Genetic Factors Contributing to the Susceptibility of Development of Prion Diseases

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Prion diseases, such as sporadic Creutzfield-Jakob disease (sCJD) in humans, are a family of neurodegenerative conditions that are derived from the misfolding of proteins (Lukic *et al*., 2014). Specifically, a GPI-anchored protein called the prion protein (PrP^{C}) which is thought to be involved in cell adhesion and inhibition of apoptosis, can be converted into a harmful variant known as PrP^{Sc}. In this abnormal isoform, PrP^{Sc} essentially aids in the conversion of other normal prion proteins, leading to an aggregation of misfolded proteins. Although the methods of conversion to PrP^{Sc} are still largely unknown, it has been determined that aggregation can lead to deficits in both neural and motor function by causing apoptosis of neurons (Dalai, Matsuo, Takeyama, Kawano & Saeki, 2016). Researchers have begun to study the genetic factors related to the likelihood of contracting a prion disease, and the prion protein gene (*PRNP*) is at the forefront of the discussion. Allelic variation in *PRNP* codon 129 in humans, and PrP^{C} codon 222 in sheep, have been associated with differences in susceptibility of developing prion diseases (Atkins, Townsend, Medlock & Galvani, 2013; Aguilar-Calvo *et al*., 2013). Scientists are also looking into how the activity of promoters, enhancers and repressors effects *PRNP* gene expression, and consequently prion formation (Dalai, Matsuo, Takeyama, Kawano & Saeki, 2016). Additionally, the extracellular matrix has been a point of interest in prion disease research because several genes associated with resistance to prion formation are related to transport of PrP^{C} into the extracellular matrix (Imerdis and Harris, 2014). While prion diseases can be acquired through mutation, they can also develop from the consumption of infected flesh, such as in Kuru and Bovine Spongiform Encephalopathy (BSE), more commonly known as ‘Mad Cow Disease’. Regardless of the method of contraction, prion diseases can be extremely detrimental to one’s quality of life and may eventually lead to death (Atkins, Townsend, Medlock & Galvani, 2013). Research into the genetic causes of susceptibility to prion

disease contraction has pointed to variation in *PRNP* and PrP^C, as well as genes associated with the extracellular matrix, as the keys to developing future treatments for prion disease.

Specific codons in the *PRNP* gene have been examined in relation to susceptibility and resistance to prion diseases. Prior research has signified codon 129 in *PRNP* as a potential factor in the progression of prion disease development (Atkins, Townsend, Medlock & Galvani, 2013). There are two possible alleles at codon 129, amino acids methionine or valine. Therefore, it is possible to be homozygous for either one of the amino acids, or heterozygous and have both of the amino acids. To study the relevance of *PRNP* codon 129 in one's susceptibility to prion disease, residents of the Fore population of Papua New Guinea who were exposed to the 1950's Kuru epidemic were evaluated. Kuru is a prion disease that spread quickly through the small tribal population due to ritualistic consumption of the flesh of members who had died. Although cannibalism was banned in the population in 1959, deaths due to Kuru are still occurring due to the lengthy incubation period of the disease. Therefore, studying the genomes of the epidemic's survivors has the potential to provide information about genetic factors that contributed to their decreased susceptibility to Kuru contraction. Using a population genetics approach, researchers studied data regarding the frequency of genotypes using various models and mortality data to determine whether there was an advantage to allelic homozygosity or heterozygosity at codon 129. The results of the study concluded that of those exposed to the Kuru epidemic in Papua New Guinea, heterozygotes for codon 129 contracted the disease less frequently than homozygotes at the locus. In addition, heterozygotes for codon 129 had longer incubation rates if they did develop the disease, which in turn increased life longevity (Atkins, Townsend, Medlock & Galvani, 2013). This means that the presence of both methionine and valine at codon 129 in the *PRNP* gene is advantageous to having just one of the two amino acids for both alleles, and is

related to a decreased genetic susceptibility to the prion disease Kuru. These findings exhibit that variation at the codon level can be attributed to the susceptibility of developing Kuru, and should be expanded to other prion diseases to generate a potentially wider impact in future treatments. While it is unlikely that prion susceptibility is limited to variance at just one codon in the *PRNP* gene, this study could be a starting point for determining how resistance to prion diseases can be attained.

