Creutzfeldt-Jakob disease in Hungary

Gábor G. Kovács, Katalin Majtényi

Department of Neuropathology, National Institute of Psychiatry and Neurology, Budapest, Hungary

Abstract

Human prion diseases or transmissible spongiform encephalopathies are progressive fatal neuropsychiatric diseases. In addition to the evaluation of clinical features, a common diagnostic procedure includes examination of the protein 14-3-3 in the cerebrospinal fluid, performing EEG to detect periodic sharp wave complexes with triphasic morphology, and cranial MRI to demonstrate high signal intensity in the basal ganglia or thalamus. The definite diagnosis requires a neuropathological examination. The analysis of the prion protein gene (PRNP) is initiated mainly after suspicion of a positive family history or an atypical presentation. In Hungary collecting data and setting up the neuropathological diagnosis in suspect prion disease cases originates from the late 1960s. Systematic surveillance was established in 1994 and since 2001 reporting of Creutzfeldt-Jakob disease has been compulsory. According to our database, the incidence of genetic prion disease is increased in Hungary. The most frequent mutation in the PRNP is at codon 200. This might be linked to migration from the Slovakian focus. Acquired forms of prion disease were not detected in our country. The surveillance system is based on referrals from clinicians and pathologists and the aim is to perform the neuropathological examination and analysis of the PRNP on the majority of suspect cases.

Key words: Creutzfeldt-Jakob disease, prion protein, E200K mutation.

Introduction

Prion diseases (PrDs) or human transmissible spongiform encephalopathies are progressive fatal diseases characterised by neurological and psychiatric symptoms. Accumulation of a disease-associated, pathological form of prion protein (PrP^{Sc}) predominantly, but not exclusively, in the central nervous system is a common disease marker [29]. The most frequent human PrD is Creutzfeldt-Jakob disease (CJD). The majority of cases are thought to present as a sporadic disorder without specified etiology [31]. The worldwide incidence of sporadic CJD is between 0.5 and 1.5 per million per year [31]. Acquired forms include iatrogenic CJD and variant CJD [29,31]. The latter is related to bovine spongiform encephalopathy. In genetic PrDs, disease-specific point or insertional mutations in the prion protein gene (PRNP) have been demonstrated [15]. The codon 129 polymorphism (methionine/valine, MV) has been proven to influence the phenotype in different etiological types of PrDs. Together with the Western blot pattern of the protease resistant PrP, it is an important factor in the molecular classification of PrDs [6,27].

A recent multicentric study based on standardised data on genetic PrD cases indicated that point and insertional mutations in the *PRNP* gene varies

Communicating author:

Gábor G. Kovács, MD PhD, Department of Neuropathology, National Institute of Psychiatry and Neurology, H-1021 Budapest, Hüvösvölgyi út 116, Hungary. Tel.: +36-1-391-5400; fax: +36-1-391-5409, e-mail: kovacsgg@opni.hu
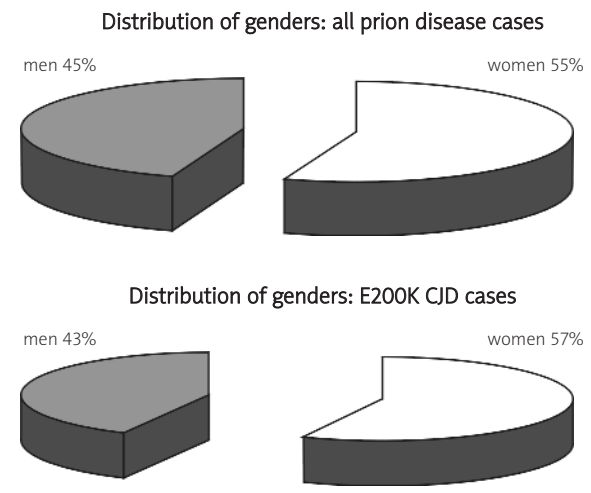
significantly in frequency between countries, furthermore, family history is frequently lacking, thus the term 'genetic' was preferred to 'familial' [14].
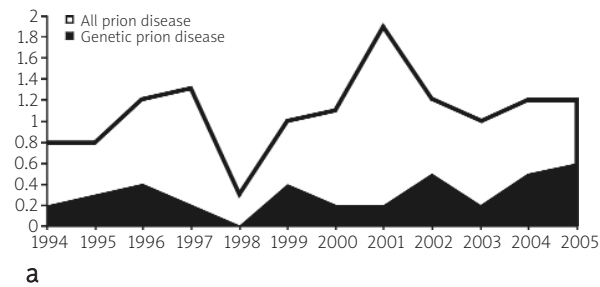
a

