Progress on low susceptibility mechanisms of transmissible spongiform encephalopathies

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Abstract: Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are a group of fatal neurodegenerative diseases detected in a wide range of mammalian species. The "protein-only" hypothesis of TSE suggests that prions are transmissible particles devoid of nucleic acid and the primary pathogenic event is thought to be the conversion of cellular prion protein (PrP^C) into the disease-associated isoform (PrP^{Sc}). According to susceptibility to TSEs, animals can be classified into susceptible species and low susceptibility species. In this review we focus on several species with low susceptibility to TSEs: dogs, rabbits, horses and buffaloes. We summarize recent studies into the characteristics of low susceptibility regarding protein structure, and biochemical and genetic properties.

Keywords: Transmissible spongiform encephalopathy; Low susceptibility; Dog; Rabbit; Horse; Buffalo; PRNP; SPRN

Transmissible spongiform encephalopathy (TSE), or prion disease, is an invariably fatal neurodegenerative disease detected in a wide range of mammalian species, including Scrapie in goats (Capra hircus) and sheep (Ovis aries); bovine spongiform encephalopathy (BSE) in cattle (Bos taurus); chronic wasting disease (CWD) in elaphure (Elaphurus davidianus) and moose (Alces americanus); feline spongiform encephalopathy (FSE) in cats (Felis catus); transmissible mink encephalopathy (TME) in minks (Mustla vison); and Creutzfeldt-Jakob disease (CJD), variant Creutzfeldt-Jakob disease (vCJD), fatal familial insomnia (FFI), Gerstmann-Straussler-Scheinker syndrome (GSS) and Kuru in humans (Homo sapiens) (Collins et al, 2004; Prusiner, 1982). Humans and other animals infected with TSE are clinically and pathologically characterized with neuronal progressive vacuolation, stellate cell gliosis, spongiform lesions in gray matter, amyloid deposition and eventually disastrous degeneration and death (Prusiner, 1998). No effective treatments have been found and the World Health Organization has named TSE and AIDS as two major health problems of the 21st century.

The "protein-only" hypothesis of TSE suggests that the pathogenic factors of TSE are not bacteria or a virus, but a protein devoid of nucleic acid, which has been named prion protein (PrP). PrP is encoded by the prion protein gene (PRNP) (Prusiner, 1982). Normal cellular PrP (PrP^C) expresses in the cells of mammalian species and the number of amino acid residues varies from 253 to 264 across species (Wopfner et al, 1999) and are all highly conserved (Figure 1). PrP has two signal peptide sequences, a N-terminal and a C-terminal. Mature PrP has an intra-molecular disulfide bond and two glycosylation sites, and is anchored on the cell membrane surface via glycosylphosphatidylinositol (GPI) at the C-terminal (Aguzzi et al, 2008). According to the protein-only hypothesis TSE is a conformational disease and under certain circumstances cellular PrP^C mistakenly

