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Codon 129 polymorphism of the *PRNP* gene in normal Polish population and in Creutzfeldt-Jakob disease, and the search for new mutations in *PRNP* gene

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Abstract. Polymorphism at codon 129 of the prion protein gene (*PRNP*) is implicated both in susceptibility and phenotype of human prion diseases. We characterized the valine and methionine allele frequency at codon 129 in 109 individuals representing the normal Polish population and in 15 Polish CJD cases. The distribution of the genotype was 45% Met/Met, 39% Met/Val, and 16% Val/Val in the control group whereas, of the CJD cases, 73.3% were homozygous for methionine, 13.3% homozygous for valine and 13.3% were heterozygous. The novel missense mutation (ATG→ACG) at codon 232 was identified in one of the samples with a GSS phenotype.

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INTRODUCTION

Prion diseases are a group of fatal neurodegenerative disorders that afflict both humans and animals (Prusiner 1996). They are characterized by the deposition of an abnormal protease-resistant isoform of the prion protein, PrP, which is a cellular glycoprotein of unknown function (Prusiner and DeArmond 1994). Prion protein (PrP) is encoded by a gene, (*PRNP*), which in humans is located on the short arm of chromosome 20 (Brown et al. 1991, Prusiner 1996).

Prion diseases include kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI). More than 85% of CJD cases are classified as sporadic and iatrogenic and they are not associated with any mutation of *PRNP* open reading frame (ORF). However, there is a polymorphism at codon 129 of *PRNP*, which may occur as either ATG (methionine) or a GTG (valine), and seems to be involved in susceptibility to sporadic and iatrogenic CJD (Owen et al. 1989, Miyazono et al. 1992, Prusiner 1996, Alperovitch 1999).

Genotype of codon 129 affects susceptibility to sporadic and iatrogenic forms (Collinge et al. 1991, Palmer et al. 1991) as well as phenotypic expression of mutations elsewhere within the ORF, such as the mutation at codon 178 which coupled with methionine at codon 129 is linked to FFI and coupled with valine at codon 129 results in a familial CJD (Goldfarb et al. 1992). Both clinical course and neuropathology of GSS (associated with the mutation P102L) in patients with 129^{Val} at the same allele are significantly different from that observed in P102L 129^{Met} patients (Young et al. 1997).

Variations at this locus may influence disease expression: for example, early age of onset or shorter duration of disease in homozygous individuals with familial CJD; and the occurrence (due either to differences in susceptibility and/or incubation period) of sporadic and iatrogenic CJD (Salvatore 1994). It is of note that, so far, all cases of a variant CJD (vCJD) are homozygous for methionine at codon 129 (Collinge et al. 1996).

METHODS

DNA of 15 Polish cases of CJD and one case with GSS phenotype was extracted from formalin-fixed and paraffin-embedded brain tissue. These sporadic CJD cases were collected over last few years and every sample was stained by routine neuropathological methods and PrP immunohistochemistry; thus they all should be classified as definitive CJD. The tissue was cut and transferred to 1.5 ml tubes and washed twice with xylene followed by washing with 95% ethanol twice and with 70% ethanol once. The pellet was dried and resuspended in 500 µl of extraction buffer (1% SDS, 10 mM EDTA, 0.2 M TRIS pH 8.0) and digested with proteinase K (5 mg/ml) overnight at 56°C. DNA was then isolated by phenol:chloroform extraction and salt-ethanol precipitation. DNA of 109 control subjects was extracted from leukocytes of the peripheral blood according to a standard procedure (Sambrook et al. 1985). As control specimens, we used randomly selected adult individuals of both sexes. First, the region from 85 to 840 residue (according to GeneBank access No. M13899) of ORF was amplified by the polymerase chain reaction (PCR) with primers oligo2 and oligo3. The PCR cycling conditions were 94°C for 30 s, 57°C for 30 s, 72°C for 1 min for 35 cycles. To obtain a sufficient copy number of DNA samples extracted from paraffin-embedded brain tissue, nested PCR was performed with Ex 1 and Ex 2 primers. To analyze the polymorphic codon 129, the amplified DNA samples were precipitated and digested with the restriction enzymes *Nsp* I and *Mae* II. The digested product was analyzed by 2% agarose gel electrophoresis. To verify the PCR-RFLP technique the PCR product was also sequenced by the Sanger dideoxy-mediated chain-termination method with sequencing primer P1.

