEASE WITH E200K MUTATION IN SLOVAKIA: CHARACTERIZATION AND DEVELOPMENT

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Summary. – Creutzfeldt-Jakob disease (CJD), the most important human prion disease, occurs in sporadic, iatrogenic and familial form. Except Slovakia and Israel, the recorded familial cases have never exceeded 10–15%. In the Slovak CJD group 95 out of 136 CJD cases (74.2%) carried a CJD-specific mutation in the prion protein gene (PRNP) at codon 200 (mutation E200K). All CJD^{E200K} patients carried a heterozygous E200K mutation within the alelle with methionine at codon 129. No more than 53.7% were typical familial cases. The penetrance of the E200K mutation in 1975–2000 was 59.5%. The distribution of codon 129 polymorphism showed 78.6% of methionine-homozygous and 21.4% of methionine/valine-heterozygous patients. Genetic analysis performed on 278 CJD patient relatives demonstrated the E200K mutation in 97 (34.8%) of healthy relatives tested. The E200K mutation carriers were methionine-homozygous in 64% and methionine/valine-heterozygous in 36%. The relatives without the mutation showed a 54.9% methionine homozygosity, 10.4% valine homozygosity and 34.7% methionine/valine heterozygosity. Analysis of the E200K carriers provided evidence that the methionine homozygosity is a CJD risk factor, more efficient in CJD patients than in asymptomatic relatives. Th influence of both the E200K mutation and methionine homozygosity at codon 129 was evident in the duration of the clinical stage of CJD and in the immunoreactivity pattern of PrP resistant to proteases (PrP^{res}). In the CJD^{E200K} methionine-homozygous patients the mean duration of the disease was significantly shorter (3.7±2.0 months) than in the methionine/valine-heterozygous patients (7.84±7.3 months). Comparison of the PrP^{res} positivity in the cerebellum of familial and sporadic CJD using specific polyclonal and monoclonal antibodies (MAbs) to PrP showed less conspicuous immune reaction in CJD^{E200K} cases. Methionine-homozygous CJD patients were characteristic mainly by synaptic pattern of staining, while methionine/valine-heterozygous patients by PrP^{res} granules and plaque-like structures. Most of numerous plaque-like PrP^{res} deposits were found in sporadic valine/valine-homozygous cases. Potential professional risk was excluded in health facility workers. The percentage of professions related to farming was significantly higher in CJD^{E200K} (48%) and sporadic CJD (44%) cases as compared to the employed population (9%).

Key words: Creutzfeldt-Jakob disease; genetic form; E200K mutation; penetrance; potential professional risk; Slovakia

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Abbreviations: BSE = bovine spongiform encephalopathy; CJD = Creutzfeldt-Jakob disease; PrP = prion protein; PrP^{res} = PrP resistant to proteases; PrP^{sen} = PrP sensitive to proteases; PRNP = prion protein gene; E200K = mutation of PRNP at codon 200; CJD^{E200K} = CJD with E200K mutation; GSS = Gerstmann-Streussler syndrome; wtPrP = wild type PrP; ctmPrP = transmembrane form of PrP with carboxy terminus in the endoplasmic reticulum; PCR = polymerase chain reaction; SAF = scrapie-associated fibrils; TSE = transmissible spongiform encephalopathies

Introduction

Transmissible spongiform encephalopathies (TSE), also called prion diseases, are rare fatal neurodegenerative disorders affecting humans and animals. Recently, due to the epidemic of bovine spongiform encephalopathy (BSE), their public health importance has significantly increased. In all affected hosts a patognomic insoluble glykoprotein, prion (PrP) is present. As partially resistant to proteases, it

has been designated PrP^{res}. The precursor of PrP^{res} is a soluble proteases-sensitive PrP^{sen}. In humans it is encoded by the PrP gene (PRNP) on the chromosome 20. There is convincing evidence that genetic control plays a decisive role in the spread of the disease. In humans the most important TSE is CJD. It occurs as a classical variant (CJD) described in 1920–1921 and a new variant (nvCJD) related to BSE and described in 1996 (Will *et al.*, 1996). CJD is known as sporadic (85%), iatrogenic (1–3%) and familial. Familial CJD follows autosomal dominant pattern with the occurrence of 10–15% recorded worldwide. Slovakia is an exception, since as many as 95 of 128 (74.2%) CJD patients carry on the PRNP gene a CJD-specific mutation at codon 200 (mutation E200K). In the mutant allele the amino acid lysine is substituted by glutamic acid. The E200K mutation spread worldwide (France, Germany, Chile, Israel, Italy, Japan, and Slovakia) is the most frequent TSE-specific mutation, in occurring 60% according to Alperovitch *et al.* (1999) and in 70% of TSE cases according to Goldfarb *et al.* (1991). In affected families, besides CJD patients also asymptomatic carriers of this mutation have been found. It has not yet been proved and/or explained whether and how this mutation influences the clinical onset and manifestation of the disease. The potential risk to develop the disease in "healthy" persons carrying the mutation or to transmit the disease by tissue donation has not yet been determined. As it has been already published, familial CJD shares some similarities with sporadic CJD (Brown, 1992; Lee *et al.*, 1999). On the other side, there are obvious differences between familial CJD and other familial TSEs. More detailed studies of E200K carriers could render a useful contribution to a better understanding of this important CJD subgroup as well as to a general knowledge of human TSE.

