



Review

Genetic risk factors for Creutzfeldt-Jakob disease

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A B S T R A C T

Prion diseases are a group of fatal neurodegenerative disorders of mammals that share a central role for prion protein (PrP, gene *PRNP*) in their pathogenesis. Prions are infectious agents that account for the observed transmission of prion diseases between humans and animals in certain circumstances. The prion mechanism invokes a misfolded and multimeric assembly of PrP (a prion) that grows by templating of the normal protein and propagates by fission. Aside from the medical and public health notoriety of acquired prion diseases, the conditions have attracted interest as it has been realized that common neurodegenerative disorders share so-called prion-like mechanisms. In this article we will expand on recent evidence for new genetic loci that alter the risk of human prion disease. The most common human prion disease, sporadic Creutzfeldt-Jakob disease (sCJD), is characterized by the seemingly spontaneous appearance of prions in the brain. Genetic variation within *PRNP* is associated with all types of prion diseases, in particular, heterozygous genotypes at codons 129 and 219 have long been known to be strong protective factors against sCJD. A large number of rare mutations have been described in *PRNP* that cause autosomal dominant inherited prion diseases. Two loci recently identified by genome-wide association study increase sCJD risk, including variants in or near to *STX6* and *GAL3ST1*. *STX6* encodes syntaxin-6, a component of SNARE complexes with cellular roles that include the fusion of intracellular vesicles with target membranes. *GAL3ST1* encodes cerebroside sulfotransferase, the only enzyme that sulfates sphingolipids to make sulfatides, a major lipid component of myelin. We discuss how these roles may modify the pathogenesis of prion diseases and their relevance for other neurodegenerative disorders.

1. Introduction and overview

Prion diseases are invariably fatal, generally rapidly progressive neurodegenerative diseases characterized neuropathologically by deposition of abnormal prion protein, spongiform vacuolation, neuronal loss and astrocyte proliferation (Budka et al., 1995). In humans, the disease has three aetiological types: sporadic, inherited and acquired. In animals, prion diseases include scrapie in sheep and goats, chronic wasting disease (CWD) in cervids and bovine spongiform encephalopathy (BSE) in cattle (recently reviewed (Houston and Androletti, 2019)). Molecular pathogenesis of all types involves misfolding and aggregation of the cellular prion protein (PrP^C) into a number of disease-associated forms including proteinase K (PK)-resistant scrapie forms termed PrP^{Sc}.

PrP^C is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein ubiquitously expressed in mammals with highest expression in the central nervous system, but present in most other tissue and cell types (cellular prion protein biology recently reviewed (Castle and Gill, 2017)). PrP^C has an apparently unstructured N-terminal domain in solution, comprising a signal peptide followed by a nonapeptide and three or four octapeptides, copper-binding repeats, a central hydrophobic domain and a predominantly α -helical C-terminal domain that includes two variably occupied glycosylation sites, a disulphide bridge and a site for GPI-anchor attachment. Following synthesis, PrP^C is first

translocated into the endoplasmic reticulum where glycosylation and addition of a GPI anchor occurs, as well as cleavage of N- and C-terminal peptides. After correctly folding, the protein is then trafficked through the Golgi cisternae for further post-translational modifications of the glycosylation sites and GPI anchor, before being trafficked to the plasma membrane where most steady-state PrP^C is located. PrP^C is concentrated in cholesterol rich lipid rafts and is thought to constitutively recycle through the endosomal system.

Sporadic Creutzfeldt-Jakob disease (sCJD) is the most common human prion disease with 1–2 cases per million population per year, accounting for approximately 85% of annual incidence in most countries (Ladogana et al., 2005; NCJDRSU, 2018). Diagnostic criteria stipulate that there is no known genetic aetiology or environmental exposure to prions, instead, the cause is hypothesized to be a stochastic misfolding event of PrP^C or somatic *PRNP* mutation in a single or clade of cells. Due to the transmissibility of prion diseases, several acquired forms of the disease have been described. The first and to date largest recognized outbreak, involving over 3000 cases, was kuru, which affected the Fore ethnic group and their neighbours in the Eastern Highlands Province of Papua New Guinea. Kuru was acquired through ritual endocannibalism (Collinge et al., 2006). More recently, variant CJD (vCJD) was caused by dietary transmission of BSE prions, resulting in a zoonotic outbreak of over 230 confirmed cases (Creutzfeldt-Jakob Disease International Surveillance Network, 2019; Will et al., 1996).