b

c

Fig. 1.
a. Incidence of all (black line) and genetic (gray) prion disease cases between 1st January, 1994 and 25th October, 2005.
b. Distribution of age at death in all prion disease cases.
c. Distribution of genders in all prion disease cases (left side of figure) and in cases with mutation at codon 200 (right side of figure)

Based on neuropathological observations three main groups of genetic PrD are distinguished, including genetic CJD, fatal familial insomnia (FFI), and Gerstmann-Sträussler-Scheinker disease (GSS) [16,31]. Only 5-15% of PrD is thought to be related to any mutation in the *PRNP*, except for Slovakia, where it is above 70% [14,24,31].

Diagnostic criteria for surveillance distinguish definite, probable, and possible categories for different types of PrDs [31]. In addition to the evaluation of clinical features, a common diagnostic procedure includes examination of the protein 14-3-3 in the cerebrospinal fluid (CSF), performing EEG to detect the characteristic periodic sharp wave complexes with triphasic morphology, and cranial MRI to demonstrate high signal intensity in the basal ganglia or thalamus [30-32]. The analysis of the *PRNP* is more commonly initiated after suspicion of a positive family history or an atypical presentation.

CJD Surveillance in Hungary

Collecting data and setting up the neuropathological diagnosis in suspect CJD cases originates from the late 1960s. In 1994 letters to the heads of Neurological departments were sent and the Department of Neuropathology of the National Institute of Psychiatry and Neurology (NIPN) was the Institute responsible for the neuropathological diagnosis. Occasionally, examinations were performed in other neuropathology laboratories, but this was followed by a consultation or reporting to the NIPN. In 2001 the Ministry of Health decreed the reporting of CJD. Systematic surveillance was carried out without any respect to familial cases in the selection. Currently our Department is contacted if suspicion of PrD is raised by clinicians. Mainly neurologists and less frequently psychiatrists refer the patients. Occassionally pathologists send post mortem material in cases of uncleared progressive dementia. After consultation, CSF is sent for examination of protein 14-3-3 which is performed either in Vienna or Bratislava, and, in case of consent, blood is submitted for the genetic analysis. As a rapid test RFLP examination of codons 129 and 200 are performed, however, after collecting samples from more patients, all cases undergo a full length analysis of the open reading frame of the PRNP. Post mortem material is sent to our department where detailed neuropathological and

immunohistochemical evaluation is performed. The number of referrals is usually double the number of confirmed PrD cases. The main reason for a referral is rapidly progressive dementia.

Incidence of prion diseases in Hungary

The population of Hungary is approximately 10 million. The mean annual incidence of definite and probable PrD cases between 1st of January, 1994 and 25th October, 2005 is 1.18/million inhabitants (Figure 1a). Probable PrD cases include 1 sporadic CJD and 3 probable genetic PrD cases. Recently we reported that the mean annual incidence of definite genetic PrD is 0.27/million inhabitants [13]. Since the termination of that study by 30th of September, 2004, we already detected 7 genetic PrD cases (3

probable and 4 definite) further supporting our obsevations of an increased incidence of genetic PrD (Figure 1a). We also noted the lack of evidence for positive family history in around half of the cases [13].