The aim of this study was (i) to characterize the CJD^{E200K} group, (ii) to compare the data on familial CJD with those on sporadic CJD and other familial TSE, and (iii) to apply the obtained results to the assessment of CJD risk for healthy carriers of E200K mutation, all these aims applied to Slovakia only.

Patients and Methods

Patients. The prospective study of CJD in Slovakia has started in 1975 following the first experimental transmission of familial CJD to domestic cats. The Slovak National Reference Laboratory of Slow Virus Neuroinfections (SNRLSVN) was established in 1979. Since 1983, CJD reporting in Slovakia was obligatory. The total number of definite CJD patients in 1975–2001 was 136 with a man to women ratio of 0.88. Out of 128 genetically tested patients 95 carried the E200K mutation of the PRNP gene. Iatrogenic CJD and nvCJD were not recorded, one suspected nvCJD case (without autopsy) remains under investigation. Data for epidemiological and genealogical studies have been collected personally in affected families, local and parish registers.

Diagnostics. Definitive diagnosis of all clinically suspected CJD cases has been confirmed at NRLSVN SR by histopathology, later also by demonstration of scrapie-associated fibrils (SAF) and by immuno-histochemical detection of PrP using specific MAbs 3F4 (DAKO) and 6H4 (Prionics).

Detection of specific mutations in PRNP gene was performed by standard techniques in SNRLSVN. Since 1990 patients' DNAs from CJD foci as well as from extrafocal cases were sequenced in the Laboratory of Central Nervous System Studies, NIH, Bethesda, USA. The sequencing did not reveal any other PRNP mutations except E200K. Besides new patients also old cases, for which frozen brain tissues were available, were subjected to the testing. The E200K mutation was searched for in all cases, while the polymorphism at codon 129 of PRNP gene was searched for only in 60 carriers of the E200K mutation. Moreover, also 278 relatives of CJD patients were searched for both types of mutation. Total DNA was extracted from peripheral blood leukocytes or brain tissue according to Goldfarb *et al.* (1991). The PRNP coding region (717 bp) was amplified by polymerase chain reaction (PCR). The PCR products were digested with restriction endonucleases *Bsm*AI and *Mae*II which enabled detection of point mutations at codons 200 and 129, respectively. The resulting restriction fragments were separated by agarose gel electrophoresis and visualized by ethidium bromide staining (Figs. 1 and 2).

Statistical evaluation of the age and duration of the disease was calculated using the multifactor analysis of variances and the MannWhitney Wilcoxon test (Finn, 1974).

Results

Detection of E200K mutation and codon 129 polymorphism in CJD patients

The E200K mutation was present in 95 patients, representing 74.8% of verified CJD cases. All CJD^{E200K} patients carried a heterozygous E200K mutation within the codon 129 methionine alelle. A homozygous carrier of the E200K mutation was not found. Family history of CJD had 51 patients (53.6% of typical familial cases). In the rest (46.4%) of mutation E200K carriers, either family history or genealogical study did not reveal any other affected family member (sporadic-like cases). The distribution of codon 129 polymorphism was as follows: the CJD^{E200K} group – 78.6% of methionine/methionine and 21.4 % of methionine/valine, the sporadic CJD group – 55% of methionine/methionine, 35% of methionine/valine and 10% of valine/valine, the

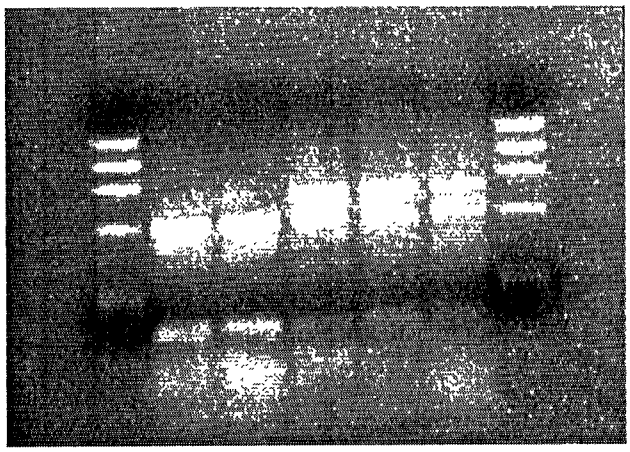

Fig. 1
***Bsm*AI restriction patterns of PCR products from CJD patients with and without E200K mutation**

The enzyme cleaves a PCR product of normal allele into 2 fragments, while the E200K mutation abolishes this cleavage site. Therefore individuals heterozygous for the mutation show a 3-band pattern composed of 2 bands (158 and 122 bp) from the cleaved normal allele and 1 band (280 bp) from the uncleaved mutated allele. CJD patients without the E200K mutation (lanes 1 and 2) and CJD patients with the E200K mutation (lanes 3–5). DNA size markers (lanes M).