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Over 450 cases of iatrogenic CJD (iCJD) have also been reported, mostly related to historical medical treatments using human cadaveric pituitary growth hormone (hGH), or use of cadaveric dura mater in surgery (Brown et al., 2012).

The human *PRNP* gene that encodes PrP^C is located on chromosome 20 and is formed of 2 exons, with the second containing the open reading frame for the 253 amino acid protein. Up to 70 variants have been identified in the *PRNP* gene comprising a variety of single nucleotide polymorphisms (SNPs), missense mutations, alterations in octapeptide repeat number, premature truncations or rarer frame-shift mutations, a number of which lead to inherited prion diseases (IPDs) (see recent review (Mead et al., 2019)) (Minikel et al., 2019). Globally, IPDs account for approximately 10–15% of annual human prion disease incidence, although this varies considerably by region. They are generally classified into groups based on clinical presentation and genetic aetiology: familial CJD, fatal familial insomnia, Gerstmann-Sträussler-Scheinker disease and PrP systemic amyloidosis. Several variants have been identified in sCJD patients without a family history. In this case, causality is much less clear and clinicians should be cautious in how results are fed-back to patients (Beck et al., 2010; Minikel et al., 2016; Mok et al., 2018).

PRNP codon 129 is a major genetic modifying factor for all aetiological types of human prion diseases; the genotype at this site influences age of clinical onset and clinical duration in a subset of IPDs and kuru and alters risk and clinical duration for sCJD and iCJD (Collinge et al., 2006; Minikel et al., 2019; Collinge et al., 1991; Mead et al., 2006; Palmer et al., 1991; Webb et al., 2008; Webb et al., 2009).

The modifying effects of codon 129 are complex in that the primary sequence of PrP determines the permissible prion strains that can propagate in a host (Collinge, 1999). Prion strains refers to distinct clinicopathological phenotypes of prion disease that can be maintained through transmission of the disease between different hosts or species. Strains are thought to be encoded in the conformation of abnormal PrP, the presence of cofactors, or stoichiometry of different glycosylation states or fragments of PrP. For example, whilst both homozygous genotypes at codon 129 confer increased risk of sCJD, in vCJD, until recently, all cases were methionine homozygous, implying that the valine homozygous genotype is strongly protective. There is good evidence from transmission studies in transgenic animals that PrP-129V, unlike for sCJD prions, cannot adopt the vCJD prion conformation (Wadsworth et al., 2004). In 2011 and 2016, two patients were diagnosed with vCJD who had a heterozygous genotype at codon 129 (Kaski et al., 2009; Mok et al., 2017), but it remains unclear whether a more substantial “second wave” of vCJD with PrP-129MV will arise. Furthermore the frequencies of codon 129 genotypes in hGH-iCJD cases and their likely incubation times vary significantly between countries, suggesting the propensity for propagation of different contaminating prion strains within growth hormone preparations is influenced by host genotype (Brandel et al., 2003; Rudge et al., 2015). A small number of additional variants have been associated with non-inherited prion diseases, for example, a valine allele at position 127 is extremely rare globally, but in kuru is completely protective (Mead et al., 2009a; Asante et al., 2015) and enriched in women exposed to kuru but surviving to old age. A variant at codon 219 found in east and south Asian populations is strongly, although not completely, protective against sCJD but has been seen in patients with acquired and genetic prion diseases including two patients with vCJD (Shibuya et al., 1998; Lukic et al., 2010; Nozaki et al., 2010). Alleles 180I and 232R may increase sCJD risk in certain populations, but this is not certain (Minikel et al., 2016; Beck et al., 2012; Qina et al., 2014).

In this article we review genetic modifiers for sCJD risk outside of the *PRNP* gene identified through large-scale human genetics studies and discuss putative roles these may play in disease biology, with particular focus on two recently identified risk loci that include the *STX6* and *GAL3ST1* genes.

