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

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Abstract

Background

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

Methods

Genome-wide association in European ancestry populations (patients diagnosed with probable or definite sCJD; identified at national CJD referral centres from 1990-2014; cases n=5208) using genotyping arrays and exome sequencing. Conditional, transcriptional and histological analyses of implicated genes/proteins in brain tissues and tests of the effects of risk variants on clinical phenotypes using deep longitudinal clinical cohort data.

Findings

We found 41 genome-wide significant single nucleotide polymorphisms and independently replicated findings at three loci associated with sCJD risk within PRNP (rs1799990; additive model P=2·68 x 10-15, OR=1·23; heterozygous model P=1.01x10-135), STX6 (rs3747957; P=9·74 x 10-9, OR=1·16) and GAL3ST1 (rs2267161; P=8·60 x 10-10, OR=1·18). Follow-up analyses suggest that associations at PRNP and GAL3ST1 are likely caused by common variants that alter the protein sequence, whereas risk variants in STX6 associate with increased expression of the

major transcripts in disease-relevant brain regions. Alteration of STX6 expression does not modify prion propagation in a neuroblastoma cell model of mouse prion infection.

Interpretation

We present the first evidence of statistically robust associations in sporadic human prion disease that implicate intracellular trafficking and sphingolipid metabolism. Risk SNPs in STX6 are shared with progressive supranuclear palsy, a neurodegenerative disease associated with misfolding of protein tau, implicating shared mechanisms in prion-like disorders.

Funding

Research in context

Evidence before this study

The rarity of the disease has been limiting in previous genome-wide association studies (GWAS) for sporadic Creutzfeldt-Jakob disease (sCJD) risk. A PubMed search with the terms (prion OR "creutzfledt*") AND ("genome wide association" OR GWAS) (without language restrictions) identified four relevant GWAS publications including two directly investigating sCJD risk through genome-wide analyses, however sample sizes were not sufficient to identify statistically significant associations outside of the known risk at *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 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, totalling 5208 sCJD patients in the two-stage study design. Through statistical fine-mapping and analysis of exome sequencing and gene expression data we propose likely causal genes and mechanisms for both novel associations. We use patient brain samples and cell-based assays to further investigate the biological implications of these in relevant systems. Two further loci at *PDIA4* and *BMERB1* were also associated with sCJD risk in gene-wide 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, with the potential to inform new therapeutic

approaches. With the shared genetic risk of variants within *STX6* and those previously identified

for tauopathy progressive supranuclear palsy, this study also provides support to the notion of

a common "prion-like" mechanism for related neurodegenerative disorders and thus potential

for shared treatments. The generation of publicly available summary statistics can be used for

further investigations by researchers.

Introduction

Prion diseases are fatal neurodegenerative conditions of 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 by exposure to prions in the diet, or through medical or surgical procedures, which may 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 (*PRNP*) gene, causes approximately 10-15% of the annual incidence 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 1 in 5000, which occurs predominantly in older adults.[4,5] Other than age and variation 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 the 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 risk factors for acquired prion diseases in and close to *Prnp*, and provided evidence for susceptibility loci on other chromosomes, yet elucidating the causal

genes has proven challenging.[3] 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. 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 joint genetic risk factors.

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] Here we conducted a GWAS in sCJD, based on samples largely derived from clinical surveillance in countries with populations of predominantly European ancestries. We performed correlations with clinical phenotypes, and transcriptional, protein and cell model-based analyses with the aim of supporting specific causal genes at risk loci and allowing propositions of molecular mechanisms. We identified new risk factors for sCJD including variants which appear to have pleiotropic effects in neurodegenerative diseases.