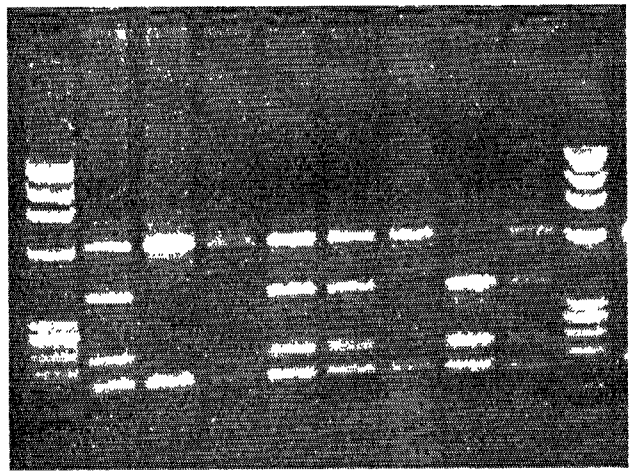

Fig. 2
Codon 129 genotypes after *Mae*II digestion

A PCR product of the methionine allele gives after the digestion 2 fragments (185 and 570 bp), while the valine allele gives 3 fragments (350, 220 and 185 bp. Methionine/valine heterozygots (lanes 1, 4, 5 and 8), methionine homozygots (lanes 2, 3, and 6), and a valine homozygot (lane 7). DNA size markers (lanes M).

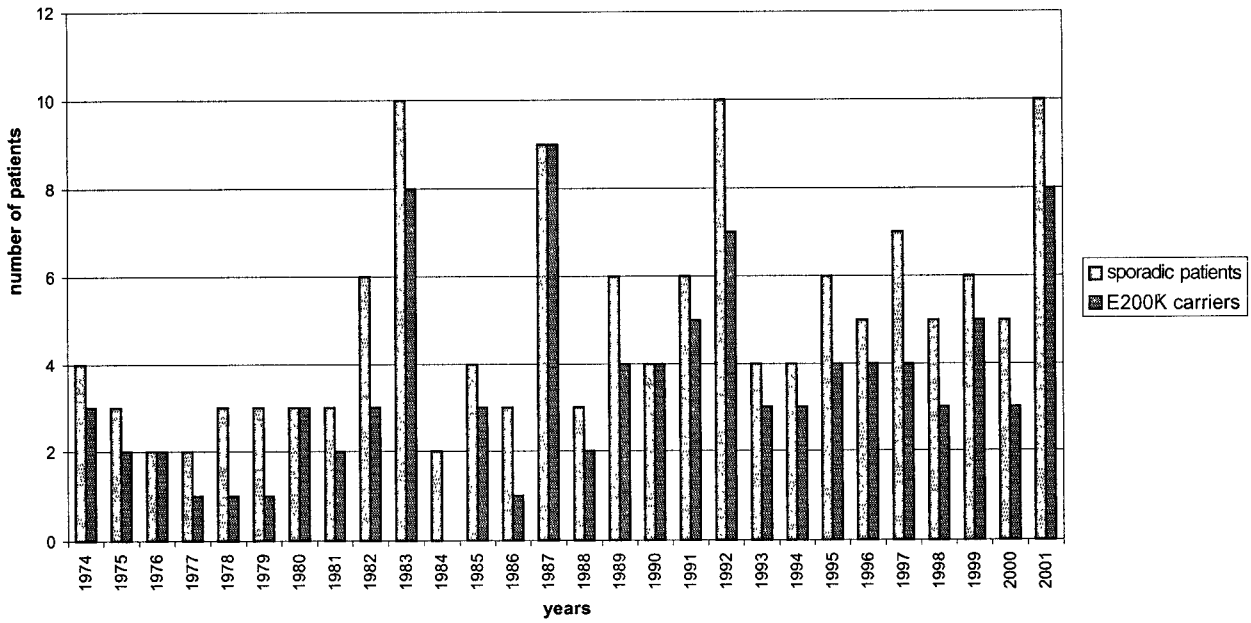

Fig. 3
Annual occurrence of CJD in Slovakia according to the death rate of the patients in 1975–2001

Table 1. The age-adjusted CJD mortality rate in Slovak population

Age group (years)	Population (thousands)		CJD patients					
			Men		Women		Total	
	Men	Women	Death	Rate	Death	Rate	Death	Rate
30–39	417	409	3	0.29	1	0.10	4	0.20
40–49	269	285	7	1.04	12	1.68	19	1.37
50–59	242	272	25	4.13	29	4.26	54	4.20
60–69	181	226	21	4.64	27	4.78	48	2.65
70–79	102	148	1	0.40	6	1.62	7	1.28
Total	1211	1340	57	2.1	75	2.49	132	1.94

control group (200 subjects tested) – 51.3% of methionine/valine, 41.4% of methionine/methionine, and 7.3% of valine/valine.

