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Genes contributing to prion pathogenesis

Gültekin Tamgüney[1], **Kurt Giles**[1,2], **David V. Glidden**[3], **Pierre Lessard**[1], **Holger Wille**[1,2], **Patrick Tremblay**[1,2,a], **Darlene F. Groth**[1], **Fruma Yehiely**[1,b], **Carsten Korth**[1,c], **Richard C. Moore**[1,d], **Jörg Tatzelt**[2,e], **Eric Rubenstein**[4], **Claude Boucheix**[4], **Xiaoping Yang**[5,f], **Pamela Stanley**[5], **Michael P. Lisanti**[6], **Raymond A. Dwek**[7], **Pauline M. Rudd**[7,g], **Jackob Moskovitz**[8], **Charles J. Epstein**[9], **Tracey Dawson Cruz**[10,h], **William A. Kuziel**[10,i], **Nobuyo Maeda**[10], **Jan Sap**[11], **Karen Hsiao Ashe**[12], **George A. Carlson**[13], **Ina Tesseur**[14,j], **Tony Wyss-Coray**[14,15], **Lennart Mucke**[2,16,17], **Karl H. Weisgraber**[17,18], **Robert W. Mahley**[19,20], **Fred E. Cohen**[1,19,20], and **Stanley B. Prusiner**[1,2,20,*]

[1]Institute for Neurodegenerative Diseases, University of California, San Francisco, California

[2]Department of Neurology, University of California, San Francisco, California

[3]Department of Epidemiology and Biostatistics, University of California, San Francisco, California

[4]INSERM, u602, and Université Paris 11, Villejuif, France

[5]Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York

[6]Department of Molecular Pharmacology and Medicine, Albert Einstein College of Medicine, Bronx, New York; Muscular and Neurodegenerative Disease Unit, University of Genova and G. Gaslini Pediatric Institute, Genova, Italy; Department of Cancer Biology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania; Albert Einstein Cancer Center, Bronx, New York

[7]Department of Biochemistry and Oxford Glycobiology Institute, University of Oxford, Oxford, United Kingdom

[8]Department of Pharmacology and Toxicology, University of Kansas, Lawrence, Kansas

[9]Institute for Human Genetics, Department of Pediatrics, University of California, San Francisco, California

[10]Department of Pathology and Laboratory Medicine, University of North Carolina Medical Center, Chapel Hill, North Carolina

[11]Biotechnology Research and Innovation Center, Faculty of Health Sciences, University of Copenhagen, Denmark

[12]Departments of Neurology, Neuroscience and Graduate Program in Neuroscience, University of Minnesota, and Geriatric Research, Education and Clinical Center, Minneapolis Veterans Affairs Medical Center, Minneapolis, Minnesota

*Corresponding author. Mailing address: Institute for Neurodegenerative Diseases, 513 Parnassus Ave., HSE-774, San Francisco, CA 94143-0518. Phone: (415)476-4482. Fax: (415) 476-8386. stanley@ind.ucsf.edu.
[a]Present addresses:
Pappas Ventures, Montreal, H3A 1X6 Quebec, Canada.
[b]Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL 60611.
[c]Institute for Neuropathology, Heinrich-Heine-University Düsseldorf, 40001 Düsseldorf, Germany.
[d]Department of Genetics and Genomics, Roslin Institute, Roslin, Midlothian EH25 PS, Scotland, United Kingdom.
[e]Department of Biochemistry, Neurobiochemistry, Ludwig-Maximilians-University Munich, Germany.
[f]University of Pennsylvania School of Medicine, Philadelphia, PA 19104.
[g]Dublin-Oxford Glycobiology Laboratory, University College, Belfield, Dublin 4, Ireland.
[h]Departments of Forensic Science and Biology, Virginia Commonwealth University, Richmond, VA 23284-3079.
[i]PDL BioPharma, Inc., Redwood City, CA 94063.
[j]Center for Human Genetics, K.U. Leuven, Department of Molecular and Developmental Genetics, 3000 Leuven, Belgium.

[13]McLaughlin Research Institute, Great Falls, Montana

[14]Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, California

[15]Geriatric Research, Education and Clinical Center, Veterans Affairs Palo Alto Health Care System, Palo Alto, California

[16]Neuroscience Graduate Program, University of California, San Francisco, California

[17]Gladstone Institute of Neurological Disease,, San Francisco, California

[18]Cardiovascular Research Institute, Department of Medicine, Department of Pathology, University of California, San Francisco, Gladstone Institute of Cardiovascular Disease, San Francisco, California

[19]Department of Cellular and Molecular Pharmacology, University of California, San Francisco, California

[20]Department of Biochemistry and Biophysics, University of California, San Francisco, California

SUMMARY

Prion diseases are caused by conversion of a normally folded, nonpathogenic isoform of the prion protein (PrP^{C}) to a misfolded, pathogenic isoform (PrP^{Sc}). Prion inoculation experiments in mice expressing homologous PrP^{C} molecules on different genetic backgrounds displayed different incubation times, indicating that the conversion reaction may be influenced by other gene products. To identify genes that contribute to prion pathogenesis, we analyzed prion incubation times in mice in which the gene product was inactivated, knocked out or overexpressed. We tested 20 gene candidates, because their products either colocalize with PrP, are associated with Alzheimer's disease, are elevated during prion disease, or function in PrP-mediated signaling, PrP glycosylation, or protein maintenance. Whereas some of the candidates tested may have a role in the normal function of PrP^{C}, our data show that many genes previously implicated in prion replication have no discernable effect on the pathogenesis of prion disease. While most genes tested did not significantly affect survival times, ablation of amyloid beta (A4) precursor protein (*App*) or interleukin 1 receptor, type I (*Il1r1*), and transgenic overexpression of human superoxide dismutase 1 (*SOD1*) prolonged incubation times by 13%, 16%, and 19%, respectively.

