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Polymorphisms in genes *CTSB, CTSD, CAPN2, KLK1* and *TGFB1* not associated with susceptibility to atypical or classical ovine scrapie

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Abstract

In the present study polymorphisms in the genes cathepsin B (*CTSB*), cathepsin D (*CTSD*), calpain, large polypeptide L2 (*CAPN2*), kallikrein 1 (*KLK1*) and transforming growth factor β1 (*TGFB1*) were investigated for association with scrapie susceptibility in sheep. Therefore single nucleotide polymorphisms in the respective genes were identified and examined for a potential impact on the gene function with different computer programs. Samples of 72 atypical and 104 classical scrapie cases as well as of 443 clinically healthy flock mates were genotyped by PCR-based screening methods. Neither allele frequencies nor genotype frequencies showed significant differences between scrapie positive sheep and control animals in any of the investigated genes.

Keywords: scrapie, cathepsin, calpain 2, kallikrein 1, transforming growth factor β1

Zusammenfassung

Polymorphismen in den Genen *CTSB, CTSD, CAPN2, KLK1* und *TGFB1* sind nicht mit Empfänglichkeit für atypische oder klassische ovine Scrapie assoziiert

In der vorliegenden Studie wurden Polymorphismen in den Genen Cathepsin B (*CTSB*), Cathepsin D (*CTSD*), Calpain, large polypeptide L2 (*CAPN2*), Kallikrein 1 (*KLK1*) und Transforming growth factor β1 (*TGFB1*) auf eine Assoziation mit Scrapieempfänglichkeit beim Schaf untersucht. Dazu wurden Einzelbasenpaaraustausche in den genannten Genen identifiziert und mit Hilfe verschiedener Computerprogramme auf einen möglichen Einfluss auf die Genfunktion analysiert. Proben von 72 atypischen und 104 klassischen Scrapiefällen sowie 443 klinisch gesunden Herdenmitgliedern wurden durch PCR-basierte Untersuchungsmethoden genotypisiert. Weder Allelfrequenzen noch Genotypfrequenzen zeigten signifikante Unterschiede zwischen Scrapie-positiven Schafen und Kontrolltieren in den untersuchten Genen.

Schlüsselwörter: Scrapie, Cathepsin, Calpain 2, Kallikrein 1, Transforming growth factor β1

Introduction

It has been indicated by several studies on quantitative trait loci (QTLs) in mouse and sheep that scrapie susceptibility is influenced by further gene loci besides the well known effects of prion protein (PrP) gene (*PRNP*) variants (LLOYD *et al.* 2001, MORENO *et al.* 2008, MORENO *et al.* 2003, STEPHENSON *et al.* 2000). This is interesting, especially in the case of atypical scrapie, where association between PrP haplotypes and susceptibility to the disease is not as stringent as in classical scrapie (LÜHKEN *et al.* 2007, MADEC *et al.* 2004, MOUM *et al.* 2005, ORGE *et al.* 2004).

The results of ARNOLD *et al.* (1995) and SUPATTAPONE *et al.* (1999, 2001) give evidence for a potential involvement of the endosomal cysteine protease cathepsin B (CTSB) in degradation of the abnormal prion protein isoform (PrP^{Sc}), which is underlined by the investigations of LUHR *et al.* (2004a, 2004b) who found out that degradation of PrP^{Sc} in scrapie infected neurons is carried out by cysteine proteases at a sour pH and that addition of a selective *CTSB* inhibitor leads to a significant increase of the PrP^{Sc} content.

Concerning the lysosomal aspartate protease cathepsin D (CTSD), several expression studies reported an elevated concentration of the respective enzyme within the course of scrapie infection in mice (BROWN *et al.* 2004, RIEMER *et al.* 2004, XIANG *et al.* 2004). Furthermore, in brains of sporadic Creutzfeldt-Jakob disease patients a co-localisation of CTSD with intra- and perineural PrP^{Sc} deposits was observed (KOVACS *et al.* 2007).

The ubiquitous cysteinprotease calpain 2 (CAPN2) as well might play a role for scrapie susceptibility, as the inhibition of calpain prevented the accumulation of PrP^{Sc} in scrapie infected mouse brain cells and resulted in reduced prion titers (YADAVALLI *et al.* 2004). Here the elevation of C2, a cleavage product of PrP^{Sc}, was most efficiently suppressed by calpain inhibitor IV, an inhibitor of calpain 2 (ANGLIKER *et al.* 1992, YADAVALLI *et al.* 2004). In this context the content of C1, a fragment of PrP^{C} which is present in the healthy brain, recovered to a normal level (YADAVALLI *et al.* 2004).