Age at death and gender distribution

The average age at death for all PrD cases is 57.9±9.8 (SD) years. The youngest patient is a 31-year-old man carrying the E200K mutation and the eldest is a 79 year-old man diagnosed with sporadic CJD. The average age at death is 53±7.1 (SD) years for definite and probable CJD cases carrying the *PRNP* mutation E200K. The eldest patient with genetic CJD is a 74-year-old woman. Figure 1b illustrates the distribution of age at death in our
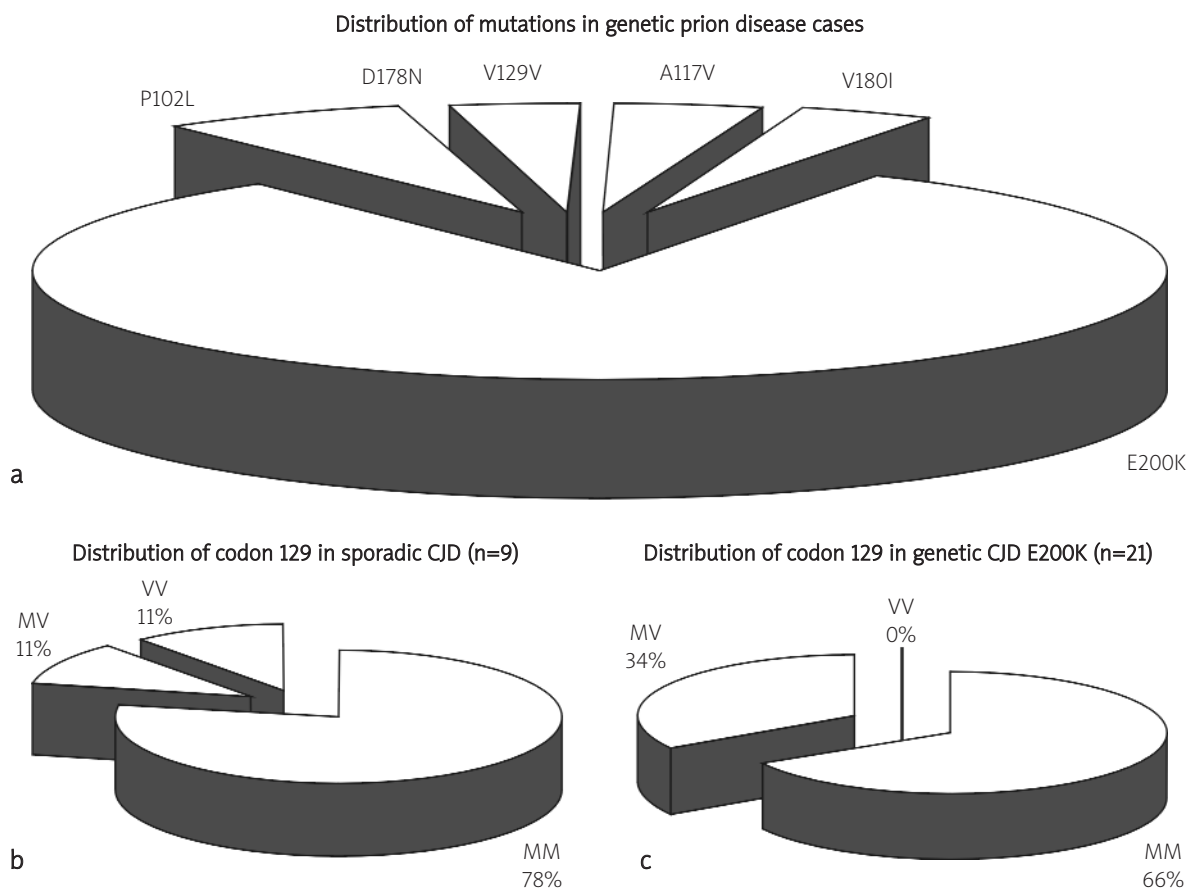

Fig. 2.
a. Distribution of mutations in genetic prion disease cases.
b. Distribution of codon 129 in sporadic Creutzfeldt-Jakob disease (CJD). MM indicates methionine, VV the valine homozygotes, and MV the heterozygotes.
c. Distribution of codon 129 in genetic CJD

series of patients. Both in sporadic and genetic (E200K) CJD we noted a female preponderance (Figure 1c). This is similar to recent and earlier studies demonstrating an excess of female patients [14,17,28].