INTRODUCTION

Prion diseases are fatal neurodegenerative disorders of humans and animals that uniquely present as sporadic, genetic, or infectious maladies (Prusiner, 2007). These diseases are caused by prions, which are formed from the conformational conversion of a ubiquitous and noninfectious isoform of the prion protein (PrP^{C}) to disease-associated and infectious isoforms (PrP^{Sc}). Prions are unprecedented pathogens because nucleic acids are not involved in their replication (Safar et al., 2005). Genetic experiments have shown that the *Prnp* gene, which encodes PrP^{C}, controls the incubation period in prioninfected mice (Carlson et al., 1986). In addition, evidence from experiments in inbred mice expressing homologous PrP^{C} molecules show` that genes other than *Prnp* modify the incubation period to some extent (Carlson et al., 1988, Stephenson et al., 2000). Similarly, studies in the yeast *Saccharomyces cerevisiae* show that chaperones of the Hsp104, Hsp70, and Hsp40 groups are critical for propagation of the yeast prions [PSI^{+}], [URE3], and [PIN^{+}] (reviewed in (Wickner et al., 2007)).

Based on these studies, any gene product involved in the formation of PrP^{Sc} should affect the onset of prion disease if knocked out, inactivated, or overexpressed as demonstrated for PrP itself (Prusiner et al., 1993, Prusiner et al., 1990). Many genes have been suggested to contribute

to prion replication, of which we tested 20 (Table 1). Mice in which the candidate gene product was inactivated, ablated or overexpressed were inoculated and incubation times relative to control mice recorded (Table 2–Table 5). To determine whether differences in incubation period were significant, we developed a novel statistical method accounting for interexperiment variability. While most of the tested genes did not play a role in prion disease, we show here that mice deficient for amyloid beta (A4) precursor protein (*App*) or interleukin 1 receptor, type I (*Il1r1*) had prolonged incubation times by 13% and 16%, respectively. Similarly, overexpression of human superoxide dismutase 1 (*SOD1*) extended disease-onset times by 19%.

METHODS

Source of mice

Most mouse lines have been described previously. *App*$^{-/-}$ mice lack amyloid beta (A4) precursor protein (App) (Zheng et al., 1995). *Aplp2*$^{-/-}$ mice lack amyloid beta (A4) precursor-like protein 2 (Aplp2) (von Koch et al., 1997). Tg(APP)6209Kahs mice, originally designated Tg(HuAPP$_{695}$.WTmyc)6209, express human APP695 (Hsiao et al., 1995). Tg(APOE3) and Tg(APOE4) mice, originally named Tg(NSE-apoE3) and Tg(NSE-apoE4), express the ε3 or the ε4 allele of human apolipoprotein E (APOE) driven by the neuron-specific enolase (NSE) promoter on a C57BL/6J-*Apoe*$^{-/-}$ (C57BL6J-*Apoe*^{tm1Unc}) background (Raber et al., 1998). Tg (Tgfb1) mice express porcine transforming growth factor-β1 (TGF-β1) driven by the glial fibrillary acidic protein (GFAP) promoter on a SJL/J background (Wyss-Coray et al., 1995). *Tgfb1*$^{+/-}$ mice with one ablated *Tgfb1* allele were generated on the NIH/Ola background (Shull et al., 1992). *Ccr2*$^{-/-}$ and *Ccr5*$^{-/-}$ mice lack chemokine (C-C motif) receptors 2 (CCR2) and CCR5, respectively (Kuziel et al., 2003, Kuziel et al., 1997). (TβRIIΔk)$^{+/-}$ × Tg(tTA)$^{+/-}$ *Prnp*$^{+/-}$ mice neuronally express a transdominant negative kinase-deficient mutant of TGF-β receptor II (TβRIIΔk) and are deficient in TGF-β signaling (Tesseur et al., 2006). *Msra*$^{-/-}$ mice lack methionine sulfoxide reductase A (Moskovitz et al., 2001). Tg(SOD1)3Cje mice overexpress human superoxide dismutase 1, soluble (SOD1); their littermate controls are (BALB/c × C57BL/6J)F1 × (C57BL/6J × DBA/2)F1 (Epstein et al., 1987). Tg(HSP70) mice express human heat shock protein 70 (Hsp70); their wt controls are (CBA × C57BL/6J)F1 mice (Plumier et al., 1995). *Mgat3*$^{-/-}$ mice lack mannoside-b1,4-*N*-acetylglucosaminyltransferase III (Yang et al., 2000). *Cd9*$^{-/-}$ mice lack the CD9 antigen (LeNaour et al., 2000). *Cav1*$^{-/-}$ mice lack caveolin-1 (Williams et al., 2003). *Fyn*$^{-/-}$ mice lack fyn proto-oncogene (Fyn) (Grant et al., 1992). Tg(Fyn) mice overexpress wt mouse Fyn directed by the calcium/calmodulin-dependent protein kinase IIα promoter on a C57BL/6 background (line N8) (Kojima et al., 1997). *Ptpra*$^{-/-}$ mice lack protein tyrosine phosphatase, receptor type, A (RPTPα) (Petrone et al., 2003). *Prnd*-ablated mice on an FVB background were produced in our laboratory as described in the online supplement.

The following mice were obtained from The Jackson Laboratory: *Apoe*$^{-/-}$ mice on C57BL/6J background (C57BL/6J-Apoe^{tm1Unc}); interleukin 10 (IL-10)–knockout mice on a C57BL/6J background, denoted B6-*IL10*$^{-/-}$ mice (B6.129P2-*Il10*^{tm1Cgn}/J); IL-10–knockout mice on a 129S6 background, denoted 129-*IL10*$^{-/-}$ mice (129-*Il10*^{tm1Cgn}/J); tumor necrosis factor–deficient mice, denoted *Tnf*$^{-/-}$ mice (B6.129S6-*Tnf*^{tm1Gkl}/J); mice lacking Tnf receptor superfamily, members 1a (TNF-R1) and 1b (TNF-R2), denoted *Tnfrsf1a*$^{-/-}$ *Tnfrsf1b*$^{-/-}$ mice (B6; 129S-*Tnfrsf1a*^{tm1Imx} *Tnfrsf1b*^{tm1Imx}/J); and Interleukin 1 receptor, type I-knockout mice, denoted *Il1r1*$^{-/-}$ mice (B6;129S1-*Il1r1*^{tm1Roml}/J). C57BL/6J,129S1/SvlmJ, B6129SF2/J, and FVB/N mice were obtained as controls. Crossing *Tnfrsf1a*$^{+/+}$ *Tnfrsf1b*$^{+/+}$ mice with B6129SF2/J mice gave *Tnfrsf1a*$^{+/-}$ *Tnfrsf1b*$^{+/-}$ mice. Crossing *Il1r1*$^{+/+}$ mice with B6129SF2/J mice yielded *Il1r1*$^{+/-}$ mice. The genetic status of the progeny was determined using protocols from the Jackson Laboratory.

