Identification of novel risk loci and causal insights for sporadic Creutzfeldt-Jakob disease: a genome-wide association study

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Summary

Background Human prion diseases are rare and usually rapidly fatal neurodegenerative disorders, the most common being sporadic Creutzfeldt-Jakob disease (sCJD). Variants in the *PRNP* gene that encodes prion protein are strong risk factors for sCJD but, although the condition has similar heritability to other neurodegenerative disorders, no other genetic risk loci have been confirmed. We aimed to discover new genetic risk factors for sCJD, and their causal mechanisms.

Methods We did a genome-wide association study of sCJD in European ancestry populations (patients diagnosed with probable or definite sCJD identified at national CJD referral centres) with a two-stage study design using genotyping arrays and exome sequencing. Conditional, transcriptional, and histological analyses of implicated genes and proteins in brain tissues, and tests of the effects of risk variants on clinical phenotypes, were done using deep longitudinal clinical cohort data. Control data from healthy individuals were obtained from publicly available datasets matched for country.

Findings Samples from 5208 cases were obtained between 1990 and 2014. We found 41 genome-wide significant single nucleotide polymorphisms (SNPs) and independently replicated findings at three loci associated with sCJD risk; within *PRNP* (rs1799990; additive model odds ratio [OR] 1·23 [95% CI 1·17–1·30], $p=2·68\times10^{-15}$; heterozygous model $p=1·01\times10^{-135}$), *STX6* (rs3747957; OR 1·16 [1·10–1·22], $p=9·74\times10^{-9}$), and *GAL3ST1* (rs2267161; OR 1·18 [1·12–1·25], $p=8·60\times10^{-10}$). Follow-up analyses showed that associations at *PRNP* and *GAL3ST1* are likely to be caused by common variants that alter the protein sequence, whereas risk variants in *STX6* are associated with increased expression of the major transcripts in disease-relevant brain regions.

Interpretation We present, to our knowledge, the first evidence of statistically robust genetic associations in sporadic human prion disease that implicate intracellular trafficking and sphingolipid metabolism as molecular causal mechanisms. Risk SNPs in *STX6* are shared with progressive supranuclear palsy, a neurodegenerative disease associated with misfolding of protein tau, indicating that sCJD might share the same causal mechanisms as prion-like disorders.

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Introduction

Prion diseases are fatal neurodegenerative conditions in humans and animals caused by the propagation of prions: atypical infectious agents comprised solely or predominantly of host prion protein.[1] Prions are thought to propagate through a process of binding to normal prion protein, induction of conformational change by templating, and fission of the polymeric assembly. Prion diseases can be acquired from exposure to prions in the diet, or through medical or surgical procedures, which can result in public health crises. The cattle prion disease, bovine spongiform encephalopathy (BSE), which transmitted to mostly young British and other European adults as variant Creutzfeldt-Jakob disease (vCJD),[2] led to enhanced clinical surveillance for all prion diseases worldwide. Inherited prion disease, caused only by mutations of the prion protein gene (*PRNP*), causes approximately 10–15% of the annual incidence of all prion diseases in most countries.[3] The most common type of human prion disease is sporadic CJD (sCJD), a rapidly progressive dementia with a lifetime risk of approximately one in 5000, which occurs predominantly in older adults.[4,5] Other than age and

Research in context

Evidence before this study

The rarity of sporadic Creutzfeldt-Jakob disease (sCJD) has been limiting in previous genome-wide association studies (GWAS) for disease risk. We searched PubMed on April 9, 2020, with the terms ("prion" OR "creutzfeldt*") AND ("genome wide association" OR "GWAS"), without language or date restrictions, and identified four relevant publications, including two directly investigating sCJD risk through genome-wide analyses. However, the sample sizes in these studies were not sufficient to identify statistically significant associations outside of the known risk at the prion protein gene (*PRNP*). Further studies into genetic risk factors for sCJD have primarily utilised targeted replication of putative risk variants or candidate gene studies to propose association.

Added value of this study

Through international collaboration of sample resources, this study is, to our knowledge, the first GWAS to identify genetic variants associated with sCJD risk outside of *PRNP*, at genome-wide significance. Two of these variants (within *STX6* and *GAL3ST1*) were statistically robust to replication in an independent cohort, with 5208 patients with sCJD in total included in the two-stage study design. Through statistical fine-mapping and analysis of exome sequencing and gene expression data, we propose genes that are likely to be causal, and mechanisms for both novel associations. We used patient brain samples and cell-based assays to further investigate the biological implications of these associations in relevant systems. Two further loci at *PDIA4* and *BMERB1* were also associated with sCJD risk in gene-based tests.

Implications of all the available evidence

Identification of two novel non-*PRNP* loci conferring sCJD risk will provide further avenues for research, with increased evidence to support a role of modified intracellular trafficking and sphingolipid metabolism within sCJD biology, providing the potential to inform new therapeutic approaches. With the shared genetic risk of variants within *STX6* and those previously identified for the tauopathy progressive supranuclear palsy, this study also supports the notion of a common so-called prion-like causal mechanism for related neurodegenerative disorders and thus potential for shared treatments.

polymorphisms at *PRNP*, no risk factors for sCJD are known, leaving only speculative explanations for sporadic prion formation.

Polymorphisms of *PRNP* at codons 127, 129, and 219 alter amino acids and are strong genetic risk factors or modifiers of the disease phenotypes.[3] Sibling or familial concurrence of sCJD has been reported, but not to the extent that chance concurrence can be eliminated as an explanation. There are no estimates of the heritability of sCJD based on family studies.[6] Animal studies have identified acquired prion disease risk factors in *Prnp* and close by, and provided evidence for susceptible loci on other chromosomes, yet elucidating the causal genes has proven to be challenging.[3] Many other neurodegenerative diseases are thought to share fundamental mechanisms with prion diseases, including template-based protein misfolding and spreading of pathology associated with abnormally aggregated proteins in diseased brain tissue. If shared mechanisms exist, this might implicate shared genetic risk factors for these diseases.

This study follows on from previous genome-wide association studies (GWAS) in human prion diseases, which have not been powerful enough to discover non-*PRNP* risk factors.[7–10] We aimed to identify specific causal genes at risk loci, to allow molecular causal mechanisms for sCJD to be proposed.

