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Hereditary form of prion disease in Poland

Postać dziedziczna choroby prionowej w Polsce

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Abstract

Background and purpose: The aim of the study was to perform molecular analysis in a group of patients affected with prion disease. Diagnosis was based on results of clinical and/or histopathological examination of the brain. This is the largest investigation of this type performed so far in Poland.
Material and methods: Analysed material contained 36 cases of prion disease, including 35 cases of Creutzfeldt-Jakob disease and one case of Gerstmann-Sträussler-Scheinker disease, as well as two familial cases initially suspected of Huntington disease and Alzheimer disease. The control group consisted of 87 subjects. The most frequent known mutations in the *PRNP* gene were looked for, namely those in codons 102, 117, 178, 200, 217 and OPRI; the polymorphism Met/Val in codon 129 was also analysed. The methods applied were PCR-RFLP and DNA sequencing.
Results: The following mutations were found: E200K in 5 families, P102L in one family (previously identified), D178N in one family and 6OPRI in one family. Overall, mutations were detected in 17 persons (including 8 preclinical ones) from 8 pedigrees. Highly significant difference of codon 129 Met/Val heterozygosity frequencies was found between the affected subjects and the controls. Frequency of the familial form of prion disease in the material analysed was 14%.
Conclusions: Screening for mutations in the *PRNP* gene should be performed in all diagnosed cases of prion disease and

Streszczenie

Wstęp i cel pracy: Celem pracy była analiza molekularna w grupie osób dotkniętych chorobą prionową, rozpoznaną na podstawie objawów klinicznych i/lub wyniku badania neuropatologicznego mózgu. Było to największe tego typu badanie przeprowadzone dotychczas w Polsce.
Materiał i metody: W skład analizowanego materiału weszło 36 przypadków choroby prionowej, w tym 35 przypadków choroby Creutzfeldta-Jakoba i jeden przypadek choroby Gerstmanna-Sträusslera-Scheinkera, a także dwa przypadki rodzinne podejrzane o chorobę Huntingtona i chorobę Alzheimera oraz grupa kontrolna (87 osób). Poszukiwano najczęstszych mutacji w genie *PRNP*: w kodonach 102, 117, 178, 200, 217 i OPRI. Kodon 129 analizowano również pod kątem zygotyczności (walina/metionina). Stosowano metodę PCR-RFLP i sekwencjonowanie.
Wyniki: Wykryto mutacje: E200K – pięć rodzin, P102L – jedna rodzina (wcześniej zidentyfikowana), D178N – jedna rodzina, 6OPRI – jedna rodzina. Łącznie stwierdzono mutację w 8 rodowodach u 17 osób, w tym u 8 osób w fazie przedobjawowej. Zaobserwowano także bardzo istotną różnicę w częstości występowania heterozygotyczności Met/Val pomiędzy grupą badaną i grupą kontrolną. Częstość dziedzicznej postaci choroby prionowej w analizowanym materiale wynosi 14%.
Wnioski: Mutacji w genie *PRNP* należy poszukiwać we wszystkich przypadkach choroby prionowej oraz w przypad-

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in cases of familial occurrence of early onset dementia of unknown aetiology. Families with identified mutations should be offered genetic counselling and informed of risks of blood and organs' donation.

Key words: prion diseases, genetics, *PRNP* gene, mutations, neuropathology.

kach rodzinnie występującego otępienia o wczesnym początku i niewyjaśnionej etiologii. Przebieg kliniczny i zmiany neuropatologiczne w niektórych przypadkach dziedzicznych chorób prionowych mogą się różnić od spotykanych najczęściej w sporadycznej postaci choroby. Rodziny ze stwierdzoną mutacją winny być objęte poradnictwem genetycznym i poinformowane o zagrożeniu związanym z dawstwem krwi i narządów do przeszczepienia.

Słowa kluczowe: choroby prionowe, genetyka, gen *PRNP*, mutacje, neuropatologia.

Introduction

Human prion diseases can be classified as the sporadic form, including the most common, Creutzfeldt-Jakob disease (CJD); the familial form associated with a mutation within the *PRNP* gene, which is inherited as an autosomal dominant trait; and the acquired form, including iatrogenic CJD, and CJD associated with the intake of infected food, as in kuru (ritual cannibalism) or in variant CJD (vCJD) transmitted through the consumption of meat of cattle affected with bovine spongiform encephalopathy (BSE).