Distribution of mutations and codon 129 constellation

DNA extracted from blood from 35 patients underwent a full length analysis of the open reading frame of the PRNP. The predominant mutation was the E200K with CJD phenotype, followed by two patients of distinct families with P102L mutation (Figure 2a). One family exhibited CJD [20], while the other GSS phenotype. One of each A117V GSS [10], D178N V129V CJD, and V180I CJD was also found (Figure 2a). The polymorphism at codon 129 showed predominance of MM homozygotes in both sporadic and genetic (E200K) CJD (Figure 2 b, c).

A recent epidemiological survey in European countries described that 67.2% of sporadic CJD cases are MM homozygotes [17]. The higher value in our series is most likely due to the small sample number. However, the distribution of codon 129 polymorphism in genetic CJD is not different compared to other surveys [14,17].

The E200K mutation is the most frequent and geographically the most widespread PRNP mutation [14]. E200K mutation has been reported not only in Europe but also in Chile, Israel, Japan, and USA [4,5,25]. Geographic or ethnic clusters of cases of genetic CJD have been found in Israel, Slovakia, Chile and Italy [1,2,9,21,22,24]. A recent study suggested that at least four independent mutational events are responsible for the geographic distribution of E200K mutation [19]. The Hungarian genetic CJD cases are proposed to belong to the Eastern European haplotype of E200K mutation cases [13,19].

Mutation at codon 178 is also frequently reported, this may present as CJD or FFI depending on the constellation of codon 129 polymorphism of the mutated allele [14,15]. The V210I PRNP mutation also contributes to a higher rate of genetic PrD detected in Italy [18]. Since the clinical presentation of genetic CJD may not differ from sporadic cases, it is important to include genetic testing in the Surveillance system. This may serve as a basis for evaluating clusters of cases or variability in the annual incidence.

Geographical distribution and occupational background of CJD cases

In our recent paper we have described that some counties in Hungary show more CJD cases when compared with the population density of a particular county [13]. As these areas overlapped with the areas where we found more genetic (E200K) CJD cases, we noted that some of these may either be related to the historical migration of the Slovakian population or they are geographically close to Slovakia. Earlier, statistically significant temporo-spatial relationship was identified in Slovakia [23]. Two areas with increased occurrence of CJD was noted, one in the southern and one in the northern parts of Central Slovakia [23].

Data about the occupational background is available from one-fourth of the cases. Four patients worked as medical doctors and one as a nurse. Seven patients worked in the agriculture (four working only with animals), while further 18 patients had variable occupations. From this information strict epidemiological conclusions cannot be set up.

Neuropathological observations

All cases in our present series have been examined in detail by traditional staining as well as with immunohistochemistry for the PrP. In our laboratory we use a three-tiered pretreatment protocol, and we usually apply monoclonal antibodies 12F10 or 3F4 [11].