To search for inserts within the octapeptide repeat region, oligo 5 and oligo 6 primers were used and the length of the PCR product was compared to that of the wild type sample after 2% agarose gel electrophoresis. To screen for any point mutations, nested PCR was performed to amplify *PRNP* regions 85-532 (oligo 3-Ex 2) and 324-840 (Ex 1-oligo2) (94°C for 30 s, 55°C for 30 s, 72°C for 30 s for 30 cycles) followed by sequencing with primers oligo 5 (101F) P 1(332F), Kin (544F), Sc 4 (795R) according to SequiTherm EXCEL™ DNA Sequencing Kit (Epicentre Technologies) protocol. The PCR cycling conditions were 94°C for 20 s, 55°C for 15 s, 72°C for 20 s for 25 cycles.

The PCR mixture for all reactions consisted of 0.5 µg genomic DNA, 25 pmol of each primer, 50 mM KCl, 10 mM TRIS-HCl (pH 9.0), 1.5 mM $MgCl_2$, 200 µM of each deoxynucleoside triphosphate, 0.1% Triton X-100, 5% dimethylsulphoxide and 2.5 U Taq polymerase. Sequences and positions of primers according to GenBank Database Accession number M13899 are placed in Table I.

Table I

Sequences of primers used to analyze *PRNP*

Primer		Sequence	Position	Reference
oligo 2	Reverse	gaa aga tgg tga aaa cag gaa gac c	840	Windl et al. 1996
oligo 3	Forward	gtg gcc aca tgg agt gac ctg ggc ctc	85	Windl et al. 1996
oligo 5	Forward	gac ctg ggc ctc tgc aag aag cgc	101	Windl et al. 1996
oligo 6	Reverse	ggc act tcc cag cat gta gcc g	449	Windl et al. 1996
Ex 1	Forward	gag gtg gca ccc aca gtc	324	Kretzschmar et al. 1995
Ex 2	Reverse	cac ttg gtt ggg gta acg ct	532	Kretzschmar et al. 1995
P 1	Forward	acc cac agt cag tgg aac aag c	332	Young et al. 1997
Sc 4	Reverse	aag atg agg aaa gag atc	795	Shibuya et al. 1998
Kin	Forward	cat gga tga gta cag caa cca g	544	Kretzschmar et al. 1995

For a comparison between our data and those collected from the literature, the Pearson Chi-square test was used.

RESULTS

The genotype frequencies at codon 129 in 109 unrelated Polish controls were 45% Met/Met, 39% Met/Val and 16% Val/Val. Among Polish CJD cases, 73.3% were Met homozygous, 13.3% were Val homozygous and 13.3% were heterozygous (Fig. 1). None of the 40 control subjects and none of 15 CJD cases carried codon 117 polymorphism. Neither insertion nor deletion was found at octapeptide repeat region. An ATG→ACG (methionine→threonine) change at codon 232 was found in a single GSS case but not in 40 healthy Polish controls (Fig. 2). This case was $129^{Met/Val}$ heterozygous and, after breaking the code of this sample, it appeared that this was a case previously published as "Sporadic Creutzfeldt-Jakob disease with a Gerstmann-Straussler--Scheinker disease phenotype but no alterations in the *PRNP* gene" (Liberski et al. 1998). In that case, the molecular analysis revealed no known mutations in the region between codons 8–221. Briefly, clinically it was characterized by cerebellar syndrome, spastic paraparesis and dementia while neuropathological examination revealed innumerable PrP-immunopositive kuru and multicentric plaques (Fig. 3). Some of these were stained also by anti Aβ-antibodies but not by anti MAP-2(τ) antibodies. The careful search toward family history was apparently negative.