2. Importance of identifying disease modifiers for sCJD

Multiple studies have demonstrated the multi-faceted role of PrP^C in prion diseases, not only for templated conversion to PrP^{Sc} but in its relationship with neurotoxicity and disease incubation time, emphasising that PrP^C is the key target for prion disease therapeutics (Brandner et al., 1996; Beler et al., 1994; Mallucci et al., 2003). Targeting PrP^C at both the mRNA and protein level after prion inoculation in mouse models is able to reduce PrP^{Sc} load and prevent, or even reverse, neuropathological changes (White et al., 2003; White et al., 2008; Raymond et al., 2019); PrP-targeting antibodies and antisense oligonucleotides are either in human use or being developed (UCLH, 2018). However, it is not yet known whether these programmes will translate into safe and effective treatments that can be used for patients and healthy but at-risk individuals. The biological functions of PrP^C are not yet clear and, although PrP-null mouse models appear to be healthy with no lethal pathogenic phenotype (most convincing evidence indicates a mild late-onset demyelinating polyneuropathy (Bremer et al., 2010)), adverse consequences of binding or depleting this protein remain possible. Therefore, it could be useful to investigate genetic modifiers of the disease and evaluate new targets.

Within the last decade the concept of a ‘prion-like’ mechanism in related neurodegenerative diseases has been increasingly acknowledged (recently reviewed (Jaunmuktane and Brandner, 2019)). A number of disease-related aggregation-prone proteins have been demonstrated to encompass various properties of bona fide prions including seeded conversion (amyloid β , tau, α -synuclein, TDP43) and propagation of distinct strains (i.e. Alzheimer's disease (AD), progressive supranuclear palsy (PSP), multiple system atrophy). If the underlying mechanism for these diseases is similar, then shared genetic aetiology could be expected, which for at least one locus appears to be the case (discussed later). An increased understanding of sCJD genetics may allow identification of common pathways which contribute to ‘prion-like’ protein propagation and thus targets relevant to a mechanism that spans multiple neurodegenerative diseases.

3. Human genetic studies of sCJD

Advances in genotyping and sequencing technologies have accelerated human genetics research allowing for large-scale studies. Over the last decade genome-wide association studies (GWAS) have proved a powerful tool for detecting genetic associations with both complex traits and diseases; however, very large sample sizes are required to achieve sufficient power to overcome the multiplicity of hypothesis testing, and allow for reliable methods to account for biases related to imperfect matching of ancestries between cases and controls. This has posed a problem for sCJD research due to the rarity of the disease limiting sample availability, and consequently a lack of statistically robust non-*PRNP* associations. Targeted replication of pre-specified genes and variants identified through GWAS led to proposals of *GRM8* as associated with sCJD risk, and variants upstream of *STMN2* and within *CYP4X1* with sCJD age of onset, although none of these associations have been clearly established (Sanchez-Juan et al., 2014; Mead et al., 2009b; Poleggi et al., 2018). Similarly a number of candidate gene studies have putatively identified genetic risk factors and disease modifiers however, whilst this work was done out of necessity, these approaches often lead to false-positive results and thus to date no robust genome-wide significant associations outside of *PRNP* have been identified.

Through international collaboration of most major human prion disease specialists working in European ancestry populations we recently performed for the first time a well-powered study to identify risk variants for sCJD (Jones et al., 2020). This two-stage study utilised 5208 cases clinically diagnosed with probable or definite sCJD from 12 European ancestries populations. The work reproducibly identified SNPs in *PRNP* (rs1799990), *STX6* (rs3747957) and *GAL3ST1*

(rs2267161) as conferring risk for the disease at genome-wide significance. Association in *GAL3ST1* comprised two SNPs including a valine to methionine missense variant at codon 29 (V29M). At the *STX6* locus a larger number of SNPs were associated which are also expression quantitative trait loci (eQTLs) for increased *STX6* expression in the putamen and caudate nuclei with the risk haplotype, brain regions particularly implicated in sCJD pathogenesis (Zerr et al., 2009); a screen for co-localisation of eQTLs associated with the GWAS signal supported this as the most likely mechanism driving the risk at this locus. Association at a genetic locus cannot definitively prove the role of a specific gene, as genetic effects can act at a distance, even on other chromosomes, however these two genes are the lead candidates at each locus based on follow up investigations.

4. Syntaxin-6 (STX6)

STX6 on human chromosome 1 encodes for syntaxin-6, a member of the SNARE (soluble NSF (N-ethylmaleimide-sensitive factor) attachment protein receptor) protein family comprised of over 38 proteins, and is characterized by the presence of a 60 to 70 residue-long conserved central coiled-coil SNARE motif (Jahn and Scheller, 2006). SNARE protein complexes are the key components for membrane fusion during the final step of vesicle transport; proteins located on vesicle membranes (v-SNARE) and their relevant target (t-SNARE) interact to form a four-helical coiled-coil which brings membranes in close proximity and drives membrane fusion. Syntaxin-6 is a t-SNARE located primarily at the trans-Golgi network (TGN) and early endosomes to mediate retrograde transport between the two compartments (Bock et al., 1997). Further classification denotes syntaxin-6 as a Qc-SNARE due to the presence of a conserved glutamine residue within the SNARE domain and its homology to the well characterized SNAP-25 protein. Syntaxin-6 has 255 amino acids and incorporates a C-terminal hydrophobic anchor and 2 coiled-coil domains, the more C-terminal of which contains the SNARE motif (Misura et al., 2002). Syntaxin-6 primarily forms a canonical SNARE complex with syntaxin-16, Vti1a and VAMP4 during retrograde transport (Kreykenbohm et al., 2002).