MORENO *et al.* (2003) found a QTL for scrapie resistance on mouse chromosome 7, in which murine paralogues of the kallikrein 1 gene (*KLK1*) as well as the transforming growth factor beta gene (*TGFB1*) are located. Results of HOU *et al.* (2005) and IWADATE *et al.* (2003) indicated that the serine protease KLK1 stimulates the release of insulin like growth factor 1 (IGF1), which plays a neuroprotective role in neurodegenerative disorders like Alzheimer disease (DORÉ *et al.* 1997). This is of interest as in the course of a scrapie infection the binding of IGF1 to its receptor is decreased (ÖSTLUND *et al.* 2001).

For TGFB1 BOCHE *et al.* (2006) claim a protective role with regard to transmissible spongiform encephalopathies. Furthermore in several models for experimental prion disorders elevated levels of TGFB1 have been detected (BAKER *et al.* 1999, WALSH *et al.* 2001).

Therefore in the present study the genes cathepsin B (*CTSB*), cathepsin D (*CTSD*), calpain, large polypeptide L2 (*CAPN2*), kallikrein 1 (*KLK1*) and transforming growth factor β1 (*TGFB1*) were investigated for sequence variants and for their association with scrapie susceptibility.

Materials and methods

Samples from 72 atypical and 104 classical scrapie positive sheep as well as 443 clinical healthy flock mates who served as control group were examined. Initially scrapie cases were identified by Bio-Rad Platelia rapid test, Prionics Check Western Blot test, or by Enfer TSE test. Scrapie diagnosis was confirmed and completed with the scrapie type by either SAF-immunoblotting, IHC or both methods (BUSCHMANN *et al.* 2004, GRETZSCHEL *et al.* 2005).

DNA extraction was performed as described earlier (LÜHKEN *et al.* 2004). For sequence analysis primers were chosen from GenBank sequences of cattle and sheep and PCR was performed (Table 1). Sequence analysis was outcared at the ABI PRISM 377 sequencer (*Applied Biosystems, Darmstadt, Germany*) according to manufacturer's instructions.

Table 1
Primers and PCR conditions used for sequence analysis of *CTSB*, *CTSD*, *CAPN2*, *KLK1* and *TGFB1*
Für die Sequenzanalyse von CTSB, CTSD, CAPN2, KLK1 und TGFB1 verwendete Primer und PCR-Bedingungen

Gene	Primer sequences (5´ —> 3´) f=forward, r=reverse	PCR conditions	Annealing temperature, °C/ elongation time, s/ number of cycles
CTSB	GGA ACA CTG ATT GGG GTG AC (f)[1] ATT CCA CAG TGG TCC TGT CC (r)[1]	Standard PCR conditions[6]	58 / 45 / 35
CTSD	CTG TGA GGC TAT CGT GGA CA (f)[2] CTC AGG CAC ACG GTC GTC (r)[2]	Standard PCR conditions[6] and 10 % (v/v) DMSO	50 / 60 / 35
CAPN2	AGT GGG AAA CCG GCT AAA GT (f)[3] TGC TGG AGT AAG GTC CCA AC (r)[3]	Standard PCR conditions[6] and 10 % (v/v) DMSO	57 / 35 / 40
KLK1	CCT GTT TGA GGA CGA AGA CAC (f)[4] GGT GTA GAC GGA GGG CTT ATT (r)[4]	Standard PCR conditions[6], 25 nmol MgO(Ac)2 added	65 / 80 / 40
TGFB1	AGA ACT GCT GTG TTC GTC AGC TC (f)[5] GTT GGA CAA CTG CTC CAC CTT G (r)[5]	Standard PCR conditions[6] and 5 % (v/v) DMSO	64 / 60 / 40

[1]NM_174 031 (Bta), [2]AF164 143 (Oar), [3]XM_864 105 (Bta), [4]AY290 705 (Bta), [5]NM_001009400 (Oar), Bta=*Bos taurus*, Oar=*Ovis aries*, [6]40-50 pmol of each primer, 30-90 ng template DNA, 1-1,75 U *Taq* DNA polymerase, 640-800 µM dNTPs in 1 fold reaction buffer supplied by the manufacturer in a total volume of 50 µl

