Public Health Genomics

Public Health Genomics 2013;16:17–24
DOI: 10.1159/000345203

Published online: March 18, 2013

The Genetics of Susceptibility to Variant Creutzfeldt-Jakob Disease

R. Saba[a] S.A. Booth[a, b]

[a]Molecular PathoBiology, National Microbiology Laboratory, Public Health Agency of Canada, and [b]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Man., Canada

Key Words
Genetics · Infection · microRNA · Mutations · Neurodegeneration · Prion disease · Single nucleotide polymorphisms

Abstract
The emergence of bovine spongiform encephalopathy (BSE) in cattle and, subsequently, its transmission to humans resulting in variant Creutzfeldt-Jakob disease (vCJD) in the UK has proved to be one of the major public health scares of the century. The oral route of infection, the long incubation period, and the incredible resistance of the transmissible infectious agent to various forms of decontamination poses unique challenges. Fortunately, despite extensive exposure of the UK population to contaminated meat, the size of the vCJD epidemic that has emerged since its initial detection is relatively low (225 worldwide). An explanation for this disparity is as yet incomplete, but the development of the disease is likely influenced by a number of factors including physical properties of the infectious agent, environmental factors such as the route and amount of exposure and individual susceptibility factors. This review focuses on current knowledge of the genetic factors that undoubtedly play a major role in influencing the development of vCJD. In terms of genetic susceptibility, the best characterised is the common single nucleotide polymorphism at codon 129 of the human prion protein gene *(PRNP)*. Moreover, several other polymorphisms and mutations have been identified that may affect susceptibility as well as other important disease characteristics such as the highly variable prion disease incubation period.

Introduction to Variant Creutzfeldt-Jakob Disease

Mammalian prion diseases, or transmissible spongiform encephalopathies (TSEs), form a unique spectrum of closely related, invariably fatal, neurodegenerative disorders of both animals and humans. The most frequently observed human prion disease is Creutzfeldt-Jakob diseases (CJD) which can be sporadic, acquired or genetic (table 1). Pathological features of the infected brain include neuronal loss, gliosis, spongiform change, and the presence of prion deposits. Neural tissue isolated from affected individuals contains an infectious agent, thus, distinguishing this disease from all other neurodegenerative diseases and disorders. The infectious agent in prion diseases is the misfolded isoform (PrP^{Sc} or PrP^{Res}) of the host encoded cellular prion protein (PrP^{c}). The abnormal isoform has the potential to aggregate and is highly neurotoxic [1, 2]. While the precise function of PrP^{c} is yet to be determined, it is, however, essential for the prion-replication process and also for neurotoxicity to occur [3]. Upon infection, α-helical rich PrP^{c} is refolded into β-sheet

KARGER

E-Mail karger@karger.com
www.karger.com/phg

1662–4246/13/0162–0017$38.00/0

Dr. Stephanie A. Booth
Molecular PathoBiology, National Microbiology Laboratory
Public Health Agency of Canada, 1015 Arlington Street
Winnipeg, MB R3E 3R2 (Canada)
E-Mail Stephanie.Booth@phac-aspc.gc.ca

Table 1. Various categories of CJD identified in humans

Categories of CJD	Examples
Sporadic	Sporadic CJD Sporadic fatal insomnia Protease-sensitive prionapathy
Acquired	Kuru Iatrogenic CJD Variant CJD
Genetic	Familial CJD Gerstmann-Straussler-Scheinker syndrome Fatal familial insomnia

rich PrP^{Sc}, initially in the presence of exogenous PrP^{Sc} and then by an autocatalytic process. In turn, this leads to the formation of aggregates that are distinguishable from PrP^{c} due to their partial resistance to protease digestion and of their insolubility in nondenaturating detergents [4].

In 1996, a new human version of prion disease, variant Creutzfeldt-Jakob disease (vCJD) was identified in the UK. It was characterised by a much younger age of onset, longer clinical course and distinct neuropathology in comparison to classical CJD [5]. Epidemiological and experimental evidence strongly suggest that the emergence of vCJD is a direct consequence of the introduction of bovine spongiform encephalopathy (BSE or 'mad cow') infected cattle into the human food chain. BSE was identified in the UK in 1985, and the numbers of infected cattle rose to epidemic proportions fuelled by the contamination of cattle feed with TSE-infected ruminant-derived meat and bone meal. It is likely that the number of infected cattle was as high as 3 million [6]. Most of these BSE infected cattle are believed to have entered the human food chain, thus exposing the majority of the population. Fortunately, an epidemic of vCJD has not manifested. As of February 2012, 225 cases of vCJD have been reported worldwide (176 in the UK); therefore, it is evident that a considerable barrier to the zoonotic transmission of BSE to humans does indeed exist.