Prion isolates and transmission

CD1 mouse–adapted RML, Me7, and 301V prions were used as inocula. RML and Me7 were originally derived from sheep with scrapie and have been serially passaged in mice for many generations; 301V was derived from a cow with BSE and passaged into VM mice (Bruce et al., 1994, Chandler, 1961, Dickinson & Meikle, 1969). Brain homogenates (10% wt/vol) in PBS (pH 7.4) were obtained by 10 repeated extrusions through syringe needles of successively smaller sizes, from 22 to 18 gauge, or alternatively by three 30-second strokes of a PowerGen homogenizer (Fisher Scientific). A final 1% (wt/vol) brain homogenate for inoculation was obtained by further diluting brain homogenates in 5% (wt/vol) bovine albumin Fraction V (ICN) and PBS. Using a 27-gauge syringe, 30 μl of 1% brain homogenate were inoculated into the right parietal lobe, or 200 μl into the peritoneal cavity of mice. Animals were monitored daily for their clinical status, while the neurologic status was assessed three times per week. Mice with progressive neurologic dysfunction were euthanized (Carlson et al., 1986).

RESULTS

The experimental paradigm of this study was to investigate the influence of 20 genes on the incubation time in prion-infected mice in which the gene product was either inactive, absent or overexpressed.

Modeling disease onset times

To determine the genotype-independent variation in incubation time, we intracerebrally (i.c.) inoculated over 400 wt FVB/N mice that were divided into 39 groups at 6 different time points over a five-year period with RML prions (Table S1, Fig. 1). Median incubation times were calculated for all 39 groups using the Kaplan-Meier function and mice with intercurrent illness were censored at the time of euthanasia (Kaplan & Meier, 1958). Although these mice were genetically identical, we observed appreciable variation in median incubation times among different groups of mice ranging between 103 and 137 days for individual groups. This variation was not restricted to wt FVB/N mice but also evident in C57BL/6J and CD1 mice (data not shown). Since the commonly used logrank test does not take into account this type of experiment-to-experiment variation among genotypically identical mice, we developed a method to better compare survival curves of prion-diseased mice. Based on our observations, disease-onset times were well fit using a Weibull regression model with the effects of genotypes following an accelerated failure time model (Table 2–Table 5) (Cox & Oakes, 1984). This model accommodates for the fact that in a group of prion-infected mice with the same genotype, all animals become sick within a very close time interval once single animals start to show symptoms. To account for experiment-to-experiment variation, we included a gamma distributed random effect term in the Weibull model that was weighted by the interexperimental variation observed in RML-inoculated, FVB/N mice (Table S1,Fig. 1) (Glidden & Vittinghoff, 2004,Nielsen et al., 1992). This improved the accuracy of our statistical evaluation and led to *p*-values (p_{WG}) that were generally higher than those obtained with the logrank test (p_L) (Table 2–Table 5). Effects of experiments in mice expressing neither, one, or both alleles of *Il1r1*, *Tnfrsf1a* and *Tnfrsf1b*, *Cd9*, and *Prnd* were also tested for trend (Vittinghoff et al., 2005). For a given gene, the trend test delivers increased significance, when incubation times are increasingly prolonged or shortened based on the number of alleles present. The relative time for each genotype shown in Table 2–Table 5 was calculated from the accelerated failure time model, which takes into account both medians and interpercentile ranges of disease-onset times from genetically modified mice and control mice. All calculations were performed with Intercooled Stata 9.2 (StataCorp).

Deficiency in App prolongs prion-incubation times

To analyze the effect of the five AD-related proteins on the incubation time in prion disease, we inoculated five different mouse models and their respective controls with RML prions (Table 2). *App*$^{-/-}$ mice deficient in App expression developed signs of prion disease at ~136 days, whereas wt mice had a median incubation time of 121 days (Fig. 2A). Taking interexperimental variation into account, this 13% protraction of disease-onset times in *App*$^{-/-}$ mice was slight but significant (p_{WG}=0.030). In contrast, lack of Aplp2 expression in *Aplp2*$^{-/-}$ mice or overexpression of human APP695 in Tg(APP)6209Kahs mice had no significant effect on incubation times (Fig. S2A and B). Similarly, expression of human *APOE3* or *APOE4* alleles in *Apoe*$^{-/-}$ mice did not result in significant differences in the incubation times when compared to *Apoe*$^{-/-}$ mice (Fig. S2C and D).

Deficiency of a functional IL-1 receptor prolongs prion incubation times

We assayed six genes associated with inflammation for their effect on the prion incubation time (Table 3). Mice with an ablated interleukin-1 receptor, type I gene (*Il1r1*$^{-/-}$) lack a functional receptor for the proinflammatory cytokines IL-1α and IL-1β and showed 16% longer incubation times compared to control mice (Fig 2B). Although small, this prolongation was statistically significant (p_{WG}=0.012).

Mice deficient of the anti-inflammatory cytokine IL-10 had a 19% reduction in the incubation period compared to wt mice. However, this reduction was observed only in *IL10*$^{-/-}$ mice on the C57BL/6J background but not on the 129S1/SvlmJ background (Fig. 2C and D).