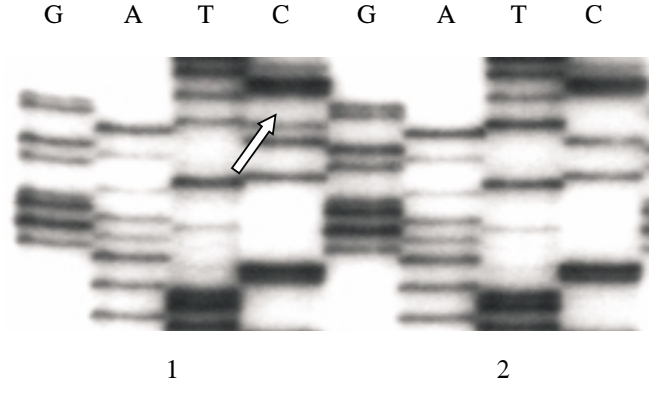


Fig. 1. Percentage distribution of 129 genotypes among unrelated Polish controls (109 subjects) and Polish CJD cases (15 subjects).

DISCUSSION

We report here that the distribution of allele frequencies among Poles does not differ significantly from that previously reported for the British (37% Met/Met, 51% Met/Val, 12% Val/Val, 0.63:0.37 Met:Val) (Collinge et al. 1991), Italian (45%Met/Met, 40% Met/Val, 15% Val/Val, 0.65:0.35 Met:Val) (Salvatore 1994), French (41% Met/Met, 49% Met/Val, 9% Val/Val, 0.66:0.34 Met:Val) (Laplanche et al. 1994), and American populations (41% Met/Met, 51% Met/Val, 8% Val/Val, 0.67:0.33 Met:Val) (Brown et al. 1994) (χ^2_8=7.677, *P*=0.446). However, it is different from that observed among the Japanese (0.96:0.04 Met:Val) (Miyazono et al. 1992).

CJD cases from Poland carried an excess of Met/Met homozygosity. This figure does not differ from that reported earlier for other populations. Collinge et al. (1991) has found that iatrogenic cases of CJD were sig-

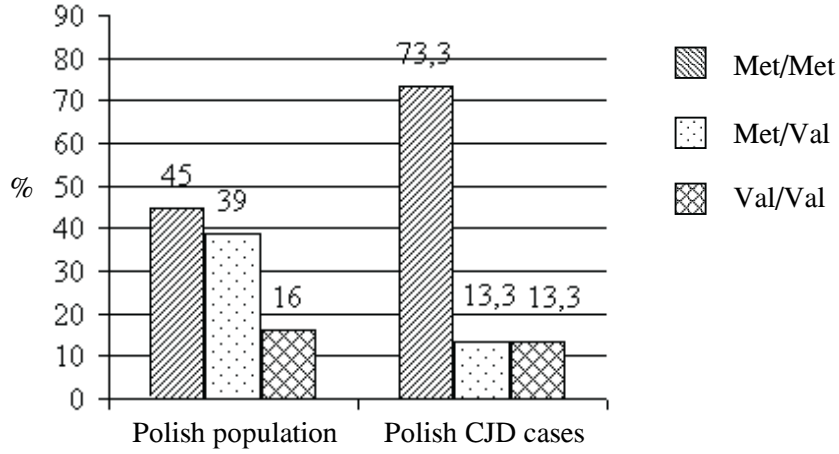
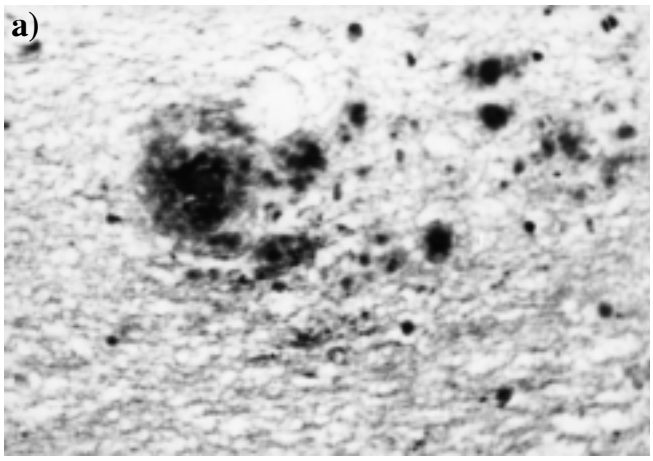