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	1 10	20	30	40	50	60	70
Human	MANLGCWMLVI	FVATWSDLGLC	KRPKPGG-WN	GG-SRYPGOG	SPGGNRYPPO	GGGGWGOPHGG	G-W 65
Chimpanzee							65
Rhesus							65
Deer	MVKSHI.S.I	WV	G				••• 68
Elk	MVKSHI.S.I	WV	G			• • • <u>•</u> • • • • • • • •	••• 68
Mouse	Y.L.A.		• • • • • • • • • • • •			<u>T</u>	••• 64
Rat	Y.L.A.	T.CT.V			• • • • • • • • • • •	S	••• 65
Pig	MVKSHI.G.I	A⊥ M V		• • • • • • • • • •	• • • • • • • • • • •	•••••	••• 68
Sneep	MVKSHT S T	M V	G			•••••	· · · · · 08 69
Rabbit	H. Y				3		66
Dog	MVKSHI.G.I.L.	v		G			68
Cat	MVKGHI.G.I	. v	G				. G . 69
House	MVKSHV.G.I						• • • 67
Cattle	MVKSHI.S.I	WV	G				• • • 68
Buffalo	MVKRHI.S.I	VMV	G		S.	•••••	• • • 68
	80	90	100	110	120	130	140
Human	GOPHGGG-WGOPH	IGGGV	ICOPHCCC-WCC	GGGTHSOWNK	PSKPKTNMKH	MAGAAAAGAVV	GI. 125
Chimpanzee							125
Rhesus				N.H.	S		125
Deer			G	–		v	• • • 128
Elk			G	–		V	• • • 128
Mouse	S	S.		N	L	v	••• 124
Rat			S.	N	L	V	••• 125
Pig		• • • • • • • • • • • • •	G	S.G		V	••• 129
Sheep	• • • • • • • • • • • • • •	• • • • • • • • • • • • •	G	··-S	• • • • • • • • • •	V	••• 128
Goat		• • • • • • • • • • • • •	· · · · · · · · · · · · · · · · · · ·	S		V	••• 128
Rabbit		· · · · · · · · · · · · · · · · · · ·		G.	N	V V	••• 126
Cat	A G	A.GG	G	GG.		• • • • • • • • • • • • •	129
House			G	S.G		V	127
Cattle		GWGOPHGG	G	G		V	136
Buffalo		GWGOPHGG	<u> </u>	_			100
				–			· · · 126
			· · · · · · · · · · · · · · · · · · ·				••• 126
	150	160	170	180	190	200	210
Human	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY	170 YRENMHRYFNQ	180 VYYRFMDEYSI	190 NONNFVHDCVN	200 NITIKOHTVTTT	210 TKG 195
Human Chimpanzee	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY	170 YRENMHRYFNQ	180 VYYRFMDEYSI	190 NQNNFVHDCVI S	200 NITIKQHTVTTT	210 TKG 195 195
Human Chimpanzee Rhesus	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN	170 YRENMHRYFNQ	180 VYYRFMDEYSI Q V.Q	190 NQNNFVHDCVN S	200 NITIKQHTVTTT	210 TKG 195 195 195
Human Chimpanzee Rhesus Deer	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN LN	170 YRENMHRYFNQ	180 VYYRFMDEYSI V.Q V.Q.N	190 NQNNFVHDCVN S	200 NITIKQHTVTTT	210 TKG 195 195 195 198
Human Chimpanzee Rhesus Deer Elk	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN LN	170 YRENMHRYFNQ Y Y Y	180 VYYRFMDEYSI V.Q V.Q.N V.Q.N	190 NQNNFVHDCVN S	200 IITIKQHTVTTT V	210 TKG 195 195 195 198 198 198
Human Chimpanzee Rhesus Deer Elk Mouse Part	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN LN LN MN.W	170 YRENMHRYFNQ Y Y Y Y Y	180 VYYRFMDEYSI V.Q V.Q.N V.Q.N V.Q.N V.Q.N	190 NQNNFVHDCVN S	200 IITIKQHTVTTT V	210 TKG 195 195 195 198 198 198 194
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN LN LN MLN.W T.	170 YRENMHRYFNQ Y Y Y Y Y Y Y Y Y Y	180 VYYRFMDEYSI V.Q V.Q.N V.Q.N V.Q.N V.Q.N V.Q.N V.Q.N	190 NQNNFVHDCVN S	200 IITIKQHTVTTT V V V	210 TKG 195 195 195 198 198 198 194 194
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheen	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN LN MN.W MLN.W LN.W	170 YRENMHRYFNQ Y Y Y Y Y Y Y	180 VYYRFMDEYSI V.Q V.Q.N V.Q.N V.Q.N V.Q. V.Q. V.Q. V.Q. V.Q.	190 NQNNFVHDCVY ST T SS.	200 IITIKQHTVTTT 	210 TKG 195 195 195 198 198 198 194 195 199
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN LN MLN.W LN. LN. L.R.N.	170 YRENMHRYFNQ Y Y Y Y Y Y Y Y Y	180 VYYRFMDEYSI V.Q V.Q.N V.Q.N V.Q.N V.Q. V.Q. V.Q. V.Q. V.Q. V.Q. V.Q.	190 NQNNFVHDCVY STT	200 IITIKQHTVTTT 	210 TKG 195 195 195 198 198 194 195 199 199 198
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN LN MLN.W LN. LN. L.R.N L.R.N	170 YRENMHRYFNQ Y YY YY. YY. Y.	180 VYYRFMDEYSI VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ.	190 NQNNFVHDCVY STT	200 IITIKQHTVTTT 	210 TKG 195 195 195 198 198 198 194 195 199 198 198 198
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN LN. MLN. MLN. LN. LN. LN. LN. LN. LN.	170 YRENMHRYFNQ Y YY YY YY YY YY YY	180 VYYRFMDEYSI VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ.	190 NQNNFVHDCVN S	200 NITIKQHTVTTT VV VV VV VV VV VV VV VV VV VV	210 TKG 195 195 195 198 198 194 194 195 199 198 198 198 198
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN LN. MLN.W MLN.W LN. LN. LN. LN. LN. LN.	170 YRENMHRYFNQ Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y	180 VYYRFMDEYSI VQ VQ.N VQ.N VQ.N VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ.	190 NQNNFVHDCVN S T S S R.	200 IITIKQHTVTTT 	210 TKG 195 195 195 198 198 198 198 199 199 198 198 198 198 199 199 199 199 199 199 195
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN LN. MLN.W MLN.W LN. LN. LN. LN. LN. LN. LN.	170 YRENMHRYFNQ Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y	180 VYYRFMDEYSI V.Q V.Q.N V.Q.N V.Q. V.Q. V.Q. V.Q. V.Q. V.Q. V.Q. V.Q. V.Q. V.Q. V.Q. V.Q. V.Q. V.Q.	190 NQNNFVHDCVN S T S S R	200 IITIKQHTVTTT 	210 TKG 195 195 195 198 198 198 198 195 198 195 198 199 198 199 199 202 197
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN LN. MN.W MLN.W LN. LN. LN. LN. LN. LN. LN. LN.	170 YRENMHRYFNQ Y Y Y Y Y Y Y Y Y Y Y	180 VYYRFMDEYSI VQ VQ.N VQ.N VQ.N VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ. VQ.	190 NQNNFVHDCVP S T S S R	200 IITIKQHTVTTT V.V.V.V V.V.V V.V V.V V.V V.V V.V	210 TKG 195 195 195 198 194 194 198 198 198 198 198 198 198 198 198 198 198 198 198 198 198 198 198 199 202 197 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN LN.W MN.W LN.W LN. LN.L LN. LN. LN. LN. LN. LN. LN. LN.	170 YRENMHRYFNQ Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y	180 VYYRFMDEYSI VQ VQ.N VQ.N VQ.N VQ. 	190 NQNNFVHDCVP S T S S R	200 IITIKQHTVTTT 	210 TKG 195 195 195 198 198 194 199 198 198 198 198 198 198 198 198 198 198 198 198 198 198 198 198 198 202 206 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN LN.W MN.W LN.W LN. LN.L LN.L LN.L LN.L LN.L LN.L LN.L	170 YRENMHRYFNQ Y	180 VYYRFMDEYSI V.Q V.Q.N V.Q.N V.Q.N V.Q. 	190 NQNNFVHDCVP S T S S S R 260	200 IITIKQHTVTTT 	210 TKG 195 195 195 198 194 194 198 198 198 198 198 198 198 198 199 198 199 198 199 198 199 202 197 206 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN LN.W MN.W LN.W LN.W LN. LN. LN. LN. LN. LN. LN. LN. LN. LN. LN.	170 YRENMHRYFNQ Y Y Y Y	180 VYYRFMDEYSI V.Q V.Q.N V.Q.N V.Q.N V.Q. 	190 NQNNFVHDCVP S T S S R 260 FVILLISFLI	200 IITIKQHTVTTT 	210 TKG 195 195 195 198 198 194 195 198 198 198 198 198 198 198 198 198 198 198 198 198 198 199 202 197 206 