Identified sequence variants in genes *CTSB*, *CTSD* and *KLK1* were genotyped in scapie positive and control animals by PCR-restriction fragment length polymorphism (RFLP) analysis with restriction enzymes *Lwe*I, *Bse*YI and *Bsm*FI respectively (Table 2). The single nucleotide polymorphism (SNP) in *TGFB1* was typed by PCR-RFLP after generation of an amplification created restriction site (ACRS) (HALIASSOS *et al.* 1989) with enzyme *Bse*GI (Table 2). For typing of the SNP in the *CAPN2* gene two allele specific PCRs were carried out, both containing the respective specific primer and the corresponding reverse primer each and additionally an extra forward primer to create a smaller control fragment (Table 2). As in some cases the allele specific PCR failed to detect the wildtype allele (G) in heterozygous animals, all probes assumed to be homozygous for TT were double checked by nested-PCR-RFLP with the enzyme *Mva*I (Table 2). Allele and genotype frequencies were calculated for the scrapie positive sheep (atypical and classical) and their healthy controls (from flocks affected by atypical or classical scrapie respectively) separately and compared using the chi square test and Fisher´s exact test (SAS v. 8.01, Institute Inc., Cary, NC, USA). Furthermore in respect to every SNP the Hardy-Weinberg equilibrium in the test population was estimated (HARDY

1908). Single nucleotide polymorphisms in exons or promoter regions of the respective genes were examined for a potential impact on the gene function by the use of computer programs MatInspector (CARTHARIUS *et al.* 2005), ESEfinder 3.0 (SMITH *et al.* 2006), and Sequence Manipulation Suite: CpG Islands (STOTHARD 2000), respectively.

Table 2
Methods used for genotyping of SNPs within the genes *CTSB*, *CTSD*, *CAPN2*, *KLK1* and *TGFB1*
Für die Genotypisierung der SNPs in den Genen CTSB, CTSD, CAPN2, KLK1 und TGFB1 verwendete Methoden

Gene	Primer sequences (5´—> 3´) f=forward, r=reverse	Genotyping method	PCR conditions	Ann. temp., °C/ Elong. time, s/ n of cycles
CTSB	GGA ACA CTG ATT GGG GTG AC (f) ATT CCA CAG TGG TCC TGT CC (r)	RFLP with enzyme *Lwe*I	Standard PCR conditions[7]	58 / 45 / 35
CTSD	GGA GGA CTA CAC GCT CAA GG (f) CTC AGG CAC ACG GTC GTC (r)	RFLP with enzyme *Bse*YI	Standard PCR conditions[7]	55 / 60 / 40
CAPN2	CCG CTT TTC CAC CGG GAT GCT CT (f)[1,6] GGT TGA GGT ACT TGA CCG CCC TCT CG (r)[1] GCT TTT CCA CCG GGA TGC TCG (f)[2,6] GGG AAG GAG GGG TCC TGG AAC AG (r)[2] CCG CGG GAC CCG GTG AAT CAT (f)[3]	Allele specific PCR[8]	Special PCR conditions[9]	PCR with Primer[1+3]: 72 / 60 / 45 PCR with Primer[2+3]: 66 / 60 / 50
CAPN2	AGT GGG AAA CCG GCT AAA GT (f)[4] TGC TGG AGT AAG GTC CCA AC (r)[4] GGA TTT GAG TCC CCG CTT TTC (f)[5] GGC AGC GGG ACT GTG AGC (r)[5]	Nested PCR-RFLP with enzyme *Mva*I	PCR No.1 with special PCR conditions[10], the amplicon was then included in PCR No.2 with special PCR conditions[11]	PCR No.1: 55.5 / 40 / 45 PCR No.2: 60.5 / 35 / 45
KLK1	CCA CAC ACA TCA GAG TTC ACG (f) GGT GTA GAC GGA GGG CTT ATT (r)	RFLP with enzyme *Bsm*FI	Standard PCR conditions[7]	65 / 45 / 45
TGFB1	GGA GAA AGA AAA GGA GAC GGA T (f)[6] CTC CCC CTG TCT TAT CTC ATT C (r)	ACRS with enzyme *Bse*GI	Standard PCR conditions[7], 15 nmol $MgO(Ac)_2$ added	63 / 30 / 45