Annual occurrence, distribution by age, gender and age at death in CJD patients with and without the E200K mutation

Annual occurrence of CJD is demonstrated in Fig. 3. An increased number of the E200K mutation carriers was observed in 1983, 1987, 1992, and 2001. The annual occurrence of patients adjusted to the regional/local population in CJD foci ranged from 21.59 to 154.63/million. The distribution of average annual age- and gender-specific mortality rates showed no death under 30 years of age (the peak was in the 60–69 age group) and over 80 years of age. In the group of sporadic CJD the peak was in the 50–59 age group. The death rate was considerably higher among females in the 70–79 age group (Table 1).

The mean age at death in CJD patients showed gradually increasing values since 1992, The increase in age at death is more pronounced and earlier in females. For the investigated period 1975–2001 the mean age at death was 58.07±8.3 (56.25±8.6 years in sporadic CJD). In the patients methionine homozygous at codon 129 the mean age was 58.76±8.1, while in the patients methionine/valine heterozygous it was 59.53±10.5 years.

Significant difference of the mean age at death was observed neither in relation to E200K mutation nor to the codon 129 polymorphism.

Geographical distribution of CJD^{E200K} patients

This characteristic according to the birthplace showed two distinct rural peaks in the northern and southern part of central Slovakia. Gradually, the E200K carrier cases appeared in the whole central region and patients as well as healthy carriers have been sporadically found also in eastern and western parts of the country. E200K carriers (patients and relatives) have been detected in the adjacent part of Hungary, among Slovak immigrants to Belgium, France, USA and Canada.

Effect of E200K mutation on the duration of clinical stage of CJD

The mean duration of the clinical stage of CJD appeared shorter in E200K carriers (5.58±5.4 months) as compared to sporadic CJD cases (10.97±17.2 months), but the difference was not significant. A significantly shorter mean duration of the disease was found in methionine homozygous (3.66±2.00 months) as compared to methionine/valine heterozygous (7.84±7.34 months) CJD^{E200K} patients. The mean difference of 4.18 months was significant. In sporadic CJD methionine homozygous cases the mean duration was 3.85± 2.11 months, while in methionine/valine heterozygous cases it was 21.85±17.42 months with a mean non-significant difference of 17.97 months. This difference was evident also when CJD-affected families were compared according to their codon 129 genotype. In families with methionine homozygous cases the range of duration was 3.5–4.0 months, while in those with methionine/valine heterozygous cases it was 7.0–12.0 months. The duration correlated with distinct genotypes even inside one family: The clinical stage of CJD in a methionine/methionine homozygous patient lasted only 3 months, while in heterozygous methionine/valine cousins it lasted 8 and 36 months (Fig. 4).

Incidence of E200K mutation in relatives of CJD^{E200K} patients

Genetic analysis performed on 278 family members detected the E200K mutation in 97 (34.8%) asymptomatic relatives. Asymptomatic "healthy" carriers have occurred in older generations with numerous (8–12) children in about 40–50% of descendants. A dramatically decreased number of children (1–2) in the last decade were followed by occurrence of families with generations free of PRNP gene mutation. In relatives with the E200K mutation the methionine homozygosity occurred in 64% and the

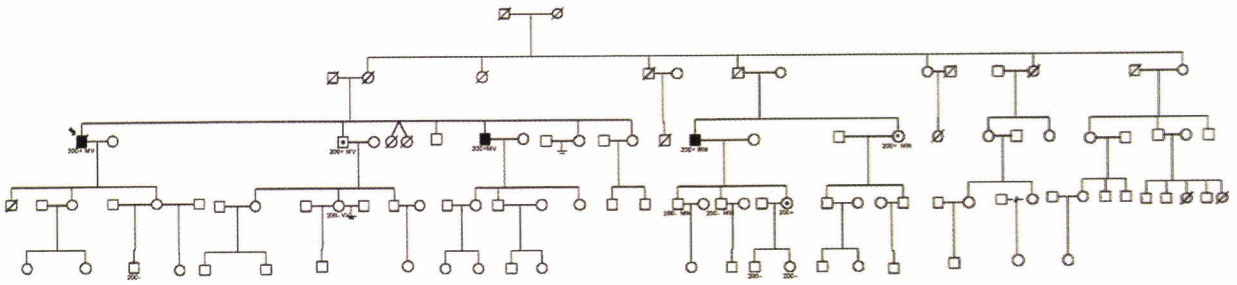

Fig. 4
Pedigree of the family with 3 members affected by CJD^{E200K}

Duration of the disease in two brothers, methionine/valine heterozygous at codon 129, was longer (8 and 36 months) than in the methionine homozygous cousin (3 months).