Prion diseases manifest with subacutely progressive dementia, cerebellar, pyramidal and extrapyramidal signs, myoclonus and typical high-voltage rhythmic, and usually symmetrical discharges in electroencephalography. Pathological studies reveal mainly spongiform degene - ration, neuronal loss, astrocytic gliosis, and specific deposits of the pathological prion protein PrP^{Sc} in nervous tissue of the brain and the brainstem. The incidence of the most common, sporadic CJD (sCJD) is estimated to be 1: 1 000 000 per year in most countries worldwide.

The familial form of prion disease, which is responsible for 10-15% of all cases [1,2], includes three clinically distinct entities: familial CJD (MIM 123400), Gerstmann-Sträussler-Scheinker (GSS) (MIM 137440), and fatal familial insomnia (FFI) (MIM 600072).

The pathological isoform of prion protein coded by the *PRNP* gene is the infectious agent in prion diseases. Pathological prion protein (PrP^{Sc}) features an altered structure (conformation) and acts as a template for conversion of the normal prion protein PrP^{C} into the pathological, infectious one [3].

The role of PrP^{C}, although studied for a long time, is unknown. Studies by Bounhar *et al.* [4] might suggest that PrP^{C} has some neuroprotective function. Experimental studies on mice suggested that the PrP^{C} expression level affects neuronal differentiation [5]. Recently, an experimental study in mice showed that PrP^{C} expression in neurons plays a crucial role in maintenance of the myelin sheath in peripheral nerves [6].

The *PRNP* gene is located on chromosome 20p12pter [7] and contains two exons [8], while the whole reading frame is comprised with the second, i.e. greater one.

Codons 51-59 of the *N*-terminal domain contain a region of five repeats; the first of them is a nonapeptide repeat and the four others are octapeptide ones. Mutations within that region of *PRNP* are octapeptide insertions. Owen *et al*. [9] were the first to describe that type of mutation in 1989. Insertion of six octapeptide repeats at the *N*-terminal of PrP was also the first documented mutation within the *PRNP* gene. Point mutations (more than 30 described so far) [10], causing the familial form of the disease, are situated in the *C*-terminal domain. It is noteworthy that the most common of those mutations, A→G in codon 200, was identified for the first time in a Polish family with CJD [11].

The most common mutations in the *PRNP* gene include E2000K, D178N, P102L, as well as OPRI, i.e. the insertion of at least three octapeptide repeats after codon 51. Additionally, it was shown that the Met/Val polymorphism within codon 129 of the *PRNP* gene markedly predisposes to the development of prion disease; it may also modify the age at onset of symptoms, duration of the disease, and other phenotypic features of the disease.

This study aimed at the molecular analysis of prion disease cases diagnosed according to the clinical features and/or histopathological brain studies performed in the Institute of Psychiatry and Neurology between 2001 and 2010. The specific goals of this study included: (1) molecular analysis of prion disease cases regarding the most common mutations of the *PRNP* gene found in the familial form of the disease; (2) pedigree analysis in familial cas-

es of prion disease; (3) assessment of the relative frequency of familial and sporadic cases of prion disease in the analysed material; (4) molecular analysis of the diagnosed cases of prion disease regarding codon 129 polymorphism – Met/Val heterozygosity, Met/Met homozygosity, and Val/Val homozygosity – as well as their comparisons with controls; (5) evaluation of the histopathological brain findings in diagnosed cases of familial form, as well as comparison of brain pathology in deceased patients with the sporadic versus familial form of prion disease.

Material and methods

Patients

This study included cases of prion disease diagnosed clinically (denoted as probable cases) or confirmed with histopathological examination of the brain (denoted as definite cases). Materials was collected at the 1st Department of Neurology, Institute of Psychiatry and Neurology, between 2001 and 2010, based on the cooperation of one of the authors (JK) with the Polish neurological wards and departments. Patients were referred to the 1st Department of Neurology, Institute of Psychiatry and Neurology with suspected prion disease. The diagnosis was then established according to the typical clinical features and/or after the histopathological examination of the brain. In some cases, the brains of patients who died were sent for evaluation from other Polish departments. Definite cases were those confirmed with brain histopathology. Probable cases included those with typical clinical features, observed at the 1st Department of Neurology, Institute of Psychiatry and Neurology or in other neurological wards and departments. Within the last decade, a total of 240 cases (100%) were referred to the 1st Department of Neurology, Institute of Psychiatry and Neurology for consultations. Among those, 117 (49%) were diagnosed with prion disease, including 63 probable cases diagnosed with clinical features, and a further 54 definite cases confirmed with neuropathological examination.

