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Codon 219 in Creutzfeldt-Jakob disease in Poland

Jolanta Bratosiewicz-Wąsik[1], **Tomasz J. Wąsik**[1] and **Paweł P. Liberski**[2]

Department of Virological Diagnostics, Chair of Molecular Biology, Biochemistry and Biopharmacy, Medical University of Silesia in Katowice, Poland, [2]Laboratory of Neuropathology and Electron Microscopy, Department of Molecular Biology, Chair of Oncology, Medical Academy Łódź, Poland

Abstract. Prion diseases are a group of etiologically heterogenous diseases. In addition to familial cases linked to mutations of *PRNP* open reading frame they include also cases of unknown etiology. One of the susceptibility factors to sporadic as well as iatrogenic prion diseases are *PRNP* polymorphisms. In the present study, we analyzed sequences of the *PRNP* gene codon 219 of 16 Polish CJD cases and we found heterozygous GAG to GAT changes on the sense strand and only wild type sequence on an antisense strand. The RFLP technique was used to verify this divergence and only wild type sequences were revealed

Correspondence should be addressed to P.P. Liberski, Email: ppliber@csk.am.lodz.pl

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Familial cases of Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS), and fatal familial insomnia (FFI) are linked to mutations within the ORF of the prion protein gene (*PRNP*). More than twenty point mutations were reported to be associated with these diseases, and several octapeptide insertions in the octapeptide repeat region have also been found in familial forms of disease (for review, Collinge 2001). Other genetic alterations involve the polymorphic codon 129, which may occur as either an ATG (methionine) or a GTG (valine) triplet (Goldfarb et al. 1989, Owen et al. 1989). Variations at this locus may influence disease susceptibility or expression: homozygotes are overrepresented in all forms of CJD (approximately 80% compared to 50% of the general population (Collinge et al. 1991, Palmer et al. 1991, Salvatore et al. 1994, Windl et al. 1996, Brown et al. 2000). Thus, heterozygosity at codon 129 may act as a protection factor against sporadic and iatrogenic CJD infection, although this protection is not absolute (Deslys et al. 1998). It is of note that, so far, all cases of variant CJD (vCJD) are homozygous for methionine at codon 129 (Collinge et al. 1996).

Another polymorphism (at codon 219) was found among the Japanese population in which guanine is replaced by adenine (Glu to Lys substitution) with 6% allele frequency (Furukawa 1995). Searching for this change among Japanese sporadic CJD cases failed to find codon 219 Glu/Lys heterozygous polymorphism. In the Japanese population, the frequency of alleles distribution is quite different (0.96:0.04 Met:Val). These results indicate that codon 219 Glu/Lys heterozygosity serves as protector against sporadic CJD among Japanese (Shibuya 1998).

To screen for point mutations among Polish CJD cases, we performed sequencing of the majority of the open reading frame of *PRNP* gene according to the SequiTherm EXCEL^{TM} DNA Sequencing Kit (Epicentre Technologies) protocol. We analyzed DNA samples of 16 Polish CJD cases. A heterozygous GAG to GAT transversion was noticed on sense strands whereas sequencing of an antisense strand revealed merely a wild type sequence (Fig. 1). At the first step of preparing DNA template for sequencing, Taq DNA polymerase was used so we decided to repeat this stage using Pfu polymerase. Pfu DNA polymerase exhibits lower error rate comparing with several other polymerases since it has 3'5' exonuclease activity. The results of amplification with Pfu DNA polymerase were the same as with Taq DNA polymerase.

To verify our results, we used mismatched primer nested PCR followed by RFLP technique. DNA was extracted from the peripheral leukocytes. Polymerase chain reaction was performed with oligo-2 (5'-gaa aga tgg tga aaa cag gaa gac c), oligo-3 (5'-gtg gcc aca tgg agt gac ctg ggc ctc) primers. To detect GAT codon mismatched primer 219R was prepared and nested PCR with 219R (5'-tct ctg gta ata ggc ctg aga ttc ccg) and Kin (5'- cat gga tga gta cag caa cca g) primer were processed. The GAT codon with mismatched primer generated restriction site for *Pvu*I enzyme whereas the GAG sequence was not recognized by this endonuclease. A pSP72 vector that possesses one site for *Pvu*I endonuclease was used as positive control. PCR product as