None of the other four inflammation-associated genes affected incubation times (Fig. S3A–F). In two mouse models, inhibited TNF-α signaling resulted in incubation times similar to controls (Fig. S3A and B). We also found that TGF-β1 signaling had no effect on prion incubation times, using three different mouse models: Tg(Tgfb1) mice overexpressing TGF-β1, heterozygous Tg(*Tgfb1*$^{+/-}$) mice, and double-transgenic Tg(TβRIIΔk)$^{+/-}$ × Tg(tTA)$^{+/-}$ *Prnp*$^{+/-}$ mice expressing a transdominant negative mutant of TGF-β receptor II that disrupts TGF-β signaling (Fig. S3C and D). Deficiency of CCR2 or CCR5 also did not influence prion incubation times (Fig. S3E and F).

Proteins associated with protein maintenance do not influence prion incubation times

We tested the influence of four genes relevant to protein expression and maintenance on prion-incubation times (Table 4). The methionine sulfoxide reductase (Msr) system consisting of MsrA and MsrB reverts methionine sulfoxide to methionine and thereby protects from oxidative stress (Moskovitz & Stadtman, 2003). We tested the effect of oxidative stress on prion replication in MsrA-knockout mice on a regular or on a selenium-depleted diet that further disables the Msr system by inactivating MsrB, which as a selenoprotein requires selenium to be fully active. *Msra*-deficient mice that were kept on a regular or selenium-depleted diet did not show incubation times significantly different from control mice (Fig. S4A). Incubation times in Tg(SOD1)3Cje mice that overexpress human superoxide dismutase 1 (SOD1) were significantly (p_{WG}=0.016) extended by 19% after infection with RML prions (Fig. 2E), compared to wt littermates. However, this prolongation was not observed when Tg(SOD1) 3Cje mice were infected with the 301V prion strain. Tg(HSP70) mice, which overexpress human Hsp70, did not show significantly altered incubation times compared to wt littermates (Fig. 2F). Finally, we addressed whether posttranslational modifications impact PrP conversion in mice lacking mannoside-b1,4-*N*-acetylglucosaminyltransferase III (Mgat3/GlcNAcT-III). In comparison to PrP^{C}, PrP^{Sc} contains decreased levels of N-glycans with a bisecting GlcNAc residue and increased levels of tri- and tetraantennary complex N-glycans, which may result from a decrease in Mgat3/GlcNAcT-III activity (Rudd et al., 1999). Incubation times in

Mgat3$^{-/-}$ mice did not differ significantly from wt littermates, even when mice were inoculated with three different prion strains: RML, ME7, and 301V (Fig. S4B).

Proteins associated with PrP signaling, doppel, and CD9 do not influence prion incubation times

We investigated the effect of three genes that are seemingly relevant to PrP signaling on prion replication (Table 5). Incubation times in caveolin-1–deficient mice inoculated either i.c. or intraperitoneally (i.p.) did not differ significantly from those of FVB/N control mice (Fig. S5A). Neither mice lacking nor overexpressing Fyn showed altered incubation times upon infection when compared to wt mice (Fig. S5B and C). *Ptpra*$^{-/-}$ mice deficient for RPTPα also did not have significantly changed incubation times (Fig. S5D). As part of this work, we created *Prnd*$^{-/-}$ mice that lack expression of the PrP paralog doppel (Dpl), which did not show any morphological or behavioral defects except for male infertility, as reported earlier (Behrens et al., 2002). Neither *Prnd*$^{+/-}$ nor *Prnd*$^{-/-}$ mice showed significantly modified incubation times when compared to wt mice (Fig. S5E). We also did not find significantly different incubation times from *Cd9*$^{-/-}$ mice, heterozygous *Cd9*$^{+/-}$ mice, or wt mice after i.c. inoculation with RML prions (Fig. S5F). Because CD9 is present on the cell surface of lymphocytes, we also inoculated *Cd9*$^{-/-}$ and wt mice i.p. with RML prions. Incubation times after i.p. inoculation were not significantly different.

DISCUSSION

The identification of genes that contribute to prion pathogenesis is of paramount importance for understanding prion diseases. Although many PrP-interacting proteins have been identified, only few have been shown to influence prion replication (Schmitt-Ulms et al., 2004, Westergard et al., In press). To prove that a gene contributes to prion pathogenesis, its inactivation, absence, or overexpression should affect prion replication as evidenced from experiments in inbred mice and yeast (Carlson et al., 1988, Stephenson et al., 2000, Wickner et al., 2007). We analyzed a series of candidate genes with importance in AD, inflammation, cell signaling, and other cellular functions for their effect on prion pathogenesis. We modeled disease-onset times after a Weibull regression model that included a gamma distributed random effect to account for interexperimental variation. Based on our statistical model, only very few from among 20 candidate genes moderately but significantly affected prion pathogenesis.

We show that knockout of *App* protracts the onset of prion disease by 13%. While the underlying mechanism is unclear, its elucidation is likely to promote our understanding of the neurodegenerative processes in prion disease as well as in AD, both of which share many common features (DeArmond, 1993). In AD, cleavage of APP gives rise to pathogenic Aβ peptides that are rich in β-sheet conformation and that can form amyloid plaques in the CNS similar to PrP^{Sc}.

Cytokines and their receptors are key mediators of inflammatory as well as anti-inflammatory responses and are often found to be upregulated in a multitude of neurodegenerative conditions, including trauma, stroke, AD, and prion disease. Of six candidate genes that had previously been reported to be upregulated during prion disease, only interruption of IL-1 signaling mildly but significantly prolonged disease-onset times by 16%. Our results agree with a study reporting prolonged incubation times in *Il1r1*-knockout mice after i.c. infection with at least ~15-fold lower concentrations of mouse-adapted 138A scrapie prions (Schultz et al., 2004). The prolonged incubation time likely occurs by delaying astrocytic gliosis, as IL-1R1 is mainly expressed on astrocytes and oligodendrocytes in the CNS and *Il1r1*$^{-/-}$ mice show reduced inflammatory responses (Brogi et al., 1997, Labow et al., 1997, Tomozawa et al., 1995).