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo Human Chimpanzee	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN LN.W MN.W LN.W LN. LN.L LN.L LN.L LN.L LN.L LN.L LN.L LN.L LN.L LN.L	170 YRENMHRYFNQ YY YY.	180 VYYRFMDEYSI V.Q V.Q.N V.Q.N V.Q.N V.Q.	190 NQNNFVHDCVP ST S S R 260 FVILLISFLII	200 IITIKQHTVTTT 	210 TKG 195 195 195 198 194 194 199 198 198 198 198 198 198 198 198 198 198 198 198 198 198 198 198 198 197 206 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo Human Chimpanzee Rhesus	150 GGYMLGSAMSRP 	160 IIHFGSDYEDRY LN.LN. LN.W. MLN.W. LN. LN. LN. LN. LN. LN. LN. LN. LN. LN. LN. LN.	170 YRENMHRYFNQ YY. Y. Y. Y. Y. Y. Y. Y. Y. Y. Y. Y	180 VYYRFMDEYSI V.Q V.Q.N 	190 NQNNFVHDCVP S T S S R 260 FVILLISFLII	200 IITIKQHTVTTT 	210 TKG 195 195 195 198 194 194 194 198 198 198 198 198 198 198 198 198 198 198 196 197 202 206 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo Human Chimpanzee Rhesus Deer	150 GGYMLGSAMSRP 	160 IIHFGSDYEDRY LN. LN. MLN.W MLN.W. LN. L	170 YRENMHRYFNQ YY YY.	180 VYYRFMDEYSI VQ. 	190 NQNNFVHDCVN S	200 NITIKQHTVTTT 	210 TKG 195 195 198 198 194 194 195 198 194 195 198 198 198 198 198 198 198 197 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo Human Chimpanzee Rhesus Deer Elk	150 GGYMLGSAMSRP 	160 IIHFGSDYEDRY LN. LN. MLN.W MLN.W. LN. L	170 YRENMHRYFNQ YY YY.	180 VYYRFMDEYSI VQ VQ.N VQ.N VQ.N VQ. 	190 NQNNFVHDCVN S T S S R 260 FVILLISFLI	200 NITIKQHTVTTT 	210 TKG 195 195 198 198 194 194 195 198 194 195 198 198 198 198 198 198 198 197 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo Human Chimpanzee Rhesus Deer Elk Mouse	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN. LN. MLN.W LN. L	170 YRENMHRYFNQ YY YY YY YY YY YY YY YY DR Y Y DR Y DR DR Z	180 VYYRFMDEYSI VQ Z50 SSSMVLFSSPI	190 NQNNFVHDCVN S S S R 260 FVILLISFLIN	200 IITIKQHTVTTT 	210 TKG 195 195 195 198 198 198 198 195 198 199 195 198 199 198 198 198 198 198 198 198 198 199 199 202 197 206 206 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo Human Chimpanzee Rhesus Deer Elk Mouse Rat	150 GGYMLGSAMSRP	160 IIHFGSDYEDRY LN. LN. MLN.W LN. L	170 YRENMHRYFNQ YY. Y. Y. Y. Y. Y. Y. Y. Y. Y. Y. Y	180 VYYRFMDEYSI VQ SSMVLFSSPI A S A S A	190 NQNNFVHDCVN S S S 260 FVILLISFLII	200 IITIKQHTVTTT 	210 TKG 195 195 195 198 198 199 199 195 198 199 199 199 198 199 198 199 202 197 206 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo	150 GGYMLGSAMSRP 	160 IIHFGSDYEDRY L. N. L. N. L. N. M. M. ML. N. W. M. L. N. W. L. N. M. L. N. M. L. N. L. N. L. N. L. N. L. N. L. N. L. N. M. L. N. M. M. M. M. M. M. M. M. M. M. M. M. M. M. M	170 YRENMHRYFNQ Y X DGR: X DGR: X X.Y	180 VYYRFMDEYSI VQ 	190 NQNNFVHDCVN S S S 260 FVILLISFLII	200 IITIKQHTVTTT 	210 TKG 195 195 195 198 198 195 198 199 198 198 198 198 198 199 199 199 199 199 197 206 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep	150 GGYMLGSAMSRP 	160 IIHFGSDYEDRY LN. LN. MLN.W LN. Q. QQ.	170 YRENMHRYFNQ YY YY YY YY YY YY YY YY Y Y Y Y X Z40 RESQAYYQR K DGR X X DGR X	180 VYYRFMDEYSI VQ. A.VI. A.VI. 	190 NQNNFVHDCVN S S S 260 FVILLISFLII	200 IITIKQHTVTTT V.V.V.V.V.V.V.V.V.V.V.V.V.V.V.V.V.	210 TKG 195 195 195 198 194 194 194 198 198 198 198 198 198 198 198 199 196 197 206 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rat Pig Sheep Goat	150 GGYMLGSAMSRP 	160 IIHFGSDYEDRY LN. LN. MN.W MLN.W. LN.L	170 YRENMHRYFNQ YY YY YY YY YY YY YY YY N DGR.: X DGR.: X DGR.: X DGR.: X Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y DGR.: X DGR.: X DGR.: X DGR.: X Z Y.	180 VYYRFMDEYSI VQ 	190 NQNNFVHDCVP ST SS 260 FVILLISFLII	200 IITIKQHTVTTT 	210 TKG 195 195 198 198 194 199 198 198 198 198 198 198 198 198 198 198 198 198 198 198 198 198 202 206 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog	150 GGYMLGSAMSRP 	160 IIHFGSDYEDRY LN. LN. MN.W. MLN.W. LN. Q. Q	170 YRENMHRYFNQ Y X DGR.: X DGR.: X DGR.: X P X P X Y X P X Y X X X X Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y X Y Y Y Y Y Y Y Y Y	180 VYYRFMDEYSI VQ. VQ.N VQ.N VQ. 	190 NQNNFVHDCVP S T S S 260 FVILLISFLIN	200 IITIKQHTVTTT V.V.V.V.V.V.V.V.V.V.V.V.V.V.V.V.V.	210 TKG 195 195 198 198 194 199 198 198 198 198 198 198 198 198 198 196 202 202 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat Rabbit Dog Cat Human Chimpanzee Rhesus Deer Elk Mouse Rat Chimpanzee Rhesus Deer Elk Mouse Rat Chimpanzee Catle Cat Cat Cat Cat Cat Cat Cat Cat Cat Cat	150 GGYMLGSAMSRP 220 ENFTETDVKMMEH I. 	160 IIHFGSDYEDRY LN. LN. MN.W. MLN.W. LN. Q. QQ.	170 YRENMHRYFNQ Y X DGR.S X DGR.S X DGR.S X X X X Y X Y X Y X Y X DGR.S X X X X X X Y X	180 VYYRFMDEYSI VQ. VQ.N VQ.N VQ. 	190 NQNNFVHDCVP S S S 260 FVILLISFLIN	200 IITIKQHTVTTT 	210 TKG 195 195 198 198 194 199 198 198 198 198 198 198 198 198 198 196 202 202 206 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cat House	150 GGYMLGSAMSRP 220 ENFTETDVKMME 1 	160 IIHFGSDYEDRY LN. LN. MLN.W MLN.W. LN. LN. LN. LN. LN. LN. LN. LN. 230 RVVEQMCITQYEI Q 	170 YRENMHRYFNQ Y YY YY YY YY YY YY YY Y Y Y XDGR. X.YE.A X.YE.A X.E X.Y X DGR. X.Y.	180 VYYRFMDEYSI V.Q V.Q.N VQ.N VQ.N VQ. 	190 NQNNFVHDCVP S S S 260 FVILLISFLIN FVILLISFLIN	200 IITIKQHTVTTT 	210 TKG 195 195 198 198 194 199 198 198 198 198 198 198 198 198 198 196 197 202 206 206
Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat House Cattle Buffalo Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat Human Chimpanzee Rhesus Deer Elk Mouse Rat Pig Sheep Goat Rabbit Dog Cat Human Chimpanzee Rhesus Deer Elk Mouse Rabbit Deer Cattle Dog Cat Human Chimpanzee Rhesus Deer Elk Mouse Rabbit Deer Cattle Rhesus Deer Elk Mouse Rabbit Dog Cat Human Chimpanzee Rhesus Deer Elk Mouse Rabbit Dog Cat Human Chimpanzee Rhesus Deer Elk Mouse Rabbit Dog Cat Rabbit Dog Cat Rabbit Deer Cattle Rhesus Deer Cattle Rhesus Cattle Rhesus Cattle Rhesus Cattle Rhesus Cattle Rhesus Cattle Rhesus Cattle Rhesus Cattle Rhesus Cattle Cattle Rhesus Cattle Rhesus Cattle Cattle Rhesus Cattle Cattle Rhesus Cattle Cattle Cattle Rhesus Cattle Cattle Cattle Chimpanzee Cattle Cattle Chimpanzee Cattle	150 GGYMLGSAMSRP 220 ENFTETDVKMMEI 	160 IIHFGSDYEDRY L. N. L. N. L. N. W ML. N. W L. N. W L. N. W L. N. L. N. L. N L. N Q	170 YRENMHRYFNQ YY YY YY YY YY YY YY YY X DGR: X DGR: X X X X X X X X X X X X X X Y X DGR: X.	180 VYYRFMDEYSI VQ.N VQ.N VQ.N VQ. 	190 NQNNFVHDCVN S	200 IITIKQHTVTTT 	210 TKG 195 195 198 198 194 199 198 198 198 198 198 198 198 198 198 199 202 197 206 206