An interesting feature of the vCJD cases recorded to date is that despite the apparent exposure of most of the population to BSE, the majority of cases are in people who are aged less than 40. This suggests that a specific susceptibility factor may be at play. Experimental transmission of prions in a number of animal models has shown that the amount of infectious agent ingested has a proportional relationship to the likelihood of transmission [7, 8]. This may indicate a greater dietary exposure by younger members of the population. There is little evidence to support this hypothesis, although one study has shown that there is a slight dietary risk factor for transmission associated with the intake of mechanically recovered beef products in vCJD cases over control cases [9]. Alternatively, there may be a developmental difference in younger individuals leading to an alteration in peripheral absorption of the agent. A precedent for this explanation exists in data from mouse studies in which it was found that younger mice develop clinical signs and reach the clinical phase of disease more quickly than older mice when inoculated at the periphery [10]. However, there is no evidence at present to confirm whether individuals infected with vCJD at an older age have longer incubation times.

Although the number of new vCJD cases in the UK appears to be slowing, it is, nevertheless, important for public health information to predict the number of cases that are currently incubating. In vCJD, prions replicate within peripheral lymphoid tissue following ingestion, and therefore, tonsil and appendix tissues are one of the earliest sites that harbour infective prions [11]. The presence of PrP^{Res} detectable by immunohistochemistry in surgically removed tonsils and appendix tissues has been determined in 2 studies to estimate the approximate number of individuals who may go on to develop the disease [12, 13]. Both suggest a prevalence of around 1 per 10,000 of the population, a number far higher than the actual number of vCJD cases in the UK would suggest. This could either mean that 'carriers' exist within the population that never progress to clinical disease, or that the incubation period in some individuals is significantly longer than in the positive patients to date. What is clear is that there are no profound occupational, dietary or other exposures to BSE prions among patients who have developed vCJD, which suggests that genetic factors might be critical. In this article, we review current knowledge regarding the factors that influence the development of vCJD with an emphasis on human genetics.

The Critical Influence of *PRNP* Codon 129 Polymorphism on vCJD Susceptibility

In humans, PrP^{c} is encoded by a single copy gene denoted as *PRNP* which is located on chromosome 20. The open reading frame of *PRNP* resides within a single large exon (exon 2 of *PRNP*) and encodes a primary translation