Another approach to examining what factors contribute to one's susceptibility to prion disease development lies in the expression of the *PRNP* gene, which regulates the impact that allelic variation can have on the function of the gene. Gene expression depends on multiple factors such as the activity at the gene's promoter or enhancer, which can be transcriptionally repressed due to methylation. A gene's promoter sequence is a stretch of DNA at which transcription begins, and is therefore an essential component of gene expression. Enhancers are located upstream of the promoter sequence, and can be bound by transcription factors to increase transcription rates, and thus intensify expression of the gene. Both enhancers and promoters can be methylated, which entails adding methyl groups to the sequences and subsequently repressing transcription of the gene by inhibiting binding of the DNA (Core, Martins, Danko, Waters, Siepel, & Lis, 2014). Areas called CpG islands are common in promoter regions, and consist of a higher number of cysteine and guanine bases than normally found in DNA. CpG islands have an increased likelihood of being methylated so that they are not expressed. A recent study looked into the methylation status of CGIs, long stretches of CpG islands, in the promoter of *PRNP* to investigate how the gene's regulation effected its' expression (Dalai, Matsuo, Takeyama, Kawano & Saeki, 2016). Using mice, researchers determined that the *PRNP* promoter sequence, located between CpGs 20-40, was completely unmethylated, while the enhancer region, which

was found to be between CpGs 1-7, had variable methylation of CpG islands. Specifically, CpG2, which typically binds to a repressor element, was found to be unmethylated when *PRNP* was not expressed. Repressors are proteins that bind to promoter sequences and restrict transcription by obstructing the binding of RNA polymerase, which inhibits transcription of the gene. Therefore, this means that when CpG2 is methylated and the repressor is unable to bind to the promoter sequence, the prion protein gene is expressed in the cell because transcription by RNA polymerase can occur. This finding has also led researchers to believe that under certain conditions, such as at the fetal stage of mouse development, the repressor typical of this binding region, *Hes1*, may be blocked from binding to the DNA due to the presence of an enhancer. Finally, the study considered the role of the gene's insulator, CTCF, which obstructs the association between the promoter and the enhancer or repressor, as well as effects gene expression through chromatin remodeling. It was determined that the insulator element was located near CpG2, which supports the notion that CpG2 methylation is an important component in *PRNP* gene expression (Dalai, Matsuo, Takeyama, Kawano & Saeki, 2016). This information has contributed to the conclusion that variable methylation of the *PRNP* promoter and enhancer could occur during different developmental periods and in different cell types. This could lead to expression of the prion protein gene in certain parts of the body during different stages of growth, and could affect one's susceptibility to developing prion disease. This new understanding of the expression of *PRNP* is a powerful addition to our comprehension of the genetic components that contribute to prion disease contraction and could aid in its eventual treatment.

While it is important to study human data regarding prion disease development, looking into related diseases in animals could be a useful tool in determining the genetic causes and

developing treatments in humans. Similar to the role of codon 129 in human prion disease, PrP^C codon 222 in sheep is thought to be a determinant of sheep's susceptibility to prion diseases such as scrapie (Aguilar-Calvo *et al*., 2013). One study looked into how amino acid variations in PrP^C affected the susceptibility of transgenic mice to various prion diseases. Researchers studied mice that were homozygous for the wild type amino acid, Q_{222} or glutamine, homozygous for the variant amino acid, K_{222} or lysine, and heterozygous for both amino acids. Mice of each genotype were exposed to scrapie, cattle BSE and goat BSE, all forms of prion disease, and their reactions were recorded. The study concluded that the mice that were homozygous for lysine, the variant amino acid, were completely resistant to infection from scrapie and cattle BSE, and heterozygotes were less susceptible to contraction than mice expressing only glutamine, the wild type amino acid. Although the mode of resistance is still unclear, it appears as if the presence of the variant amino acid at codon 222 inhibited the proliferation of prion protein (Aguilar-Calvo *et al*., 2013). Therefore, by simply having lysine instead of glutamine at both alleles of codon 222, the development of prion disease was entirely averted in the transgenic mice, similarly to how heterozygosity at codon 129 in the human *PRNP* gene greatly decreased contraction of Kuru in exposed individuals in Papua New Guinea (Atkins, Townsend, Medlock & Galvani, 2013). Although these findings are in different animals and in alternate levels of genetic material, they are both applicable to the determination of the factors that affect the susceptibility of developing prion disease. Specifically, the results of this study can be directly incorporated into selective breeding programs for prion resistance in goats, but can also be expanded to encompass other populations. If this research can be altered and applied to human prion disease as well, there is huge potential for the development of genetic resistance to prion disease in both humans and animals.