Methods

Study design and participants

We did a GWAS using samples from patients diagnosed with probable or definite sCJD according to widely accepted criteria, which were provided by specialist or national surveillance centres in countries with populations of predominantly European ancestries (appendix pp 2, 28–32). Diagnostic criteria for probable sCJD varied over the course of sample collection for the study. Using modern diagnostic methods, including real-time quaking-induced conversion assay with CSF, a probable diagnosis refuted by post-mortem examination is extremely rare; but even more than 20 years ago, probable sCJD was a highly accurate term. Patient samples were distributed across a two-stage study design: samples were genotyped using Illumina Omniexpress arrays in the discovery stage, and additional samples were genotyped at the lead variant in each hit locus using minor groove binding probes in the replication stage. Control data from healthy individuals were obtained from publicly available datasets matched for country.

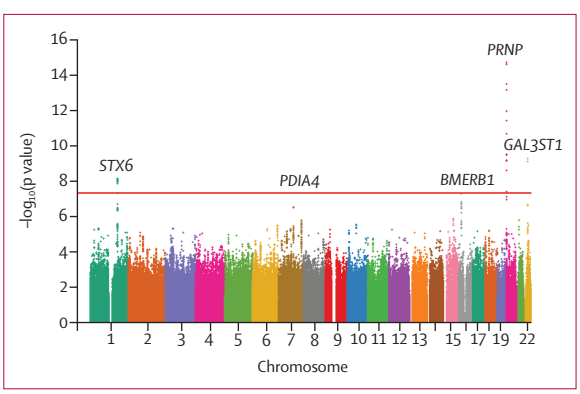

Figure 1: **Manhattan plot for significant variants**
The nearest gene to each genome-wide significant locus (significance indicated by the red horizontal line [$p<5\times10^{-8}$]) is labelled, as well as genes that were significant in gene-based tests.

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	rs1799990	rs3747957	rs2267161	rs9065	rs6498552
Nearest gene	*PRNP*	*STX6*	*GAL3ST1*	*PDIA4*	*BMERB1*
Location (in GRCh37)	20:4680251	1:180953853	22:30953295	7:148700849	16:15539901
Type of mutation	Missense exonic	Synonymous exonic	Missense exonic	3′-UTR exonic	Intronic
Risk allele	A	A	C	T	T
Minor allele	G	A	T	T	T
Discovery stage (n=4110 cases, n=13 569 controls)					
MAF cases	0·288	0·452	0·289	0·220	0·120
MAF controls	0·340	0·410	0·322	0·191	0·102
OR (95% CI)	1·23 (1·17–1·30)	1·16 (1·10–1·22)	1·18 (1·12–1·25)	1·17 (1·09–1·24)	1·27 (1·16–1·38)
p value	$2·68×10^{-15}$	$9·74×10^{-9}$	$8·60×10^{-10}$	$1·66×10^{-6}$	$5·75×10^{-8}$
Replication stage (n=1098 cases, n=498 016 controls)					
MAF cases	0·294	0·450	0·302	0·203	0·105
MAF controls	0·328	0·420	0·326	0·204	0·097
OR (95% CI)	1·15 (1·04–1·28)	1·15 (1·05–1·26)	1·11 (1·00–1·23)	1·01 (0·90–1·13)	1·11 (0·96–1·29)
p value; replication cohorts	0·0049	0·0034	0·042	0·88	0·17
p value; replication plus discovery meta-analysis	$9·61×10^{-17}$	$1·23×10^{-10}$	$1·97×10^{-10}$	$8·49×10^{-5}$	$6·45×10^{-8}$

PRNP, *STX6*, and *GAL3ST1* SNPs were successfully replicated in an independent cohort (p<0·05). OR is relative to the low-risk allele. GRCh37=Genome Reference Consortium human genome build 37. UTR=untranslated region. MAF=minor allele frequency. OR=odds ratio.

Table: **Association results of discovery and replication stages**

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Procedures and statistical analysis

Genotypes were imputed using the Michigan Imputation Server and standard sample and genotyping quality control measures were implemented, to generate 6 314 492 high-quality autosomal single nucleotide polymorphisms (SNPs) for subsequent analysis (appendix pp 28–32). SNPTEST version 2.5.2 was used to perform the association test using an additive logistic regression model. Association statistics for the replication stage were generated using PLINK version 1.9 in a fixed-effects meta-analysis of each cohort. The same model was used to study genetic association for kuru resistance (older asymptomatic individuals who were exposed to kuru compared to patients with young onset and those born after kuru exposure). Additional exome sequencing was performed on 501 CJD samples using the Illumina HiSeq2000 platform.

Further gene-based analysis was performed using MAGMA version 1.06 and VEGAS2 version 2.02, and SNP heritability estimates were calculated using SumHer with standard specifications. CAVIAR and PAINTOR were utilised to generate a credible causal set for SNPs surrounding each significant locus based on linkage disequilibrium and functional annotations.[11,12] eCAVIAR and eQTL colocalisation analysis was performed using 48 tissues included in the GTEx portal version 7.[13,14]

Short-hairpin RNAs targeting *Stx6* and *Prnp* were used to knockdown expression in N2aPK1/2 cells susceptible to infection with Chandler RML prions. Prion propagation was measured using the scrapie cell assay, as previously described.[15] Expression of each proposed gene was measured by RT-qPCR in cerebellum from ten patients with sCJD and ten neurologically healthy controls. Immunohistology for syntaxin-6 and protein disulfide isomerase family A, member 4 was done on formalin-fixed paraffin-embedded frontal cortex and cerebellum of 19 patients with sCJD and 15 non-neurological disease controls.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between 1990 and 2014, we obtained 5208 sCJD samples, of which 4110 were used in the discovery stage and 1098 were used in the replication stage.