Intracellular protein trafficking has been intensively studied in the context of prion diseases, with regards to both the cellular and scrapie forms, particularly focusing on identification of potential sites of misfolding and conversion of PrP^C into PrP^{Sc} and subsequent degradation mechanisms (Fig. 1). Both PrP^C and PrP^{Sc} are constitutively cycled between the plasma membrane and endocytic compartments (Goold et al., 2013a; Shyng et al., 1993). From the plasma membrane PrP^C is endocytosed via one of multiple possible routes to the early endosome, where it is either recycled back to the plasma membrane through the endocytic recycling compartment or via retrograde transport to the TGN, or trafficked to the late endosomes and lysosomes for degradation (Ballmer et al., 2017; Campana et al., 2005; Kang et al., 2009; Magalhes et al., 2002; Peters et al., 2003; Shyng et al., 1994). PrP^{Sc} follows the same pathway after endocytosis (the mechanism of which is also contended) for either recycling or lysosomal degradation, with the additional possibility of diversion between pathways with transport from the TGN directly to lysosomes (Goold et al., 2013a; Jen et al., 2010; Vey et al., 1996). Additional degradation mechanisms have also been identified via macroautophagy and the proteasome (Goold et al., 2013a; Heiseke et al., 2010). Studying trafficking of PrP^C and PrP^{Sc} is key to understanding disease biology due to the appreciation that direct interaction between the cellular form and misfolded protein (including cofactors) underpins prion conversion and disease progression (Horiuchi and Caughey, 1999; Telling et al., 1995).

Syntaxin-6 plays a number of different roles in various cell types which have the potential to influence prion disease biology, leading to numerous potential hypotheses as to why this gene may be related to sCJD risk (reviewed in Jung et al., 2012) and see Fig. 1). The endocytic pathway has long been hypothesized as a site of prion conversion due to the localisation of PrP^{Sc} within various intracellular compartments and

the effects of acidic pH on prion conformation, stability and intracellular localisation, leading to identification of recycling endosomes and the multivesicular body as potential sites of particular interest (Arnold et al., 1995; Borchelt et al., 1992; Hornemann and Glockshuber, 1998; Marijanovic et al., 2009; Qi et al., 2012; Yim et al., 2015). As a mediator of early endosome to TGN retrograde transport it is feasible that modified syntaxin-6 expression could alter the time both PrP^C and PrP^{Sc} are retained within the acidic endocytic compartments, potentially promoting initial prion formation or seeded conversion. A direct interaction of syntaxin-6 with either PrP^C or prion aggregates is also possible, especially with presence of all three within a number of different intracellular membranes including early, late and recycling endosomes, TGN as well as the plasma membrane depending on cell type, which could alter protein conformation or misfolding kinetics (Fig. 1) (Martin-Martin et al., 2000; Schindler et al., 2015; Wade et al., 2001; Willett et al., 2013).

Prion conversion has been shown to occur on the plasma membrane within lipid rafts (Goold et al., 2011; Goold et al., 2013b); these membrane microdomains are thought to be highly important for prion conversion as demonstrated in both cell models and protein-based aggregation assays (Goold et al., 2011; Abid et al., 2010). Inhibition of syntaxin-6 function in a fibroblast cell line inhibits transport of lipid-raft associated proteins to the cell surface. If replicated in neuronal cells this has the potential to modify the microenvironment for prion conversion (Choudhury et al., 2006). A proteomic screen in HeLa cells also identified syntaxin-6 as a cholesterol binding protein, previously shown to modulate syntaxin-6 function (Hulce et al., 2013; Reverter et al., 2014). As a key component of plasma membranes and a precursor for a number of signalling molecules, cholesterol is essential in neuronal physiology with a role in processes such as synapse formation, synaptic vesicle exocytosis and neurotransmission (Zhang and Liu, 2015). Cellular cholesterol levels also alter PrP^C folding, internalisation and degradation as well as PrP^{Sc} synthesis, therefore this lipid may act as an intermediate relating the two protein functions, through several different potential mechanisms (Marella et al., 2002; Sarnataro et al., 2004; Taraboulos et al., 1995).

