The Genetics of Susceptibility to Variant Creutzfeldt-Jakob Disease

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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 disease (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 disorders. The infectious agent in prion diseases is the misfolded isoform (PrP\textsuperscript{Sc} or PrP\textsuperscript{Res}) of the host encoded cellular prion protein (PrP\textsuperscript{c}). The abnormal isoform has the potential to aggregate and is highly neurotoxic [1, 2]. While the precise function of PrP\textsuperscript{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\textsuperscript{c} is refolded into β-sheet...
rich PrPSc, initially in the presence of exogenous PrPSc and then by an autocatalytic process. In turn, this leads to the formation of aggregates that are distinguishable from PrPc 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 proportion-

<table>
<thead>
<tr>
<th>Categories of CJD</th>
<th>Examples</th>
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<tr>
<td>Sporadic</td>
<td>Sporadic CJD</td>
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<td></td>
<td>Sporadic fatal insomnia</td>
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<tr>
<td></td>
<td>Protease-sensitive prionapathy</td>
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<tr>
<td>Acquired</td>
<td>Kuru</td>
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<td></td>
<td>Iatrogenic CJD</td>
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<td></td>
<td>Variant CJD</td>
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<tr>
<td>Genetic</td>
<td>Familial CJD</td>
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<tr>
<td></td>
<td>Gerstmann-Straussler-Scheinker syndrome</td>
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<tr>
<td></td>
<td>Fatal familial insomnia</td>
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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 PrPRes 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, PrPc 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
Genomic Alterations Contributing to vCJD Susceptibility amongst Humans

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 asparagine residues 181 and 197, and a 23 aa C-terminal sequence is removed during the attachment of a glycophasphotidylinositol anchor. Mature PrPc is found attached to the outer leaflet of the cell membrane in micro-domains known as lipid rafts. PrPc expression is almost ubiquitous albeit most abundant in neurons of the central nervous system [14, 15]. The N-terminus of PrPc is primarily unstructured, but the central and C-terminal regions form a domain comprised of 3 α-helices and 2 very short antiparallel regions of β-pleated sheet, with a disulphide bridge between helices-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 PrPc, 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

**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 isofom (PrPc). PrPc 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.
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 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\(^\text{Sc}\), 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 homozy-
Genetic Risk Factors within the Regulatory Regions of PRNP

Transgenic animal model studies have convincingly demonstrated that the level of PrP\(^\text{c}\) 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\(^\text{c}\) 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\(^\text{c}\) 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\(^\text{c}\). 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 homoyzogous 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 haplotype 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,
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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

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

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<thead>
<tr>
<th>Gene(s)</th>
<th>Comment</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Myotubularin-related protein 7 (MTMR7)</td>
<td>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.</td>
<td>[54]</td>
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<tr>
<td>Phospholipase C-delta-3 (PLCD3)</td>
<td>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.</td>
<td>[54]</td>
</tr>
<tr>
<td>Cathepsin D (CTSD)</td>
<td>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.</td>
<td>[33]</td>
</tr>
<tr>
<td>Zinc finger and BTB domain containing 38 (ZBTB38)</td>
<td>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.</td>
<td>[36]</td>
</tr>
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<td>[33]</td>
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<tr>
<td>Semaphorin-3A (SEMA3A)</td>
<td>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.</td>
<td>[36]</td>
</tr>
<tr>
<td>Chimerin 2 (CHN2)</td>
<td>Risk can be conferred by an SNP (rs1016726) with moderately strong linkage disequilibrium to codon 129.</td>
<td>[34, 36]</td>
</tr>
<tr>
<td>Retinoic acid receptor beta (RARB) and thyroid hormone receptor beta (THRB)</td>
<td>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 PrPC in cultured neurons and lymphoid tissue. Additionally, the production of PrPRes 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.</td>
<td>[36]</td>
</tr>
<tr>
<td>Shadow of prion protein (SPRN)</td>
<td>Also known as Shadoo shows homology and functional similarities to PrPC. 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.</td>
<td>[55]</td>
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<tr>
<td>HECT domain containing 2 (HECTD2)</td>
<td>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.</td>
<td>[56]</td>
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In determining the rate of susceptibility, the age of onset, the clinical manifestation, and the disease duration of further cases, requires the examination of larger cohorts of patients. Nevertheless, the association of PRNP genotype with disease is significant. Therefore, functional analyses of these genes will provide an important tool for increased understanding of numerous fundamental questions in prion biology, such as the role of PrP C and its physiological function and role in disease. Biochim Biophys Acta 2007; 1772: 629–644.


Infections that have been identified are well beyond what would have been expected by chance alone. Furthermore, the increased number of cases of CJD observed in the UK is not accounted for by a corresponding increase in the incidence of the disease. J Infect Dis 2001; 183: 192–196.