Histopathological features that led to the diagnosis of prion disease included grey matter spongiosis, astrocytic gliosis, neuronal loss and degeneration, and deposits of pathogenic PrP^{Sc} in the brain (small deposits in vacuole walls, perineuronal deposits, as well as deposits formed into kuru plaques). Deposits of PrP^{Sc} were detected with monoclonal antibodies (3F4 or, more recently, 1E4).

The probable diagnosis was made according to the presence of the characteristic clinical syndrome of various neurological abnormalities, often with cerebellar signs and rapidly progressive dementia (usually of subacute course), typical EEG abnormalities, presence of 14-3-3 protein in cerebrospinal fluid (CSF) and magnetic resonance imaging (MRI) features typical for spongious encephalopathy.

The sensitivity and specificity of the additional studies in the diagnosis of sporadic CJD is as follows: EEG, 66% and 74%, respectively [12]; brain MRI, 67% and 93%, respectively [13], protein 14-3-3 in CSF, 94% and 84%, respectively [12]. Combined probability of accurate CJD diagnosis based on those three studies is estimated to be 98%.

Control samples included anonymous DNA samples isolated from healthy subjects (61 women and 26 men), predominantly employees of the 1st Department of Neurology, Institute of Psychiatry and Neurology.

DNA analysis

DNA samples isolated from the venous blood were obtained from 36 patients diagnosed with prion disease. This group was subjected to genetic analysis regarding (1) presence or absence of the most common *PRNP* mutations typical for human prion disease, i.e. mutations in codons 102, 117, 178, 200, 217, as well as OPRI; (2) *PRNP* gene codon 129 polymorphism. Cases subjected to genetic analysis were divided into five subgroups summarized in Table 1.

The sequence containing the open reading frame of the *PRNP* gene was amplified with polymerase chain reaction using an automated thermocycler (Perkin Elmer), with the protocol described by Goldgaber *et al.* [11]. The product (amplicon) was then digested in separate reactions with the restriction enzymes matched to the analysed codons (Table 2).

Digestion products were separated on 2% agarose gel with ethidium bromide. Amplicons with the altered pattern of digestion bands were sequenced (commercial sequencing performed by Genomed sp. z o.o.).

The results were analysed with Pearson chi-square test within the statistical package Utility Programs for Statistical Genetics, designed for the analysis of genetic studies.

Results

Mutations were detected in 17 patients within 8 families affected with prion disease. Codon 200 mutation (E>K) was found in 5 families. Codon 178 mutation

Table 1. Material of the study: Group of patients in whom searching for mutations in *PRNP* gene and determination of codon 129 polymorphism was performed

Categories of subjects examined	No. of persons	*PRNP* gene	
		Search for mutations *	Examination of polymorphism in codon 129
Prion disease diagnosis based on histopathology of the brain (including one familial case of GSS reported previously, Kulczycki *et al*. [18])	20	+	+
Prion disease, diagnosis based on clinical picture, including second (not reported) familial case of GSS (the same family as mentioned above)	16	+	+
Patients suspected of HD following exclusion of mutation in *HTT* gene	63	+	np
Patients suspected of monogenic form of AD	9	+	np
Controls	87	np	+

GSS – Gerstmann-Sträussler-Scheinker disease; HD – Huntington disease; AD – Alzheimer disease.
+ DNA analysis performed; np – DNA analysis not performed.
**search for mutations in codons 102, 117, 178, 200, 217 and OPRI*

Table 2. Restriction enzymes used for mutation detection in analysed codons of *PRNP* gene

Codon	Restriction enzymes	Mutation searched for
102	*DdeI*	CCG>CTG
117	*PvuII*	GCA>GTA
129 polymorphism	*NspI/TaiI*	ATG/GTG
178	*PsyI*	GAC>AAC
200	*Alw26I*	GAG>AAG
217	*HpaII*	CAG>CGG

(D>N), codon 102 mutation (P>L), and 6-octapeptide insertion (6OPRI) were found in one family each. Testing of the first-degree relatives found mutations in eight other subjects without clinical features of the disease (Table 3).