Fig. 2. DNA sequence of *PRNP* at codon 232 region; 1-heterozygous ATG→ACG (methionine→threonine) change, 2-wild type sequence.

nificantly linked with valine homozygosity. Sporadic CJD cases are linked with both homozygous (129Met/Met and 129Val/Val) genotypes (Palmer et al. 1991, Windl et al. 1996). Thus, heterozygosity at codon 129 may act as a protection factor against sporadic and iatrogenic CJD, although this protection is not absolute (Deslys 1998). Of note, distribution of codon 129 *PRNP* genotypes in sporadic CJD cases differ significantly across the age groups; a positive linear correlation between age and the frequency of the methionine homozygosity in France, Germany, and UK, but not in Italy was reported by Alperovitch et al. (1999). The chemical basis of these effects was investigated using synthetic peptides of the prion protein (residues 118-133). Indeed, homogenous peptide amyloid (Met/Met or Val/Val) is more stable than heterogeneous amyloid (Come et al. 1993).



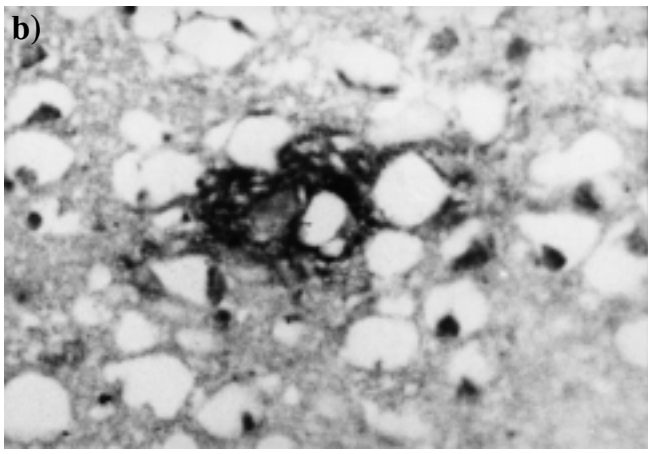


Fig. 3. (a-b) PrP immunohistochemistry of the cerebellar cortex of the GSS case with codon 232 mutation. Hydrated autoclavong; original magnification, x 1,000.

Codon 129 genotype is involved in PrP^{Sc} banding pattern on Western blots after proteinase K treatment. Type 1 pattern is seen in sporadic and iatrogenic CJD cases with $129^{Met/Met}$ phenotype, type 2 pattern is seen in $129^{Met/Met}$ as well as $129^{Met/Val}$ and $129^{Val/Val}$ sporadic CJD cases. Type 3 is limited to $129^{Met/Val}$ and $129^{Val/Val}$ phenotypes of iatrogenic CJD cases. In all vCJD cases type 4 of PrP^{Sc} is invariably found (Collinge et al. 1996, Will et al. 1996). The classification of PrP^{Sc} in sporadic CJD is still under consideration and some groups distinguish only two PrP^{Sc} types (Parchi et al. 1999).

We conclude that the sporadic form of Polish CJD cases is linked with $129^{Met/Met}$ homozygosity and this tendency is consistent with findings of the other authors. The first mutation of *PRNP* ORF linked to GSS phenotype was found in one of the Polish patients at codon 232 (Liberski et al. 1998). A different codon 232 mutation ATG→AGG (methionine→arginine) has previously been reported in three apparently sporadic Japanese patients with typical clinical and neuropathological features of CJD (Hitoshi et al. 1993). Like the Japanese cases, our case also had no family history of disease, but

unlike the Japanese cases, our case had a GSS phenotype. Thus, it is clear that not only mutations in different codons, but also different mutations of the same codon, can produce diverse phenotypic expressions. It is curious that residue 232 is located within the C-terminal segment of PrP, which is replaced by a glycophosphoinositol anchor in the mature protein, suggesting that mutation-induced conformational transition from the normal α-helix to the pathogenic β-sheet configuration occurs early in the post-translational phase of protein maturation.

Added in proof

DNA sample of son of the proband was analyzed and only wild type sequence at codon 232 was found.

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