In the discovery stage we compared genome-wide genotype data from 4110 patients with probable or definite sCJD from countries of predominantly European ancestries with 13 569 control samples from a similar range of countries (appendix pp 2–3). Imputation using the Michigan server resulted in 6 314 492 high-quality autosomal SNPs after quality control, which were used for downstream association tests in SNPTEST with ten population covariates. Genomic inflation (λ) was 1·026 (appendix p 16), indicating no significant systemic bias related to population ancestry or platforms, so no further correction was done; the threshold for genome-wide significance was $p<5×10^{-8}$. Estimated SNP heritability (LDAK model: $h^2_{SNP}=0·26$ [SD 0·014]; GCTA model: $h^2_{SNP}=0·24$ [SD 0·023]) was similar to that of common neurodegenerative diseases, in keeping with very rare reports of familial sCJD concurrence.[6,16,17]

Further to the known association at *PRNP* on chromosome 20p13, two loci achieved genome-wide significance mapping to 1q25.3 (*STX6*) and 22q12.2 (*GAL3ST1*; figure 1; table; appendix pp 17–19). Gene-based testing with VEGAS2 additionally identified *PDIA4* (p=0·040) and *BMERB1* (p=0·0014), although testing with MAGMA did not support these associations (appendix pp 20–21). No significant gene sets were found. A SNP in intron 1 of the *BMERB1* gene achieved borderline significance (rs6498552, odds ratio 1·27 [95% CI 1·16–1·38], $p=5·75×10^{-8}$, appendix p 21). Although we acknowledge that data from multiple SNPs at a locus are needed to directly replicate gene-based test results, we selected a lead SNP from the three genome-wide significant loci as well as from *PDIA4* and *BMERB1* for the replication stage.

In the replication stage we generated genotype data using minor groove binding probes from 1098 patients with probable or definite sCJD, again from multiple countries of predominantly European ancestries, and compared these with genotypes from 498 016 control samples

from the same countries (appendix pp 2–3). Association testing provided replication evidence for *PRNP* (rs1799990, heterozygous genotype and to a lesser extent the minor allele is protective), *STX6* (rs3747957, minor allele conferred risk), and *GAL3ST1* (rs2267161, minor allele was protective; table). Additionally, we explored if those loci would show an association in related prion diseases. Genotype data was generated for vCJD (acquired from exposure to BSE), iatrogenic CJD (caused by exposure to cadaveric pituitary-derived human growth hormone), or kuru (and resistance to kuru; a former epidemic of orally transmitted prion disease among people who lived in the Eastern Highlands Province of Papua New Guinea). We found no evidence for association of rs3747957 in *STX6*, or rs2267161 in *GAL3ST1* with these phenotypes ($p>0·05$), implying that these loci might confer risk specific to the sporadic form of human prion disease, although all tests were underpowered because of small sample size.[7,9]

sCJD is known to comprise a range of different clinical and pathological phenotypes, broadly correlating with prion molecular strain types, the latter including categorisation by different proportions of three glycoforms and the apparent molecular weight of abnormal prion protein by western blotting.[18] The National Prion Clinic London, UK has done longitudinal observational cohort studies of CJD involving systematic clinical assessments of patients, resulting in deep phenotype data.[19,20] We tested rs1799990, rs3747957, and rs2267161 for association with age at clinical onset, clinical duration, and the slope of decline in a functional measure of disease severity, along with 27 other phenotypic variables (appendix p 4). As expected, rs1799990 in *PRNP* showed associations with several clinical and biomarker traits (ten associations in 30 tested hypotheses). We found no evidence for epistasis between discovered loci and genotypes at rs1799990, which is known to be a major determinant of clinical phenotype.

Because association in a genomic region might not be mediated through the nearest gene, we investigated the potential mechanisms underlying associations with *PRNP*, *STX6*, and *GAL3ST1*. We used CAVIAR to fine-map the association signal at a locus through joint modelling of association statistics for all variants at a locus and estimation of a conditional posterior probability of causality while allowing for multiple plausibly functional SNPs.[12] Around *PRNP* most of the SNPs identified tagged rs1799990. Unexpectedly, a cluster of SNPs located

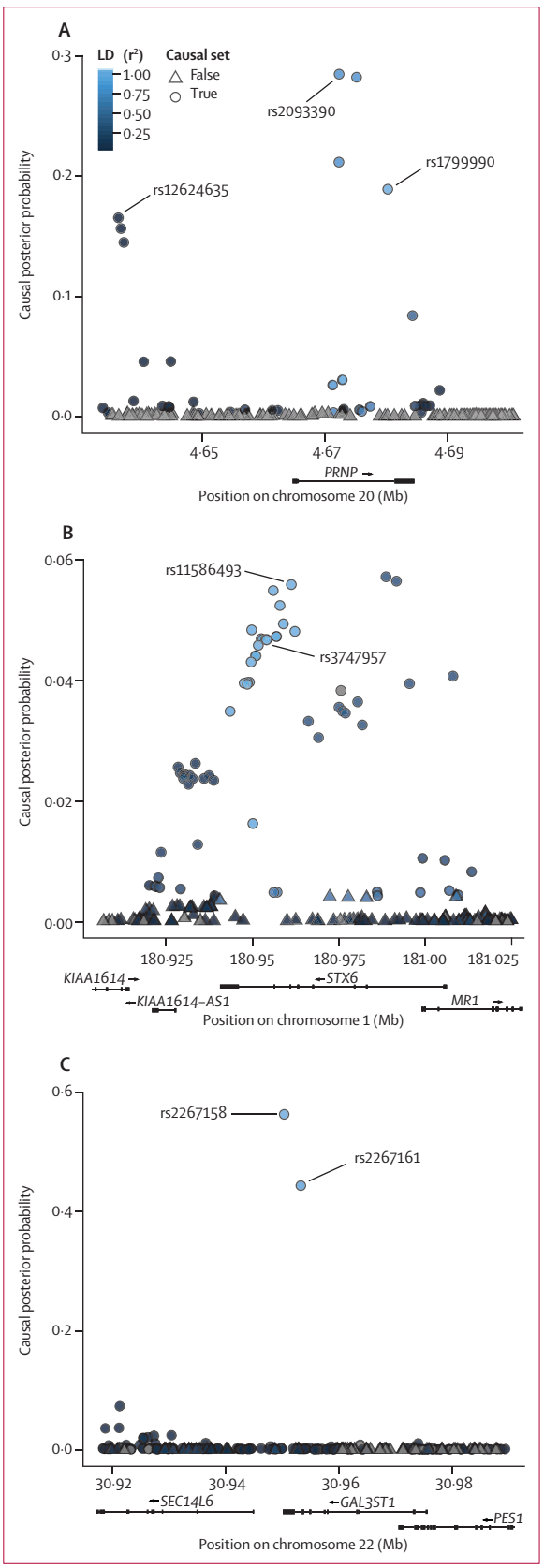

Figure 2: **Statistical fine-mapping using CAVIAR**
CAVIAR utilises summary statistics and LD structure to predict the probability of each variant being causal, producing a causal set with 95% probability of containing the causal SNP, while allowing for the possibility of multiple causal SNPs. Each locus was defined as 100 variants upstream and downstream of the top SNP. Plots show causal posterior probability of each variant at *PRNP* (A), *STX6* (B), and *GAL3ST1* (C), coloured by LD (derived from 1000 Genomes Project European populations data) with the top SNP. Circles indicate variants within the 95% causal set. Triangles highlight other SNPs not predicted to be in the causal set. Mb=megabases. LD=linkage disequilibrium. SNP=single nucleotide polymorphism.

