PRION DISEASE

New techniques developed for prion detection in blood and cerebrospinal fluid

Research teams based in the UK and Japan have developed new assays in blood and cerebrospinal fluid (CSF), respectively, to detect the misfolded prion proteins that cause Creutzfeldt–Jakob disease (CJD). Their findings could have important ramifications for the antemortem diagnosis of CJD.

The blood-based assay was developed by John Collinge, Graham Jackson and colleagues at the Medical Research Council (MRC) Prion Unit, London, UK. “The ability to diagnose prion disease has been a long-term aim of the MRC Prion Unit and National Prion Clinic,” says Collinge. “The ultimate aim has been to develop a straightforward noninvasive method—ideally using blood as an analyte—that could be used for screening purposes in addition to clinical care.”

The blood-based technique involves the use of stainless steel particles to capture disease-associated prions from whole-blood samples. First, the sensitivity of the assay was evaluated through analysis of serial dilutions of brain homogenate from patients with variant (vCJD). The researchers found that even at a 10^{10} -fold dilution, the vCJD samples

were distinguishable from normal brain homogenate.

The team went on to test their assay in 21 patients with vCJD, 27 patients with sporadic CJD, 42 patients with other neurological diseases, and 100 healthy controls. Of the 190 blood samples, 15, all of which were from patients with vCJD, were found to be prion-positive. The researchers concluded that their assay had a sensitivity of 71.4% and a specificity of 100% for the detection of vCJD.

“The most significant finding was that vCJD infection could be detected in blood at all,” explains Collinge. “Although there are well-documented cases of secondary vCJD resulting from exposure to contaminated blood, the general view in the field was that a blood test would not be possible due to the low prion concentration in this analyte.”

In the Japanese study, Ryuichiro Atarashi and his colleagues from Nagasaki University used an *in vitro* amplification technique—known as real-time quaking-induced conversion (RT-QUIC)—to detect the abnormal prion PrP^{Sc} in CSF samples. RT-QUIC is a more-advanced version of the original QUIC assay, which was developed by Atarashi’s team in 2008.

“QUIC assays involve the use of soluble recombinant PrP as a substrate, which is seeded with PrP^{Sc} and then subjected to intermittent automated shaking,” explains Atarashi. “We further refined the QUIC test to improve its sensitivity and practicability.” Preliminary tests showed that RT-QUIC was capable of detecting as little as 1 fg of PrP^{Sc} in diluted brain homogenate from patients with CJD.

Atarashi’s team initially trialed RT-QUIC in CSF samples from 18 patients with CJD and 35 controls with other neurodegenerative diseases. Of the 18 CJD samples, 15 (83.3%) tested positive for PrP^{Sc}, and no false positives were observed in the control group.

To further validate the RT-QUIC assay, the researchers conducted a blind trial involving 30 CSF samples—16 cases of definite CJD and 14 non-CJD controls—from the Australian National CJD Registry. PrP^{Sc} was detected in 14 (87.5%) of the definite CJD samples, compared with none of the controls.

Atarashi believes that RT-QUIC could allow definitive antemortem confirmation of CJD, which currently relies on detection of PrP^{Sc} in biopsy specimens—an approach that is both invasive for the patient and potentially risky for the health-care personnel performing the procedure.

On the basis of the data obtained so far, RT-QUIC seems to have a sensitivity of over 80% and a specificity of 100% for the diagnosis of CJD, but additional studies will be required to rule out the possibility of false positives. “A much larger sample should be evaluated using the RT-QUIC assay under blind conditions,” says Atarashi. He also emphasizes the importance of international collaboration to enable the technique to be developed further.

Like RT-QUIC, the blood-based assay developed at the MRC Prion Unit will require further validation before it can be introduced into routine clinical practice. “Our immediate aim is to test larger numbers of controls to confirm what we believe is high specificity,” says Collinge. “Further research is ongoing in the Prion Unit, with the aim of improving the current assay and developing confirmatory assays.”

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Original articles Edgeworth, J. A. *et al.* Detection of prion infection in variant Creutzfeldt–Jakob disease: a blood-based assay. *Lancet* 377, 487–493 (2011) | Atarashi, R. *et al.* Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nat. Med.* 17, 175–178 (2011)

Further reading Atarashi, R. *et al.* Simplified ultrasensitive prion detection by recombinant PrP conversion with shaking. *Nat. Methods* 5, 211–212 (2008)

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