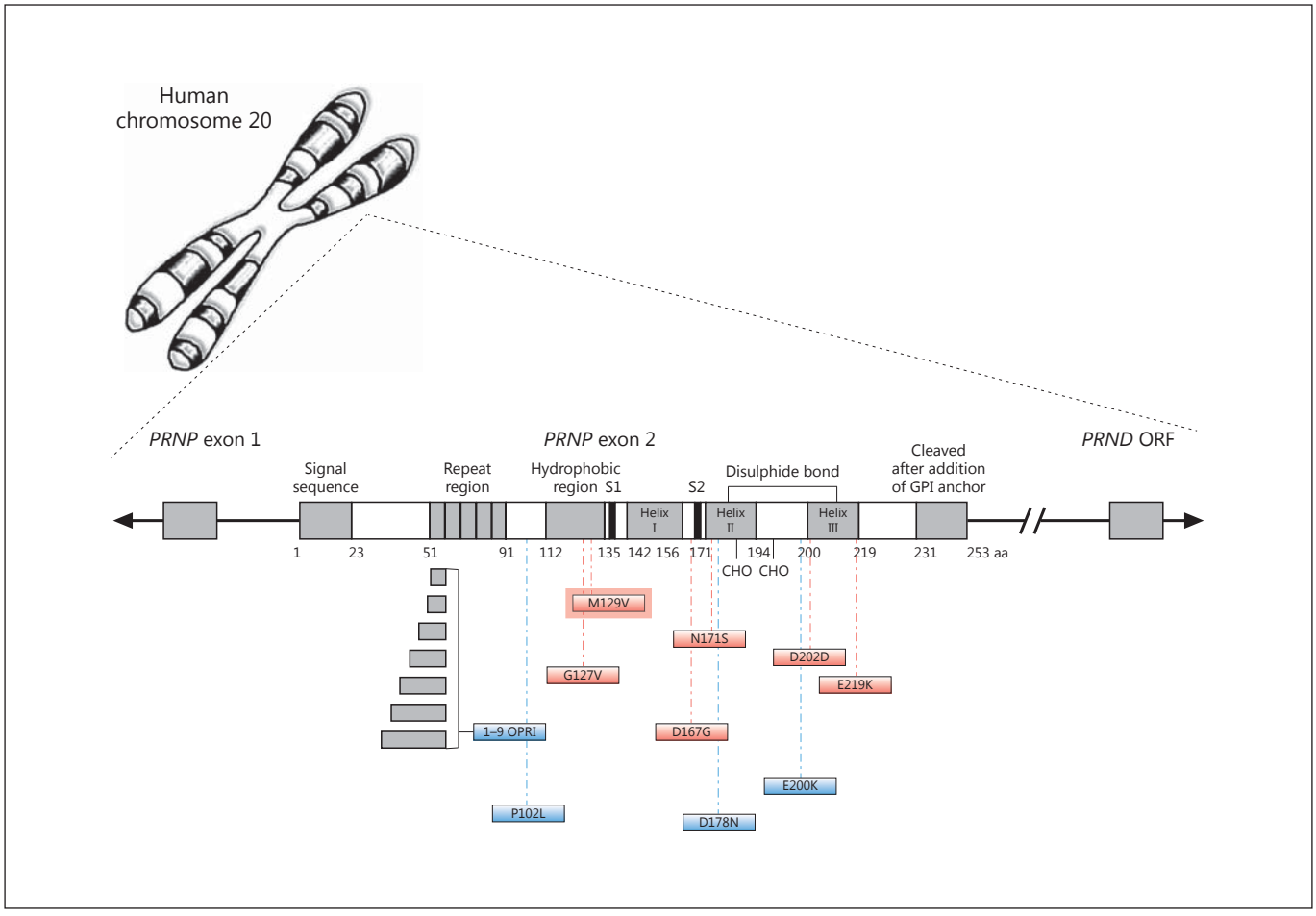

Fig. 1. A schematic illustrating the location of polymorphisms and mutations of the human prion protein gene *(PRNP)*. The 762 base pair (bp) open reading frame of PRNP encodes the 253 amino acid protease sensitive, cellular isoform (PrP^c). PrP^c consists of 3 α-helicies (H1, H2 and H3) and 2 β-strands (S1 and S2). Asn-linked glycosylation sites (CHO) occur at residues 181 and 197. The octapeptide repeat segments extend between residues 51 and 91. Some of the polymorphisms (red) and mutations (blue) of the *PRNP* discussed in this review are represented below the schematic.

product of 253 amino acids (aa) (fig. 1). A 22 aa N-terminal sequence targets the nascent chain to the secretory pathway where it is glycosylated at aspargine residues 181 and 197, and a 23 aa C-terminal sequence is removed during the attachment of a glycophosphotidylinositol anchor. Mature PrP^c is found attached to the outer leaflet of the cell membrane in micro-domains known as lipid rafts. PrP^c expression is almost ubiquitous albeit most abundant in neurons of the central nervous system [14, 15]. The N-terminus of PrP^c is primarily unstructured, but the central and C-terminal regions form a domain comprised of 3 α-helicies and 2 very short antiparallel regions of β-pleated sheet, with a disulphide bridge between helicies-2 and -3 [16].

A known genetic factor that contributes to the risk of developing vCJD is the single nucleotide polymorphism (SNP) at codon 129 of the *PRNP* gene (rs1799990) that encodes either methionine (M = ATG) or valine (V = GTG) [17]. Although 129M or 129V do not have any discernible effects on the biochemical properties of PrP^c, nor on the phenotype of individuals carrying the alternate forms, they can nevertheless have profound effects on susceptibility to human prion diseases. In contrast to the normal Caucasian population in which 40% are homozygous for the more frequent methionine allele, 50% are heterozygous and 10% homozygous for valine, there is a predominance of homozygotes amongst sufferers of both vCJD and sCJD, particularly for methionine. In the case

of vCJD, almost all pathologically positive cases in the UK that have undergone genetic testing are homozygous M/M at codon 129. Only one probable case of vCJD has so far been reported in a heterozygotic (M/V) individual [18].

It is as yet unclear whether codon 129 heterozygosity confers resistance to the disease or whether this results in a lengthening of the incubation period [19, 20]. Some evidence for increased survival of M/V heterozygotes exists from observations on the only other human prion disease acquired by oral transmission, kuru. Kuru is a disease that was recognized in the 1950s in inhabitants of a remote group of villages in Papua New Guinea who practiced ritual funerary cannibalism. This disease has a mean incubation period of around 12 years although some clinical cases are known to have developed as long as 50 years after initial exposure [21]. Retrospective DNA sequencing has revealed that those patients with the short incubation period were, for the most part, 129 homozygous for M/M or V/V. The majority of recent cases in the elderly, who contracted the disease more than 40 years after the funerary feasts were outlawed, were M/V heterozygotes at codon 129 [22, 23].

Further evidence of the involvement of codon 129 in the lengthening of incubation comes from transmission experiments in transgenic mice expressing human *PRNP* genes. Mice expressing *PRNP* carrying the M/V 129 codon were still susceptible to vCJD infection albeit with less efficiency than 129M homozygotes and possibly with longer incubation times [24, 25], while transgenic mice homozygous for human PrP 129 valine show the most pronounced transmission barrier [24–26]. Interestingly, in the study to detect prions in anonymous appendices previously mentioned, 2 of the 3 positive samples had the V/V genotype [9]. Taking all these data into account, it seems that vCJD can be transmitted to individuals carrying all possible 129 genotypes; however, it is still unclear whether a lengthy preclinical phase of disease will precede disease resulting in a second wave of infected individuals, or perhaps, a subclinical carrier state may exist that could still contribute to secondary transmission [27].