Ann. temp.=Annealing temperature, Elong. t.=Elongation time, No. cycles=Number of cycles, [1]specific forward primer (f) used for amplification of the allele T and corresponding reverse primer (r), [2]specific forward primer (f) used for amplification of the allele G and corresponding reverse primer (r), [3]forward primer (f) set in both reactions to create a control fragment with the respective reverse primer, [4]outer primer, [5]inner primer, [6]base pair mismatches are indicated by lower case letters, [7]15 pmol of each primer, 7 -10 ng template DNA, 0,3- 0,6 U *Taq* DNA polymerase, 640-800 µM dNTPs in a 1 fold reaction buffer supplied by the manufacturer in a total volume of 15 µl, [8]two independent reactions for amplification of the T allele, G allele respectively, [9]24 pmol of respective specific forward and corresponding reverse primer each, 12 pmol of extra forward primer to create control fragment, 8 ng template DNA, 0,5 U *Taq* DNA polymerase, 800 µM dNTPs, 10 % (v/v) DMSO in a 1 fold reaction buffer supplied by the manufacturer in a total volume of 12 µl, [10]10 pmol of each outer primer, 0,35 U *Taq* DNA polymerase, 800 µM dNTPs, 10 % (v/v) DMSO in a 1 fold reaction buffer in a total volume of 10 µl, [11]16 pmol of each inner primer, 0,5 U *Taq* DNA polymerase, 800 µM dNTPs, 10 % (v/v) DMSO in a 1 fold reaction buffer supplied by the manufacturer in a total volume of 16 µl

Results and discussion

Sequence analyses led to the identification of several SNPs in the investigated genes, of which one per gene was used for the association analysis (Table 3).

Table 3
Genotype frequencies (%) of atypical and classical scrapie cases and control animals regarding SNPs in the genes *CTSB*, *CTSD*, *CAPN2*, *KLK1* and *TGFB1*
Genotypfrequenzen (%) der atypischen und klassischen Scrapiefälle und der Kontrolltiere im Bezug auf die SNPs in den Genen CTSB, CTSD, CAPN2, KLK1 und TGFB1

Gene and position[1] of SNP	GenBank Acc. No.	Genotype	Atypical scrapie		Classical scrapie	
			cases	controls	cases	controls
CTSB	GQ355936	CC	60.6	62.1	67.0	67.4
Intron9 (403)		CT	31.8	32.5	32.0	29.0
C>T		TT	7.6	5.4	1.0	3.6
CTSD	GQ355937	CC	31.3	33.5	20.2	31.4
Exon9 (385)		CT	46.3	45.9	54.5	44.0
C>T		TT	22.4	20.6	25.3	24.6
CAPN2	GQ355935	GG	55.9	51.3	50.5	55.4
5´-UTR (26)		GT	36.8	38.8	38.4	36.7
G>T		TT	7.3	9.9	11.1	7.9
KLK1	GQ355938	CC	60.9	61.6	69.7	79.1
Intron4 (889)		CT	34.8	31.7	27.3	20.1
C>T		TT	4.3	6.7	3.0	0.8
TGFB1	GQ355939	GG	88.2	81.8	94.2	96.3
Intron6 (225) G>T		GT	10.3	17.2	5.8	3.7
		TT	1.5	1.0	0.0	0.0

[1]position in respective GenBank sequence

At position 12 of *CTSD* exon IX a silent mutation (C>T) was detected. In case of the *C* allele we detected a potential exon splicing enhancer (ESE) (GGC CGG G) at this location. The location near the exon-intron boundary is typical for exonic splice control elements (MAJEWSKI and OTT 2002). In case of the *T* allele the score for this ESE drops below the threshold. As ESEs are of great importance for correct splicing, it is possible that the destruction of the respective ESE causes an exon skipping of exon IX as it has been described for a number of silent mutations in other genes before (CARTEGNI *et al.* 2002).

In ovine *CAPN2* the promoter and part of the first exon were investigated. Computer analysis revealed that the whole region spanned a CpG island including two G/C boxes. In the promoter region at position 191 in front of exon I a single nucleotide polymorphism (G>T) was detected. In case of the *G* allele a potential binding site (TGC CCG GGG TGC T) for a transcription repressor was identified, which could not be detected for the mutated *T* allele as the conserved »GGGG« core is essential for it's binding activity (BRESLIN *et al.* 2002). At position 391 of *CTSB* intron 9, position 392 of *KLK1* intron 4 and position 206 in front of exon VII in intron 6, respectively, a single nucleotide polymorphism (C>T), (C>T), (G>T), respectively, was detected. No evidence could be found for any of these mutations to have a potential impact on the gene function. Significant differences between scrapie positive

sheep and control animals were identified neither in allele nor in genotype frequencies for any of the investigated genes.