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See **Online** for appendix

For more on **SNP heritability** see http://dougspeed.com/sumher

For more on **CAVIAR** see http://genetics.cs.ucla.edu/caviar

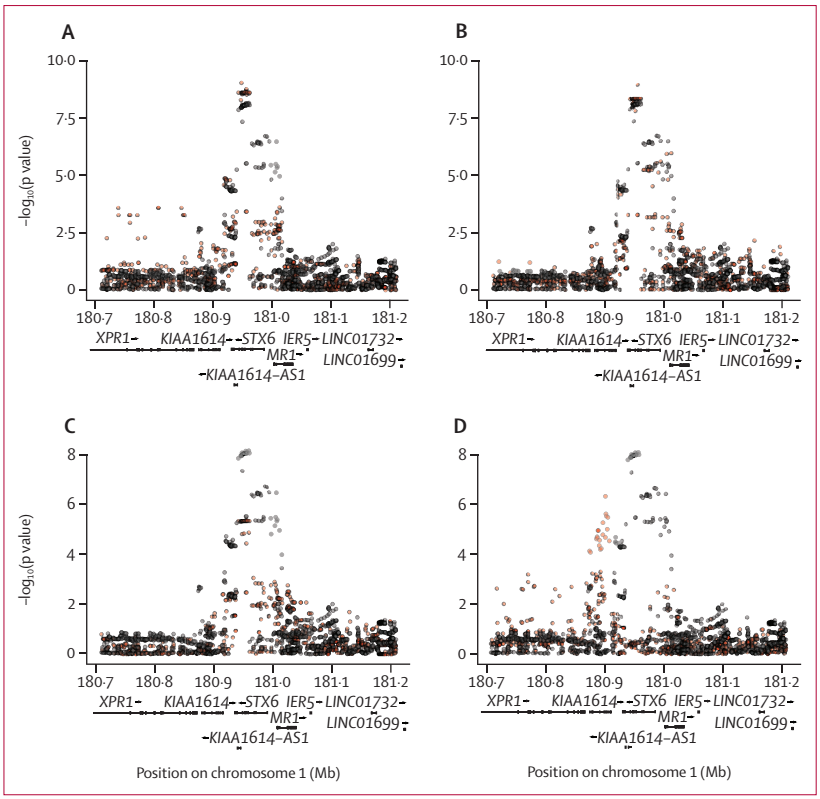

Figure 3: **Colocalisation of GWAS results at *STX6* locus with expression quantitative trait loci**
Plot of $-\log_{10}$ of p values from the GWAS analysis at the *STX6* locus (black) and the expression quantitative trait locus association analysis from the GTEx dataset (red) for: *STX6* expression in the caudate (A), *STX6* expression in the putamen (B), *STX6* expression in the hypothalamus (C), and *KIAA1614* expression in the tibial artery (D). Peaks correspond to the colocalisation posterior probability in the expression quantitative trait locus and GWAS CAVIAR analysis, with a higher degree of colocalisation with increasing colocalisation posterior probability (appendix p 12). GWAS=genome-wide association study. Mb=megabases.

For more on **PAINTOR** see https://hpc.nih.gov/apps/PAINTOR.html

5′ to those tagging rs1799990 (lead SNP rs12624635, not an eQTL) with low levels of linkage disequilibrium to rs1799990 were also putatively causal, suggesting a potential additional signal at this locus (figure 2A). Previous studies have reported that variants at the *PRNP* locus might confer an increased risk for sCJD, independently of rs1799990.[21–24] To further delineate the genetic architecture of the *PRNP* risk locus, we first performed an association analysis under a heterozygous model, which is more appropriate for the known mechanism, and confirmed rs1799990 as the lead SNP ($p=1{\cdot}01\times10^{-135}$; appendix p 22). In a conditional analysis, adjusting for heterozygosity at rs1799990, the lead SNP was rs6139515 ($p=8{\cdot}98\times10^{-4}$). This SNP, which is in low linkage disequilibrium with rs1799990 ($r^2=0{\cdot}04$), is correlated with *PRNP* transcript levels in tibial nerve in the GTEx eQTL database[14] ($p=1{\cdot}8\times10^{-6}$; appendix pp 5, 23). The conditional analysis provided no substantive evidence of an independent association signal at the CAVIAR lead SNP, rs12624635 (p=0·03; appendix pp 5, 23).

The region of high linkage disequilibrium surrounding rs3747957 in *STX6* resulted in a large causal set, making identification of a single causal variant more difficult (figure 2B). Subsequently, using eCAVIAR[13], GTEx[14], and other eQTL databases, we identified a strong correlation between sCJD risk and increased expression of *STX6* mRNA in multiple brain regions, particularly in the caudate and putamen nuclei of the brain (putamen: rs3747957, $p=2{\cdot}3\times10^{-13}$, GTEx; figure 3). Both the caudate and putamen nuclei are key regions implicated in sCJD and are the most commonly abnormal brain regions at diagnostic brain MRI.[25] Correlations between lead SNPs in *STX6*, rs11586493 and rs3747957, and other genes at the locus or within other tissues were absent or less strong (figure 3, appendix p 12). These results suggest that increased expression of *STX6* in brain regions confers an increased risk of sCJD. Using PAINTOR, a tool that integrates functional genomic annotation with association statistics, we next identified three SNPs (rs12754041, rs10797664, and rs6425657; each in strong linkage disequilibrium with lead SNP rs3747957) with high posterior probability of being causal because they were members of one of four functional annotation groups (RoadMap_Assayed_NarrowPeak; Maurano_Science2012_DHS; RoadMap_Enhancers; Roadmap_ChromeHMM_15state).[11]

