

and its subsequent amplification was repeated nine times. The product from the tenth round was diluted 1:10 with PBS and inoculated intracerebrally (20 µl per mouse) into tga20 mice (Fischer *et al.*, 1996) that overexpress mouse PrP^C. Infectivity of the PMCA product from the tenth round of amplification of a PrP^{Sc}-positive WBC sample from macaque no. 7 obtained at dissection 1127 days p.i. was also examined. The PMCA product from the tenth round of amplification of no-seed sample was inoculated as negative control. In addition to the PMCA products, 10% brain homogenate of a c-BSE infected cow was also inoculated into tga20 mice to compare infectivity. The bioassay experiments were approved by the Animal Care and Use Committee of the National Institute of Animal Health (approval ID: 09-44) and were conducted in accordance with the guidelines for animal transmissible spongiform encephalopathy experiments of the Ministry of Agriculture, Forestry and Fisheries of Japan.

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REFERENCES

- Atarashi, R., Moore, R. A., Sim, V. L., Hughson, A. G., Dorward, D. W., Onwubiko, H. A., Priola, S. A. & Caughey, B. (2007). Ultrasensitive detection of scrapie prion protein using seeded conversion of recombinant prion protein. *Nat Methods* **4**, 645–650.
- Atarashi, R., Satoh, K., Sano, K., Fuse, T., Yamaguchi, N., Ishibashi, D., Matsubara, T., Nakagaki, T., Yamanaka, H. & other authors (2011). Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nat Med* **17**, 175–178.
- Belay, E. D. (1999). Transmissible spongiform encephalopathies in humans. *Annu Rev Microbiol* **53**, 283–314.
- Brown, P., Rohwer, R. G., Dunstan, B. C., MacAuley, C., Gajdusek, D. C. & Drohan, W. N. (1998). The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathy. *Transfusion* **38**, 810–816.
- Castilla, J., Gonzalez-Romero, D., Saá, P., Morales, R., De Castro, J. & Soto, C. (2008). Crossing the species barrier by PrP^(Sc) replication in vitro generates unique infectious prions. *Cell* **134**, 757–768.
- Caughey, B. W., Dong, A., Bhat, K. S., Ernst, D., Hayes, S. F. & Caughey, W. S. (1991). Secondary structure analysis of the scrapie-associated protein PrP 27–30 in water by infrared spectroscopy. *Biochemistry* **30**, 7672–7680.
- Collinge, J. (2001). Prion diseases of humans and animals: their causes and molecular basis. *Annu Rev Neurosci* **24**, 519–550.
- Comoy, E. E., Casalone, C., Lescoutra-Etcheagaray, N., Zanusso, G., Freire, S., Marcé, D., Auvré, F., Ruchoux, M. M., Ferrari, S. & other authors (2008). Atypical BSE (BASE) transmitted from asymptomatic aging cattle to a primate. *PLoS ONE* **3**, e3017.
- Deleault, N. R., Harris, B. T., Rees, J. R. & Supattapone, S. (2007). Formation of native prions from minimal components in vitro. *Proc Natl Acad Sci U S A* **104**, 9741–9746.
- Edgeworth, J. A., Farmer, M., Sicilia, A., Tavares, P., Beck, J., Campbell, T., Lowe, J., Mead, S., Rudge, P. & other authors (2011). Detection of prion infection in variant Creutzfeldt-Jakob disease: a blood-based assay. *Lancet* **377**, 487–493.
- Fischer, M., Rüllicke, T., Raeber, A., Sailer, A., Moser, M., Oesch, B., Brandner, S., Aguzzi, A. & Weissmann, C. (1996). Prion protein (PrP) with amino-proximal deletions restoring susceptibility of PrP knockout mice to scrapie. *EMBO J* **15**, 1255–1264.