The genotype frequency of polymorphic codon 129 in controls was as follows: Met/Met 36.8%, Met/Val 54.0% and Val/Val 9.2% (allele frequency: Met 0.638 and Val 0.362). This is in agreement with the Hardy-Weinberg law and does not differ significantly from the frequencies found in Poland [14] or in other Caucasian populations – British [9], French [15], American [16] or Italian [17].

The genotype frequency in patients differed significantly from controls and was as follows: Met/Met 85.7% (24/28), Met/Val 10.7% (3/28), and Val/Val 3.6% (1/28) (allele frequency: Met 0.9107 and Val 0.0893) (Table 4).

Table 3. Mutations detected in 17 persons of 8 families

Pedigree No.	Diagnosis	Mutation	Codon 129
1/I	CJD	E200K	Met/Val
2/I	Preclinical	E200K	Met/Val
3/I	Preclinical	E200K	Met/Val
4/I	Preclinical	E200K	Met/Met
5/I	Preclinical	E200K	Met/Val
6/II	CJD	E200K	Met/Val
7/II	CJD	E200K	Met/Val
8/III	CJD	E200K	Met/Met
9/IV	CJD	E200K	Met/Met
10/V	CJD*	E200K	Met/Val
11/VI	GSS**	P102L	Met/Val
12/VII	CJD**	OPRI	Met/Met
13/VIII	CJD	D178N	Met/Met
14/VIII	Preclinical	D178N	Met/Met
15/VIII	Preclinical	D178N	Met-Met
16/VIII	Preclinical	D178N	Met-Val
17/VIII	Preclinical	D178N	Met-Val

CJD – Creutzfeldt-Jakob disease, GSS – Gerstmann-Sträussler-Scheinker disease
** initially suspected of Huntington disease, ** a case of GSS reported in same family [18], *** initially suspected of Alzheimer disease*

Figure 1 presents an example of the pedigree in familial prion disease caused by the 6OPRI mutation in the *PRNP* gene.