Although all of the studies discussed thus far have focused on the specific *PRNP* and PrP^{C} codons that play a role in the development and resistance to prion diseases, it is also important to consider alternate genes that could affect one's susceptibility to contraction. Imberdis and Harris (2014) reviewed a study completed by Marbiah *et al.* (2014) in which the cellular roles of genes associated with prion disease were analyzed. Remarkably, the study showed that many of the genes that were related to prion resistance were also associated with the extracellular matrix. The extracellular matrix surrounds cells and consists of an assortment of extracellular components that assist the cell both structurally and developmentally by regulating tissue specific homeostasis (Mouw, Ou, & Weaver, 2014). One of the genes of interest from the study, *Fn1*, was found to have a negative relationship with the amount of PrP^{Sc} present in the extracellular matrix when it was expressed. Researchers concluded that inhibition of *Fn1* and a related gene, *Papss2*, led to an increase in the amount of PrP^{C} discovered in the extracellular matrix. An increased presence of PrP^{C} in the extracellular matrix means that there is more protein available to be converted to the isoform, PrP^{Sc}, which therefore increases the cells' susceptibility to developing prion disease. Therefore, increasing the expression of genes such as *Fn1* and *Papss2* through the utilization of transcriptional equipment, such as the genes' enhancers and promoters, could impede the progression of prion disease by limiting the availability of normal prion protein that can be converted to the harmful variant. Consequently, this study has shown that the extracellular matrix and the genes that are associated with it have the potential to be hubs for drug treatments of prion diseases due to their relationship with the conversion of the prion protein to its isoform (Imerdis and Harris, 2014). Therefore, the future of prion resistance studies should not be limited to only research on the *PRNP* gene or PrP^{C} protein,

but also explore the roles of genes related to the conversion of PrP^{C} to PrP^{Sc} in the extracellular matrix.

Although much research must still be done to determine all of the genetic factors that effect one's susceptibility to prion disease formation, the future of genetically based treatment of prion diseases seems to lie in the allelic variation and regulation at multiple gene loci. Research on human prion disease data has pointed to variation in *PRNP* codon 129 as a factor in prion disease formation, and animal research has denoted PrP^{C} codon 222 as another topic of interest that should be investigated further (Atkins, Townsend, Medlock & Galvani, 2013; Aguilar-Calvo *et al*., 2013). In addition to specific variable components of the *PRNP* coding gene, there seems to be promise in the methylation patterns of non-coding regulatory regions of *PRNP* as a probable contributor to prion disease manifestation (Dalai, Matsuo, Takeyama, Kawano & Saeki, 2016). Conversely, studies have also investigated the expression of other genes, such as *Fn1* and *Papss2*, which are associated with the extracellular matrix and may be related to the propagation of the variant version of the prion protein. This relationship points to the extracellular matrix, and the proteins that effect its behavior, as potential targets for future drug treatment research (Imberdis & Harris, 2014). In recent years, scientists have also begun to question whether or not the site of conversion of PrP^{C} to PrP^{Sc} is relevant to prion disease progression, which could be another promising component of the picture to explore (Cracco *et al*., 2017). While our understanding of many of the genetic components that effect susceptibility to prion disease is advancing, more research must be conducted before development of a treatment for this debilitating family of diseases can begin. Additionally, due to the likeness of more common neurodegenerative ailments such as Parkinson's and Alzheimer's diseases to prion disease, it is

possible that any findings regarding the genetic susceptibility of developing one of the diseases may be applicable to treatment of the others, and therefore benefit a wider range of individuals.

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