In contrast to the genes analyzed above, significant differences had been observed between *PRNP* genotype frequencies of the classical and of the atypical scrapie positive sheep and their healthy flock mates in a previous study (LÜHKEN *et al.* 2007), which included the samples of the present study. German sheep breeds differ in their *PRNP* genotype frequencies (DRÖGEMÜLLER *et al.* 2001); thus the breed was considered when samples from healthy flock mates were selected in order to minimize stratification effects. However, any of the five analyzed genes is located on chromosome 13, where *PRNP* has been mapped (LÜHKEN *et al.* 2006). Therefore no genetic linkage between *PRNP* and the analyzed genes could have affected the results of the association analysis. In fact, the population was found to be completely in Hardy Weinberg equilibrium for all analyzed SNPs.

In conclusion, no evidence for an association of SNPs in the genes *CTSB*, *CTSD*, *CAPN2*, *KLK1* and *TGFB1* with scrapie susceptibility could be detected. Further studies may show if other sequence variants in these candidate genes have an impact on the trait under study.

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References

Angliker H, Anagli J, Shaw E (1992) Inactivation of calpain by peptidyl fluoromethyl ketones. J Med Chem 35, 216-20

Arnold JE, Tipler C, Laszlo L, Hope J, Landon M, Mayer RJ (1995) The abnormal isoform of the prion protein accumulates in late-endosome-like organelles in scrapie-infected mouse brain. J Pathol 176, 403-11

Baker CA, Lu ZY, Zaitsev I, Manuelidis L (1999) Microglial activation varies in different models of Creutzfeldt-Jakob disease. J Virol 73, 5089-97

Boche D, Cunningham C, Docagne F, Scott H, Perry VH (2006) TGFbeta1 regulates the inflammatory response during chronic neurodegeneration. Neurobiol Dis 22, 638-50

Breslin MB, Zhu M, Notkins AL, Lan MS (2002) Neuroendocrine differentiation factor, IA-1, is a transcriptional repressor and contains a specific DNA-binding domain: identification of consensus IA-1 binding sequence. Nucleic Acids Res 30, 1038-45

Brown AR, Webb J, Rebus S, Williams A, Fazakerley JK (2004) Identification of up-regulated genes by array analysis in scrapie-infected mouse brains. Neuropathol Appl Neurobiol 30, 555-67

Buschmann A, Biacabe AG, Ziegler U, Bencsik A, Madec JY, Erhardt G, Lühken G, Baron T, Groschup MH (2004) Atypical scrapie cases in Germany and France are identified by discrepant reaction patterns in BSE rapid tests. J Virol Methods 117, 27-36

Cartegni L, Chew SL, Krainer AR (2002) Listening to silence and understanding nonsense: exonic mutations that affect splicing. Nat Rev Genet 3, 285-98

Cartharius K, Frech K, Grote K, Klocke B, Haltmeier M, Klingenhoff A, Frisch M, Bayerlein M, Werner T (2005) MatInspector and beyond: promoter analysis based on transcription factor binding sites. Bioinformatics 21, 2933-42

Doré S, Kar S, Quirion R (1997) Rediscovering an old friend, IGF-I: potential use in the treatment of neurodegenerative diseases. Trends Neurosci 20, 326-31

Drögemüller C, Leeb T, Distl O. (2001) Breeding for resistance to scrapie: detection of polymorphisms in the prion protein gene in German sheep breeds. Arch Tierz 44, Special Issue, 280-7

Gretzschel A, Buschmann A, Eiden M, Ziegler U, Lühken G, Erhardt G, Groschup MH (2005) Strain typing of German transmissible spongiform encephalopathies field cases in small ruminants by biochemical methods. J Vet Med B Infect Dis Vet Public Health 52, 55-63

Haliassos A, Chomel JC, Tesson L, Baudis M, Kruh J, Kaplan JC, Kitzis A (1989) Modification of enzymatically amplified DNA for the detection of point mutations. Nucleic Acids Res 17, 3606