- Gajdusek, D. C., Gibbs, C. J. & Alpers, M. (1966). Experimental transmission of a Kuru-like syndrome to chimpanzees. *Nature* **209**, 794–796.
- Geissen, M., Krasemann, S., Matschke, J. & Glatzel, M. (2007). Understanding the natural variability of prion diseases. *Vaccine* **25**, 5631–5636.
- Gibbs, C. J., Jr, Gajdusek, D. C., Asher, D. M., Alpers, M. P., Beck, E., Daniel, P. M. & Matthews, W. B. (1968). Creutzfeldt-Jakob disease (spongiform encephalopathy): transmission to the chimpanzee. *Science* **161**, 388–389.
- Glatzel, M., Rogivue, C., Ghani, A., Streffer, J. R., Amsler, L. & Aguzzi, A. (2002). Incidence of Creutzfeldt-Jakob disease in Switzerland. *Lancet* **360**, 139–141.
- Gough, K. C., Baker, C. A., Rees, H. C., Terry, L. A., Spiropoulos, J., Thorne, L. & Maddison, B. C. (2012). The oral secretion of infectious scrapie prions occurs in preclinical sheep with a range of PRNP genotypes. *J Virol* **86**, 566–571.
- Green, K. M., Castilla, J., Seward, T. S., Napier, D. L., Jewell, J. E., Soto, C. & Telling, G. C. (2008). Accelerated high fidelity prion amplification within and across prion species barriers. *PLoS Pathog* **4**, e1000139.
- Haley, N. J., Mathiason, C. K., Zabel, M. D., Telling, G. C. & Hoover, E. A. (2009a). Detection of sub-clinical CWD infection in conventional test-negative deer long after oral exposure to urine and feces from CWD+ deer. *PLoS ONE* **4**, e7990.
- Haley, N. J., Seelig, D. M., Zabel, M. D., Telling, G. C. & Hoover, E. A. (2009b). Detection of CWD prions in urine and saliva of deer by transgenic mouse bioassay. *PLoS ONE* **4**, e4848.
- Haley, N. J., Mathiason, C. K., Carver, S., Zabel, M., Telling, G. C. & Hoover, E. A. (2011). Detection of chronic wasting disease prions in salivary, urinary, and intestinal tissues of deer: potential mechanisms of prion shedding and transmission. *J Virol* **85**, 6309–6318.
- Hayashi, H., Takata, M., Iwamaru, Y., Ushiki, Y., Kimura, K. M., Tagawa, Y., Shinagawa, M. & Yokoyama, T. (2004). Effect of tissue deterioration on postmortem BSE diagnosis by immunobiochemical detection of an abnormal isoform of prion protein. *J Vet Med Sci* **66**, 515–520.
- Hill, A. F., Desbruslais, M., Joiner, S., Sidle, K. C., Gowland, I., Collinge, J., Doey, L. J. & Lantos, P. (1997). The same prion strain causes vCJD and BSE. *Nature* **389**, 448–450, 526.
- Hilton, D. A., Sutak, J., Smith, M. E., Penney, M., Conyers, L., Edwards, P., McCardle, L., Ritchie, D., Head, M. W. & other authors (2004). Specificity of lymphoreticular accumulation of prion protein for variant Creutzfeldt-Jakob disease. *J Clin Pathol* **57**, 300–302.
- Holada, K., Simak, J., Brown, P. & Vostal, J. G. (2007). Divergent expression of cellular prion protein on blood cells of human and nonhuman primates. *Transfusion* **47**, 2223–2232.
- Honjo, S. (1985). The Japanese Tsukuba Primate Center for Medical Science (TPC): an outline. *J Med Primatol* **14**, 75–89.
- Houston, F., McCutcheon, S., Goldmann, W., Chong, A., Foster, J., Sisó, S., González, L., Jeffrey, M. & Hunter, N. (2008). Prion diseases are efficiently transmitted by blood transfusion in sheep. *Blood* **112**, 4739–4745.
- Ironside, J. W. (1998). Prion diseases in man. *J Pathol* **186**, 227–234.
- Ironside, J. W. (2010). Variant Creutzfeldt-Jakob disease. *Haemophilia* **16** (Suppl 5), 175–180.
- Iwata, N., Sato, Y., Higuchi, Y., Nohtomi, K., Nagata, N., Hasegawa, H., Tobiume, M., Nakamura, Y., Hagiwara, K. & other authors (2006).