The molecular mechanisms that contribute to the effect of codon 129 polymorphisms on disease susceptibility are not completely understood. Confounding the discovery is that the stability, dynamics, metal ion binding capabilities, and 3D-structure of PrP^{c}, with either 129M or 129V, is indistinguishable precluding a simple explanation based directly on a structural determinant [28–30]. However, one study has shown that 129M homozygosity is consistent with the formation of effective 'steric-zippers' made up of a pair of self-complementary β-sheets devoid of water and possessing side chains with the potential to interdigitate [31]. These structures have previously been found to be characteristic features of aggregated amyloids and prion proteins, with the potential to serve as nuclei during PrP^{Sc} propagation. Additionally, 129M homozygosity has the potential to influence the formation of ordered amyloid fibrils of partially denatured α-helical fold of the human PrP, in contrast to 129V homozygotes [30]. These 2 studies suggest that the human M/V polymorphism may act by influencing the kinetics of amyloid formation. Nevertheless, other polymorphisms within *PRNP* and adjacent regions, susceptibility genes and mutations have also been identified in prion diseases, and their potential synergistic contribution to the overall susceptibility to vCJD may be important.

Alternate *PRNP* Polymorphisms and Their Potential Role in vCJD Susceptibility

A number of other polymorphisms within the coding region of *PRNP* that may contribute to CJD susceptibility have also been reported in literature. Many of these are rare and show significant variability between geographically distinct populations, a stark contrast to the 129 M/V polymorphism. These include polymorphisms N171S [32], D202D [33], D167G [33], 24 base pair deletions [33], G127V [34], and E219K [35]. The polymorphism at codon 127 has been identified in several 129M homozygotes exposed to prions, specifically kuru, but have nevertheless shown prolonged survival. This polymorphism has been suggested to possibly represent a resistance marker against the acquired prion disease [34]. Similarly, transmission studies in transgenic knock-in mice for the 219 polymorphism suggest that a heterozygous state at codon 219 may confer reduced susceptibility to prion transmission [35]. Many of these polymorphic sites, however, have not been evaluated to the large extent the 129 polymorphism has. Indeed, in a recent study with a larger cohort of 2,000 human prion patients, no SNPs within *PRNP* other than at codon 129 were determined to be significant [36]. Further work must be performed to determine the prevalence and potential role of *PRNP* locus SNPs, but it seems likely that they may have a modest overall effect.

Genetic Risk Factors within the Regulatory Regions of *PRNP*

Transgenic animal model studies have convincingly demonstrated that the level of PrP^c expression has significant effect on the initiation and progression of prion diseases [37–40]. Moreover, studies have also shown that the development and severity of prion diseases is dependent on prion gene dosage [41]. Therefore, genetic variation at loci outside the *PRNP* coding region, specifically in 5′- and 3′-regulatory regions, can potentially influence the susceptibility of an individual to vCJD and could also go a long way to provide a plausible explanation for the observed susceptibility of 129 heterozygotes and/or 129V homozygotes to vCJD. In an analysis of ~25 kb regions within the *PRNP* locus, 56 polymorphic sites were identified [42]. These included sites within the *PRNP* promoter and also 3′-untranslated region (3′UTR). Association studies involving sCJD subjects with healthy individuals further identified a significant association between a SNP upstream of *PRNP* exon 1 (SNP 1368) and sCJD. SNP 1368 was suspected to be a risk factor independent of codon 129 [42]. However, follow-up studies have both confirmed [43] and disputed [44] these claims. It is interesting to note, however, that a significantly smaller number of cohorts were examined in the study refuting the independent association status of SNP 1368 and whether this association would be evident in a larger cohort study is yet to be resolved. Three SNPs in *PRNP* at positions 101 bp upstream of exon 1 and at 310 bp and 385 bp downstream of exon 1, which are within and adjacent to the regulatory regions of *PRNP*, respectively, have also been suggested to show a codon 129 independent association with CJD [45]. Interestingly, the –101 C to G polymorphism is overrepresented among sCJD subjects who are also heterozygous at codon 129, suggesting that this regulatory region polymorphism may be a risk factor for these individuals by potentially weakening the protective effect conferred by codon 129 heterozygosity [46].

The 3′ UTR is known to contain sequences that regulate translation efficiency, mRNA stability and polyadenylation signals. These sites also contain binding regions for microRNAs (miRNAs), a potent class of gene regulatory molecules. In general, miRNAs are genome encoded RNAs ~18–25 nucleotides long that regulate gene expression by binding to sequence complementary regions in the 3′UTR of protein coding transcripts. miRNAs are also highly expressed in the brain where they have been identified to play vital functional roles in all aspects of the post-mitotic neuron [47]. It is possible that genetic variation present in miRNA binding sites within PrP^c and/or other susceptibility genes can lead to alterations in transcript regulation. However, to date, this type of effect has remained an unexplored mechanism of genetic susceptibility in prion diseases.