Methods

Study design and participants

Samples from patients diagnosed with probable or definite sporadic CJD according to widely accepted criteria were provided by specialist or national surveillance centres in countries with populations of predominantly European ancestries (see appendix p 2 and Supplementary Methods appendix pp 28-32). Probable sporadic CJD criteria have varied over the course of sample collection for the study. Using modern diagnostic methods, including real-time quaking induced conversion assay with cerebrospinal fluid, a probable diagnosis refuted by post-mortem examination is extremely rare; but even >20 years ago probable sporadic CJD was highly accurate term. A total of 5208 cases were obtained from numerous countries/regions which were distributed across a two-stage study design: 4110 cases were genotyped using Illumina Omniexpress array in the discovery phase and an additional 1098 cases were genotyped at the lead variant in each hit locus using minor groove binding probes in the replication phase. Control data was obtained from publicly available datasets matched for country.

Procedures and statistical analysis

Genotypes were imputed using the Michigan Imputation Server and standard sample and genotyping quality control measures implemented to generate 6,314,492 high quality autosomal SNPs for subsequent analysis (see Supplementary Methods appendix pp 28-32). SNPTEST (v2.5.2) was used to perform the association test using an additive logistic regression model. Association statistics for the replication phase were generated using PLINK 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 exposed to kuru compared to young onset cases and those born after kuru exposure). Additional exome sequencing was performed on 501 CJD cases using the Illumina HiSeq2000 platform.

Further gene-based analysis was performed using MAGMA (v1.06) and VEGAS2 (v2.02) and SNP heritability estimates 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 (LD) and functional annotations.[11,12] eCAVIAR and eQTL colocalisation analysis was performed using 48 tissues included in the GTEx portal (v7).[13,14]

Short-hairpin RNAs (shRNAs) 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 10 sCJD cases and 10 neurologically healthy controls. Immunohistology for syntaxin-6 and protein disulfide isomerase family A member 4 was performed on formalin-fixed paraffin-embedded frontal cortex and cerebellum of 19 sCJD cases and 15 non-neurological disease controls.

Data availability

Summary statistics are available through the GWAS catalog at NHGRI-EBI via study accession number GCST90001389. Further data available upon request.

Results

In the discovery phase 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 countries matched for population (appendix pp 2-3). Imputation using the Michigan server resulted in 6,314,492 high quality autosomal SNPs after QC which were used for downstream association tests in SNPTEST with 10 population covariates. Genomic inflation (λ) was 1.026 (appendix p 16), indicating no significant and systemic bias related to population ancestry or platforms, so no further correction was done; the threshold for genome-wide significance was P<5x10^{-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 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 1; 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, P=5·75 x 10^{-8}, appendix p 21). Whilst 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 phase.

In the replication phase we generated genotype data using minor groove binding probes from 1098 patients with probable or definite CJD, again from multiple countries of predominantly European ancestries, and compared 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 1). Next, we explored if those loci would also 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 may confer risk specific to the sporadic form of human prion disease, although all tests were underpowered due to small sample size.[7,9]

Sporadic CJD 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 PrP by western blotting.[18] The National Prion Clinic London has conducted 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, 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 (10/30 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*. First 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 whilst allowing for multiple plausibly functional SNPs.[12] Around *PRNP* most of the SNPs identified tagged rs1799990. Unexpectedly, a cluster of SNPs located 5' to those tagging rs1799990 (lead SNP rs12624635, not an eQTL) with low levels of LD 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 may 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, $P=1.01 \times 10^{-135}$ (appendix p 22). Next in a conditional analysis, adjusting for heterozygosity at rs1799990, the lead SNP was rs6139515 ($P=8.98 \times 10^{-4}$). This SNP, which is in low LD with rs1799990 ($r^2=0.04$), is correlated with *PRNP* transcript levels in tibial nerve in the GTEx eQTL database[25]; $P=1.8 \times 10^{-6}$ (appendix p 23; appendix p 5). The

conditional analysis provided no substantive evidence of an independent association signal at the CAVIAR lead SNP, rs12624635, P=0.03 (appendix p 23; appendix p 5).