- Distribution of PrP^{Sc} in cattle with bovine spongiform encephalopathy slaughtered at abattoirs in Japan. *Jpn J Infect Dis* 59, 100–107.
- Knight, R. (2010).** The risk of transmitting prion disease by blood or plasma products. *Transfus Apheresis Sci* 43, 387–391.
- Kurt, T. D., Perrott, M. R., Wilusz, C. J., Wilusz, J., Supattapone, S., Telling, G. C., Zabel, M. D. & Hoover, E. A. (2007).** Efficient *in vitro* amplification of chronic wasting disease PrP^{RES}. *J Virol* 81, 9605–9608.
- Kurt, T. D., Seelig, D. M., Schneider, J. R., Johnson, C. J., Telling, G. C., Heisey, D. M. & Hoover, E. A. (2011).** Alteration of the chronic wasting disease species barrier by *in vitro* prion amplification. *J Virol* 85, 8528–8537.
- Lasmézas, C. I., Deslys, J. P., Demaimay, R., Adjou, K. T., Lamoury, F., Dormont, D., Robain, O., Ironside, J. & Hauw, J. J. (1996).** BSE transmission to macaques. *Nature* 381, 743–744.
- Lasmézas, C. I., Fournier, J. G., Nouvel, V., Boe, H., Marcé, D., Lamoury, F., Kopp, N., Hauw, J. J., Ironside, J. & other authors (2001).** Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt–Jakob disease: implications for human health. *Proc Natl Acad Sci U S A* 98, 4142–4147.
- Lasmézas, C. I., Comoy, E., Hawkins, S., Herzog, C., Mouthon, F., Konold, T., Auvré, F., Correia, E., Lescoutra-Etcheagaray, N. & other authors (2005).** Risk of oral infection with bovine spongiform encephalopathy agent in primates. *Lancet* 365, 781–783.
- Maddison, B. C., Baker, C. A., Rees, H. C., Terry, L. A., Thorne, L., Bellworthy, S. J., Whitelam, G. C. & Gough, K. C. (2009).** Prions are secreted in milk from clinically normal scrapie-exposed sheep. *J Virol* 83, 8293–8296.
- Maddison, B. C., Rees, H. C., Baker, C. A., Taema, M., Bellworthy, S. J., Thorne, L., Terry, L. A. & Gough, K. C. (2010).** Prions are secreted into the oral cavity in sheep with preclinical scrapie. *J Infect Dis* 201, 1672–1676.
- Masujin, K., Shu, Y., Yamakawa, Y., Hagiwara, K., Sata, T., Matsuura, Y., Iwamaru, Y., Imamura, M., Okada, H. & other authors (2008).** Biological and biochemical characterization of L-type-like bovine spongiform encephalopathy (BSE) detected in Japanese black beef cattle. *Prion* 2, 123–128.
- Mathiason, C. K., Powers, J. G., Dahmes, S. J., Osborn, D. A., Miller, K. V., Warren, R. J., Mason, G. L., Hays, S. A., Hayes-Klug, J. & other authors (2006).** Infectious prions in the saliva and blood of deer with chronic wasting disease. *Science* 314, 133–136.
- Mathiason, C. K., Hayes-Klug, J., Hays, S. A., Powers, J., Osborn, D. A., Dahmes, S. J., Miller, K. V., Warren, R. J., Mason, G. L. & other authors (2010).** B cells and platelets harbor prion infectivity in the blood of deer infected with chronic wasting disease. *J Virol* 84, 5097–5107.
- Murayama, Y., Yoshioka, M., Okada, H., Takata, M., Yokoyama, T. & Mohri, S. (2007).** Urinary excretion and blood level of prions in scrapie-infected hamsters. *J Gen Virol* 88, 2890–2898.
- Murayama, Y., Yoshioka, M., Masujin, K., Okada, H., Iwamaru, Y., Imamura, M., Matsuura, Y., Fukuda, S., Onoe, S. & other authors (2010).** Sulfated dextrans enhance *in vitro* amplification of bovine spongiform encephalopathy PrP^{Sc} and enable ultrasensitive detection of bovine PrP^{Sc}. *PLoS ONE* 5, e13152.
- Murayama, Y., Imamura, M., Masujin, K., Shimozaki, N., Yoshioka, M., Mohri, S. & Yokoyama, T. (2012).** Ultrasensitive detection of scrapie prion protein derived from ARQ and AHQ homozygote sheep by interspecies *in vitro* amplification. *Microbiol Immunol* 56, 541–547.