As the GWAS signal is associated with only two SNPs at *GAL3ST1* (in strong linkage disequilibrium with each other but low linkage disequilibrium with all surrounding variants), these SNPs define the causal set, yet they are statistically indistinguishable from each other (figure 2C). Using GTEx, neither SNP correlated with expression of genes at the locus in brain tissues. One of the SNPs, rs2267161, is a non-synonymous variant of *GAL3ST1* p.V29M. Close to p.V29M resides p.V34M (rs55674628, allele frequency=0·02; linkage disquilibrium with rs2267161, $r^2=0{\cdot}01$, $D'=1{\cdot}00$, discovery p=0·18), the only common non-synonymous variants in European ancestries populations. These polymorphisms form three common haplotypes, rs2267161-C/rs55674628-C (CC; frequency in the combined case-control dataset of 0·667), CT (frequency of 0·018), and TC (frequency of 0·315). We found no evidence of an association driven by the rs55674628-T allele using a haplotype-based test (appendix p 13). Furthermore, analyses of 501 CJD samples by exome sequencing[26] did not identify additional rare variants in *GAL3ST1* or *STX6*.

Expression of *STX6*, *GAL3ST1*, *PDIA4*, and *BMERB1* mRNA was slightly reduced in bulk analysis of post-mortem cerebellar brain tissue from patients with sCJD, but only to a similar extent as genes that have been suggested as good comparators (appendix p 24).[27] Immunohistology of frontal cortex (in 19 patients with sCJD and 15 controls) showed that syntaxin-6 expression is restricted to neurons of different sizes, although other cell types, probably astrocytes or oligodendrocytes, were less consistently stained. In the cerebellum, syntaxin-6 staining was observed in Purkinje cells and in large neurons of the dentate nucleus, and a fine granular staining was seen in the molecular layer (appendix p 25). In all neuron populations of cerebellum and forebrain,

the staining pattern was fine granular, and was located in the cytoplasm, but did not extend into the processes. The staining pattern was compatible with the predicted target, the Golgi apparatus. The pattern for both syntaxin-6 and PDIA4 was indistinguishable between patients with CJD and controls (appendix p 26).

Based on GTEx data, we hypothesised that increased expression of *STX6* in deep brain nuclei increases risk of prion disease. To test whether this might be conferred through facilitating prion propagation in mammalian neuronal cells, we depleted prion-susceptible mouse neuroblastoma-derived cells (N2aPK1/2)[28] of *Stx6* expression using RNA interference. Using the automated scrapie cell assay we measured the impact of *Stx6* knockdown on prion propagation[15,28] using *Prnp* knockdown cells, known to inhibit prion propagation in this assay, as positive controls.[29] Figure 4 shows that *Stx6* depletion, unlike *Prnp* depletion, does not consistently reduce the ability of N2aPK1/2 cells to propagate RML prions.

Discussion

We report, to our knowledge, the first GWAS in a human prion disease powered to detect alleles with the modest effect sizes typical of complex diseases. We identified new risk factors for sCJD, including variants which appear to have pleiotropic effects in neurodegenerative diseases. Further to the known effects at *PRNP* codon 129, we report two independently replicated loci and evidence to support the conclusion that risk variants modify the primary sequence of the encoded protein (*GAL3ST1*) or increase expression in brain tissues (*STX6*). Although a multitude of potential binding partners for prion protein and mechanisms for the modification of prion infection have been proposed, GWAS discoveries have great value because risk variants identified are implicitly causal in the human disease.[30] Therapeutic targets underpinned by genetic evidence have better chances of successful drug development, further encouraging research into the mechanisms that underpin these signals.[30]

Risk variants in sCJD might act at different disease stages: increasing the chance of the spontaneous generation