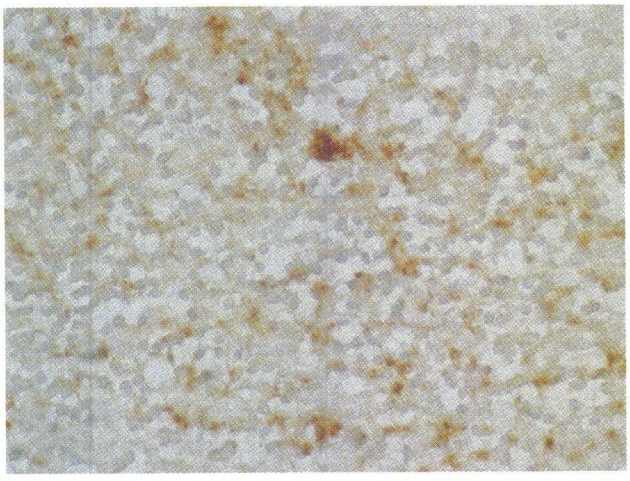

Fig. 5
A familial CJD^{E200K} patient, methionine/valine heterozygous at codon 129 of the PRNP gene

A few scattered PrP^{E200K} granules with one plaque-like in the cerebellum. PrP^{res} antibodies (3F4). Magnification 10 x 40.

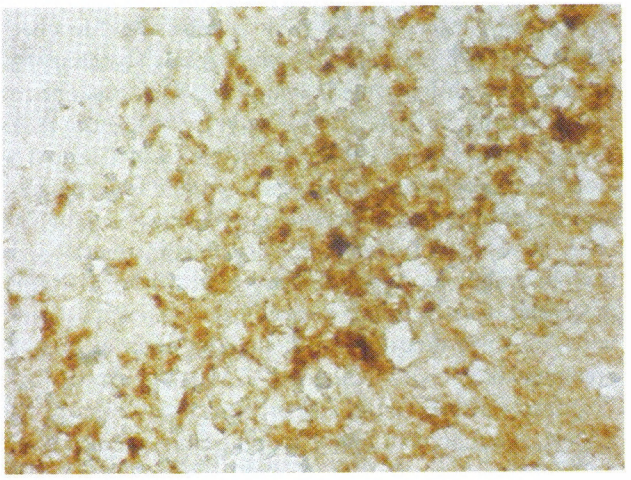

Fig. 6
A sporadic CJD^{E200K} patient, methionine/valine heterozygous at codon 129 of the PRNP gene

PrP^{res} immune reaction in the granular layer of the cerebellum, numerous PrP granules and a few plaque-like structures. PrP^{res} antibodies (3F4) used. Magnification 10 x 40.

methionine/valine heterozygosity in 36%. No valine homozygous person was found. Relatives without the mutation (65%) showed a 54.9% methionine homozygosity, a 34.7% methionine/valine heterozygosity, and a 10.4% valine homozygosity (data not shown).

Neuroimmunohistochemical detection of PrP in CJD patients with and without E200K mutation

Detection of PrP^{res} was performed in the cerebellum of CJD patients. A comparison of 20 patients with and 14 patients without the E200K mutation revealed striking difference in the positivity pattern, considering both the E200K mutation and the codon 129 polymorphism. The PrP positivity, observed more in granular and less in molecular layer, was more conspicuous in sporadic CJD cases than in CJD^{E200K} cases. CJD^{E200K} methionine homozygous patients showed mainly synaptic type of immune PrP^{res} positivity, while in methionine/valine heterozygous cases a small number of PrP^{res} granules and unique plaque-like structures could be found (Fig. 5). Methionine/valine heterozygous sporadic CJD cases were characteristic by widely spread PrP^{res} granules and plaque-like structures of different size (Fig. 6). PrP^{res} deposits were evidently most numerous in the granular layer in valine homozygous sporadic cases (data not shown).

Possible professional risk of CJD

Unexpectedly, it occurred in 7.29% of health facilities workers. Since it correlated with the percentage of health workers in the employed population (6%) and because of prevailing typical familial cases without professional contact with CJD patients of the affected health facilities workers, the professional risk of CJD was not confirmed. A high proportion of agricultural workers (48% of CJD^{E200K} cases and 44% of sporadic CJD cases), most of them involved in animal breeding, did not correlate with agricultural workers in the employed population (9%) and has not yet been explained.