- Nemecsek, J., Nag, N., Carlson, C. M., Schneider, J. R., Heisey, D. M., Johnson, C. J., Asher, D. M. & Gregori, L. (2013).** Red-backed vole brain promotes highly efficient *in vitro* amplification of abnormal prion protein from macaque and human brains infected with variant Creutzfeldt–Jakob disease agent. *PLoS ONE* 8, e78710.
- Notari, S., Molerés, F. J., Hunter, S. B., Belay, E. D., Schonberger, L. B., Cali, I., Parchi, P., Shieh, W. J., Brown, P. & other authors (2010).** Multiorgan detection and characterization of protease-resistant prion protein in a case of variant CJD examined in the United States. *PLoS ONE* 5, e8765.
- Ono, F., Terao, K., Tase, N., Hiyaoka, A., Ohyama, A., Tezuka, Y., Wada, N., Kurosawa, A., Sato, Y. & other authors (2011a).** Experimental transmission of bovine spongiform encephalopathy (BSE) to cynomolgus macaques, a non-human primate. *Jpn J Infect Dis* 64, 50–54.
- Ono, F., Tase, N., Kurosawa, A., Hiyaoka, A., Ohyama, A., Tezuka, Y., Wada, N., Sato, Y., Tobiume, M. & other authors (2011b).** Atypical L-type bovine spongiform encephalopathy (L-BSE) transmission to cynomolgus macaques, a non-human primate. *Jpn J Infect Dis* 64, 81–84.
- Orrú, C. D., Wilham, J. M., Hughson, A. G., Raymond, L. D., McNally, K. L., Bossers, A., Ligios, C. & Caughey, B. (2009).** Human variant Creutzfeldt–Jakob disease and sheep scrapie PrP^{Sc} detection using seeded conversion of recombinant prion protein. *Protein Eng Des Sel* 22, 515–521.
- Pan, K. M., Baldwin, M., Nguyen, J., Gasset, M., Serban, A., Groth, D., Mehlhorn, I., Huang, Z., Fletterick, R. J. & Cohen, F. E. (1993).** Conversion of α -helices into β -sheets features in the formation of the scrapie prion proteins. *Proc Natl Acad Sci U S A* 90, 10962–10966.
- Peden, A. H., Ritchie, D. L., Head, M. W. & Ironside, J. W. (2006).** Detection and localization of PrP^{Sc} in the skeletal muscle of patients with variant, iatrogenic, and sporadic forms of Creutzfeldt–Jakob disease. *Am J Pathol* 168, 927–935.
- Peden, A. H., McGuire, L. I., Appleford, N. E., Mallinson, G., Wilham, J. M., Orrú, C. D., Caughey, B., Ironside, J. W., Knight, R. S. & other authors (2012).** Sensitive and specific detection of sporadic Creutzfeldt–Jakob disease brain prion protein using real-time quaking-induced conversion. *J Gen Virol* 93, 438–449.
- Primate Society of Japan (1986).** Guiding principles for animal experiments using nonhuman primates. *Primate Research* 2, 111–113.
- Prusiner, S. B. (1991).** Molecular biology of prion diseases. *Science* 252, 1515–1522.
- Prusiner, S. B. (1998).** Prions. *Proc Natl Acad Sci U S A* 95, 13363–13383.
- Rubenstein, R. & Chang, B. (2013).** Re-assessment of PrP^{Sc} distribution in sporadic and variant CJD. *PLoS ONE* 8, e66352.
- Saá, P., Castilla, J. & Soto, C. (2006).** Presymptomatic detection of prions in blood. *Science* 313, 92–94.
- Saborio, G. P., Permanne, B. & Soto, C. (2001).** Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature* 411, 810–813.
- Scott, M. R., Safar, J., Telling, G., Nguyen, O., Groth, D., Torchia, M., Koehler, R., Tremblay, P., Walther, D. & other authors (1997).** Identification of a prion protein epitope modulating transmission of bovine spongiform encephalopathy prions to transgenic mice. *Proc Natl Acad Sci U S A* 94, 14279–14284.
- Shimizu, Y., Kaku-Ushiki, Y., Iwamaru, Y., Muramoto, T., Kitamoto, T., Yokoyama, T., Mohri, S. & Tagawa, Y. (2010).** A novel anti-prion protein monoclonal antibody and its single-chain fragment variable derivative with ability to inhibit abnormal prion protein accumulation in cultured cells. *Microbiol Immunol* 54, 112–121.
- Tattum, M. H., Jones, S., Pal, S., Collinge, J. & Jackson, G. S. (2010).** Discrimination between prion-infected and normal blood samples by protein misfolding cyclic amplification. *Transfusion* 50, 996–1002.

