A pitfall in diagnosis of human prion diseases using detection of protease-resistant prion protein in urine: contamination with bacterial outer membrane proteins

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Abstract

To evaluate diagnostic usefulness and reliability of the detection of protease-resistant prion protein in urine, we extensively analyzed proteinase K (PK)-resistant protein in patients affected with prion diseases and the control subjects by Western blot, a coupled liquid chromatography and mass spectrometry analysis, and N-terminal sequence analysis. The PK-resistant signal migrating around 32 kDa previously reported by Shaked et al. was not observed in this study. Instead, discrete protein bands with an apparent molecular mass of approximately 37 kDa were detected in the urine of many patients affected with prion diseases and two diseased controls. Although these proteins also gave strong signals in the Western blot using a variety of anti-PrP antibodies as a primary antibody, we found that the signals were still detectable by the incubation of secondary antibodies alone, i.e., in the absence of the primary anti-PrP antibodies. Mass spectrometry and N-terminal protein sequencing analysis revealed that majority of the PK-resistant 37 kDa proteins in patients’ urine
were outer membrane proteins (OMPs) of Enterobacterial species. OMPs isolated from these bacteria were resistant to PK and the PK-resistant OMPs from Enterobacterial species migrated around 37 kDa on SDS-PAGE. Furthermore, non-specific binding of OMPs to antibodies could be mistaken for PrP^Sc. These findings caution that bacterial contamination can affect the immunological detection of prion protein. Therefore, the presence of Enterobacterial species should be excluded in the immunological tests for PrP^Sc in clinical samples, in particular, urine.