Discussion

As soon as a linkage between the E200K mutation and two largest clusters of CJD (Slovak and Israeli) has been confirmed (Goldfarb *et al.*, 1991), further small accumulation of patients with the same mutation has been described in France (Chatelain *et al.*, 1998), Italy (D´Alessandro *et al.*, 1998), Japan (Miyakawa *et al.*, 1998) and Chile. The Goldfab's hypothesis of a common origin of this mutation and its spread through Sephardic Jewish migrants (Goldfarb *et al.*, 1991) from the beginning was in contradiction with the following historical and epidemiological data. (1) Despite of careful search, in the Slovak CJD cluster no patient with a Jewish ancestor was found. In addition, highly predominating Jewish immigrants to Slovakia, especially to CJD foci were Ashkenazi and not Sephardic Jews. (2) The E200K mutation was found in indigenous population of Japan (Miyakawa *et al.*, 1998; Seno *et al.*, 2000), where, unlike in Chile, immigration of Jewish carriers of the mutation has not been reported.

Eight years later the abovementioned hypothesis was abandoned (Lee *et al.*, 1999). The latter authors have undertaken a molecular genetic characterization of three major haplotypes in carriers of the E200K mutation. Two of the described subtypes, the Mediterranean (Spanish, Libyan, Tunisian, Chilean, and Italian) and the Eastern European inclusive of the Japanese carry the mutation within the methionine alelle at codon 129. The third, the Western European haplotype (Austrian, German, and Sicilian) has the mutation on the alelle with valine at the codon 129. The confirmed multifocal origin of this most frequent human TSE mutation underlined the importance of further investigative efforts focused on the E200K carriers, patients as well as asymptomatic subjects (relatives) representing the genetic CJD risk group.

Slovak CJD^{E200K} patients were classified by Lee *et al.* (1999) as the "Eastern European" haplotype. A majority of this subgroup (95 cases) originated from Slovakia, 1 from Poland, 4 from Hungary; 1 case verified in France and assumed to originate from Hungary has been found to come from northern Romania, the possible origin of Slovak carriers. Considering the above listed "birth place" distribution of all known CJD^{E200K} cases and the distance from that region to the eastern border of Europe, we propose "Central European" as a geographically correct designation of this haplotype.

Identification of multiple haplotypes in E200K carriers correlates with certain distinctions and characteristic features of the described subgroups. Slovak rural CJD accumulation is closely related to traditional genetic isolates (Ferák and Kroupová, 1977) preserved until the end of the 2nd World War. Genealogical studies identified consanguinity but also marriages between non-related CJD-affected families with the E200K mutation (Mitrová, 1991). As an example may serve a locality with 2000 inhabitants and 16 verified CJD cases, where two socially and economically distinct "clusters of 4–5 families", each with numerous inter-familial marriages could be recognized. Despite of this and unlike the Israeli cases, no patient homozygous for the mutation has been found. The mutation was in all cases in the alelle with methionine at codon 129.

In the group of 278 genetically tested relatives 34.8% had the mutation. They represented a genetic CJD risk group. In the course of investigation the genetic CJD risk group revealed (i) a decreasing occurrence in affected families and (ii) spread thorough the whole country. The Slovak CJD^{E200K} group did not behave in many respects like typical familial TSE. Comparing to the Gerstmann-Streussler-Scheinker syndrome (GSS) with a 100% penetrance, there is increasing evidence that not all asymptomatic carriers of the E200K mutation develop the disease. Such a "healthy" carriers of the mutation have been found not only above the mean age of CJD but also above the age of the oldest CJD patients. The penetrance of this mutation was for the first time estimated by Goldfarb *et al.* (1991), but that time with a limited number of data. The results showed a low penetrance (0.54). More complete data of patients of the same CJD group and time period (1975–1991) and the data of all Slovak patients recorded in 1975–2000 have been recently reevaluated. The obtained results have (i) confirmed that the "Central European" haplotype of the mutation E200K had a reduced incomplete penetrance (59.5%), (ii) showed a slightly increasing penetrance in the course of 25 years of investigation (from 0.54 to 0.59), and (iii) demonstrated a similarity with the Italian cluster (De´Alessandro *et al.*, 1998) and considerable dissimilarity to Libyan Jewish patients. In that cluster of CJD^{E200K} a life table analysis has shown as high as an 0.89 penetrance by age 80 (Chapman *et al.*, 1994).