Figure 1 Amino acid sequences of PrP in 16 mammals (data from GenBank)

Human (NM_000311.3), chimpanzee (NM_001009093.3), Rhesus (NM_001047152.1), deer (AY330343.1), elk (EU082291.1), mouse (NM_011170.3), rat (NM_012631.2), pig (NM_001008687.1), sheep (NM_001009481.1), goat(JF729302.1), rabbit (NM_001082021.1), dog (NM_001013423.1), cat (EU341499.1), horse(NM_001143798.1), cattle (NM_181015.2), buffalo (KC.1). 137634.

converts into the disease-associated isoform (PrP^{Sc}). Although the primary structures of PrP^C and PrP^{Sc} are the same, their secondary structures are quite different. PrP^C is enriched with α -helix (42% are α -helix, 3% are β -fold), whereas PrP^{Sc} is enriched with β -fold (43% are β -fold, 30% are α -helix) and is protease resistant (McKinley et al, 1983; Pan et al, 1993; Prusiner, 1982). The massive intracellular accumulation of PrP^{Sc} induces formations of oligomer and amyloid fibrils, and eventually neuronal degeneration (Barron et al, 2007; Caughey et al, 2009). PrP plays vital roles in the pathological process of TSE. Knockout and low expression of PrP effectively abolishes or reduces susceptibility to TSEs, respectively (Brandner et al, 1996; Büeler et al, 1993), whereas high expression is associated with susceptibility and a shortened incubation time for disease development (Manson et al, 1994).