PrP immunostaining patterns characteristic of PrDs include fine deposition (diffuse/synaptic pattern); pericellular deposits of immunoreactivity around the neuronal perikarya; coarser depositions (these include the granular, the patchy/perivacuolar deposits); and plaques either with amyloid characteristic, eg. kuru-type, multicentric in GSS, and florid plaques in variant CJD, or without amyloid characteristic, as plaque-like deposits or so called focal PrP deposits [12]. These immunostaining patterns may be characteristic of certain molecular subtypes of sporadic CJD, and a mixture of patterns may reflect coexistence of different isotypes of protease-resistant PrP within the same brain [11,27]. Genetic PrDs represent further morphological variants of PrP immunoreactivity [15]. GSS is defined by multicentric amyloid plaques (Figure 3 a-c), however, there is a considerable variety of morphological appearance according to the mutation.
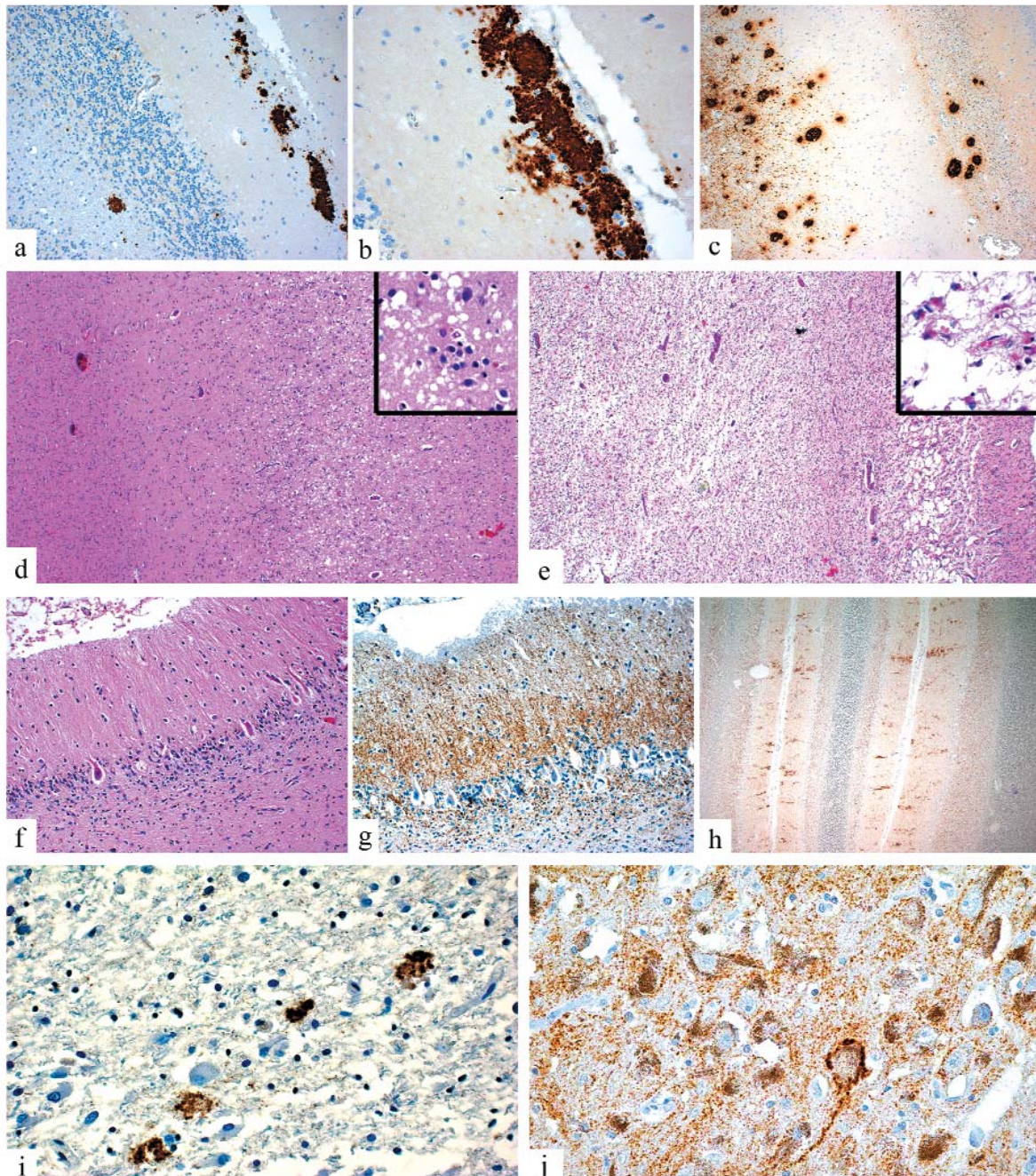
Fig. 3. Immunohistochemistry for PrP using monoclonal antibody 12F10 in the cerebellum (**a:** x100, **b:** x400) showing multicentric amyloid plaques in the white matter and molecular layer in Gerstmann-Sträussler-Scheinker disease (mutation A117V). Similar plaques with granular immunodeposits are seen in the subiculum (**c:** x100). Relatively preserved white matter in sporadic CJD (**d:** x100) is contrasted by severe damage observed in the 'panencephalopathic' type of CJD (**e:** x100). Note the difference in the degree of spongiform change in the cortex enlarged (x400) in the upper right insets. Severe loss of neurons in the granular layer of the cerebellum (**f:** x100) accompanied by the diffuse/synaptic type of PrP immunopositivity (**g:** x100) in all cortical layers. Stripe-like PrP immunostaining pattern in the molecular layer of the cerebellum in a case with E200K mutation (**h:** x40). Focal PrP immunopositivity (**i:** x400) is occasionally seen in the white matter in cases of 'panencephalopathic' CJD. Diffuse/synaptic and perineuronal PrP immunoreactivity in the substantia nigra (**j:** x400)