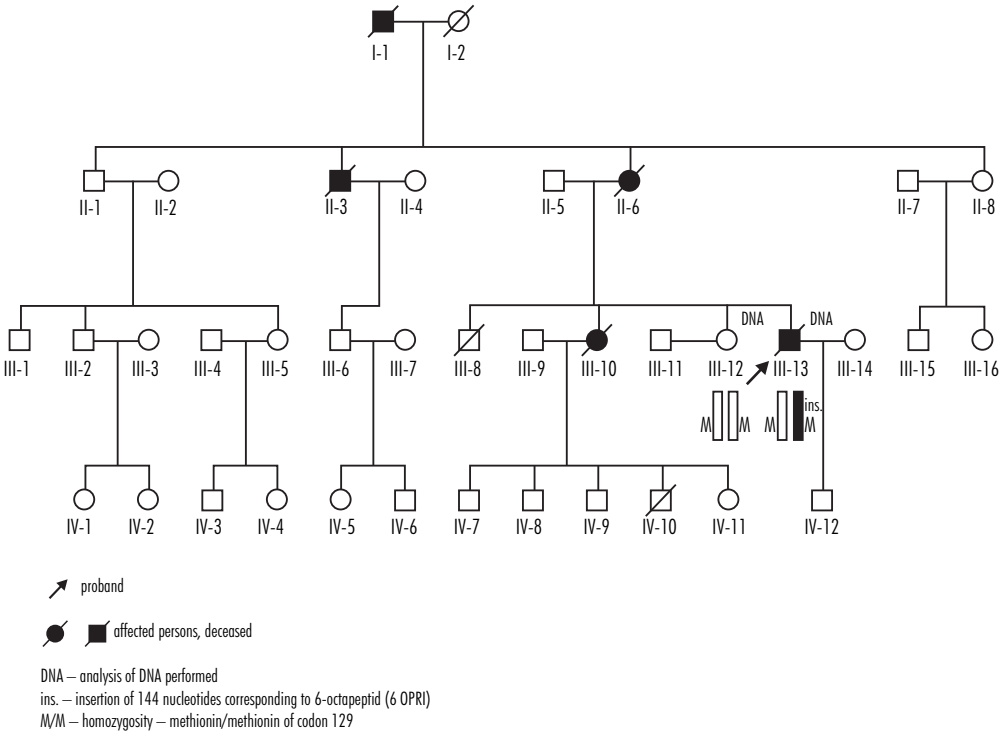

Fig. 1. Pedigree with 6OPRI mutation, found in III-13 (see Table 3). The mutation was excluded in unaffected sister III-12. DNA samples from other family members were not available. Course of the disease in 4 other affected subjects was similar to that in proband III-13

Neuropathological examination was carried out in 54 cases, but the genetic analysis was performed in 20 of them only (Table 1); we did not have DNA samples of the other 34 subjects. This paper deals mostly with genetic testing of *PRNP* mutations in familial cases. Thus, we limit our report of the histological findings to the characteristic or typical abnormalities found in some hereditary prion diseases, especially in cases with codon 102 mutation (GSS case reported previously from our centre, [18]) (Fig. 2), and in the first Polish case of OPRI mutation (Fig. 3), reported previously in 2009 at the meeting of the Neuropathology and Neurooncology Section, Committee of Neurological Sciences, Polish Academy of Sciences (PAN) [19].

In both those cases, peculiar histological findings included the distribution and shape of PrP deposits within the brain structures, while the features of spongious encephalopathy (spongiosis, neuronal loss, astrocytosis) were all within the spectrum of the CJD picture.

The detailed analysis of clinical features in patients affected with the familial form of prion disease is beyond the scope of the paper. We would like to point out that the age at onset of symptoms was advanced in most familial cases, and the duration of the disease was short, similarly to the most common sporadic form of prion disease. For example, in six cases of familial CJD with E200K mutation identified in this study, mean age at onset of symptoms was 59.7 years (range, 51-74 years). It is noteworthy that within one of the reported families, one sister (6/II) developed clinical symptoms at the age of 51 (soon after head trauma with brain concussion),

Table 4. Polymorphism of codon 129: Met/Met, Val/Val, Met/Val in group of 36 patients affected with prion disease compared with 87 controls ($p < 0.001$)

Codon 129 polymorphism	Affected *n* (%)	Unaffected *n* (%)
Met/Met	31 (86.1%)	32 (36.8%)
Val/Val	1 (2.8%)	8 (9.2%)
Met/Val	4 (11.4%)	47 (54.0%)
Total	36 (100%)	87 (100%)

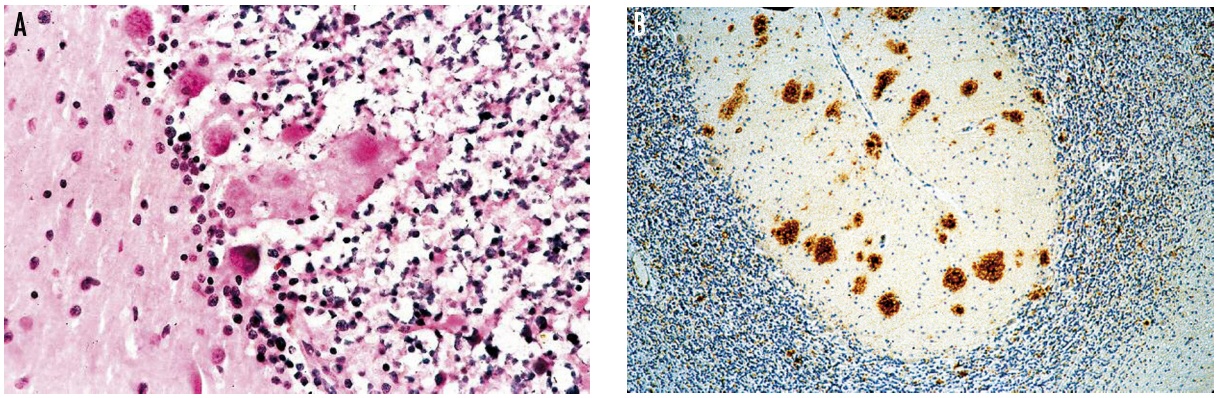

Fig. 2. Deposits of PrP in the cerebellum in a case of Gerstmann-Sträussler-Scheinker disease. A) Multicentric (or confluent) deposits in granular layer of cerebellar cortex. H&E staining, approx. × 100; B) deposits of PrP in molecular layer of cerebellar cortex. Staining with monoclonal antibody 3F4, approx. × 100