TSE susceptibility is species-specific. Previous studies show high susceptibility in hamsters (Mesocricetus *auratus*) to TSE, as they can be infected by various PrP^{Sc} virus strains isolated from human, cattle, goats, mice (Mus musculus) and minks (Bessen & Marsh, 1992; Gibbs & Gajdusek, 1973; Kimberlin & Walker, 1977; Thomzig et al, 2006). Similar high susceptibility is also found in mice (Chandler, 1961; Gibbs & Gajdusek, 1973; Hill et al. 2000; Lasmézas et al. 1997; Thomzig et al. 2006). However, rabbits could not be infected by PrP^{Sc} strains isolated from human, goats and mice (Barlow & Rennie, 1976; Gibbs & Gajdusek, 1973). During the outbreak of BSE in the UK, infections in humans and several species of feline were reported, but no infection was found in dogs (Canis familiaris) or horses (Equus caballus) (Aldhous, 1990; Kirkwood & Cunningham, 1994). Collectively, species with confirmed susceptibility to TSE include humans, rhesus monkeys (Macaca *mulatta*), hamsters, mice, minks, elaphures, moose, goats, sheep, cattle and raccoons (Procyon lotor) (Imran & Mahmood, 2011). Only a few species, such as dogs (Canis familiaris), rabbits (Orvctolagus cuniculus) and horses (Equus caballus) have been recognized as TSE resistant (Fernandez-Funez et al, 2011; Yuan et al, 2013; Zhang, 2011a). Interestingly, although more than 190,000 cattle were infected by BSE, and buffaloes (Bubalus bubalis) and cattle are closely related, no buffalo has been reported with BSE infection (http://www.oie.int) and are of low susceptibility to BSE (Zhao et al, 2012). In this review, based on TSE susceptibility, animals have been classified into TSE susceptible animals and TSE low susceptible animals.

The pathological mechanisms of TSE are yet to be clarified. Although highly susceptible animals are important to understanding this disease, studies on anim. als with low susceptibility provide a new angle from which to examine TSE. Here, we review recent research developments on protein structures, biochemical characteristics and genetic features of four animals (dogs, rabbits, horses and buffaloes) with low susceptibly to TSE.

Dogs

During the outbreak of BSE in the UK, several species of feline were reportedly infected, including cheetahs (Acinonyx jubatus), pumas (Pumaconcolor) and cats (Kirkwood & Cunningham, 1994). Since 1990, about 100 cats and 29 captive felines, including 15 cheetahs, four lions (Panthera leo), three leopard cats (Prionailurus bengalensis), three pumas, three tigers (Panthera tigris) and one Asian golden cat (Catopuma temminckii), have been diagnosed with FSE (Imran & Mahmood, 2011). The presumed infection source was PrP^{Sc}-contaminated food; however, dogs and cats are provided similar food and no dogs were reported with TSE (Imran & Mahmood, 2011; Kirkwood & Cunningham, 1994; Wopfner et al, 1999). With further laboratory cell experiments, dogs have been recognized as a species with low TSE susceptibility. For example, when Madin-Daby canine kidney cells (MDCK) were infected with brain tissue homogenates from CJD patients or RML prion strain isolated from scrapie animals, although the biosynthesis and processing of PrP^C in MDCK are similar with those in N2aPK1 cells of murine neuroblastoma, which are highly susceptible to TSE, no PrP^{Sc} was found in MDCK. When infected MDCK were used to infect N2aPK1 cells, no PrP^{Sc} was found in N2aPK1 cells either (Ploymenidou et al, 2008; Zhang & Liu, 2011).

The gene polymorphism of *PRNP* is correlated with TSE susceptibility (Westaway et al, 1994). In humans, at least 30 mutations of *PRNP* are intertwined with TSE susceptibility (Lloyd et al, 2011). In dogs, the amino acid residue 187 and 229 of the PrP sequence are histidine and glycine, respectively, whereas, they both are arginine in cats (Wopfner et al, 1999). No FSE-related polymorphic site was found by screening encoding sequences of *PRNP* in 609 animals (including 15 FSE infected cases) and 29 species from 22 genera of the Order Carnivora, but Stewart et al (2012) did notice that amino acid residue 163 in all canines is either aspartate or glutamic acid, indicating this locus may have some

connection to TSE susceptibility.