Potential for the Contribution of *PRNP* Mutations to vCJD Susceptibility

In conjunction with the polymorphism observed at codon 129, certain *PRNP* mutations that have been noted in other types of CJD (table 1) may have the potential to contribute to vCJD, perhaps by modifying the variability of the observed clinical phenotype. This assumption, however, has to undergo rigorous analysis prior to drawing any definite conclusions. To date, numerous *PRNP* mutations have been described and these may be grouped into 2 types: (i) alterations in the number of octapeptide repeats in the N-terminal domain of PrP^c and (ii) missense mutations resulting in a premature stop codon or amino-acid variant in the C-terminal domain. The most common worldwide *PRNP* mutations are octapeptide repeat insertions (OPRI), E200K, D178N, and P102L [48]. OPRI mutations are caused by the insertion of more than 3 additional octapeptide repeats in the N-terminal region of PrP^c. Some OPRI mutations have been shown to be linked to codon 129 homozygosity in familial CJD [49]. Insert carriers who are codon 129 heterozygous are more likely to show delayed age of onset of disease by ~10 years in comparison to 129 homozygous individuals. E200K mutation is the most common cause of inherited prion diseases worldwide. Interestingly, E200K carriers with 129M homozygosity show a perpendicular strike like prion deposit in the molecular layer of the cerebellum [50]. The E200K mutation shows a highly variable expressivity, manifesting in a wide age range of onset of the disease. In asymptomatic mutation carriers, the 129 polymorphism may play a role in the manifestation of the disease [51]. The D178N mutation is involved in fatal familial insomnia. A haplotypic relationship has been established between codons 178 and 129, whereby the mutation on a 129M chromosome leads to fatal familial insomnia and the mutation on a 129V chromosome leads to familial CJD [52]. More recently, however, this type of relationship has been questioned as some cases do not obey this rule. The P102L mutation is typical of Gerstmann-Straussler-Scheinker syndrome. In contrast to the other mutations discussed,

Table 2. Genetic variation in genes other than *PRNP* that may contribute to the susceptibility of an individual to prion diseases

Gene(s)	Comment	Reference
Myotubularin-related protein 7 *(MTMR7)*	A CNS expressed gene involved in the phosphatidylinositol pathway. Intronic regions possess an SNP (rs4921542) that has been found with increased prevalence in vCJD subjects.	[54]
Phospholipase C-delta-3 *(PLCD3)*	Enzyme of the phosphatidylinositol pathway that has been identified indirectly through linkage disequilibrium with the prion disease specific SNPs rs7565981 and rs17024792. Involved in the hydrolysis of phosphatidylinositol 4,5-bisphosphate.	[54]
Cathepsin D *(CTSD)*	A polymorphism (rs17571) that alters the protease activity of the enzyme, leading to alterations in the amyloid processing activity of the enzyme in Alzheimer's disease, also appears to be associated with increased risk for vCJD in certain sets of populations.	[33]
Zinc finger and BTB domain containing 38 *(ZBTB38)*	An SNP (rs9857275) is found intronic to this zinc finger transcriptional activator that binds methylated DNA and is expressed in the brain with potential implications for determining adult height.	[36]
Semaphorin-3A *(SEMA3A)*	A SNP (rs488333) upstream of this gene which is a secreted protein with chemo-attractive or repulsive functions, such as inhibition of axonal growth or stimulation of the growth of apical dendrites, has also been implicated in CJD.	[36]
Chimerin 2 *(CHN2)*	Risk can be conferred by an SNP (rs1016726) with moderately strong linkage disequilibrium to codon 129.	[34, 36]
Retinoic acid receptor beta *(RARB)* and thyroid hormone receptor beta *(THRB)*	An SNP (rs6794719) in the intergenic region between *RARB* and *THRB* has been identified to confer risk. *RARB* is particularly interesting since retinoic acid regulates the expression of PrP^{c} in cultured neurons and lymphoid tissue. Additionally, the production of PrP^{Res} is increased in vitro by retinoic acid treatment. Whether retinoic acid acts specifically through the receptor encoded by *RARB* for these biological activities is not yet known.	[36]
Shadow of prion protein *(SPRN)*	Also known as Shadoo shows homology and functional similarities to PrP^{c}. Genetic variation in *SPRN* has been evaluated as a potential risk factor for prion diseases as a frameshift mutation (caused by the insertion of a single nucleotide at codon 46) has been detected in several vCJD subjects.	[55]
HECT domain containing 2 *(HECTD2)*	An SNP (rs12249854) located in this gene has shown successful significant association with several forms of acquired prion disease. *HECTD2* is an E3 ubiquitin ligase and is involved in the ubiquitin-proteasome pathway which has been implicated in prion diseases and a number of other neurodegenerative diseases.	[56]

codon 129 appears to have only limited modifying effect; nevertheless, P102L mutations are in chromosomal phase with 129M coding [53].