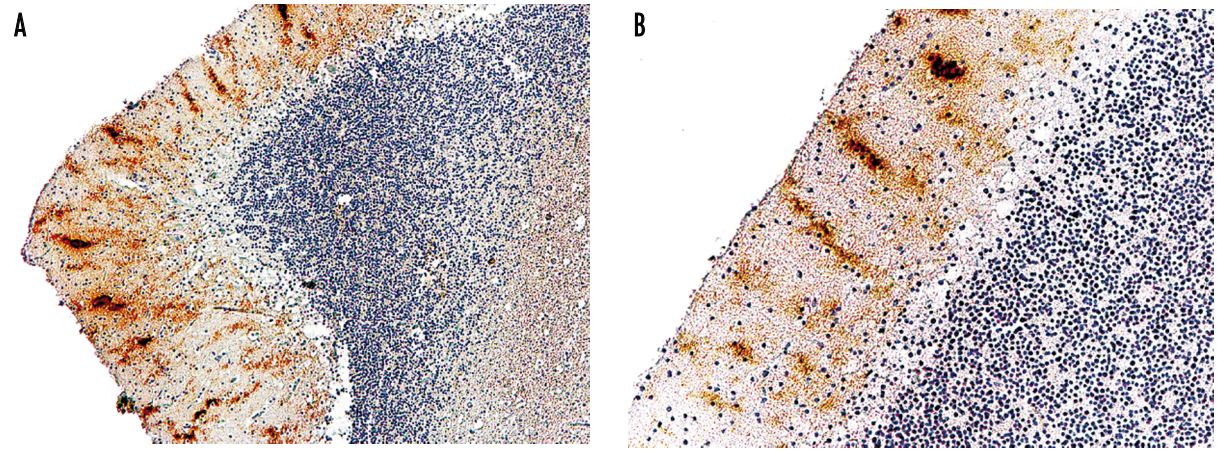

Fig. 3. Deposits of PrP in the cerebellum of the patient with 6OPRI mutation. Deposits situated only in molecular layer of cortex form stripes perpendicular to the surface of cerebellum. Staining with monoclonal antibody 3F4. A) approx. × 100, B) approx. × 200

and the other one (7/II) developed the symptoms at the age of 61 (Table 3); both sisters featured Met/Val heterozygosity at codon 129.

A completely different clinical course of the disease was noted in patient 12/VII (Table 3), in whom the 6OPRI mutation (6-octapeptide insertion) was found. He was previously suspected of having a monogenic type of Alzheimer disease or other monogenic type of dementia, which were all excluded by the team of C. Żekanowski (mutations in *APP*, *PSEN1*, *PSEN2*, *MAP1*, *PGRN1* genes were excluded). The patient developed clinical symptoms at the age of 28, and died at the age of 36 in terminal cachexia. The duration of the disease was therefore 8 years. As suggested by the family history of the three generations in that family, the clinical course was very similar in four other family members, who were already deceased (Fig. 1). The patient's mother, for example, developed clinical symptoms at the age of 35, and died at 52. The disease lasted therefore 17 years. DNA samples were obtained from the patient (proband) and his healthy sister only.

Discussion

It is difficult to state clearly the prevalence of familial cases with *PRNP* mutation in Poland according to such a small sample (DNA analysis of 36 cases). After the exclusion of three pedigrees (V, VI, and VII) (Table 3), suspected previously of having Huntington or Alzheimer disease, and after the exclusion of a family with a previously detected P102L mutation, the prevalence of mutations in the *PRNP* gene among studied subjects is 14% (5/35). It is in agreement with the preva-

lence of 10-15% reported by other authors [1,2]. A particularly high percentage of familial prion disease (25.5%) is noted in Hungary. It is assumed to be related to the migration from Slovakia, where the prevalence of E200K is particularly high [20].