Consistent with other studies, abrogated TNF-α or TGF-β signaling did not affect prion pathogenesis after i.c. inoculation (Klein et al., 1997, Mabbott et al., 2000). In *Il10*$^{-/-}$ mice, we observed a 19% reduction in the incubation time on a C57BL/6J background, but no change on a 129S1/SvlmJ background. Differences in the genetic background of these two inbred mouse strains likely explain the different incubation times we observed: While all *Il10*$^{-/-}$ mice are anemic, growth retarded and develop chronic enterocolitis to intestinal antigens (Kuhn et al., 1993), *Il10*$^{-/-}$ mice on the C56BL/6J background have less inflammation and fewer adenocarcinomas than mice on the 129Sv background (Berg et al., 1996). Our results make it difficult to draw a firm conclusion about the role of IL-10 in prion pathogenesis but severely contrast an earlier report in which a >50% reduction in incubation time was reported for *Il10*$^{-/-}$ mice on a 129S1/SvlmJ background (Thackray et al., 2004).

Prion disease is accompanied by activation and recruitment of microglia to sites of prion replication (Guiroy et al., 1994). CCR2 and CCR5 are expressed on microglia and functional in their activation and recruitment through interaction with chemokine (C-C motif) ligand 2 (CCL2) and CCL5 (Albright et al., 1999, El Khoury et al., 2007). Although survival times of *Ccl2*$^{-/-}$ mice infected with ME7 prions were reported to be prolonged (Felton et al., 2005), we failed to detect an effect on survival in *Ccr2*$^{-/-}$ and *Ccr5*$^{-/-}$ mice infected with RML prions. How ablation of *Ccl2* but not *Ccr2* can affect survival times in prion disease is unclear, since CCR2 is the only established high-affinity receptor for CCL2 (Charo et al., 1994). This disparity may be attributed to infection with different prion strains of different titers or to problematic statistical analysis of survival times in *Ccl2*$^{-/-}$ mice. It remains to be determined if either CCR2 or CCR5 can compensate for the other's activity in microglial activation and if incubation times would be prolonged in *Ccr2–Ccr5* double-knockout mice.

Our studies also clearly show that the formation of methionine oxides does not play a critical role in prion pathogenesis. Because certain methionine residues in PrP^{C} can be oxidized, it was suggested that PrP^{C} may protect from oxidative stress (Wong et al., 1999). Disabling the Msr system, which reverts methionine sulfoxide to methionine, increases sensitivity to oxidative stress resulting in reduced life span, elevated levels of hippocampal neurodegeneration, Aβ deposition and tau phosphorylation (Pal et al., 2007). MsrA-knockout mice without a functional Msr system (Moskovitz & Stadtman, 2003) showed incubation times similar to controls.

Furthermore, our studies show that the production of free radicals may affect the progression of disease in a strain-dependent fashion. PrP^{C} has been controversially discussed to have SOD activity and that loss of this putative function by its conversion to PrP^{Sc} may contribute to prion disease (Hutter et al., 2003, Wong et al., 1999). While disabling the Msr system did not affect prion pathogenesis, Tg(SOD)3Cje mice overexpressing human SOD1 showed prolonged incubation times when inoculated with RML prions, but not when injected with 301V prions. In contrast to RML prions, the 301V strain replicates very slowly in mice expressing mouse PrP-A and PrP^{Sc} deposits accumulate only relatively late during pathogenesis. This may cause less immediate inflammation and thus less free radical production (Farquhar et al., 1996). A protective effect of increased SOD activity may only be visible with a fast-replicating strain, such as RML prions where PrP^{Sc} deposits, inflammation, and free-radical production appear relatively early after infection.

While CD9 ablation did not affect incubation times, we cannot exclude that CD81, which shares approximately 65% sequence similarity with CD9, can compensate for the PrP-related functions in *Cd9*$^{-/-}$ mice. In this case, a *Cd9–Cd81* double-knockout mouse might provide some insight into the role of CD9 and/or CD81 during PrP conversion. While tetraspanins such as CD9 are known for their exceptional ability to associate with other surface proteins, no association of CD9 with PrP could be observed (E. Rubinstein, unpublished data).