Terry, L. A., Howells, L., Hawthorn, J., Edwards, J. C., Moore, S. J., Bellworthy, S. J., Simmons, H., Lizano, S., Estey, L. & other authors (2009). Detection of PrP^{Sc} in blood from sheep infected with the scrapie and bovine spongiform encephalopathy agents. *J Virol* **83**, 12552–12558.

Thorne, L. & Terry, L. A. (2008). *In vitro* amplification of PrP^{Sc} derived from the brain and blood of sheep infected with scrapie. *J Gen Virol* **89**, 3177–3184.

Wadsworth, J. D., Joiner, S., Hill, A. F., Campbell, T. A., Desbruslais, M., Luthert, P. J. & Collinge, J. (2001). Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. *Lancet* **358**, 171–180.

Wang, F., Wang, X., Yuan, C. G. & Ma, J. (2010). Generating a prion with bacterially expressed recombinant prion protein. *Science* **327**, 1132–1135.

Will, R. G., Ironside, J. W., Zeidler, M., Estibeiro, K., Cousens, S. N., Smith, P. G., Alperovitch, A., Poser, S., Pocchiari, M. & Hofman, A.

(1996). A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* **347**, 921–925.

Wroe, S. J., Pal, S., Siddique, D., Hyare, H., Macfarlane, R., Joiner, S., Linehan, J. M., Brandner, S., Wadsworth, J. D. & other authors (2006). Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. *Lancet* **368**, 2061–2067.

Yoshioka, M., Imamura, M., Okada, H., Shimozaki, N., Murayama, Y., Yokoyama, T. & Mohri, S. (2011). Sc237 hamster PrP^{Sc} and Sc237-derived mouse PrP^{Sc} generated by interspecies *in vitro* amplification exhibit distinct pathological and biochemical properties in tga20 transgenic mice. *Microbiol Immunol* **55**, 331–340.

Yutzey, B., Holznagel, E., Coulibaly, C., Stuke, A., Hahmann, U., Deslys, J. P., Hunsmann, G. & Löwer, J. (2007). Time-course studies of 14-3-3 protein isoforms in cerebrospinal fluid and brain of primates after oral or intracerebral infection with bovine spongiform encephalopathy agent. *J Gen Virol* **88**, 3469–3478.