Conclusion

Understanding the full extent of human genetic susceptibility to prions is not only important for modelling the size of a potential epidemic of vCJD, but also for identifying high-risk individuals who might be in a presymptomatic phase of illness. Such individuals represent a potential risk of transmitting human prions to the general public. Nevertheless, to date, the only definite susceptibility marker of disease appears to be the M/V polymorphism observed at codon 129 of *PRNP*. However, it is difficult to fathom that it is the sole determinant of a very complex neurological disease that encompasses multiple genes and is intertwined in many neuronal regulatory networks. Genetic variation in genes other than *PRNP* may contribute additive effects to the susceptibility of an individual to prion diseases, and, therefore, to conclude that no other genes are involved in modifying such a risk would be premature. Although none of the genes and loci that have been investigated via genome wide association studies have shown a similarly strong and/or universal

association as the *PRNP* locus. Nevertheless, the associations that have been identified are well beyond what would have been expected by chance alone. Furthermore, their association with the disease, and the identification of further genes, requires the examination of larger cohorts, which is a major challenge when working with such a rare disease. A brief description of these genes is provided in table 2. Taken as a whole, we believe that numerous as yet poorly characterized genetic factors are involved in determining the rate of susceptibility, the age of onset, the clinical manifestation, and the disease duration. Very likely genetic risk factors will be found in genes whose products contribute to expression, maturation and/or function of the prion protein. Therefore, functional analyses of these genes will provide an important inroad for increased understanding of numerous fundamental questions in prion biology, such as the role of PrP^{C} and the cellular processes and pathways that play key roles in disease pathogenesis.

References

1 Prusiner SB: Prions. Proc Natl Acad Sci USA 1998;95:13363–13383.
2 Aguzzi A, Heikenwalder M, Polymenidou M: Insights into prion strains and neurotoxicity. Nat Rev Mol Cell Biol 2007;8:552–561.
3 Collinge J, Clarke AR: A general model of prion strains and their pathogenicity. Science 2007;318:930–936.
4 Prusiner SB: Novel proteinaceous infectious particles cause scrapie. Science 1982;216:136–144.
5 Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, Poser S, Pocchiari M, Hofman A, Smith PG: A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 1996;347:921–925.
6 Smith PG, Bradley R: Bovine spongiform encephalopathy (BSE) and its epidemiology. Br Med Bull 2003;66:185–198.
7 Baier M, Norley S, Schultz J, Burwinkel M, Schwarz A, Riemer C: Prion diseases: infectious and lethal doses following oral challenge. J Gen Virol 2003;84:1927–1929.
8 Lasmézas CI, Comoy E, Hawkins S, Herzog C, Mouthon F, Konold T, Auvré F, Correia E, Lescoutra-Etchegaray N, Salès N, Wells G, Brown P, Deslys JP: Risk of oral infection with bovine spongiform encephalopathy agent in primates. Lancet 2005;365:781–783.
9 Ward HJ: Evidence of a new human genotype susceptible to variant CJD. Euro Surveill 2006;11:E060601.3.
10 Avrahami D, Gabizon R: Age-related alterations affect the susceptibility of mice to prion infection. Neurobiol Aging 2011;32:2006–2015.
11 Hilton DA, Ghani AC, Conyers L, Edwards P, McCardle L, Ritchie D, Penney M, Hegazy D, Ironside JW: Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. J Pathol 2004;203:733–739.
12 Clewley JP, Kelly CM, Andrews N, Vogliqi K, Mallinson G, Kaisar M, Hilton DA, Ironside JW, Edwards P, McCardle LM, Ritchie DL, Dabaghian R, Ambrose HE, Gill ON: Prevalence of disease related prion protein in anonymous tonsil specimens in Britain: cross sectional opportunistic survey. BMJ 2009;338:b1442.
13 Peden A, McCardle L, Head MW, Love S, Ward HJ, Cousens SN, Keeling DM, Millar CM, Hill FG, Ironside JW: Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia. Haemophilia 2010;16:296–304.
14 Morris RJ, Parkyn CJ, Jen A: Traffic of prion protein between different compartments on the neuronal surface, and the propagation of prion disease. FEBS Lett 2006;580:5565–5571.
15 Westergard L, Christensen HM, Harris DA: The cellular prion protein (PrP(C)): its physiological function and role in disease. Biochim Biophys Acta 2007;1772:629–644.
16 Zahn R, Liu A, Lührs T, Riek R, von Schroetter C, López García F, Billeter M, Calzolai L, Wider G, Wüthrich K: NMR solution structure of the human prion protein. Proc Natl Acad Sci USA 2000;97:145–150.
17 Collinge J, Palmer MS, Dryden AJ: Genetic predisposition to iatrogenic Creutzfeldt-Jakob disease. Lancet 1991;337:1441–1442.
18 Kaski D, Mead S, Hyare H, Cooper S, Jampana R, Overell J, Knight R, Collinge J, Rudge P: Variant CJD in an individual heterozygous for *PRNP* codon 129. Lancet 2009;374:2128.
19 Huillard d'Aignaux J, Costagliola D, Maccario J, Billette de Villemeur T, Brandel JP, Deslys JP, Hauw JJ, Chaussain JL, Agid Y, Dormont D, Alpérovitch A: Incubation period of Creutzfeldt-Jakob disease in human growth hormone recipients in France. Neurology 1999;53:1197–1201.
20 Lee HS, Brown P, Cervenáková L, Garruto RM, Alpers MP, Gajdusek DC, Goldfarb LG: Increased susceptibility to Kuru of carriers of the *PRNP* 129 methionine/methionine genotype. J Infect Dis 2001;183:192–196.
21 Wadsworth JD, Joiner S, Linehan JM, Asante EA, Brandner S,Collinge J. Review. The origin of the prion agent of kuru: molecular and biological strain typing. Philos Trans R Soc Lond B Biol Sci 2008;363:3747–3753.
22 Mead S, Stumpf MP, Whitfield J, Beck JA, Poulter M, Campbell T, Uphill JB, Goldstein D, Alpers M, Fisher EM, Collinge J: Balancing selection at the prion protein gene consistent with prehistoric kurulike epidemics. Science 2003;300:640–643.
23 Collinge J, Whitfield J, McKintosh E, Beck J, Mead S, Thomas DJ, Alpers MP: Kuru in the 21st century–an acquired human prion disease with very long incubation periods. Lancet 2006;367:2068–2074.
24 Wadsworth JD, Asante EA, Desbruslais M, Linehan JM, Joiner S, Gowland I, Welch J, Stone L, Lloyd SE, Hill AF, Brandner S, Collinge J: Human prion protein with valine 129 prevents expression of variant CJD phenotype. Science 2004;306:1793–1796.
25 Bishop MT, Hart P, Aitchison L, Baybutt HN, Plinston C, Thomson V, Tuzi NL, Head MW, Ironside JW, Will RG, Manson JC: Predicting susceptibility and incubation time of human-to-human transmission of vCJD. Lancet Neurol 2006;5:393–398.
26 Asano M, Mohri S, Ironside JW, Ito M, Tamaoki N, Kitamoto T: vCJD prion acquires altered virulence through trans-species infection. Biochem Biophys Res Commun 2006;342:293–299.
27 Garske T, Ghani AC: Uncertainty in the tail of the variant Creutzfeldt-Jakob disease epidemic in the UK. PLoS One 2010;5:e15626.
28 Riek R, Wider G, Billeter M, Hornemann S, Glockshuber R, Wüthrich K: Prion protein NMR structure and familial human spongiform encephalopathies. Proc Natl Acad Sci USA 1998;95:11667–11672.