In conclusion, our studies underscore the importance of screening genes that may contribute to prion pathogenesis in *in vivo* models to establish their function in PrP conversion and pathogenesis. While all candidate genes tested here were implicated to have a role in prion pathogenesis in previous studies, only three were shown to affect disease-onset times. We note that many factors influence these findings, such as the genetic background of the animal model, the prion strain, inoculum titers, the route of infection, and importantly, the statistical analysis of incubation times. By using appropriate controls and a rigorous statistical analysis that takes interexperimental variation into account, we were able to rule out many candidate genes, which otherwise would have scored as "significant" based on the logrank test alone. Clearly, the conversion of PrP^{C} to PrP^{Sc} involves a complex pathway, the elucidation of which would benefit treatment of these devastating diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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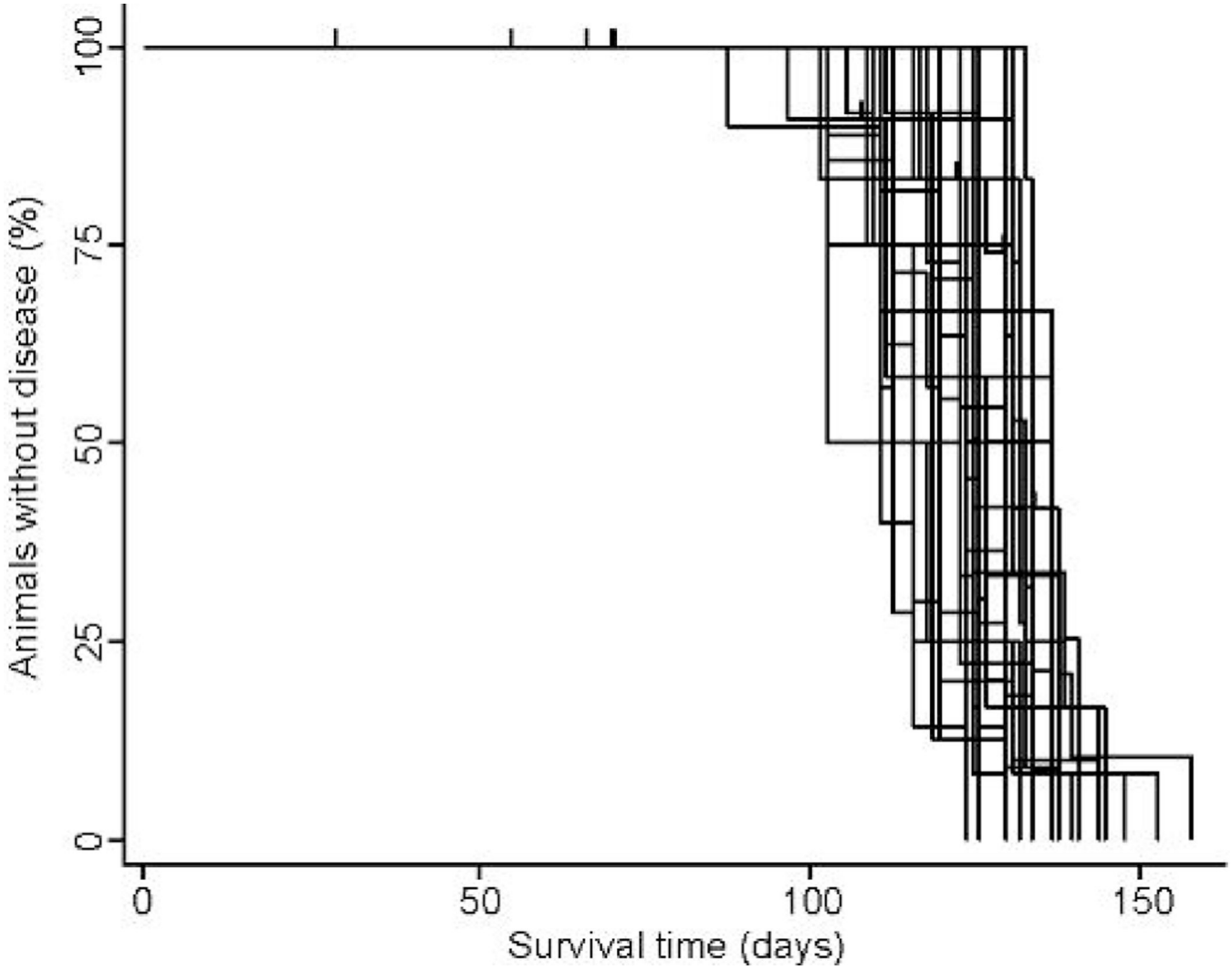

Fig. 1.
Survival curves from transmissions of RML prions to six sets of FVB/N mice. Animals in each set were inoculated on the same day. Median incubation times ranged between 103 and 137 days.

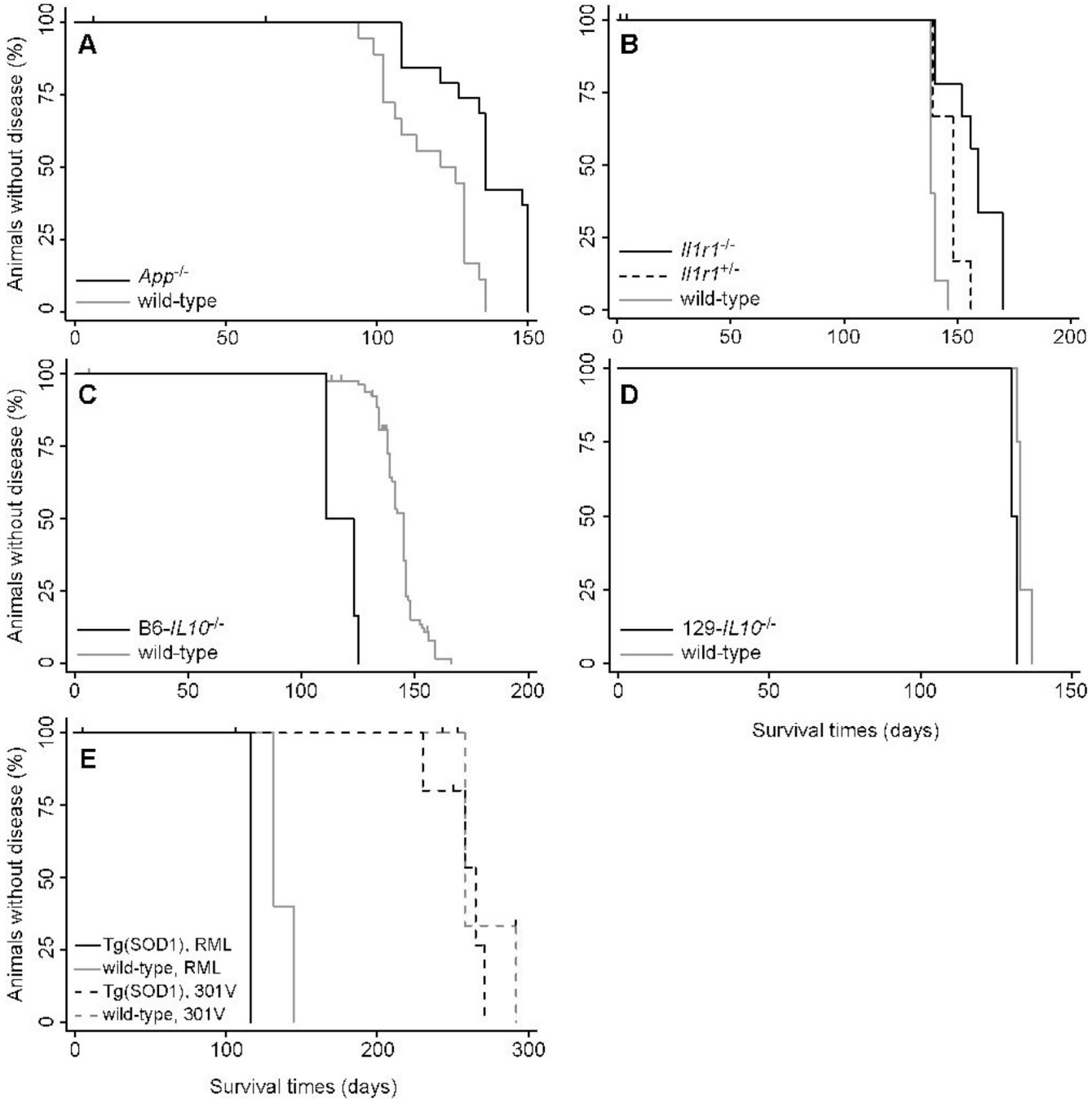

Fig. 2.