In 2006, Mead put together 492 published cases; according to his estimates, the most common *PRNP* mutations worldwide are E200K, D178N, P102L, and OPRI [10]. The commonest of those (> 70%) is E200K mutation [21]. Our study brought a similar estimate: E200K mutation was found in 5 out of 8 pedigrees with a *PRNP* gene mutation.

As mentioned above, we detected 6OPRI mutation (with 6-octapeptide insertion) in one pedigree. This mutation is the most common one in the United Kingdom [9,10,22]. According to the data compiled by Mead, median age at onset of the disease was 35 (range, 21-82) in 129 cases with more than three repeats. The median duration of the disease was 7 years, and ranged between 3 months and 21 years [10]. This diversity was related both to the number of octapeptide repeats and to the zygosity at codon 129. According to Poulter *et al*. [22], the heterozygosity Met/Val at that codon was associated with the age at onset delayed for about 10 years when compared with the homozygosity Met/Met, which was also the genotypic combination noted in our case (pedigree VII, Table 3, Fig. 1).

Both clinical features and neuropathological findings are very diverse. Features similar to our case (Fig. 3), including stripe-like deposits of PrP^{Sc} in the granular layer of the cerebellum, were reported by Ironside (personal communicaton), and also by Vital *et al.* [23] and King *et al.* [24], but only in cases with OPRI insertions with less than 8 octapeptide repeats. It is interesting that in cases with a small number of repeats (< 4), age at onset was paradoxically more advanced, and the duration of the disease was shorter [25]. This negative correlation is explained by some authors with reference to the declining ability of the aging brain to degrade the assumed toxic factor, possibly such as PrP^{Sc} [26].

Specific neuropathological features of a GSS case within the same pedigree that was detected recently (11/VI, Table 3) were described in 2001 [18] (Fig. 2). In our pedigree, we detected P102L mutation of the *PRNP* gene, which is the most characteristic mutation for GSS. GSS may be, however, associated with other, less frequently seen mutations within this phenotypic variant of prion disease. In 2000, for example, Bratosiewicz *et al.* [27] described a case of GSS with a novel point mutation in codon M232T. This mutation is included in the schema of mutations related to 'certain or suspected pathogenicity' published by Mead [10].

In one pedigree, we found D178N mutation in an affected person (13/VIII, Table 3); age at onset was 59 years. This mutation seems to be more prevalent than the others in Germany [2] and particularly in Finland [28]. This mutation was observed both in familial CJD and in FFI, even within the same pedigrees [29]. Among 72 cases reviewed by Mead, median age at onset of symptoms in patients with the above-mentioned mutation was 50 (range, 20-71), and the mean duration of the disease was 11 months (range, 5 months – 4 years) [10].

The presence of mutation within the *PRNP* gene is not equivalent to being affected with the disease. Thus, we deal with the incomplete penetrance of the mutated gene. It is confirmed by observations that quite commonly (about 40%) prion disease occurs as an isolated case (family history does not suggest presence of other affected family members) [30]. The incomplete penetrance of the mutated gene might be attested by the single case reports; e.g. E200K was found in a 75-year-old mother of a patient affected with CJD in Slovakia [31]. Goldfarb *et al*. [32] estimated the penetrance of the most common E200K mutation to be 0.56. According to Chapman *et al*. [33], cumulated penetrance among Libyan and Tunisian Jews was 50% at the age of 60, and 80% at the age of 80.

Our sample contained eight subjects, mutation carriers, who are healthy so far (Table 3), but it cannot be considered as proof of the incomplete penetrance of the mutated gene, as the majority of those subjects did not reach the age associated with the highest risk of incident disease.

From the clinical point of view, diagnosis of dementia of unknown aetiology is the major issue related to the genetics of prion diseases. We highlight the fact that two cases in our sample were patients with previously suspected Huntington or Alzheimer disease. This is not an exception. A family with Huntington disease-like disorder (MIM 603218) was described, with the autosomal dominant pattern of inheritance, and symptomatology that included dementia, dysarthria and ataxia, but also choreiform involuntary movements and atrophy of the basal ganglia [34]. A case of familial CJD with a long duration of the disease, initially thought to be Alzheimer disease, and finally diagnosed with the 6OPRI mutation (12/VII, Table 3, Fig. 1), is also very instructive. Unusual neuropathological findings in that case might be considered as typical for OPRI mutations (Fig. 3) but only in cases with insertions of a specific number of octapeptide repeats [23,24]. Early onset of symptoms in that patient might be related to the Met/Met homozygosity

at codon 129. The patient's mother, very probably affected with the same disease, developed symptoms later in her life and the disease lasted even longer, which might be related to the heterozygosity at codon 129.