The region of high LD surrounding rs3747957 in *STX6* resulted in a large causal set, making identification of a single causal variant more complex (Figure 2b). Subsequently, using eCAVIAR[13], GTEx[25] 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=2·3 x 10^{-13}, GTEx; Figure 3). Both are key regions implicated in sCJD and are the most frequently abnormal brain regions at diagnostic brain magnetic resonance imaging.[26] 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 elevated 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 LD with lead SNP rs3747957) with high posterior probability of being causal in four 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 LD with each other but low LD with all surrounding variants) these SNPs predominantly 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; LD with rs2267161, r^2=0·01, D'=1·00, 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), CT and TC with frequencies in the combined case-control dataset of 0·667, 0·018 and 0·315, respectively. 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 cases by exome sequencing[27] 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 sCJD cases but only to a similar extent as genes suggested to be good comparators (appendix p 24).[28] Immunohistology of frontal cortex (19 sCJD; 15 controls) showed that syntaxin-6 expression is restricted to neurons of different sizes, whilst other cell types, probably astrocytes or oligodendrocytes, were less consistently stained. In the cerebellum, syntaxin-6 staining is observed in Purkinje cells and in large neurons of the dentate nucleus, whilst a fine granular staining is seen in the molecular layer (appendix p 25). In all neuron populations of cerebellum and forebrain, the staining pattern is fine granular, and is located in the cytoplasm, but does not extend into the processes. The staining pattern is compatible with the predicted target, the Golgi apparatus. The pattern for both syntaxin-6 and *PDIA4* was indistinguishable between CJD cases and controls (appendix p 26).

Based on GTEx data, we hypothesized 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)[29] of *Stx6* expression using RNA interference. Using the

Automated Scrapie Cell Assay we measured the impact of *Stx6* knockdown on prion

propagation[15,29] using *Prnp* knockdown cells, known to inhibit prion propagation in this assay,

as positive control.[30] Figure 4 shows that *Stx6* depletion, unlike *Prnp*, does not consistently

reduces the ability of N2aPK1/2 cells to propagate RML prions.

Discussion

We report the first GWAS in a human prion disease powered to detect alleles with the modest effects typical of complex 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*). Whilst a multitude of potential binding partners for PrP and mechanisms for the modification of prion infection have been proposed, GWAS discoveries have special value because risk variants are implicitly causal in the human disease.[31] Therapeutic targets underpinned by genetic evidence have better chances of successful drug development, further encouraging research into the mechanisms that underpin these signals.[31]

Risk variants in sCJD might act at different disease stages: increasing the chance of the spontaneous generation of prions, reducing their 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, yet 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 PrP. Our functional data therefore point to a role early in the disease process, perhaps by altering the risk of spontaneous prion formation in the brain, but studies in other models are warranted.

STX6 encodes syntaxin-6, an 11 exon, 255 amino-acid protein that localises to the trans-Golgi network, 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.[32] The intracellular location of abnormal PrP in prion-infected cells involves the plasma membrane where conversion is primarily thought to occur,[30] as well as early and recycling endosomes, late endosomes, and the perinuclear region.[33] Other studies implicate the endocytic-recycling compartments (ERCs) and/or multivesicular bodies (MVBs) for the sites of generation of prions, and dysregulation of trafficking genes by sCJD.[34,35] Intracellular trafficking has also been implicated in the degradation of prions.[36] The modification of trafficking of normal and/or abnormal PrP by syntaxin-6 might be a focus for future investigation (see our review for detailed discussion[37]).