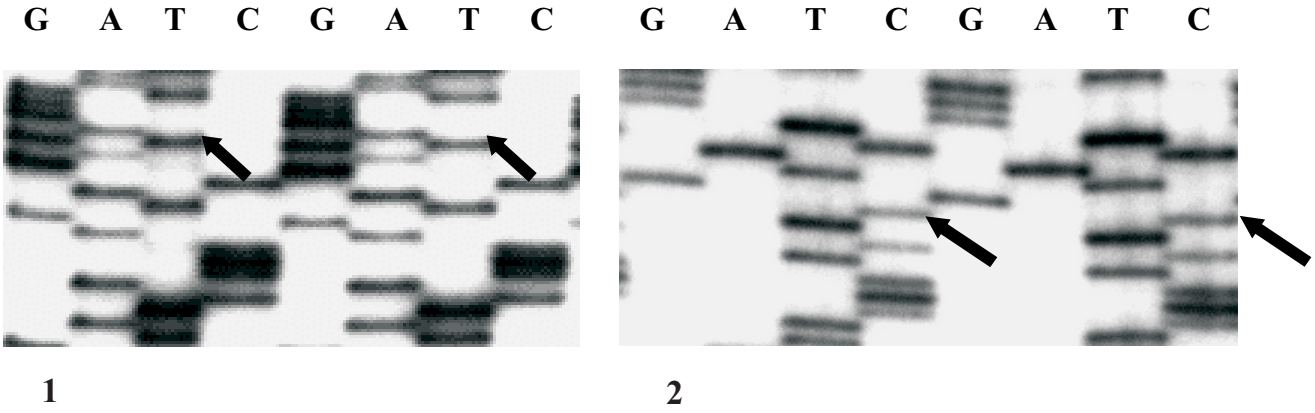

Fig. 1. DNA sequence of PRNP at codon 219; (1) sense strand showing heterozygous change GAGGAT (glutamic acidaspartic acid), (2) antisense strand showing wild type sequence CTC. A GAGAAG (glutamic acidlysine) substitution was reported from Japan.

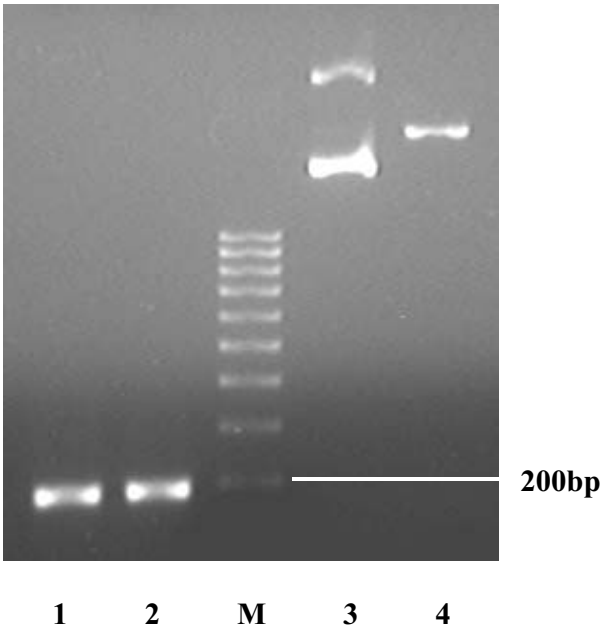

Fig. 2. Restriction enzyme digestion. Line 1shows PCR product not digested, line 2 PCR product digested with PvuI, line 3 pSP72 not digested, and line 4 pSP72 digested with PvuI.

well as pSP72 was digested for 1 hour in 37°C using 1 U of enzyme per 1 μg DNA.

None of the PCR products were cut by *Pvu*I enzyme whereas we have seen linearyzation of the pSP72 vector (Fig. 2). Thus, we may conclude that only the wild type of sequence is present at codon 219 of our samples.

These results incline us to use caution in the interpretation of the findings to avoid false positive results. It is advisable to sequence both strands of DNA to exclude error. Even a high fidelity PCR system does not protect from merging of incorrect nucleotides. The probably reason for this error is the template conformational properties that allow polymerase to perform misincorporation.

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