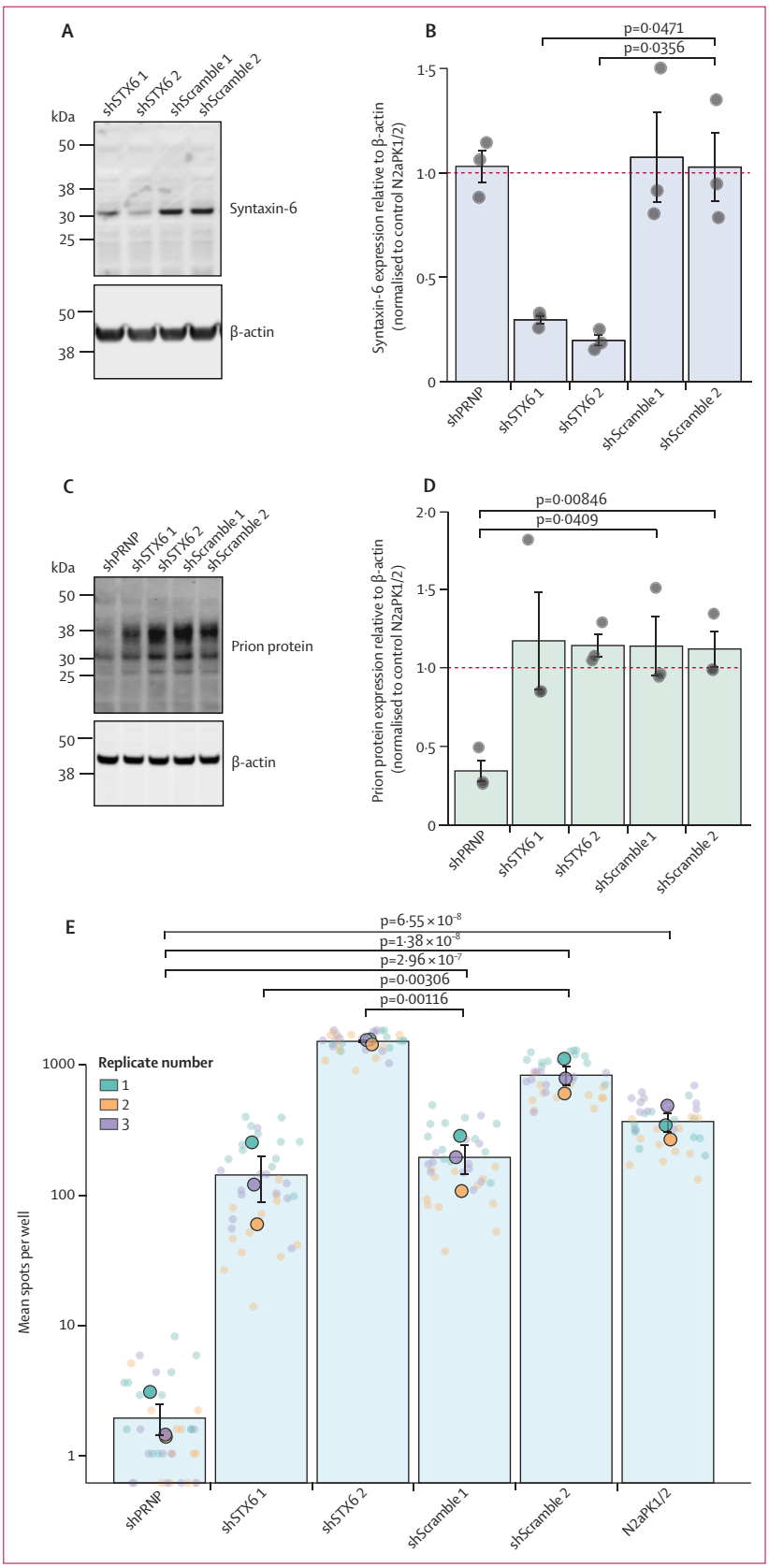

Figure 4: **Scrapie cell assay to measure prion propagation in N2aPK1/2 cells with modified *Stx6* expression**

N2aPK1/2 cells were transfected with pRetroSuper vectors containing *Stx6* (shSTX6 1, shSTX6 2) or *Prnp* (shPRNP) targeting short-hairpin RNAs or a scrambled non-silencing shRNA sequence (shScramble 1, shScramble 2) for controls. Samples were taken before scrapie cell assay for immunoblot (n=3) and expression normalised to untransfected N2aPK1/2 (indicated by dashed line). (A, B) Knockdown of syntaxin-6 protein determined by immunoblot with anti-syntaxin-6 antibody (A), with band intensity measured relative to β-actin loading control (Student's *t* test; B). (C, D) Knockdown of cellular prion protein determined by immunoblot with anti-prion-protein antibody ICSM18 (C), with band intensity measured relative to β-actin loading control (Student's *t* test; D). (E) Average spot count of infected cell number after fourth split in scrapie cell assay following infection with RML at 3×10^{-6} dilution (one-way analysis of variance with Tukey's post-hoc test on log-transformed data). Statistical associations of knockdown lines relative to controls are indicated; other results have been omitted for clarity. All error bars show mean plus and minus SEM. kDa=kilodaltons.

of prions, reducing prion clearance, enabling prion propagation throughout brain tissue, or modifying the downstream toxic effects of prion propagation on brain cells. We did not find any evidence of a role for risk variants in the modification of clinical or pathological disease phenotypes, or in modified expression of risk genes at the end stage of the disease, but it is too early to draw confident conclusions in this respect. Altering the expression of *Stx6* in a cellular model of prion infection did not modify the susceptibility of mouse cells to infection or the accumulation of abnormal forms of prion protein. Our functional data therefore point to a role early in the disease process, perhaps in altering the risk of spontaneous prion formation in the brain, but studies in other models are warranted.

STX6 encodes syntaxin-6, an eight exon, 255 amino-acid protein that localises to the trans-Golgi network, and recycling and early endosomes. Syntaxin-6 is thought to form part of the t-SNARE complex involved in the decision of a target membrane to accept the fusion of a vesicle.[31] The intracellular location of abnormal prion protein in prion-infected cells involves the plasma membrane where conversion is primarily thought to occur,[29] as well as early and recycling endosomes, late endosomes, and the perinuclear region.[32] Other studies implicate the endocytic-recycling compartment or multivesicular bodies as sites of generation of prions, and dysregulation of trafficking genes by sCJD.[33,34] Intracellular trafficking has also been implicated in the degradation of prions.[35] The modification of trafficking of normal or abnormal prion protein by syntaxin-6 might be a focus for future investigation.[36]

There has been considerable recent discussion about the extent to which neurodegenerative diseases associated with the accumulation of misfolded proteins or peptides are similar to prion diseases in their pathogenesis.[37] This concept provokes the suggestion that prion diseases and prion-like disorders might share genetic risk factors. Progressive supranuclear palsy is an uncommon neurodegenerative cognitive and movement disorder associated with the accumulation of abnormal forms of microtubule-associated protein tau with four repeats.[38,39] Variants in *STX6* are in a haplotype with SNPs previously identified as associated with progressive supranuclear palsy, with shared risk alleles (appendix p 14).[39,40] Pleiotropic effects at this locus shared between prion diseases and a tauopathy lend support to the concept of prion-like disorders and indicate the possibility of genetically inspired interventions across multiple neurodegenerative disorders.

GAL3ST1 encodes galactose-3-O-sulfotransferase 1, a 423 amino-acid protein that localises to the Golgi network in oligodendrocytes, and is the sole enzyme responsible for the sulfation of membrane sphingolipids to form sulfatides—a major brain lipid and component of the myelin sheath.[41] Degradation of sulfatides is catalysed by ARSA in the lysosome; recessive defects in this enzyme cause metachromatic leukodystrophy: a lysosomal storage disorder associated with profound central and peripheral demyelination.[42] Knockout of *Gal3st1* in mice results in a neurological phenotype associated with abnormal myelin maintenance with age, histological abnormalities at the paranodal junctions, and abnormal diffusion tensor imaging.[43] Furthermore, in a GWAS of UK Biobank participants, rs2267161 in *GAL3ST1* was significantly associated with multiple changes in white matter microstructure measured using brain diffusion tensor imaging.[44] Sphingolipid metabolism and myelin maintenance have both been previously implicated in prion protein function and prion diseases.[45,46] Multiple genes in the sphingolipid metabolic pathways are dysregulated early in the pathogenesis of mouse prion diseases, a finding consistent between inbred mouse lines and prion strains.[47] Knockout of prion protein in mice, or naturally in goats, results in a demyelinating neuropathy, which in goats is associated with abnormal sphingolipid metabolism.[36,48–50]

PDIA4 and *BMERB1* loci, identified in the discovery stage by gene-based analysis, were not replicated at their lead SNPs; however, the replication sample was necessarily limited by the rarity of the disease, and the lead SNPs had a lower allele frequency than at other risk loci. Further attempts to replicate are justified as gene-based test results are driven by multiple SNPs at each locus.