There has been considerable recent discussion about the extent to which neurodegenerative diseases associated with the accumulation of misfolded proteins or peptides are "prion-like" in their pathogenesis.[38] This concept provokes the suggestion that prion and prion-like disorders might share genetic risk factors. Progressive supranuclear palsy (PSP) is an uncommon neurodegenerative movement disorder associated with the accumulation of abnormal forms of microtubule-associated protein tau with four repeats.[39,40] Variants in *STX6* are in a haplotype with SNPs previously identified as associated with PSP with shared risk alleles (appendix p 14).[40,41] Pleiotropic effects at this locus between prion diseases and a tauopathy lend support to the concept of prion-like disorders and raise the prospect for 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, the sole enzyme responsible for the sulfation of membrane sphingolipids to form sulfatides, a major brain lipid and component of the myelin sheath.[42] 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.[43] 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 (DTI).[44] Furthermore, in a GWAS of UK Biobank participants, rs2267161 in *GAL3ST1* was significantly associated with multiple changes in white matter microstructure measured using brain DTI.[45] Sphingolipid metabolism and myelin maintenance have both been previously implicated in PrP and prion diseases.[46,47] 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.[48] Knockout of PrP in mouse, or naturally in goats, results in a demyelinating neuropathy, which in goats is associated with abnormal sphingolipid metabolism (see review for further discussion[37]).[49-51]

PDIA4 and *BMERB1* loci identified in the discovery phase by gene-wide analysis, were not replicated by single SNP tests, 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 are justified as gene-based test results are driven by multiple SNPs at each locus.

In summary, we present the first evidence of statistically robust associations in sporadic human prion disease that implicate intracellular trafficking and sphingolipid metabolism. Future work might further test 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.

Conflicts of Interest

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		rs1799990	rs3747957	rs2267161	rs9065	rs6498552
	Nearest gene	*PRNP*	*STX6*	*GAL3ST1*	*PDIA4*	*BMERB1*
	Location (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	**No. cases**	4110	4110	4110	4110	4110
	No. controls	13569	13569	13569	13569	13569
	MAF cases	0.288	0.452	0.289	0.220	0.120
	MAF controls	0.340	0.410	0.322	0.191	0.102
	P-value	2.68×10^{-15}	9.74×10^{-9}	8.60×10^{-10}	1.66×10^{-6}	5.75×10^{-8}
	Odds ratio (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)
Replication stage	**No. cases**	1,098	1,098	1,098	1,098	1,098
	No. controls	498,016	498,016	498,016	498,016	498,016
	MAF cases	0.294	0.450	0.302	0.203	0.105
	MAF controls	0.328	0.420	0.326	0.204	0.0972
	P-value; replication cohorts	**0.0049**	**0.0034**	**0.042**	0.88	0.17
	Odds ratio	1.15	1.14	1.11	1.01	1.11
	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}