While in GSS all cases were familial, in the analyzed groups of CJD^{E200K} not only Slovak but also Libyan-Jewish,

Table 2. Age-adjusted mortality rate in CJD patients with and without E200K mutation in Slovakia (1975–2000)

Age group (years)	CJD patients E200K+						CJD patients E200K–					
	Men		Women		Total		Men		Women		Total	
	Death	Rate	Death	Rate	Death	Rate	Death	Rate	Death	Rate	Death	Rate
30–39	2	0.19	1	0.10	3	0.29	1	0.10	0	0	1	0.05
40–49	5	0.74	7	0.98	12	0.82	1	0.15	5	0.70	6	0.47
50–59	18	2.97	17	2.50	35	2 80	7	1.16	11	1.62	18	1.40
60–69	16	3.53	20	3.54	37	3.63	5	1.10	6	1.06	11	1.08
70–79	1	0.27	4	1.08	6	0 96	0	0	1	0.27	1	0.16
Total	43	1.57	52	1.76	95	1.72	14	0.62	23	0.91	37	0.63

Table 3. Age distribution of CDJ patients in Slovakia (1975–2001)

Time interval	Mutation E200K	Mean age at death			
		Men	Women	Mean	Mean (total CJD)
1975–1979	E200K+	55.4	55.0	55.2	53.5
	E200K–	51.1	50.6	50.9	
1980–1984	E200K+	58.3	54.0	56.1	54.8
	E200K–	54.3	53.0	53.7	
1985–1989	E200K+	51.9	60.7	56.3	55.1
	E200K–		53.8	53.8	
1990–1994	E200K+	60.5	60.3	60.4	56.9
	E200K–	50.0	56.5	53.3	
1995–2001	E200K+	56.6	62.8	59.7	60.2
	E200K–	60.1	61.3	60.7	

French and Italian verified familial cases did not exceed 65%. A more careful investigation might detect another affected relative of some patients, but there were always CJD^{E200K} cases, where neither a detailed family history nor a genealogical study succeeded to find another CJD case in a family. It is doubtful whether having the present knowledge all CJD^{E200K} cases could be classified as familial.

In our CJD^{E200K} group reported here we differentiated "familial" and "sporadic-like" patients. Sporadic-like carriers of the E200K mutation partially explain the discrepancy between low occurrence (10–15 %) of familial CJD cases quoted world wide and recent results of genetic testing of CJD. The latters demonstrate that the proportion of patients with TSE-specific mutation was evidently underestimated and a more complete genetic data will soon enforce a correction concerning the familial form either to higher values or to a reclassification.

Another interesting dissimilarity between CJD^{E200K} and GSS concerns experiments with transgenic animals. Mice over expressing a murine PrP transgene with GSS mutation P102L develop spontaneously an infectious scrapie-like disease (Hsiao *et al.*, 1994), while the E200K mutation does not cause illness (Telling *et al.*, 1996). The mean age at death, usually lower in familial TSE, appears to be not significantly influenced by the E200K mutation. Unexpectedly, in our study, it was not reduced but slightly increased as compared to sporadic CJD patients. The increased mean age at death observed by us in the Slovak CJD^{E200K} group correlates in time with the data on sporadic CJD from England (Cousens *et al.*, 1997) and France (Hullard *et al.*, 2000).

In addition to dissimilarities with familial TSE mentioned above, transmission of typical familial CJD^{E200K} has been successfully performed not only to primates (Brown, 1992) but also to cats and rodents (Mitrová and Mayer, 1977). Considering the incubation period and the percentage of successful trasmissions, the results were comparable or in some experiments even better than in sporadic cases.

It is generally accepted that homozygosity of PRNP gene at codon 129 is predisposing to iatrogenic, sporadic and nvCJD (Palmer *et al.*, 1991; Alperovitch *et al.*, 1999). Methionine homozygots are in increased risk for sporadic and nvCJD. The CJD^{E200K} group in our study also showed high proportion (78%) of methionine homozygous patients, similar (71%) to that found by Alperovitch *et al.* (1999) in sporadic CJD. A similar distribution was found in tested Slovak asymptomatic relatives with the E200K mutation (64% of Met/Met and 36% of Met/Val). The E200K carriers under study provided evidence that the methionine homozygosity at this locus is a CJD risk factor, more in CJD patients than in asymptomatic relatives. The influence of the E200K mutation and the methionine homozygosity at codon 129 was reflected also in the duration of the disease, with the shortest value in the methionine homozygous E200K carriers and in the PrP immunohistochemistry pattern.