Survival curves from transmissions of RML prions to **(A)** *App*$^{-/-}$ mice; **(B)** *Il1rr*$^{-/-}$ mice (black line) and *Il1rI*$^{+/-}$ mice (dashed line); **(C)** B6-*IL10*$^{-/-}$ mice; **(D)** 129Sv-*IL10*$^{-/-}$ mice; and **(E)** Tg(SOD1)3Cje (solid lines). In panel E, Tg(SOD1)3Cje mice were also inoculated with 301V prions (dashed lines). Survival curves of controls are shown by gray lines. Tick marks signify censored animals.

Table 1

Proteins tested for their effect on prion replication.

Protein tested	Reason of interest	Reference	Results
Amyloid beta (A4) precursor protein (App) Human APP695 Amyloid beta (A4) precursor-like protein (Aplp2) Human Apolipoprotein E (APOE), ε3 and ε4 alleles	mouse orthologs colocalize with PrP^C; human orthologs are associated with AD	(Farrer et al., 1997, Schmitt-Ulms et al., 2004)	Table 2
Interleukin 10 (IL-10)	elevated in CJD patients	(Stoeck et al.,2005)	Table 3
Interleukin-1 receptor, type I (IL-1R1) Tumor necrosis factor (TNF-α)	elevated levels of IL-1α, IL-1β, and TNF-α in prion-infected mice and CJD patients	(Campbell et al., 1994, Sharief et al., 1999)	
Transforming growth factor-β1 (TGF-β1)	elevated in prion-infected mice	(Baker et al., 1999)	
Chemokine (C-C motif) receptor 2 (CCR2) Chemokine (C-C motif) receptor 5 (CCR5)	associated with microglial activation in prion disease; elevated levels of CCL2, CCL5, and CCR5 in prion-infected mice	(Baker et al., 1999, Felton et al., 2005, Lee et al., 2005, Marella & Chabry, 2004)	
Methionine sulfoxide reductase A (MsrA) Methionine sulfoxide reductase B (MsrB)	putative SOD activity of PrP^C	(Moskovitz & Stadtman, 2003, Wong et al., 1999)	Table 4
Human superoxide dismutase 1 (SOD1)	putative SOD activity of PrP^C	(Wong et al., 1999)	
Human heat shock protein 70 (Hsp70)	elevated in prion-infected mice and Purkinje cells of CJD patients	(Kenward et al., 1994, Kovacs et al., 2001)	
Mannoside-b1,4-*N*-acetylglucosaminyltransferase III (Mgat3)	affects PrP glycosylation in prion disease; associated with AD	(Fiala et al., 2007, Rudd et al., 1999)	
Caveolin-1 Fyn kinase Receptor protein tyrosine phosphatase α (RPTPα)	involved in PrP-mediated cellular signaling	(Mouillet-Richard et al., 2000, Santuccione et al., 2005)	Table 5
Doppel (DpI)	paralog of PrP; induces ataxia when	(Moore et al., 1999)	

Protein tested	Reason of interest	Reference	Results
	cerebrally expressed in *Prnp*$^{-/-}$ mice		
CD9	elevated in prion-infected mice and CJD patients	(Doh-ura et al., 2000)	

Table 2

Prion transmission to mouse models relevant to Alzheimer's disease.*

Protein	Mice	Strain	Median incubation time (days)	$n^{\dagger}$	p_{L}	p_{WG}	Relative time (95% c.i.)
App	*App*$^{-/-}$	C57BL/6J	136 20		<0.001	0.030	1.133 (1.012–1.269)
	wt	C57BL/6J	121	19			
Aplp2	*Aplp*$^{-/-}$	C57BL/6J	125 22		0.966	0.879	1.009 (0.902–1.129)
	wt	C57BL/6J	135	22			
Human APP695	Tg(APP)6209Kahs	FVB/N	123 6		0.753	0.772	1.019 (0.898–1.155)
	wt	FVB/N	126	9			
Human APOE, ε3 and ε4 alleles	Tg(APOE3)	C57BL/6J	168 20		0.061	0.772	1.017 (0.907–1.140)
	Tg(APOE4)	C57BL/6J	161 20		0.082	0.959	0.997 (0.890–1.117)
	Apoe$^{-/-}$	C57BL/6J	141	19‡			

*Mice were inoculated i.c. with RML prions.

†*n*, number of inoculated mice.

‡Data from (Tatzelt et al., 1996).

Table 3

Prion transmission to mouse models relevant to inflammation.*

Protein	Mice	Strain	Median incubation time (days)	$n^{\dagger}$	p_L	p_{WG}	Relative time (95% c.i.)
IL-10	B6-*IL10*$^{-/-}$ C	57BL/6J	111	6	<0.001	<0.001	0.814 (0.724–0.916)
	wt	C57BL/6J	145	82‡			
	129-*IL10*$^{-/-}$ 129S	6	130	8	0.001	0.718	0.978 (0.867–1.103)
	wt	129S1/SvlmJ	133	8			
IL-1R1	*Il1r1*$^{-/-}$	129S1/Sv and C57BL/6	159	10	<0.001	0.012	1.164 (1.035–1.309)
	Il1r1$^{+/-}$	129S1/Sv, C57BL/6, and B6129SF2/J	148 10		0.009	0.332	1.061 (0.941–1.197)
	wt	B6129SF2/J	138	10§			
TNF-α	*Tnf*$^{-/-}$ C	57BL/6	149	15	0.008	0.432	0.954 (0.848–1.073)
	wt	C57BL/6J	162	12			
TNF-R1 and TNF-R2	*Tnfrsf1a*$^{-/-}$ *Tnfrsf1b*$^{-/-}$	129S7/SvEvBrd and C57BL/6	126	9	0.001	0.341	0.944 (0.839–1.063)
	Tnfrsf1a$^{+/-}$ *Tnfrsf1b*$^{+/-}$	129S7/SvEvBrd, C57BL/6, and B6129SF2/J	132 10		<0.001	0.315	0.942 (0.838–1.059)
	wt	B6129SF2/J	138	10§			
TGF-β1	Tg(Tgfb1) S	JL/J	99	5			
	wt	SJL/J		5			
	Tgfb1$^{+/-}$ N	IH/Ola	124	8	0.990	0.353	1.060 (0.937–1.200)
	wt	NIH/Ola	124	6			
TGF-β type II receptor, kinase deficient (TβRIIΔk)	Tg(TβRIIΔk)$^{+/-}$ × Tg(tTA)$^{+/-}$ *Prnp*$^{+/-}$	C57BL/6J and FVB/N	355	7	0.523	0.857	1.011 (0.894–1.145)

