However, the normal function(s) of PrP^C are not well understood. We have used our *prp1; prp2* compound homozygous knockout mutant zebrafish to identify potentially conserved, cross-species functions of PrP^C using RNA-sequencing analysis in addition to exploring the increasing evidence for PrP^C involvement in cell adhesion. **Methods**: Three-day post fertilization wild-type and $prp1^{ua5003/ua5003}$; $prp2^{ua5001/ua5001}$ zebrafish larvae underwent PE100-125 HiDeq2500 RNA-sequencing. Three biological replicates each consisting of 50 pooled larvae were used for each genotype. Analysis of sequencing results was processed using Geneious R9/10 and R packages cufflinks and cummeRbund. Alterations in transcript abundance were confirmed using RT-qPCR for selected genes of interest. Further cell adhesion studies were carried out through *in situ* hybridization analysis and immunohistochemistry.
Results: RNA-sequencing analysis of mutant fish show large changes in transcript abundance (log_2 fold change of 0.5 or greater) of 1,249 genes compared to wild-type fish. Of interest are genes involved in cell adhesion, including *st8sia2, ncam1a* and members of the protocadherin family, which are thought to be involved during early development and continued maintenance of the central nervous system (CNS). Through search and comparison with published transcriptomes from embryonic PrP^C knockout mice, we have identified many common affected biological processes in our mutant zebrafish including apoptosis, proteolysis, oxidative stress and cell proliferation, and adhesion. Further analysis on cell adhesion through RT-qPCR of *ncam1a* and *st8sia2* shows a significant reduction in transcript abundance between wild-type and mutant fish, with *in situ* hybridization of *ncam1a* confirming a decreased distribution of mRNA across the CNS.
Conclusions: Our transcriptomic sequencing analysis supports a role for PrP^C during early development and sustainment of the CNS, seen through significant changes in transcript abundance in members of the protocadherin family and other genes responsible for cell adhesion and proliferation. We propose cell adhesion is an evolutionary conserved function of PrP^C which will be of important consideration regarding how PrP^C function may be affected during disease.

KEYWORDS Cellular prion protein; cell adhesion; RNA-sequencing; zebrafish

49. The agent of transmissible mink encephalopathy passaged in sheep is similar to BSE-L

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ABSTRACT

Introduction: Transmissible mink encephalopathy (TME) is a fatal neurologic prion disease of farmed mink. Epidemiologic and experimental evidence following a Wisconsin outbreak in 1985 has linked TME to low-type bovine spongiform encephalopathy (BSE-L). Evidence suggests that farmed mink were likely exposed through feeding of BSE-L infected downer cattle. The interspecies transmission of TME to cattle has been documented. Recently, we demonstrated the susceptibility of sheep to cattle passaged TME by intracranial inoculation. The aim of the present study was to compare ovine passaged cattle TME to other prion diseases of food-producing animals. Using a bovine transgenic mouse model, we compared the disease phenotype of sheep TME to BSE-C and BSE-L.
Materials and Methods: Separate inoculants of sheep passaged TME were derived from animals with the VRQ/VRQ (VV_{136}) and ARQ/VRQ (AV_{136}) prion protein genotype. Transgenic bovinized mice (TgBovXV) were intracranially inoculated with 20 µl of 1% w/v brain homogenate. The disease phenotypes were characterized by comparing the attack rates, incubation periods, and vacuolation profiles in TgBovXV mice.
Results: The attack rate for BSE-C (13/13), BSE-L (18/18), and TME_{VV} (21/21) was 100%; whereas, the TME_{AV} group (15/19) had an incomplete attack rate. The average incubation periods were 299, 280, 310, and 541 days, respectively. The vacuolation profiles of BSE-L and TME_{VV} were most similar with mild differences observed in the thalamus and medulla. Vacuolation profiles from the BSE-C and TME_{AV} experimental groups were different than TME_{VV} and BSE-L.
Conclusion: Overall the phenotype of disease in TME inoculated transgenic mice was dependent on the sheep donor genotype (VV vs AV). The results of the present study indicate that TME isolated from VRQ/VRQ sheep is similar to BSE-L with regards to incubation period, attack rate, and vacuolation profile. Our findings are in agreement with previous research that found phenotypic similarities between

BSE-L and cattle passaged TME in an ovine transgenic rodent model. In this study, the similarities between ovine TME and BSE-L are maintained after multiple interspecies passages.

50. Cellular Prion protein on human leucocytes is associated with iron metabolism

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ABSTRACT

Introduction: The function of the prion protein remains elusive. Several findings indicate a role in iron metabolism. In this study, we wanted to investigate an association between indicators of iron storage and the expression of prion protein on human leucocytes in glaucoma patients.
Materials and Methods: Patients were recruited at the Department of Ophthalmology at the Medical University Graz. Twenty patients with glaucoma were included. The expression of prion protein on CD3+ CD4+, CD3+ CD8+ and CD14+ leukocytes was investigated by FACS with POM1 antibody.
Results: The expression of Prion Protein correlated significantly with soluble transferrin receptor, haemoglobin, and serum iron (Pearson $r > 0.6$; p-value < 0.01). No association was found between expression of prion protein and glaucoma progression.
Conclusions: We found a strong correlation between iron metabolism and the expression of prion protein on human leucocytes. This presents add to our understanding of the function and regulation of the cellular prion protein.

KEYWORDS: Prion protein; human leucocytes; soluble transferrin receptor; haemoglobin

51. Cryptic prion strains/variants appear when anti-prion systems are disabled

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ABSTRACT

The yeast prions [PSI+] and [URE3] are in-register parallel amyloids of Sup35p and Ure2p, respectively. Ssb1,2p are ribosome-associated Hsp70s that ~10-fold repress [PSI+] generation. Upf1,2,3p are factors essential for mRNA nonsense-mediated decay that, at normal levels, cure >90% of [PSI+] variants arising in any *upf* mutant. The formation of the Upf1,2,3-Sup35 complex is critical for the curing process. The *upf* mutants show 10–15 fold elevation in frequency of spontaneous or induced [PSI+] generation. By controlling cellular levels of 5-pyrophospho-inositol pentaphosphate, Siw14p prevents the propagation of about half of the [PSI+] variants arising in its absence. Normal levels of the disaggregating chaperone Hsp104 cures about half of the [PSI+] variants that arise in an $hsp104^{T160M}$ mutant defective in the Hsp104 overproduction curing activity. Normal levels of Btn2p and Cur1p are paralogs that cure ~90% of [URE3] prion strains/variants arising in a *btn2*Δ *cur1*Δ strain, preferentially those with low propagon numbers. Btn2p (but not Cur1p) cures by collecting Ure2p amyloid filaments at a single site in the cell co-inciding with Btn2p itself. Cell division results in frequent curing of one of the daughter cells.

The common thread of most of these systems is that prion strains that would not survive in a wild type strain are detected in mutants defective in one or more of these anti-prion systems. We infer that the normal cell experiences a large load of nascent prions, most of which are quickly eliminated by one or more of these systems. The mechanistic details, insofar as we know them, argue that each of these systems are distinct. The excess prion strains/variants detected in cells defective in one anti-prion system are evidently immune to curing by the other anti-prion systems, indicating that each anti-prion system defines a new class of prion strains/variants. It is likely that some prion/prion-like diseases are a consequence of breakdown of homologous/analogous human anti-prion systems.

Future work will address the following questions:

- What other components are involved in each of these processes? Is there any overlap?
- Do cells defective in multiple anti-prion systems show even higher prion generation frequencies?
- What other anti-prion systems are there?