The three-dimensional structure of PrP^C may be another tool in resolving the puzzle of TSE susceptibility (Lin & Wen, 2011). To understand the structural differences of PrP^C in animals with low and high susceptibility to TSE, Lysek et al (2005) carried out a study on nuclear magnetic resonance (NMR) structures of PrP^C in dogs (canine PrP, cPrP), cats (feline PrP, fPrP), pigs (sus scrofa PrP, scPrP) and goats (ovine PrP, ovPrP). Their overall three-dimensional structures are quite close, consisting of a N-terminal (constituted of about 100 amino acid residues in random coil) and a globular domain in the C-terminal (including three α -helixes and a pair of short, reverse paralleled β-folds constituting about 100 amino acid residues). The globular domain in the Cterminal is species-specific, e.g., four amino acids (Asp159Asn, Arg177His, Lys185 Arg and Gly229Arg) are different between cPrP^C and fPrP^C; Asp-159 and Arg-177 in cPrP^C make it unique in potential distribution; in fPrP^C, scPrP^C and ovPrP^C same positive potential distribution patterns are observed in their C-terminals.

The conversion of PrP^{C} into PrP^{Sc} is critical in TSE pathogenesis. Besides PrP^{C} and PrP^{Sc} , the existence of

the third state- β intermediate state, consisting mainly of β-fold in circular dichroism (CD) (Hornemann & Glockshuber, 1998), has raised attention due to its capability in introducing TSE (Collinge & Clarke, 2007). Khan et al (2010) claimed that in different species, the propensities in forming the β intermediate state are one of the vital factors influencing TSE susceptibility. Using dual wavelength CD, Khan et al (2010) compared the structures of the globular domain in high-susceptible (hamsters and mice) and low-susceptible species to TSE (rabbits, horses and dogs) and found that under inducing conditions with different pH values and urea concentrations, the propensities of forming the β intermediate state vary in different species. At pH 7.0, urea concentrations have no effect on PrP^{C} and no β intermediate state is observed in all five species; at pH 5.0, the structure of hamster PrP^C (hPrP^C) is most unstable and easily forms the β intermediate state; at pH 4.0, the PrP^C in all five species is unstable and easily forms the β intermediate state, the lowest concentration of β intermediate state occurs in dogs (Figure 2). The propensities in forming the β intermediate state (hamsters > mice > rabbits > horses > dogs) can also be adopted in evaluating



Figure 2 Propensities of conversions of PrP^{C} into the β intermediate state in different species at pH 7.0 (A), 5.0 (B), 4.5 (C) and 4.0 (D) and different concentrations of urea (modified from Khan et al, 2010)

species' susceptibilities to TSE (Fernandez-Funez et al, 2011). Using molecular kinetic methods, Zhang & Liu (2011) found stable molecular structures of wild cPrP^C under both neutral and acidic pH conditions, and in the neutral condition the salt bridge between D177 and R163 improves structural stability. These studies help to explain the mechanism of low TSE susceptibility in dogs and general TSE pathogenesis.

Rabbits

No cases of spontaneous TSE infection in rabbits have been reported to date. In 1973, Gibbs & Gajdusek failed to infect rabbits with either brain tissues from human CJD or Kuru patients or brain tissues from scrapie animals and minks with TME. In 1976, Barlow & Rennie failed to infect rabbits with ME7 strains isolated from animals with scrapie. By constructing rabbit PrP^C (RaPrP^C) over-expressed murine neuroblastoma tumor cell lines, Vorberg et al (2003) confirmed that RaPrP^C can neither be infected by RML strains nor convert into the PrPSc. The in vivo experiments conducted by Fernandez-Funez et al (2010) support the view that rabbits are TSE resistant species. Fernandez-Funez et al (2010) expressed full length PrP of hamsters, mice and rabbits in drosophilae voided of endogenous PrP, and found that cavernous transformation and isomers similar with PrPsc can only be found in the brains of transgenic drosophilae expressing shPrP^C and mouse PrP^C (moPrP^C), but not in drosophilae expressing RaPrP^C. Moreover, Bellotti & Chiti (2008) reported that TSE correlates with the deposition of amyloid fibrils. Zhou et al (2011) found that Ficoll 70 and dextran 70 significantly accelerate the fibration of hPrP^C and bPrP^C, but prevent fibration of RaPrP^C; however, different from hPrP^C and bPrP^C, RaPrP^C does not have fragments resisting protease K digestion.

Initially it was presumed that one of the possible reasons rabbits are resistant to TSE infection is that certain transforming factors are lacking in their cellular environment or some inhibitory factors are expressed and the conversion of PrP^C to PrP^{Sc} is prevented. This presumption was later proved wrong. When PK13 cells, expressing shPrP^C, moPrP^C and vole (*Microtus spp.*) PrP^C, respectively, were infected with shPrP^{Sc}, moPrP^{Sc} and vole PrP^{Sc} were observed, indicating that factors necessary for PrP conversion exist in rabbit cells (Courageot et al, 2008;