In conclusion, we present the first evidence of statistically robust genetic associations in sporadic human prion disease that implicate intracellular trafficking and sphingolipid metabolism as molecular causal mechanisms. Future work might further test the hypotheses derived from these discoveries in prion disease model systems, and examine the effects of genome-wide genetic variation on clinical, pathological, and molecular phenotypes in sporadic and inherited prion diseases.

Contributors

EJ, HH, EV, and JU did the main data collection and analysis. AD, HS, TC, PN, LQ, JW, JL, ZJ, SBr, PJ, AN, THM, and PAh contributed specific sections of data collection and analysis. SCo, CS, SS, GGK, MDG, AG, KF, HB, AA, HK-C, SJvdL, CAI-V, CMvD, BS, EG, PPL, MC, OC, PS-J, AS, FM-T, EB-A, SH, J-LL, J-PB, PAm, J-CL, PP, AB-S, SCa, AP, AL, MP, SA, GM, RK, SZ, IZ, SBo, MBC, GHJ, KG, JB, PG, JS, and BA contributed to sample collection and phenotyping. JC and SM had overall supervision of the study and obtained funding. SM and EJ drafted the text and figures for the manuscript. All authors contributed to editing of the text and figures for the manuscript.

Declaration of interests

HB reports grants from Federal Office for Health, Swiss Government, during the conduct of the study. SH reports grants from Santé Publique France, during the conduct of the study; grants from LFB Biomedicaments, Institut de Recherche Servier, and MedDay Pharmaceuticals, outside the submitted work; and has a patent method for treating prion diseases (PCT/EP2019/070457) pending. PAm reports personal fees from Fondation Alzheimer, and personal fees and other from Genoscreen, outside the submitted work. BA reports grants from Centers for Disease Control and Prevention, during the conduct of the study. KF reports grants from Ono Pharmaceuticals, outside the submitted work. SM reports grants from Medical Research Council and National Institute of Health Research's Biomedical Research Centre at University College London Hospitals NHS Foundation Trust, during the conduct of the study. GGK reports personal fees from Biogen, outside the submitted work. JC reports grants from Medical Research Council and National Institute of Health Research's Biomedical Research Centre at University College London Hospitals NHS Foundation Trust, during the conduct of

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Data sharing

Summary statistics are available through the GWAS catalog at National Human Genome Research Institute-European Bioinformatics Institute via study accession number GCST90001389. Further data are available upon request to the corresponding author.

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For more on the **Wellcome Trust Case-Control Consortium** see https://www.wtccc.org.uk

References

1 Collinge J. Prion diseases of humans and animals: their causes and molecular basis. *Annu Rev Neurosci* 2001; **24:** 519–50.
2 Collinge J. Variant Creutzfeldt-Jakob disease. *Lancet* 1999; **354:** 317–23.
3 Mead S, Lloyd S, Collinge J. Genetic factors in mammalian prion diseases. *Annu Rev Genet* 2019; **53:** 117–47.
4 National CJD Research and Surveillance Unit. Twenty-sixth annual report. 2017. https://www.cjd.ed.ac.uk/sites/default/files/report26.pdf (accessed June 1, 2020).
5 Maddox RA, Person MK, Blevins JE, et al. Prion disease incidence in the United States: 2003–2015. *Neurology* 2020; **94:** e153–57.
6 Webb TE, Pal S, Siddique D, et al. First report of Creutzfeldt-Jakob disease occurring in 2 siblings unexplained by *PRNP* mutation. *J Neuropathol Exp Neurol* 2008; **67:** 838–41.
7 Mead S, Uphill J, Beck J, et al. Genome-wide association study in multiple human prion diseases suggests genetic risk factors additional to *PRNP*. *Hum Mol Genet* 2012; **21:** 1897–906.
8 Sanchez-Juan P, Bishop MT, Kovacs GG, et al. A genome wide association study links glutamate receptor pathway to sporadic Creutzfeldt-Jakob disease risk. *PLoS One* 2015; **10:** e0123654.
9 Mead S, Poulter M, Uphill J, et al. Genetic risk factors for variant Creutzfeldt-Jakob disease: a genome-wide association study. *Lancet Neurol* 2009; **8:** 57–66.
10 Sanchez-Juan P, Bishop MT, Aulchenko YS, et al. Genome-wide study links *MTMR7* gene to variant Creutzfeldt-Jakob risk. *Neurobiol Aging* 2012; **33:** 1487 e21–28.
11 Kichaev G, Yang WY, Lindstrom S, et al. Integrating functional data to prioritize causal variants in statistical fine-mapping studies. *PLoS Genet* 2014; **10:** e1004722.

12 Hormozdiari F, Kostem E, Kang EY, Pasaniuc B, Eskin E. Identifying causal variants at loci with multiple signals of association. *Genetics* 2014; **198:** 497–508.

13 Hormozdiari F, van de Bunt M, Segrè AV, et al. Colocalization of GWAS and eQTL signals detects target genes. *Am J Hum Genet* 2016; **99:** 1245–60.

14 Lonsdale J, Thomas J, Salvatore M, et al. The Genotype-Tissue Expression (GTEx) project. *Nat Genet* 2013; **45:** 580–85.

15 Klöhn PC, Stoltze L, Flechsig E, Enari M, Weissmann C. A quantitative, highly sensitive cell-based infectivity assay for mouse scrapie prions. *Proc Natl Acad Sci USA* 2003; **100:** 11666–71.

16 Speed D, Balding DJ. SumHer better estimates the SNP heritability of complex traits from summary statistics. *Nat Genet* 2019; **51:** 277–84.

17 Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet* 2011; **88:** 76–82.

18 Collinge J, Clarke AR. A general model of prion strains and their pathogenicity. *Science* 2007; **318:** 930–36.

19 Mead S, Burnell M, Lowe J, et al. Clinical trial simulations based on genetic stratification and the natural history of a functional outcome measure in Creutzfeldt-Jakob Disease. *JAMA Neurol* 2016; **73:** 447–55.