Protein	Mice	Strain	Median incubation time (days)	$n^{\dagger}$	p_L	p_{WG}	Relative time (95% c.i.)
	Tg(TβRIIΔk)$^{+/-}$ × Tg(tTA)$^{-/-}$ *Prnp*$^{+/-}$	C57BL/6J and FVB/N	287	6			
CCR2	*Ccr2*$^{-/-}$ C	57BL/6J	141	20	0.827	0.456	1.04 (0.934–1.166)
	wt	C57BL/6J	145	82‡			
CCR5	*Ccr5*$^{-/-}$ C	57BL/6J	141	31	0.968	0.891	0.992 (0.889–1.108)
	wt	C57BL/6J	145	82‡			

*Mice were inoculated i.c. with RML prions.

†*n*, number of inoculated mice.

‡§Experiments were listed more than once for better comparison.

Table 4

Prion transmission to mouse models relevant to protein maintenance.*

Protein	Mice	Strain	Median incubation time (days)	$n^{\dagger}$	p_{L}	p_{WG}	Relative time (95% c.i.)
MsrA and MsrB	*Msra*$^{-/-}$	129/SvJ and C57BL/6J	134	9	0.507	0.888	0.991 (0.879–1.119)
	wt	129/SvJ and C57BL/6J	124	10			
	Msra$^{-/-}$	129/SvJ and C57BL/6J	134‡	7	0.949	0.997	1.000 (0.887–1.128)
	wt	129/SvJ and C57BL/6J	131‡	10			
Human SOD1	Tg(SOD1)3Cje	BALB/c, C57BL/6J, and DBA/2	132	5	0.014	0.016	1.188 (1.033–1.367)
	wt	BALB/c, C57BL/6J, and DBA/2	117	4			
	Tg(SOD1)3Cje	BALB/c, C57BL/6J, and DBA/2	265§	5	0.441	0.267	0.923 (0.800–1.064)
	wt	BALB/c, C57BL/6J, and DBA/2	258§	5			
Human Hsp70	Tg(HSP70)	CBA and C57BL/6	132	10	0.007	0.271	1.070 (0.948–1.207)
	wt	CBA and C57BL/6	118	10			
Mgat3	*Mgat3*$^{-/-}$	C57BL/6 and CD1	161	7	0.001	0.079	1.114 (0.988–1.255)
	wt	C57BL/6 and CD1	140	11			
	Mgat3$^{-/-}$	C57BL/6 and CD1	165¶	6	0.116	0.067	1.122 (0.992–1.269)
	wt	C57BL/6 and CD1	147¶	10			
	Mgat3$^{-/-}$	C57BL/6 and CD1	237§	9	0.218	0.378	1.059 (0.933–1.201)
	wt	C57BL/6 and CD1	225§	8			

[*] Unless indicated, mice were inoculated i.c. with RML prions.

[†] *n*, number of inoculated mice.

[‡] Mice were fed a selenium-depleted diet.

[§] Mice were inoculated with 301V prions.

[¶] Mice were inoculated with Me7 prions.

Table 5

Prion transmission to mouse models relevant to cell signaling.*

Protein	Mice	Strain	Median incubation time (days)	$n^{\dagger}$	p_{L}	p_{WG}	Relative time (95% c.i.)
Caveolin-1	*Cav1*$^{-/-}$ FV	B/N	120	8	0.006	0.081	0.901 (0.802–1.013)
	wt	FVB/N	131	181			
	Cav$^{-/-}$ F	VB/N	182‡	8	0.015	0.613	1.031 (0.915–1.163)
	wt	FVB/N	176‡	10			
Fyn	*Fyn*$^{-/-}$	129S7/SvEvBrd and C57BL/6J	133	8	0.416	0.592	1.034 (0.915–1.169)
	wt	129S1/SvlmJ	133	8			
	Tg(Fyn) C	57BL/6J	139	12	0.611	0.996	1.000 (0.888–1.126)
	wt	C57BL/6J	134	8			
RPTPα	*Ptpra*$^{-/-}$ C	57BL/6J	137 8		0.018	0.381	0.950 (0.847–1.066)
	wt	C57BL/6J	145	82			
Dpl	*Prnd*$^{-/-}$ FV	B/N	124	10	0.009	0.148	0.920 (0.822–1.030)
	Prnd$^{+/-}$ FV	B/N	124	10	0.002	0.243	0.936 (0.837–1.046)
	wt	FVB/N	131	181			
CD9	*Cd9*$^{-/-}$ C	57BL/6	144	9	0.083	0.526	1.040 (0.922–1.172)
	Cd9$^{+/-}$ C	57BL/6	151	10	0.065	0.329	1.060 (0.943–1.193)
	wt	C57BL/6	128	9			
	Cd9$^{-/-}$ C	57BL/6	196‡	10	0.032	0.535	1.038 (0.922–1.169)
	wt	C57BL/6	194‡	10			

*Unless indicated, mice were inoculated i.c. with RML prions.

† *n*, number of inoculated mice.

‡ Mice were inoculated intraperitoneally.