Whereas the E200K mutation appears to reduce the amount of PrP^{res} deposits, the codon 129 polymorphism appears to affect their type and pattern. The methionine homozygous group showed mainly the synaptic type of immune positivity, while PrP^{res} granules and plaque-like structures could be found in methionine/valine heterozygous cases. They were most frequent in valine homozygous patients. Lack of correlation between the PrP^{res}deposits and the severity of lesions suggests that the neurodegeneration less dependent on PrP^{res} accumulation could be involved in the pathogenesis of CJD ^{E200K}. It could be probably related to transmembrane forms of PrP, mainly to that termed ^{Ctm}PrP with carboxy-terminus located in the lumen of endoplasmic reticulum and the amino-terminus located in the cytosol. These data suggest that mutation favors the synthesis of ^{Ctm}PrP, which is associated with scrapie-like neuropathological changes but not with accumulation of PrP^{res} (Hedge *et al.*, 1999; Zahn, 1999). The codon 129 homozygosity was shown to be a key determinant of genetic susceptibility to acquired and sporadic CJD. Alperovitch (2001) assumes that it is only one of multiple factors which influence the initiation and course of the pathological process causing the disease. In our study, the CJD^{E200K} group with a high percentage of methionine homozygous cases and an incomplete penetrance of the mutation clearly demonstrated both an important role of the methionine homozygosity also in patients with the CJD-specific mutation and a multifactorial character of CJD pathogenesis, where some other factor besides the codon 129 polymorphism are needed for the clinical manifestation of the disease.

Finally, the Slovak CJD group was characteristic by a high percentage of agricultural professions (48% of CJD^{E200K}, 44% of sporadic CJD, and 9% of control). This finding is still not fully understood. The methionine homozygosity at codon 129 in agricultural workers (76%) was similar to that in CJD ^{E200K}. Unlike the excluded potential risk for health facilities workers (Mitrová and Belay, 2000) the question of the CJD risk for these professions warrants further study. The unusually high proportion of patients involved in some way in animal breeding compared to the general population, and the significantly increased percentage of familial cases compared to the worldwide occurrence were striking from the beginning of our studies of CJD in Slovakia. The first observations have been characterized as "a CJD cluster caused by a coincidence of genetic and environmental (possible professional) risks" (Mitrová, 1988). That time there have not been available any data concerning scrapie in Slovakia. Later on, scrapie has been confirmed by a specific diagnostic method (Mitrová *et al.*, 1991) but found as a rare disease in the country. Epidemiological studies have never confirmed a link between scrapie in sheep and CJD in humans. However, an indirect link seems to develop only recently, since sheep infected with scrapie play most probably an important role at the beginning of the BSE epidemic. Lasmézas *et al.* (2001) have demonstrated identical lesion profiles in C57BL/6 mice after transmission of sporadic CJD and scrapie. According to the authors "There is still a possibility that, in some instances, TSE strains infecting humans do share a common origin with scrapie."

According to Cohen *et al.* (1994) characteristic mutations favor a spontaneous conversion of the prion protein to the PrP^{res} isoform. The question whether only a mutant PrP^{E200K} is converted into PrP^{res} or also the wild type PrP (wtPrP) participates in the pathological process has been partially answered in an experimental study of the brain from a heterozygous E200K carrier (Gabizon *et al.*, 1996; Chen *et al.*, 1997). It has been found that whereas only PrP ^{E200K} acquired protease resistance, wtPrP become insoluble in detergents, what is also a characteristic of PrP^{res}

Recent studies demonstrating that there is no difference in the neurodegeneration patterns between sporadic and genetic TSE (Telling *et al.*, 1996; Hedge *et al.*, 1999) support the view that once initiated, the sporadic CJD follows a path similar to the germline-inherited CJD. Data suggesting that none of the mutations affect the global protein structure indicate that these mutations do not cause the PrP^{res}-like formation "*per se*" (Zahn, 1999).

Considering related experimental data and specific features of the Central European haplotype of the E200K mutation (incomplete penetrance, less that 100% of familial cases, persisting high proportion of agricultural professions, and similarities with sporadic CJD) the question "why should be the E200K carrier resistant to exogenous PrP^{res} ?" could not be avoided.

Our findings of 34.8% of asymptomatic E200K carriers in affected families (the genetic risk group in Slovak population) and their progressive spread thought the country call attention to the possible source of iatrogenic CJD. There is a lack of information concerning the risk carried by "the genetic risk group", but experimental data showing a slower degradation of the mutant PrP and its accumulation in the brain and lymphatic tissue (Meiner *et al.*, 1992) are warning. Since there are no available methods for detection of the preclinical stage of CJD, the awareness of this character is even more necessary. No doubt that recognizing both the patients and "healthy" carriers by genetic testing, is the first step to preventive measures. It renders a chance for (i) prevention of iatrogenic CJD, mainly of the risk caused by tissue (organ) donation. (Corneal transplantation as a cause of iatrogenic CJD has been confirmed. An increasing number of corneal transplantations and of verified genetic CJD risk group in Slovak population triggered in 2002 a preventive genetic testing of all corneal donors for E200K mutation.), (ii) prevention of consanguinity and of marriages between asymptomatic E200K carriers, and (iii) early effective treatment of CJD (prevention of clinical manifestation of CJD) as soon as a TSE therapy will be available.

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