The transport of GPI-anchored proteins to lipid rafts in the plasma membrane is also regulated by syntaxin-6 (Choudhury et al., 2006). PrP^C harbours a GPI anchor so it is feasible that syntaxin-6 plays a role in transport of this protein to these locations; the level of PrP^C within the plasma membrane is important for prion conversion (Enari et al., 2001). After plasma membrane conversion PrP^{Sc} is rapidly endocytosed and directed for lysosomal degradation along with PrP^C. Through regulating the trafficking of proteins to lipid rafts, syntaxin-6 subsequently modulates caveolae-mediated endocytosis through which internalisation of both PrP^C and PrP^{Sc} may occur (Choudhury et al., 2006). Altering PrP^C or PrP^{Sc} internalisation could further alter their plasma membrane concentrations as well as uptake of seed-competent protein into cells.

Syntaxin-6 function has been implicated in the lysosomal degradation pathway through localisation of distinct SNARE complexes in late endosomes in a melanoma cell line and interaction with ubiquitin ligases MARCH-II and MARCH-III in tagging proteins for degradation (Wade et al., 2001; Fukuda et al., 2006; Nakamura, 2005); ubiquitination by MARCH-II has been established as a regulatory mechanism for lysosomal targeting of cystic fibrosis transmembrane conductance regulator (CFTR) (Cheng and Guggino, 2013). If the same mechanism occurs through syntaxin-6 interaction with either PrP^C or PrP^{Sc} it is possible this provides an additional regulatory step in protein degradation, and modified syntaxin-6 expression could feasibly interfere with this process. Additionally, alternative targeting of late endosomes to the plasma membrane for exosome release is a likely method of cell-to-cell spreading of prions, for which a role of syntaxin-6 has also been proposed (Peak et al., 2020; Fevrier et al., 2004).

The possibility of a common underlying mechanism in related neurodegenerative diseases is supported by association of the same