Table 1. Main results of discovery and replication stages. *PRNP*, *STX6* and *GAL3ST1* SNPs were successfully replicated in an independent cohort (P<0.05) with a similar effect as the discovery phase. [1]Replication minor allele frequencies (MAF) were estimated as a weighted mean of each cohort based on case sample number. Odds ratio shown relative to the risk allele.
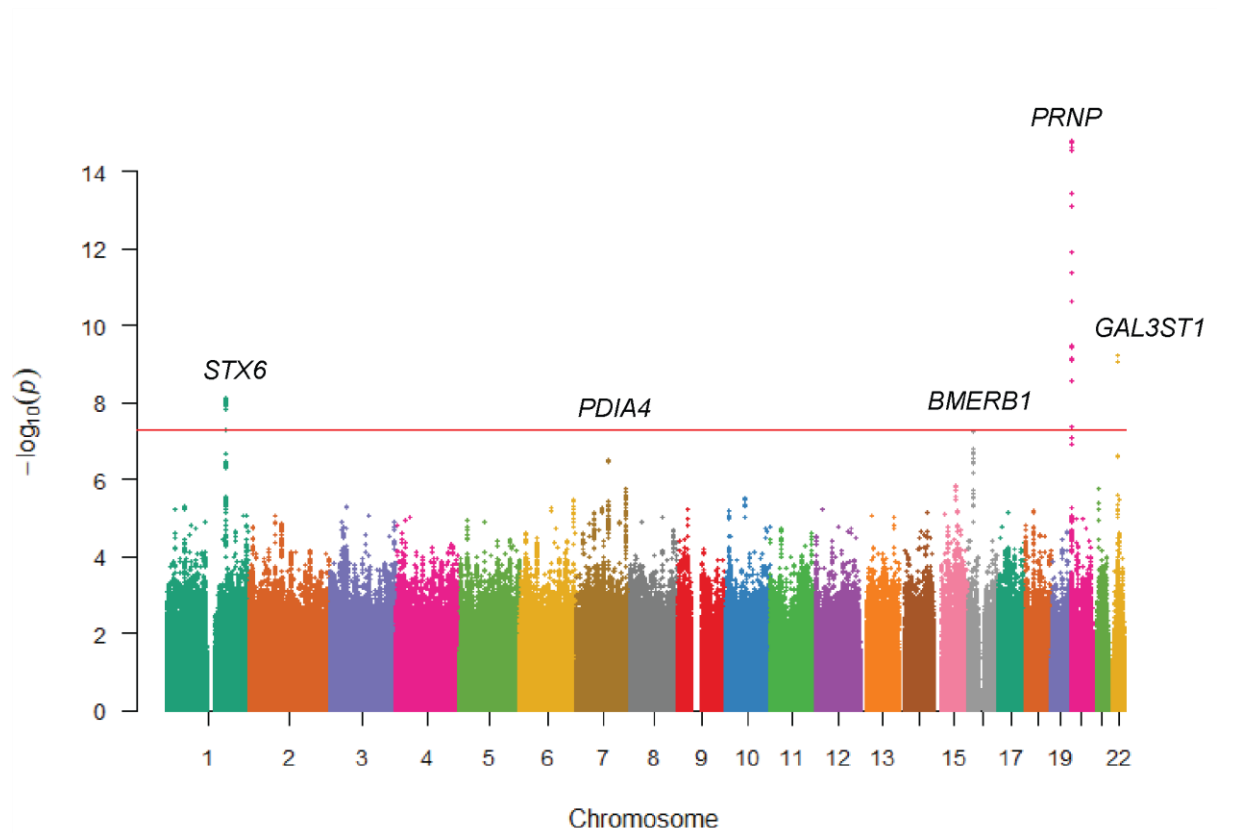
Figure 1. Manhattan plot for significant variants. The nearest gene to each genome-wide significant locus (indicated by the red horizontal line) is labelled as well as genes significant in gene-wide tests.
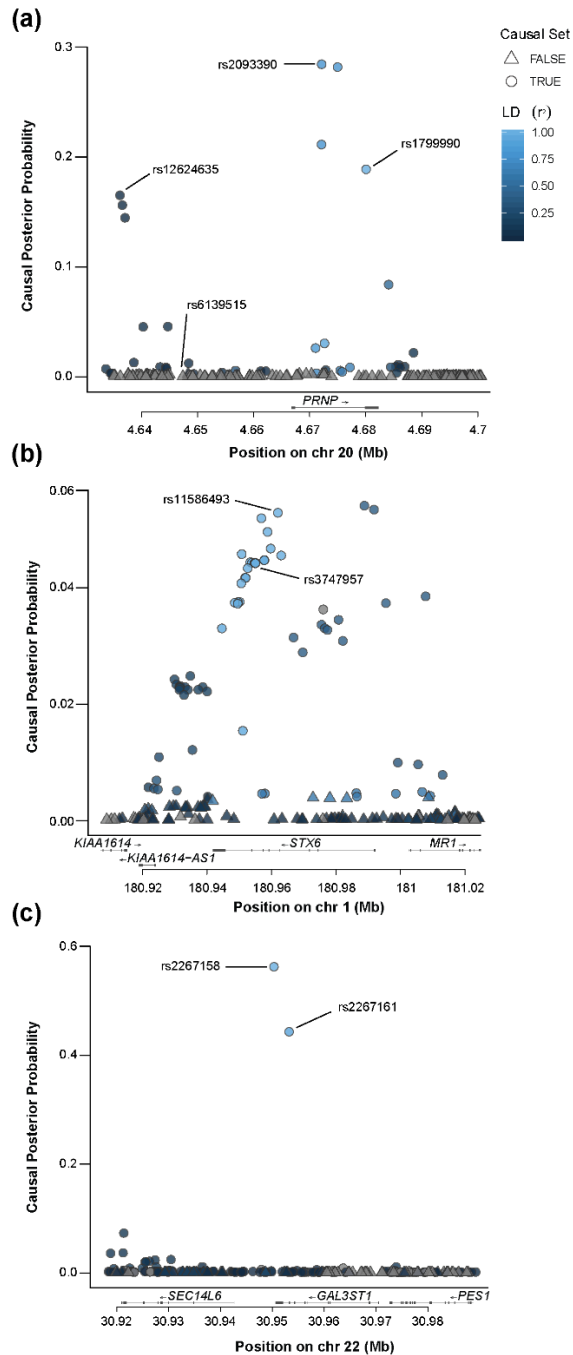