Cases with base pair insertions in *the PRNP* may also show peculiar PrP deposits in the cerebellar molecular layer. In CJD associated with the *PRNP* mutation E200K we demonstrated that PrP immunoreactivity reminiscent of stripes perpendicular to the surface of the cerebellar molecular layer is highly characteristic (Figure 3h) [8]. In genetic CJD associated with E200K mutation, PrP immunoreactive stripes perpendicular to the surface of the molecular layer were noted in 73% of cases, all MM homozygotes at the codon 129 [13]. Thus, examination of the cerebellum is of outmost help in classification of PrD. In our series, in addition to the stripe-like deposits, we observed diffuse/synaptic, kuru type amyloid plaques, and plaque-like PrP immunopositivities.

We also noted severe white matter destruction in four cases, one carrying the E200K mutation. Earlier these cases were named as 'panencephalopathic' CJD. The reason why this term was introduced is in this type both the gray and white matter shows changes, in contrast to others which affect the gray matter exclusively (Figure 3 d, e) [26]. However, according to the molecular analysis, it was suggested that the panencephalopathic variant of CJD is rather an aspecific end-stage condition displayed by most if not all CJD variants in individual patients with an unusually prolonged course, than a specific molecular subtype [3]. We also noted that the PrP immunostaining pattern is not specific to these cases (Figure 3 f, g), although we observed focal PrP deposits in the white matter also (Figure 3i) which is unusual in other forms. It may be that the prolonged course is related to another, yet unidentified, genetic or epigenetic influence.

In the brainstem we noted variability according to subtypes. Some cases show more prominent PrP deposition (e.g. substantia nigra, Figure 3j), without an obvious neuronal loss, while in some cases we noted a prominent nigra lesion without PrP deposits. A recent study demonstrated that in the brainstem the neuronal loss is relatively prominent in the pontine nucleus and less so in the substantia nigra and inferior olivary nucleus, while motor nuclei of the brainstem tegmentum are well preserved [7]. PrP deposition is usually identified in the substantia nigra, quadrigeminal body, pontine nucleus, and inferior olivary nucleus [7].

In addition to the confirmed PrD cases, the neuropathological examination of cases with clinically suspect CJD included Alzheimer disease, diffuse Lewy body disease, alcoholic encephalopathy, multiple cortical lung carcinoma micrometastases, and tuberculous meningitis.

Conclusions

The incidence of genetic PrD is increased in Hungary. The most frequent mutation in the *PRNP* is at codon 200. This might be linked to historical migration from the Slovakian focus. Acquired forms of PrDs were not detected in our country. The Surveillance system is based on referrals from clinicians and pathologists and the aim is to perform a post mortem examination and analysis of the PRNP on the majority of suspect cases.

References

1. Chapman J, Ben-Israel J, Goldhammer Y, Korczyn AD. The risk of developing Creutzfeldt-Jakob disease in subjects with the PRNP gene codon 200 point mutation. Neurology 1994; 44: 1683-1686.
2. D'Alessandro M, Petraroli R, Ladogana A, Pocchiari M. High incidence of Creutzfeldt-Jakob disease in rural Calabria, Italy. Lancet 1998; 352: 1989-1990.
3. Ghorayeb I, Series C, Parchi P, Sawan B, Guez S, Laplanche JL, Capellari S, Gambetti P, Vital C. Creutzfeldt-Jakob disease with long duration and panencephalopathic lesions: molecular analysis of one case. Neurology 1998; 51: 271-274.
4. Goldfarb LG, Brown P, Mitrova E, Cervenakova L, Goldin L, Korczyn AD, Chapman J, Galvez S, Cartier L, Rubenstein R, Gajdusek DC. Creutzfeldt-Jacob disease associated with the PRNP codon 200Lys mutation: an analysis of 45 families. Eur J Epidemiol 1991; 7: 477-486.
5. Goldfarb LG, Mitrova E, Brown P, Toh BK, Gajdusek DC. Mutation in codon 200 of scrapie amyloid protein gene in two clusters of Creutzfeldt-Jakob disease in Slovakia. Lancet 1990; 336: 514-515.
6. Hill AF, Joiner S, Wadsworth JD, Sidle KC, Bell JE, Budka H, Ironside JW, Collinge J. Molecular classification of sporadic Creutzfeldt-Jakob disease. Brain 2003; 126: 1333-1346.
7. Iwasaki Y, Hashizume Y, Yoshida M, Kitamoto T, Sobue G. Neuropathologic characteristics of brainstem lesions in