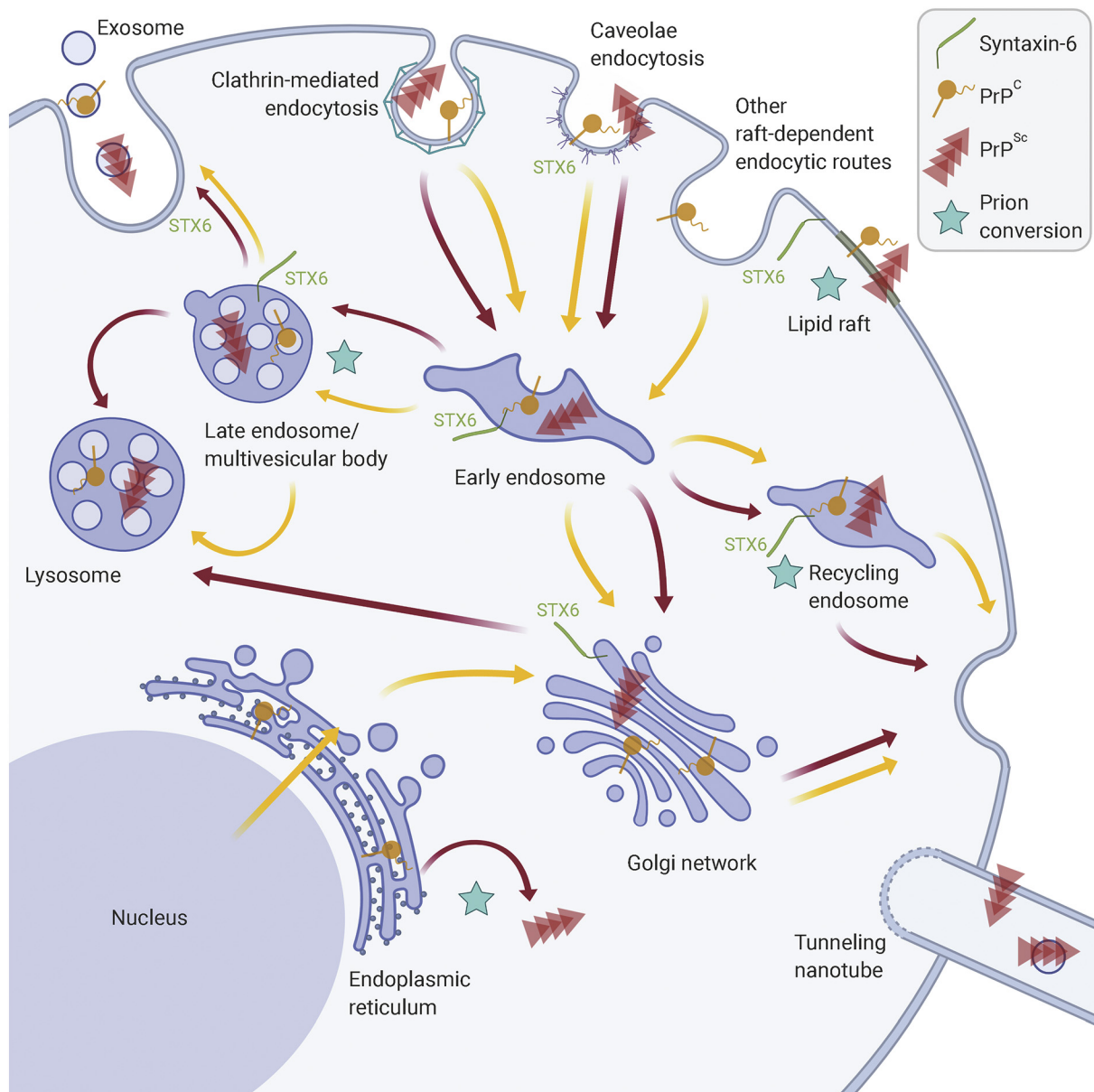


Fig. 1. Trafficking of PrP^{Sc} and PrP^C through endocytic and secretory pathways and relationship with SNARE protein syntaxin-6. Both PrP^C and PK-resistant aggregates (PrP^{Sc}) are constitutively trafficked between the plasma membrane (PM) and endocytic compartments in cell models. PrP^C (yellow arrows) is synthesised in the ER and trafficked through the Golgi to lipid rafts on the PM. Endocytosis of PrP^C occurs via clathrin-, caveolae- or other raft-dependent routes to early endosomes (EE) where it is either recycled back to the PM via retrograde transport through the trans-Golgi network (TGN) or via recycling endosomes (RE). PrP^C is alternatively trafficked to late endosomes (LE) and lysosomes for degradation. PrP^{Sc} is primarily formed on the PM (indicated by the star symbol) in association with lipid rafts before internalisation (red arrows) via caveolae- or clathrin-mediated endocytosis to EE. It can then be recycled to the PM via the TGN or RE (where additional conversion may occur), or from TGN or EE to LE/multivesicular body (MVB) (another potential conversion site) and lysosomes. Retrotranslocation from the ER to the cytosol during synthesis may also promote aggregation. Cell-to-cell PrP^{Sc} transmission has been demonstrated through exosomes and tunneling nanotubes. Syntaxin-6 (STX6) is primarily localised to the TGN and EE but has also been detected on PM, RE and LE. It is further implicated indirectly in exosomal transport and caveolae-mediated endocytosis. Figure created with [Biorender.com](https://www.biorender.com). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sCJD risk variants and direction of effect in the tauopathy progressive supranuclear palsy (PSP) in a GWAS of 2165 patients, suggesting the 'prion-like' aetiology of other diseases may extend to genetic risk (Höglinger et al., 2011; Ferrari et al., 2014). Multiple neurodegeneration-associated proteins, including tau, have been shown to induce templated conversion of cellular protein in a 'prion-like' manner, therefore the previously discussed mechanisms by which syntaxin-6 could contribute to prion conversion and propagation may well increase the propensity of misfolding or templated conversion of additional aggregation-prone proteins (Jaunmuktane and Brandner, 2019).