Figure 2. Statistical fine-mapping using CAVIAR. CAVIAR utilizes summary statistics and LD structure to predict the probability of each variant being causal, producing a 'causal set' with 95% probability of containing the causal SNP, whilst allowing for the possibility of multiple causal SNPs. Each locus was defined as 100 variants upstream and downstream of the top SNP. Plots show causal posterior probability of each variant at (**a**) *PRNP*, (**b**) *STX6* and (**c**) *GAL3ST1* coloured by LD (1000G; EUR) with the top SNP. Circles indicate variants within the 95% causal set.
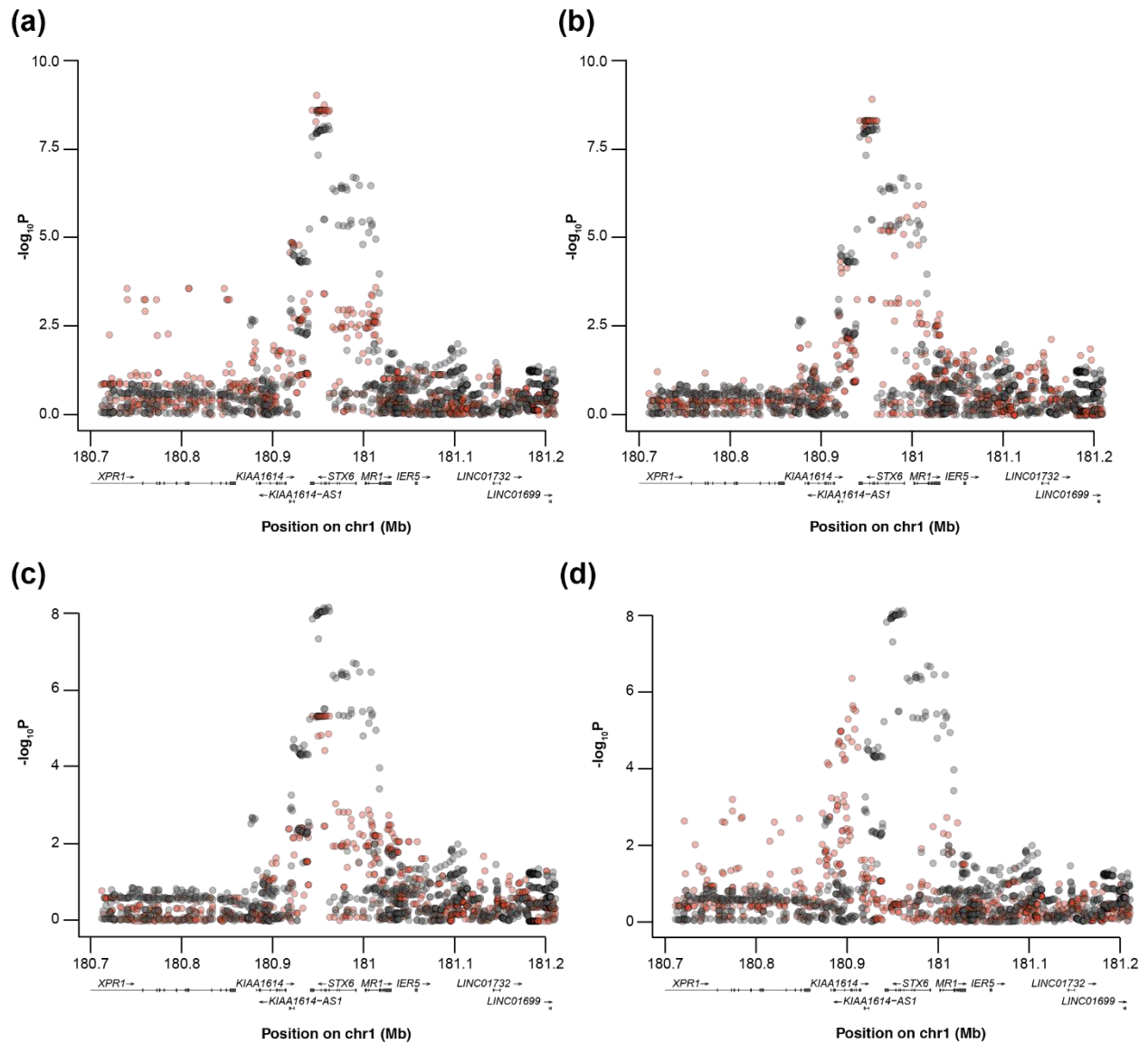