29 Hosszu LL, Jackson GS, Trevitt CR, Jones S, Batchelor M, Bhelt D, Prodromidou K, Clarke AR, Waltho JP, Collinge J: The residue 129 polymorphism in human prion protein does not confer susceptibility to Creutzfeldt-Jakob disease by altering the structure or global stability of PrPC. J Biol Chem 2004;279:28515–28521.

30 Lewis PA, Tattum MH, Jones S, Bhelt D, Batchelor M, Clarke AR, Collinge J, Jackson GS: Codon 129 polymorphism of the human prion protein influences the kinetics of amyloid formation. J Gen Virol 2006;87:2443–2449.

31 Apostol MI, Sawaya MR, Cascio D, Eisenberg D: Crystallographic studies of prion protein (PrP) segments suggest how structural changes encoded by polymorphism at residue 129 modulate susceptibility to human prion disease. J Biol Chem 2010;285:29671–29675.

32 Samaia HB, Mari JJ, Vallada HP, Moura RP, Simpson AJ, Brentani RR: A prion-linked psychiatric disorder. Nature 1997;390:241.

33 Bishop MT, Pennington C, Heath CA, Will RG, Knight RS: *PRNP* variation in UK sporadic and variant Creutzfeldt Jakob disease highlights genetic risk factors and a novel non-synonymous polymorphism. BMC Med Genet 2009;10:146.

34 Mead S, Whitfield J, Poulter M, Shah P, Uphill J, Campbell T, Al-Dujaily H, Hummerich H, Beck J, Mein CA, Verzilli C, Whittaker J, Alpers MP, Collinge J: A novel protective prion protein variant that colocalizes with kuru exposure. N Engl J Med 2009;361:2056–2065.

35 Hizume M, Kobayashi A, Teruya K, Ohashi H, Ironside JW, Mohri S, Kitamoto T: Human prion protein (PrP) 219K is converted to PrP-Sc but shows heterozygous inhibition in variant Creutzfeldt-Jakob disease infection. J Biol Chem 2009;284:3603–3609.

36 Mead S, Uphill J, Beck J, Poulter M, Campbell T, Lowe J, Adamson G, Hummerich H, Klopp N, Rückert IM, Wichmann HE, Azazi D, Plagnol V, Pako WH, Whitfield J, Alpers MP, Whittaker J, Balding DJ, Zerr I, Kretzschmar H, Collinge J: Genome-wide association study in multiple human prion diseases suggests genetic risk factors additional to *PRNP*. Hum Mol Genet 2012;21:1897–1906.

37 Scott M, Foster D, Mirenda C, Serban D, Coufal F, Wälchli M, Torchia M, Groth D, Carlson G, DeArmond SJ, Westaway D, Prusiner SB: Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. Cell 1989;59:847–857.

38 Westaway D, Mirenda CA, Foster D, Zebarjadian Y, Scott M, Torchia M, Yang SL, Serban H, DeArmond SJ, Ebeling C: Paradoxical shortening of scrapie incubation times by expression of prion protein transgenes derived from long incubation period mice. Neuron 1991;7:59–68.