20 Thompson AG, Lowe J, Fox Z, et al. The Medical Research Council prion disease rating scale: a new outcome measure for prion disease therapeutic trials developed and validated using systematic observational studies. *Brain* 2013; **136:** 1116–27.

21 Mead S, Mahal SP, Beck J, et al. Sporadic—but not variant—Creutzfeldt-Jakob disease is associated with polymorphisms upstream of *PRNP* exon 1. *Am J Hum Genet* 2001; **69:** 1225–35.

22 Bratosiewicz-Wąsik J, Smoleń-Dzirba J, Rozemuller AJ, et al. Association between the *PRNP* 1368 polymorphism and the occurrence of sporadic Creutzfeldt-Jakob disease. *Prion* 2012; **6:** 413–16.

23 Sanchez-Juan P, Bishop MT, Croes EA, et al. A polymorphism in the regulatory region of *PRNP* is associated with increased risk of sporadic Creutzfeldt-Jakob disease. *BMC Med Genet* 2011; **12:** 73.

24 Vollmert C, Windl O, Xiang W, et al. 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.

25 Meissner B, Kallenberg K, Sanchez-Juan P, et al. MRI and clinical syndrome in dura mater-related Creutzfeldt-Jakob disease. *J Neurol* 2009; **256:** 355–63.

26 Koriath C, Kenny J, Adamson G, et al. Predictors for a dementia gene mutation based on gene-panel next-generation sequencing of a large dementia referral series. *Mol Psychiatry* 2018; published online Oct 2. https://doi.org/10.1038/s41380-018-0224-0.

27 Rydbirk R, Folke J, Winge K, Aznar S, Pakkenberg B, Brudek T. Assessment of brain reference genes for RT-qPCR studies in neurodegenerative diseases. *Sci Rep* 2016; **6:** 37116.

28 Brown CA, Schmidt C, Poulter M, et al. In vitro screen of prion disease susceptibility genes using the scrapie cell assay. *Hum Mol Genet* 2014; **23:** 5102–08.

29 Goold R, Rabbanian S, Sutton L, et al. Rapid cell-surface prion protein conversion revealed using a novel cell system. *Nat Commun* 2011; **2:** 281.

30 Claussnitzer M, Cho JH, Collins R, et al. A brief history of human disease genetics. *Nature* 2020; **577:** 179–89.

31 Wendler F, Tooze S. Syntaxin 6: the promiscuous behaviour of a SNARE protein. *Traffic* 2001; **2:** 606–11.

32 Yamasaki T, Suzuki A, Hasebe R, Horiuchi M. Retrograde transport by clathrin-coated vesicles is involved in intracellular transport of PrP^{Sc} in persistently prion-infected cells. *Sci Rep* 2018; **8:** 12241.

33 Yim YI, Park BC, Yadavalli R, Zhao X, Eisenberg E, Greene LE. The multivesicular body is the major internal site of prion conversion. *J Cell Sci* 2015; **128:** 1434–43.

34 Bartoletti-Stella A, Corrado P, Mometto N, et al. Analysis of RNA expression profiles identifies dysregulated vesicle trafficking pathways in Creutzfeldt-Jakob disease. *Mol Neurobiol* 2019; **56:** 5009–24.

35 Goold R, McKinnon C, Tabrizi SJ. Prion degradation pathways: potential for therapeutic intervention. *Mol Cell Neurosci* 2015; **66:** 12–20.

36 Jones E, Mead S. Genetic risk factors for Creutzfeldt-Jakob disease. *Neurobiol Dis* 2020; **142:** 104973.

37 Collinge J. Mammalian prions and their wider relevance in neurodegenerative diseases. *Nature* 2016; **539:** 217–26.

38 Colin M, Dujardin S, Schraen-Maschke S, et al. From the prion-like propagation hypothesis to therapeutic strategies of anti-tau immunotherapy. *Acta Neuropathol* 2020; **139:** 3–25.

39 Chen JA, Chen Z, Won H, et al. Joint genome-wide association study of progressive supranuclear palsy identifies novel susceptibility loci and genetic correlation to neurodegenerative diseases. *Mol Neurodegener* 2018; **13:** 41.

40 Höglinger GU, Melhem NM, Dickson DW, et al. Identification of common variants influencing risk of the tauopathy progressive supranuclear palsy. *Nat Genet* 2011; **43:** 699–705.

41 Takahashi T, Suzuki T. Role of sulfatide in normal and pathological cells and tissues. *J Lipid Res* 2012; **53:** 1437–50.

42 Platt FM, d'Azzo A, Davidson BL, Neufeld EF, Tifft CJ. Lysosomal storage diseases. *Nat Rev Dis Primers* 2018; **4:** 27.

43 Honke K, Hirahara Y, Dupree J, et al. Paranodal junction formation and spermatogenesis require sulfoglycolipids. *Proc Natl Acad Sci USA* 2002; **99:** 4227–32.

44 Zhao B, Zhang J, Ibrahim JG, et al. Large-scale GWAS reveals genetic architecture of brain white matter microstructure and genetic overlap with cognitive and mental health traits (n=17706). *Mol Psychiatry* 2019; published online Oct 30. https://doi.org/10.1038/s41380-019-0569-z.

45 Klein TR, Kirsch D, Kaufmann R, Riesner D. Prion rods contain small amounts of two host sphingolipids as revealed by thin-layer chromatography and mass spectrometry. *Biol Chem* 1998; **379:** 655–66.

46 Agostini F, Dotti CG, Pérez-Cañamás A, Ledesma MD, Benetti F, Legname G. Prion protein accumulation in lipid rafts of mouse aging brain. *PLoS One* 2013; **8:** e74244.

47 Hwang D, Lee IY, Yoo H, et al. A systems approach to prion disease. *Mol Syst Biol* 2009; **5:** 252.

48 Bremer J, Baumann F, Tiberi C, et al. Axonal prion protein is required for peripheral myelin maintenance. *Nat Neurosci* 2010; **13:** 310–18.

49 Küffer A, Lakkaraju AK, Mogha A, et al. The prion protein is an agonistic ligand of the G protein-coupled receptor Adgrg6. *Nature* 2016; **536:** 464–68.

50 Skedsmo FS, Malachin G, Våge DI, et al. Demyelinating polyneuropathy in goats lacking prion protein. *FASEB J* 2020; **34:** 2359–75.