Figure 3. Co-localisation of GWAS results at *STX6* locus with eQTLs for *STX6* expression in the caudate, putamen and hypothalamus and *KIAA1614* expression in the tibial artery. Plot of −log₁₀ of P-values from the GWAS association analysis at the *STX6* locus (black) and the eQTL association analysis from the GTEx dataset (v7) (red) for: (**a**) *STX6* expression in the caudate, (**b**) *STX6* expression in the putamen, (**c**) *STX6* expression in the hypothalamus, (**d**) *KIAA1614* expression in the tibial artery. Peaks correspond to the CLPP in the eCAVIAR analysis with a higher degree of colocalisation with increasing CLPP (see appendix p 12).
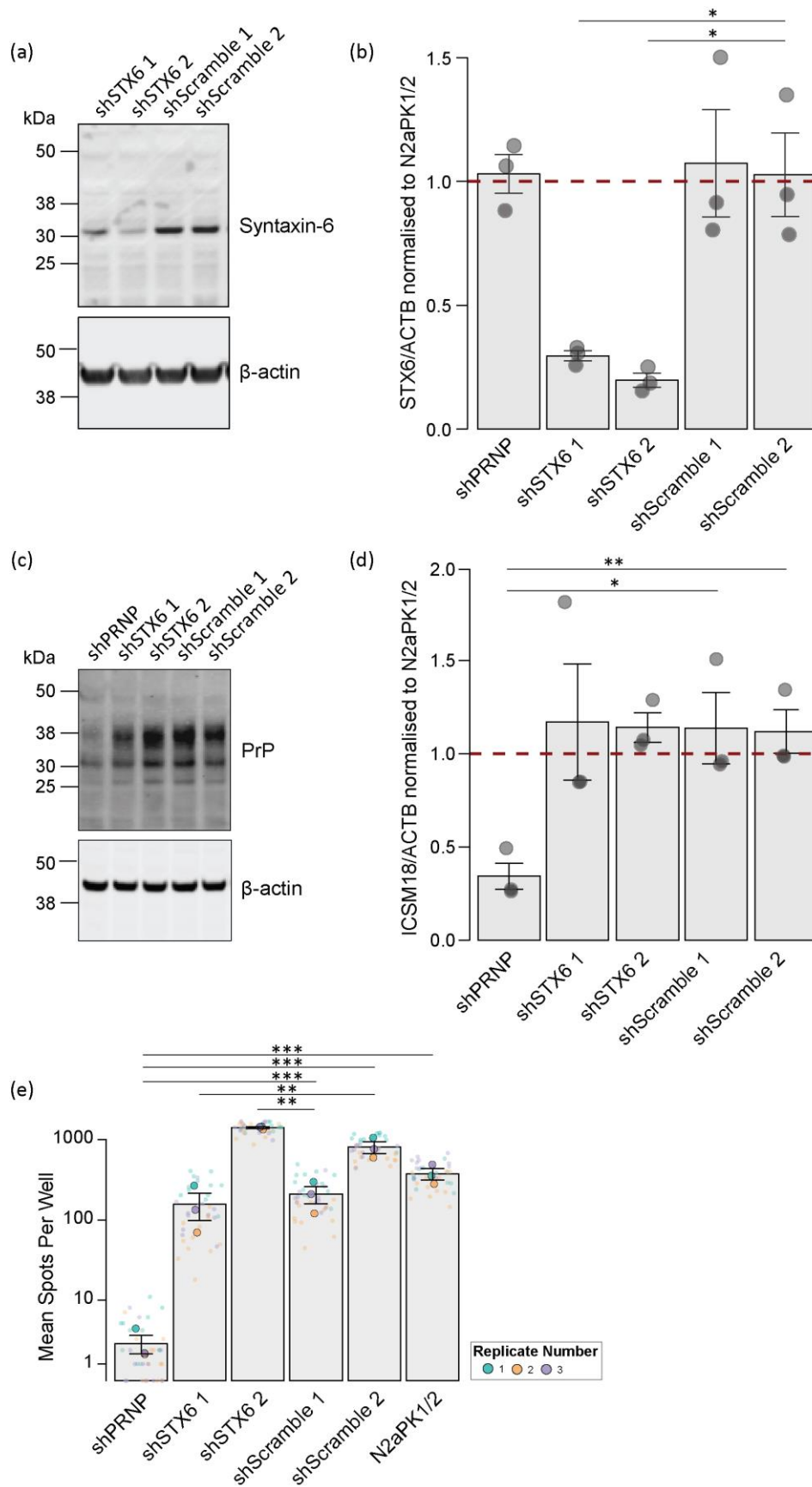
(a)