Vilette et al, 2001). Rabbits and mice share 87% similarity in amino acid sequences (33 amino acids are different, including 22 in mature peptides). When amino acid residues 99, 108, 173 and 214 in moPrP^C were mutated into the corresponding amino acid residues in RaPrP (Asn99Gly, Leu108Met, Asn173Ser and Val214Ile, respectively) and were overexpressed in mouse neuroblastoma (MNB), and then RML prion strains were used to infect MNB, the mutants of moPrP^C could not convert into PrPSc, indicating that several amino acid residues in RaPrP^C can prevent the replication of isomers of PrP^{Sc} (Vorberg et al, 2003). About 33% of the different amino acids in moPrP^C and RaPrP^C locate around the attachment site of the GPI-anchor. Nisbet et al (2010) stably transfected RK13 cells voided of endogenous PrP^C with a constructed double-mutant (Ser230Gly and Ser231Val) model of moPrP^C, MoPrP-RbGPI, and then infected RK13 cells expressing MoPrP-RbGPI using human prion strains M1000 or MU-02 isolated from mice brain homogenates. The results showed that MoPrP-RbGPI could change neither the attachment of the GPI-anchor nor the location of PrP^C on cells, but no PrP^{Sc} produce either, indicating that rabbit-specific amino acids may interfere with PrP^{Sc} and PrP^C contact and eventually prevent conversion of PrP^C to PrP^{Sc} (Nisbet et al, 2010). These findings indicate that the resistance of rabbits to TSE may be attributable to their unique RaPrP^C structure (Lin & Wen, 2011). Wen et al (2010a) adopted NMR techniques to study the solution structure of RaPrP^C and found that compared to hPrP^C, $mPrP^{C}$ and $bPrP^{C}$. $RaPrP^{C}$ features a unique charge distribution pattern. A large consecutive positive potential area exists on the protein surface of RaPrP^C which may interfere to interact with molecules such as chaperon protein X, prevents the proliferation of PrP^{Sc} (Wen et al, 2010a). Khan et al (2010) found a critical helix-capping motif interacting with the third α -helix and regulating the β -intermediate state by exploring the crystal structure of RaPrP^C. As we mentioned earlier, the complexities of PrP^{C} forming the β -intermediate state in different species are correlated with their susceptibility to TSE. Compared with $ShPrP^{C}$ and $moPrP^{C}$, $RaPrP^{C}$ is difficult to transform into the β -intermediate state (Figure 2) (Khan et al, 2010). The irregular curling fragment, called an $\alpha 2$ - $\beta 2$ loop, locates between the second β -fold and the second α -helix (165-172). The epitope consisting of the $\alpha 2$ - $\beta 2$ loop and the C-terminal of the third α -helix is considered capable of recognizing protein X and

regulating progression of TSE (Kaneko et al, 1997). Protein dynamics analysis shows that RaPrP^C has a constructively highly ordered $\beta 2-\alpha 2$ loop (Wen et al, 2010a) which may function as a species barrier for TSE dissemination (Lin & Wen, 2011). However, compared with wild RaPrP^C, S173N and I124V mutations affect the interactions of the $\beta 2-\alpha 2$ loop with the third α -helix, and thereafter decrease the stability of the entire construct (Wen et al, 2010a, b). In addition, when the salt bridges between D202-R156 and D178-R164 were removed in hPrP^C and moPrP^C, although secondary protein structures remained intact, the helix structures of RaPrP^C were destroyed, indicating that salt bridges are important to the stability of RaPrP^C (Zhang, 2009, 2010, 2011a).

Recently, Joaquin Castilla's research group has raised questions about the view that rabbits are resistant to TSE. They amplified rabbit brain homogenates using serial automated protein misfolding cyclic amplification (saPMCA) and then inoculated this in vitro novel PrP into the brains of three other rabbits. One rabbit was found with TSE symptoms 766 days after inoculation even although no exogenous PrPSc was involved. Then the brain homogenates from this infected rabbit could 100% infect RaPrP^C over-expressed transgenic mice. Therefore, Chianini et al (2012) claims that rabbits are not TSE resistant. Furthermore, using saPMCA, when the amplified proteins from mixtures of rabbit brain homogenates and BSE prion strains were inoculated into the brains of RaPrP^C over-expressed transgenic mice, the resultant strains similar to BSE prion strains were discovered (Vidal et al, 2013). Fernández-Borges et al (2012) claims that in vivo infective experiments are imperfect when forming the conclusion that rabbits are resistant to TSE, especially when supported only by the observation that rabbits can not be infected with TSE naturally.

Horses

As there is no reports of horses being naturally infected with TSE, horses are recognized as low susceptibility species (Zhang, 2011a). Relative to dogs and rabbits, fewer studies have looked at low susceptibility in horses. Studies on the conversion of PrP^{C} into the β intermediate state show that under unstable conditions at pH 4, the PrP^{C} of hamsters, mice, rabbits, dogs and horses can convert into the β intermediate state, but the lowest level of β intermediate state is found in horses (Figure 2), indicating that equus caballus PrP^C (ecPrP^C) is relatively stable (Khan et al, 2010). Structural NMR on ecPrP^C found two horse-specific amino acid alterations in its $\beta 2-\alpha 2$ loop (Ser-167 and Lys-173, respectively), among which, S167 affects the highly ordered solution structure of the $\beta 2-\alpha 2$ loop and may influence the low susceptibility of horses to TSE (Pérez et al, 2010). However, when amino acid residue 167 in moPrP^C was mutated from asparagine into ecPrP^Cspecific serine (MoPrP^{D167S}), although MoPrP^{D167S} and ecPrP^C share similar NMR structures and their $\beta 2-\alpha 2$ loops in solutions are both relatively highly ordered, spongiform lesions were found in mice expressing MoPrP^{D167S} and neural diseases can be induced with the accumulation of PrP^{Sc} in the brain (Sigurdson et al. 2011). Therefore, the ordered state of the $\beta 2-\alpha 2$ loop in solution alone does not fully explain different susceptibilities to TSE (Lin & Wen, 2011). Moreover, as the salt bridge in RaPrP^C stabilizes protein structures, similar salt bridges consisting of GLU196-ARG156-HIS187, ARG156-ASP202 and GLU211-HIS177 are also found in ecPrP^C (Zhang, 2011b). The structures of $ecPrP^{C}$ and $cPrP^{C}$ are stable under both neutral and acidic conditions (Zhang, 2011a). A common phenomenon found among RaPrP^C, ecPrP^C and cPrP^C, is the salt bridge ASP177-ARG163 connects with the $\beta 2-\alpha 2$ loop of PrP and is probably correlated with TSE susceptibility (Zhang, 2011a). Nevertheless, the low susceptibility of horses to TSE requires further work.