sporadic Creutzfeldt-Jakob disease. Acta Neuropathol (Berl) 2005; 109: 557-566.

8. Jarius C, Kovács GG, Belay G, Hainfellner JA, Mitrova E, Budka H. Distinctive cerebellar immunoreactivity for the prion protein in familial (E200K) Creutzfeldt-Jakob disease. Acta Neuropathol (Berl) 2003; 105: 449-454.

9. Kahana E, Alter M, Braham J, Sofer D. Creutzfeldt-Jakob disease: focus among Libyan Jews in Israel. Science 1974; 183: 90-91.

10. Kovács GG, Ertsey C, Majtenyi C, Jelencsik I, László L, Flicker H, Strain L, Szirmai I, Budka H. Inherited prion disease with A117V mutation of the prion protein gene: a novel Hungarian family. J Neurol Neurosurg Psychiatry 2001; 70: 802-805.

11. Kovács GG, Head MW, Hegyi I, Bunn TJ, Flicker H, Hainfellner JA, McCardle L, László L, Jarius C, Ironside JW, Budka H. Immunohistochemistry for the prion protein: comparison of different monoclonal antibodies in human prion disease subtypes. Brain Pathol 2002; 12: 1-11.

12. Kovács GG, Kalev O, Budka H. Contribution of neuropathology to the understanding of human prion disease. Folia Neuropathol 2004; 42 (suppl A): 69-76.

13. Kovács GG, László L, Bakos Á, Minárovits J, Bishop MT, Ströbel T, Vajna B, Mitrova E, Majtényi K. Increased incidence of genetic human prion disease in Hungary. Neurology 2005; 65: 1666-1669.

14. Kovács GG, Puopolo M, Ladogana A, Pocchiari M, Budka H, van Duijn C, Collins SJ, Boyd A, Giulivi A, Coulthart M, Delasnerie-Laupretre N, Brandel JP, Zerr I, Kretzschmar HA, de Pedro-Cuesta J, Calero-Lara M, Glatzel M, Aguzzi A, Bishop M, Knight R, Belay G, Will R, Mitrova E. Genetic prion disease: the EUROCJD experience. Hum Genet 2005; 118: 166-174.

15. Kovács GG, Trabattoni G, Hainfellner JA, Ironside JW, Knight RS, Budka H. Mutations of the prion protein gene phenotypic spectrum. J Neurol 2002; 249: 1567-1582.

16. Kovács GG, Voigtlander T, Gelpi E, Budka H. Rationale for diagnosing human prion disease. World J Biol Psychiatry 2004; 5: 83-91.

17. Ladogana A, Puopolo M, Croes EA, Budka H, Jarius C, Collins S, Klug GM, Sutcliffe T, Giulivi A, Alperovitch A, Delasnerie-Laupretre N, Brandel JP, Poser S, Kretzschmar H, Rietveld I, Mitrova E, Cuesta J de P, Martinez-Martin P, Glatzel M, Aguzzi A, Knight R, Ward H, Pocchiari M, van Duijn CM, Will RG, Zerr I. Mortality from Creutzfeldt-Jakob disease and related disorders in Europe, Australia, and Canada. Neurology 2005; 64: 1586-1591.

18. Ladogana A, Puopolo M, Poleggi A, Almonti S, Mellina V, Equestre M, Pocchiari M. High incidence of genetic human transmissible spongiform encephalopathies in Italy. Neurology 2005; 64: 1592-1597.