Finckh *et al*. [35] performed genetic testing in 36 patients with early onset familial dementia. Their analysis included *PSEN1*, *PSEN2*, *APP* and *PRNP* genes. Mutations were found in 12 cases, and were related to *PRNP* in 4 cases (one-third). We support the view of Malluci *et al.* [36], who suggested that "indeed, inherited prion disease should be excluded in any individual presenting with atypical presenile dementia or neuropsychiatric features and ataxia, including suspected cases of new variant CJD".

We have already mentioned that the polymorphism of Met and Val at codon 129 greatly influences susceptibility to the disease and modifies its clinical picture. Similarly to other studies, the difference between patients and controls is striking (Table 4). It cannot be excluded that besides genotype (type of mutation and polymorphisms), some environmental factors also affect the time of disease onset. It may be suggested by the great difference of age at onset between two sisters affected with E200K mutation (6II and 7II in Table 3). The one that had earlier onset of symptoms suffered from a major head injury shortly before the beginning of symptoms.

Genetic counselling is a major issue in genetic testing of prion diseases. In particular, it should include families with *PRNP* gene mutations detected with molecular studies. According to our experience, not all subjects who are at high risk of having a mutated gene decide to have such studies performed, while the results of testing might enable the appropriate prophylactic measures to avoid transmission of the disease to subsequent generations. According to the accepted bioethical principles, any decision made by the members of families at risk of inherited prion disease should be accepted. Each adult has the right to know whether he or she inherited the causative gene, but could decline testing as well, at least in the case of incurable disease.

The peculiar aspect of genetic counselling in relation to inherited prion diseases is the infectiousness of the pathological form of prion protein PrP^{Sc}. Healthy persons with a diagnosed *PRNP* gene mutation or those who are at risk of prion disease but who did not consent to genetic testing should be informed that they can be neither blood donors nor organ donors. Ostrowski [37] raised that problem in his monograph related to the transmission of disease through transplants. At the end of the special conference dedicated to that problem, he concluded: "we should resign from donors who were affected with the subacute encephalitis when alive, as well as from those in whose families Creutzfeldt-Jakob disease or other prion disease was diagnosed" [38].

Conclusions

1. Fourteen percent of the 36 analysed cases of prion disease were familial cases caused by mutations within the *PRNP* gene. Other cases were probably sporadic ones.
2. Mutation at codon 200 within the *PRNP* gene was the most commonly detected mutation (5 out of 8 pedigrees with the detected mutation). This is in agreement with observations made in other countries.
3. As expected, homozygosity Met/Met and Val/Val is significantly more common in patients with prion disease than in controls. It is valid especially in sporadic cases of the disease. This confirms the protective role of codon 129 heterozygosity and suggests the importance of that polymorphism in the pathogenesis of human prion disease.
4. The course of familial prion disease might differ widely from the most common sporadic disease, which is attested by the early onset of the disease in some patients and by the long duration of the disease in one of the analysed cases with 6OPRI mutation in the *PRNP* gene.
5. The diagnosis of prion disease should be considered in familial occurrence of neurological disorders with dementia, especially in cases with early onset of symptoms, as suggested by two familial cases, initially suspected of Huntington disease and monogenic form of Alzheimer disease, in whom mutations in the *PRNP* gene were found.
6. Histopathological findings in brains of patients affected with the familial form of prion disease might be divergent from those typical for the sporadic form. This may be associated with the characteristics of particular mutations in the *PRNP* gene and with prolonged, long-term course of the disease in some familial cases.
7. Genetic counselling is a major issue in prion diseases. Families with detected mutations in the *PRNP* gene should be informed that the carriers of those mutations are not allowed to be donors of blood or organs for transplantations.

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Disclosure

Authors report no conflict of interest.

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