Buffalo

BSE was initially found in the UK in 1986, rapidly spread to over 25 countries, and caused major economic losses (Harman & Silva, 2009; Wells et al, 1987). BSE can also infect humans via the food chain and cause human vCJD (Collinge et al, 1996; Hill et al, 1997). The multiple pathogenic pathways of TSE, including spontaneous mutant, inheritance and infection (Nicholson et al, 2008), may explain why even after meat and bone meal was strictly forbidden, more than 15 000 BSE infected cattle were found in the UK (http://www.oie.int). Worldwide, there were over 190 000 taurus cattle, 1 Bos indicus, and 1 Bos indicus × Bos taurus cross reported with BSE infections (data of OIE, Novakofski et al, 2005; Seuberlich et al, 2006). Although buffaloes and cattle are quite close phylogenetically, no case of BSE infected buffalo was ever reported, suggesting that genetic factors

are crucial to BSE susceptibility (Zhao et al, 2012). The expression level of PrP is closely correlated with BSE susceptibility. Studies show that the PRNP gene of cattle has two indel (insertion and deletion) polymorphisms (a 23-bp indel in putative promoter, and a 12-bp indel in intron 1). These Indel polymorphisms affect gene expression (Msalya et al, 2011; Sander et al, 2005) and eventually BSE susceptibility (Haase et al, 2007; Juling et al, 2006; Sander et al, 2004). Studies on polymorphisms in buffalo in Anatolia (Oztabak et al, 2009), Pakistan (Imran et al, 2012), Indonesia and Thailand (Uchida et al, 2014) show signifycant differences in frequency distributions between buffaloes and cattle. Recently, genotyping analysis on Chinese buffalo showed that the distribution frequencies of BSE susceptibility related to genotypes and alleles, including the 23-bp deletion allele (D_{23}) and 12-bp deletion allele (D_{12}) , were significantly lower than those of healthy cattle and BSE infected cattle, indicating that the low PrP expressed in buffalo may influence BSE susceptibility. Our later experiments proved that in tissue of the cerebellum, brain stem, mesenteric lymph nodes and bronchial lymph nodes, the expression of PrP is lower in buffalo than in cattle (submitted data).

Although PRNP play a vital role in the pathogenesis of TSE, the underlying pathological mechanisms of TSE remain unclear. Some propose that other than prions, there may be other factors or proteins regulating the pathogenesis and pathological process of TSE (Daude & Westaway, 2011; Watts et al, 2007). The SPRN (shadow of prion protein) gene and its encoded protein Shadoo (Sho) have drawn lots of attention due to their roles in the pathogenesis of TSE. Comparative genomics analysis indicates that Sho is a newly discovered member of the prion protein family. Sho has been found in mammals such as mice and humans and is highly conserved from fish to mammals (Premzl et al, 2003). Sho and PrP^C have a lot in common regarding structure and expression (Wang et al, 2014). In PrPSc infected animal brains or nervous cell, with increasing PrPSc expression, the level of Sho decreases dramatically (Watts et al, 2007, 2011; Westaway et al, 2011). Beck et al (2008) reported that the insertion of a base (heterozygous) within the encoding area of SPRN induces a frame-shift mutation which is correlated with vCJD. So, it is highly possible that Sho regulates the process of TSE by functioning as an inhibitory factor (Daude & Westaway, 2011). Our analysis of differences in the genetics and expression of SPRN between buffalo and cattle show that in the

hydrophobic domain (HD) within the encoding area, cattle have a 12-bp indel polymorphism which induces insertion/deletion of four amino acids. However, this phenomenon was not observed in buffalo (Zhao et al, 2012). The HD of Sho not only protects against physiological stressors in nervous cell, but also helps Sho to interconnect with PrP^C (Wang et al, 2010). This interconnection is a prerequisite of Sho regulating the pathogenesis of disease (Wang et al, 2010). The exploration of the indel polymorphism within the Sho HD structural area is critical in fully understanding underlying mechanisms of TSE. Our luciferase reporter and immuno-blotting experiments confirm that compared to cattle, buffaloes have higher promoter activity and higher Sho expression, consistent with our prediction that buffalos have more transcription factor binding sites than cattle (Zhao et al, 2012). These findings suggest that the low susceptibility of buffalos to BSE is probably attributable to significant genetic differences in SPRN.

Further Research

Joaquin Castilla's research group denies there are TSE resistant mammals, and believes that with improvements in detection any species can be found to be at risk of TSE infection (Fernandez-Borges et al, 2012). However, from available data, TSE susceptibility does vary between species and we can classify animals as high susceptibility species and low susceptibility species. Scientists worldwide have applied various techniques to the study of TSE pathogenesis and have mainly focused on the genetic polymorphism of *PRNP*, expression levels of PRNP/PrP, three-dimensional structure and stability of PrP^C and dynamics of *PRNP*. However, the pathogenesis of TSE remains unclear. Lin & Wen (2011) claim that the of PrP^{Sc} three-dimensional structure and the physiological function of PrP^C are keys to resolving this puzzle but these two research directions have proved extremely difficult, even after two decades of attention. With breakthroughs in novel technologies and methods however, progress is likely. Studies on TSE low susceptibility molecules and newly discovered SPRN (Wang et al, 2014) provide important clues about the formation of PrPSc and our understanding of TSE pathogenesis. Due to similarities in Sho and PrP^C regarding structure and function, especially their important roles in the pathogenesis and development of TSE, exploration of the biological functions of Sho and its regulatory effect on TSE will be vital.

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