Furthermore neurite outgrowth is impacted in models for multiple neurodegenerative diseases including prion disease, AD and Parkinson's disease, a process shown to be somewhat mediated by syntaxin-6 in response to nerve growth factor signalling (Fahnestock et al., 2001; Hu et al., 2019; Kabayama et al., 2008; Mogi et al., 1999). Syntaxin-6 also regulates trafficking of insulin-responsive proteins GLUT4 and IRAP (Kumudu et al., 2003; Watson and Pessin, 2008); insulin-resistance in the brain is related to cognitive impairment and neurodegeneration and has been associated with models of prion disease and multiple other neurodegenerative diseases (Chiu et al., 2008; de Brito et al., 2017;

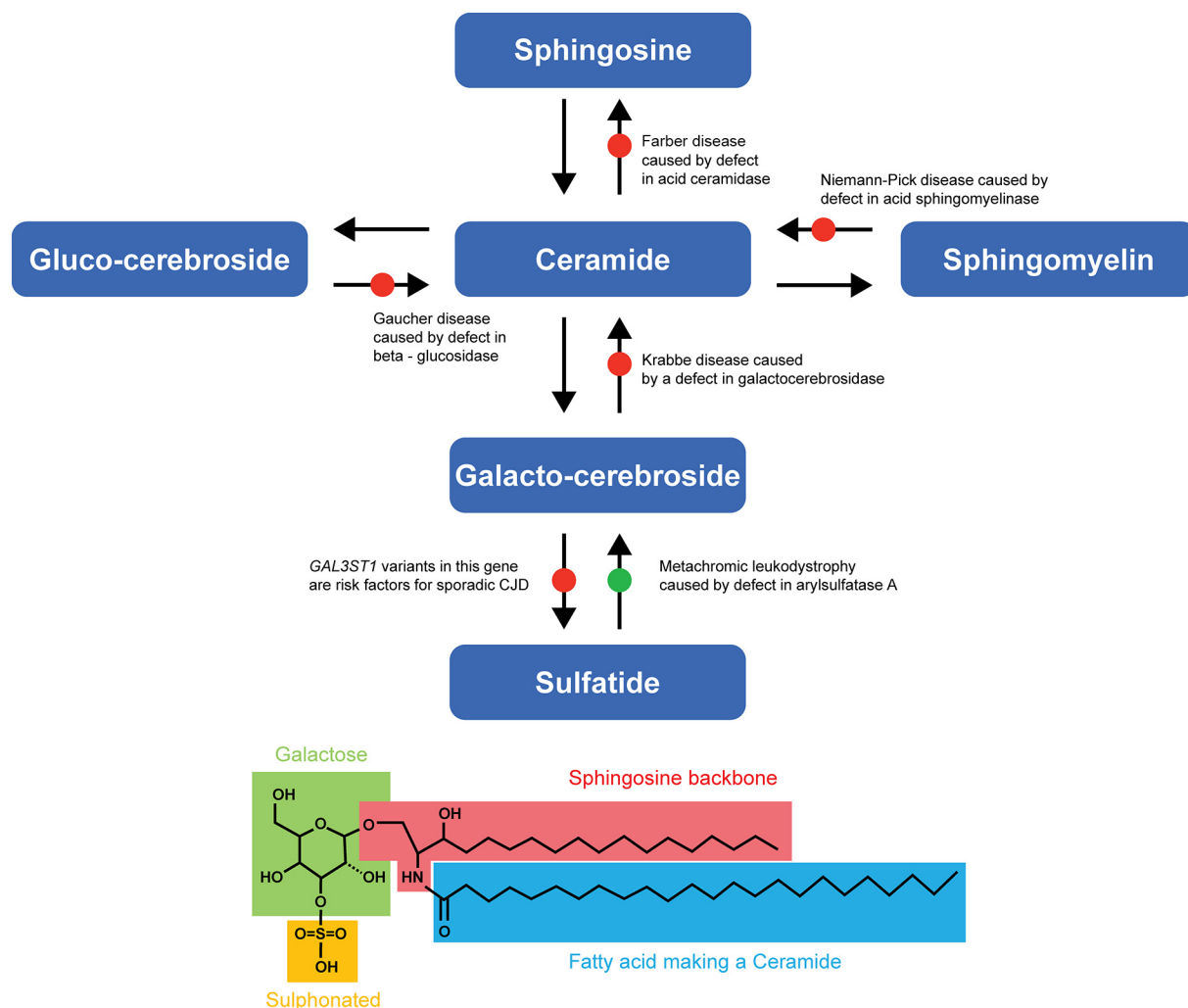


Fig. 2. Premetabolites of sulfatide and defects in enzymes that lead to lysosomal storage disorders.

Rivera et al., 2005). The possible roles for syntaxin-6 function in prion biology are numerous and direct investigation of these discussed hypotheses will be required to determine any potential relationship.