19. Lee HS, Sambuughin N, Cervenakova L, Chapman J, Pocchiari M, Litvak S, Qi HY, Budka H, del Ser T, Furukawa H, Brown P, Gajdusek DC, Long JC, Korczyn AD, Goldfarb LG. Ancestral origins and worldwide distribution of the PRNP 200K mutation causing familial Creutzfeldt-Jakob disease. Am J Hum Genet 1999; 64: 1063-1070.

20. Majtényi C, Brown P, Cervenakova L, Goldfarb LG, Tateishi J. A three-sister sibship of Gerstmann-Straussler-Scheinker disease with a CJD phenotype. Neurology 2000; 54: 2133-2137.

21. Mayer V, Orolin D, Mitrova E. Cluster of Creutzfeldt-Jakob disease and presenile dementia. Lancet 1977; 2: 256.

22. Meiner Z, Gabizon R, Prusiner SB. Familial Creutzfeldt-Jakob disease. Codon 200 prion disease in Libyan Jews. Medicine (Baltimore) 1997; 76: 227-237.

23. Mitrova E. Some new aspects of CJD epidemiology in Slovakia. Eur J Epidemiol 1991; 7: 439-449.

24. Mitrova E, Belay G. Creutzfeldt-Jakob disease with E200K mutation in Slovakia: characterization and development. Acta Virol 2002; 46: 31-39.

25. Miyakawa T, Inoue K, Iseki E, Kawanishi C, Sugiyama N, Onishi H, Yamada Y, Suzuki K, Iwabuchi K, Kosaka K. Japanese Creutzfeldt-Jakob disease patients exhibiting high incidence of the E200K PRNP mutation and located in the basin of a river. Neurol Res 1998; 20: 684-588.

26. Mizutani T. Panencephalopathic type of Creutzfeldt-Jakob disease. In: Mizutani T, Shiraki H (eds.). Clinicopathological aspects of Creutzfeldt-Jakob disease. Elsevier/Nishimura, Amsterdam/Niigata, 1985, pp. 123-163.

27. Parchi P, Giese A, Capellari S, Brown P, Schulz-Schaeffer W, Windl O, Zerr I, Budka H, Kopp N, Piccardo P, Poser S, Rojiani A, Streichemberger N, Julien J,Vital C, Ghetti B, Gambetti P, Kretzschmar H. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann Neurol 1999; 46: 224-233.

28. Pocchiari M, Puopolo M, Croes EA, Budka H, Gelpi E, Collins S, Lewis V, Sutcliffe T, Guilivi A, Delasnerie-Laupretre N, Brandel JP, Alperovitch A, Zerr I, Poser S, Kretzschmar HA, Ladogana A, Rietvald I, Mitrova E, Martinez-Martin P, de Pedro-Cuesta J, Glatzel M, Aguzzi A, Cooper S, Mackenzie J, van Duijn CM, Will RG. Predictors of survival in sporadic Creutzfeldt-Jakob disease and other human transmissible spongiform encephalopathies. Brain 2004; 127: 2348-2359.

29. Prusiner SB. Prion diseases and the BSE crisis. Science 1997; 278: 245-251.

30. Tschampa HJ, Kallenberg K, Urbach H, Meissner B, Nicolay C, Kretzschmar HA, Knauth M, Zerr I. MRI in the diagnosis of sporadic Creutzfeldt-Jakob disease: a study on inter-observer agreement. Brain 2005; 128: 2026-2033.

31. WHO manual for surveillance of human transmissible spongiform encephalopathies including variant Creutzfeldt-Jakob disease. WHO, Geneva, 2003.

32. Zerr I, Pocchiari M, Collins S, Brandel JP, de Pedro Cuesta J, Knight RS, Bernheimer H, Cardone F, Delasnerie-Laupretre N, Cuadrado Corrales N, Ladogana A, Bodemer M, Fletcher A, Awan T, Ruiz Bremon A, Budka H, Laplanche JL, Will RG, Poser S. Analysis of EEG and CSF 14-3-3 proteins as aids to the diagnosis of Creutzfeldt-Jakob disease. Neurology 2000; 55: 811-815.