39 Bueler H, Aguzzi A, Sailer A, Greiner RA, Autenried P, Aguet M, Weissmann C: Mice devoid of PrP are resistant to scrapie. Cell 1993;73:1339–1347.

40 Manson JC, Clarke AR, McBride PA, McConnell I, Hope J: PrP gene dosage determines the timing but not the final intensity or distribution of lesions in scrapie pathology. Neurodegeneration 1994;3:331–340.

41 Westaway D, DeArmond SJ, Cayetano-Canlas J, Groth D, Foster D, Yang SL, Torchia M, Carlson GA, Prusiner SB: Degeneration of skeletal muscle, peripheral nerves, and the central nervous system in transgenic mice overexpressing wild-type prion proteins. Cell 1994;76:117–129.

42 Mead S, Mahal SP, Beck J, Campbell T, Farrall M, Fisher E, Collinge J: Sporadic–but not variant–Creutzfeldt-Jakob disease is associated with polymorphisms upstream of *PRNP* exon 1. Am J Hum Genet 2001;69:1225–1235.

43 Vollmert C, Windl O, Xiang W, Rosenberger A, Zerr I, Wichmann HE, Bickeböller H, Illig T; KORA group, Kretzschmar HA: Significant association of a M129V independent polymorphism in the 5′ UTR of the *PRNP* gene with sporadic Creutzfeldt-Jakob disease in a large German case-control study. J Med Genet 2006;43:e53.

44 Croes EA, Alizadeh BZ, Bertoli-Avella AM, Rademaker T, Vergeer-Drop J, Dermaut B, Houwing-Duistermaat JJ, Wientjens DP, Hofman A, Van Broeckhoven C, van Duijn CM: Polymorphisms in the prion protein gene and in the doppel gene increase susceptibility for Creutzfeldt-Jakob disease. Eur J Hum Genet 2004;12:389–394.

45 McCormack JE, Baybutt HN, Everington D, Will RG, Ironside JW, Manson JC: *PRNP* contains both intronic and upstream regulatory regions that may influence susceptibility to Creutzfeldt-Jakob disease. Gene 2002;288:139–146.

46 Bratosiewicz-Wasik J, Liberski PP, Golanska E, Jansen GH, Wasik TJ: Regulatory sequences of the *PRNP* gene influence susceptibility to sporadic Creutzfeldt-Jakob disease. Neurosci Lett 2007;411:163–167.

47 Saba R, Schratt GM: MicroRNAs in neuronal development, function and dysfunction. Brain Res 2010;1338:3–13.

48 Mead S: Prion disease genetics. Eur J Hum Genet 2006;14:273–281.

49 Poulter M, Baker HF, Frith CD, Leach M, Lofthouse R, Ridley RM, Shah T, Owen F, Collinge J, Brown J, et al: Inherited prion disease with 144 base pair gene insertion. 1. Genealogical and molecular studies. Brain 1992;115(Pt 3):675–685.

50 Jarius C, Kovacs GG, Belay G, Hainfellner JA, Mitrova E, Budka H: Distinctive cerebellar immunoreactivity for the prion protein in familial (E200K) Creutzfeldt-Jakob disease. Acta Neuropathol 2003;105:449–454.

51 Mitrová E, Belay G: Creutzfeldt-Jakob disease with E200K mutation in Slovakia: characterization and development. Acta Virol 2002;46:31–39.

52 Goldfarb LG, Petersen RB, Tabaton M, Brown P, LeBlanc AC, Montagna P, Cortelli P, Julien J, Vital C, Pendelbury WW, et al: Fatal familial insomnia and familial Creutzfeldt-Jakob disease: disease phenotype determined by a DNA polymorphism. Science 1992;258:806–808.

53 Brown K, Mastrianni JA: The prion diseases. J Geriatr Psychiatry Neurol 2010;23:277–298.

54 Sanchez-Juan P, Bishop MT, Aulchenko YS, Brandel JP, Rivadeneira F, Struchalin M, Lambert JC, Amouyel P, Combarros O, Sainz J, Carracedo A, Uitterlinden AG, Hofman A, Zerr I, Kretzschmar HA, Laplanche JL, Knight RS, Will RG, van Duijn CM: Genome-wide study links *MTMR7* gene to variant Creutzfeldt-Jakob risk. Neurobiol Aging 2012;33:1487.e21–1487.e28.

55 Beck JA, Campbell TA, Adamson G, Poulter M, Uphill JB, Molou E, Collinge J, Mead S: Association of a null allele of *SPRN* with variant Creutzfeldt-Jakob disease. J Med Genet 2008;45:813–817.

56 Lloyd SE, Maytham EG, Pota H, Grizenkova J, Molou E, Uphill J, Hummerich H, Whitfield J, Alpers MP, Mead S, Collinge J: *HECTD2* is associated with susceptibility to mouse and human prion disease. PLoS Genet 2009;5:e1000383.