kDa

50
38
30
25

Syntaxin-6

50
38

β-actin

(b)

STX6/ACTB normalised to N2aPK1/2

shPRNP   shSTX6 1   shSTX6 2   shScramble 1   shScramble 2

(c)

kDa

50
38
30
25

PrP

50
38

β-actin

(d)

ICSM18/ACTB normalised to N2aPK1/2

shPRNP   shSTX6 1   shSTX6 2   shScramble 1   shScramble 2

(e)

Mean Spots Per Well

shPRNP   shSTX6 1   shSTX6 2   shScramble 1   shScramble 2   N2aPK1/2

Replicate Number
1   2   3

Figure 4: Scrapie cell assay (SCA) 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 shRNAs or a scrambled non-silencing ('shScramble 1', 'shScramble 2') shRNA sequence for controls. Samples were taken prior to SCA for immunoblot (n=3) and expression normalised to untransfected N2aPK1/2 (indicated by dashed line). (**a**-**b**) Knockdown of syntaxin-6 protein determined by (**a**) immunoblot with anti-syntaxin-6 antibody with (**b**) band intensity measured relative to β-actin loading control (Student's t-test). (**c**-**d**) Knockdown of PrP^C determined by (**c**) immunoblot with anti-PrP antibody ICSM18 with (**d**) band intensity measured relative to β-actin loading control (Student's t-test). (**e**) Average spot count of infected cell number post-4th split in SCA following infection with RML at 3x10^{-6} dilution (one-way ANOVA with Tukey's post-hoc test on log-transformed data). Statistical associations of knockdown lines relative to controls indicated; other results omitted for clarity. All error bars show mean ± SEM; *P<0·05, ** P<0·01, *** P<0·001.