5. Cerebroside sulfotransferase (*GAL3ST1*)

Sphingolipids are a major class of membrane lipids. The class structure is typically based on an 18-carbon amine alcohol, often conjugated with a fatty acid and a sugar residue to make a cerebroside (Fig. 2). Cerebrosides in vertebrates may be sulphated by the cerebroside sulfotransferase enzyme (encoded by the *GAL3ST1* gene) to make sulfatide, a dominant component of the myelin sheath in the nervous system. Abnormal metabolism of sulfatide is directly implicated in neurological disease as mutations in the enzyme that degrades sulfatide are associated with metachromic leukodystrophy (MLD), a rare lysosomal storage disorder (Platt et al., 2018) (Fig. 2). MLD is caused by recessive defects in either the arylsulfatase A and prosaposin proteins leading to the accumulation of sulfatides in the lysosome, and consequently ataxia, weakness, loss of speech, behavioural problems and psychomotor regression and cognitive decline.

There is little specific literature on sulfatides and prion diseases (zero results from search terms “sulfatide” AND “prion” using PubMed). However, sulfatide metabolism has been implicated in early Alzheimer's disease. Severe sulfatide deficiency has been repeatedly documented in Alzheimer's disease (brain or CSF) (Gonzalez de San Roman et al., 2017; Han, 2007), which may relate to the transport of

lipids by the protein product of *APOE*, variants of which are the major genetic risk factor for typical late onset Alzheimer's disease. The risk factor identified at *GAL3ST1* is not however shared with other neurodegenerative disorders (no hits in the GWAS catalogue). In prion diseases, speculation about a possible role for sulfatides must therefore be indirect.

PrP^C is known to reside on cholesterol- and sphingolipid-rich lipid rafts (or detergent-resistant membranes), their composition being affected by age, which could in turn lead to changes in the proximity of PrP^C molecules and risks for conformational reactions that require interactions between proteins (Agostini et al., 2013). Enzymes of sphingolipid metabolism (though not including *GAL3ST1* specifically) are altered early in the course of mouse prion disease (Hwang et al., 2009). Purified prion rods have been found to contain low concentrations of galactosylceramide and sphingomyelin implicating co-localisation of prions with these lipids (Klein et al., 1998). In summary, there is a rationale and some evidence to implicate sphingolipid metabolism in prion disease pathogenesis, possibly through modification of the composition of lipid rafts.

Whilst for *STX6* there is evidence that the most common transcripts are increased in expression in disease-critical brain regions, for *GAL3ST1*, and its homodimeric enzyme product, initial follow up questions will focus on the effects of the V29M polymorphism on enzyme activity and localisation. A role for sulfatides or related sphingolipid pre-metabolites in lipid rafts and concentration of PrP^C is possible, but several alternatives might be put forward. For example,

sulfatides or pre-metabolites might have a direct interaction with or alter trafficking of PrP^C or PrP^{Sc}. It would be interesting to learn of whether sulfatides or pre-metabolites are altered in prion diseases in a similar way to early Alzheimer's disease.

6. Conclusions and future perspectives

Identification of additional genetic modifiers for sCJD has potential to increase our understanding of prion disease biology. Whilst a paucity of partners and pathways have been identified in the literature, genetic association implicates a causal effect in humans and therefore a priority for functional work. Although large-scale genetic studies are powerful for discovering disease-associated variants, the translation of these into causal genes and underlying mechanisms is not straightforward. Statistical fine-mapping and functional annotations are useful for minimizing potential candidates, however experimental work in relevant disease models will now be pivotal.

Future studies might focus on establishing more precisely which mechanisms at these risk loci are driving the genetic association. Although it is likely polymorphisms in *STX6* increase the expression of this protein in disease-associated brain regions, without understanding the consequences of this for prion disease pathogenesis it cannot be easily translated into therapeutic possibilities. The unusual association with a coding change at the *GAL3ST1* locus is advantageous for elucidating the causal gene, however without an understanding of the ramifications for enzyme function the utility of this is also yet to be determined.

Development of large sample resources and genome-wide genetic data which are sufficiently powered to detect variants with small effect sizes provides additional opportunity for further analysis beyond disease risk. Integration of genetic data with clinical parameters will allow us to investigate genetic aetiology for other aspects of the disease such as age of onset and rate of clinical decline; this has the potential for even greater utility, as disease modifiers acting after onset of symptoms may pose more realistic therapeutic targets. Furthermore the shared genetic risk variants with PSP, for which similarities with sCJD can extend beyond mechanistic to shared symptoms and diagnostic classification, provides support to a common 'prion-like' mechanism underlying related neurodegenerative diseases, highlighting the possibility for shared targets (Josephs et al., 2004).

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