Hardy GH (1908) Mendelian Proportions In A Mixed Population. Science 28, 49-50

Hou J, Clemmons DR, Smeekens S (2005) Expression and characterization of a serine protease that preferentially cleaves insulin-like growth factor binding protein-5. J Cell Biochem 94, 470-84

Iwadate H, Sugisaki T, Kudo M, Kizuki K (2003) Actions of insulin-like growth factor binding protein-5 (IGFBP-5) are potentially regulated by tissue kallikrein in rat brains. Life Sci 73, 3149-58

Kovacs GG, Gelpi E, Strobel T, Ricken G, Nyengaard JR, Bernheimer H, Budka H (2007) Involvement of the endosomal-lysosomal system correlates with regional pathology in Creutzfeldt-Jakob disease. J Neuropathol Exp Neurol 66, 628-36

Lloyd SE, Onwuazor ON, Beck JA, Mallinson G, Farrall M, Targonski P, Collinge J, Fisher EM (2001) Identification of multiple quantitative trait loci linked to prion disease incubation period in mice. Proc Natl Acad Sci U S A 98, 6279-83

Lühken G, Buschmann A, Brandt H, Eiden M, Groschup MH, Erhardt G (2007) Epidemiological and genetical differences between classical and atypical scrapie cases. Vet Res 38, 65-80

Lühken G, Brandt HR, Buschmann A, Groschup MH, Erhardt G (2006) Microsatellite CTSBJ12 is located distal to the ovine prion protein gene on OAR13 and is not associated with scrapie susceptibility. Anim Genet 37, 426-27

Lühken G, Buschmann A, Groschup MH, Erhardt G (2004) Prion protein allele A136 H154Q171 is associated with high susceptibility to scrapie in purebred and crossbred German Merinoland sheep. Arch Virol 149, 1571-80

Luhr KM, Nordstrom EK, Low P, Kristensson K (2004a) Cathepsin B and L are involved in degradation of prions in GT1-1 neuronal cells. Neuroreportv 15, 1663-7

Luhr KM, Nordstrom EK, Low P, Ljunggren HG, Taraboulos A, Kristensson K (2004b) Scrapie protein degradation by cysteine proteases in CD11c+ dendritic cells and GT1-1 neuronal cells. J Virol 78, 4776-82

Madec JY, Simon S, Lezmi S, Bencsik A, Grassi J, Baron T (2004) Abnormal prion protein in genetically resistant sheep from a scrapie-infected flock. J Gen Virol 85, 3483-6

Majewski J, Ott J (2002) Distribution and characterization of regulatory elements in the human genome. Genome Res 12, 1827-36

Moreno CR, Cosseddu GM, Schibler L, Roig A, Moazami-Goudarzi K, Andreoletti O, Eychenne F, Lajous D, Schelcher F, Cribiu EP, Laurent P, Vaiman D, Elsen JM (2008) Identification of new quantitative trait Loci (other than the PRNP gene) modulating the scrapie incubation period in sheep. Genetics 179, 723-6

Moreno CR, Lantier F, Lantier I, Sarradin P, Elsen JM (2003) Detection of new quantitative trait Loci for susceptibility to transmissible spongiform encephalopathies in mice. Genetics 165, 2085-91

Moum T, Olsaker I, Hopp P, Moldal T, Valheim M, Benestad SL (2005) Polymorphisms at codons 141 and 154 in the ovine prion protein gene are associated with scrapie Nor98 cases. J Gen Virol 86, 231-5

Orge L, Galo A, Machado C, Lima C, Ochoa C, Silva J, Ramos M, Simas JP (2004) Identification of putative atypical scrapie in sheep in Portugal. J Gen Virol 85, 3487-91

Östlund P, Lindegren H, Pettersson C, Bedecs K (2001) Up-regulation of functionally impaired insulin-like growth factor-1 receptor in scrapie-infected neuroblastoma cells. J Biol Chem 276, 36110-5

Riemer C, Neidhold S, Burwinkel M, Schwarz A, Schultz J, Kratzschmar J, Monning U, Baier M (2004) Gene expression profiling of scrapie-infected brain tissue. Biochem Biophys Res Commun 323, 556-64

Smith PJ, Zhang C, Wang J, Chew SL, Zhang MQ, Krainer AR (2006) An increased specificity score matrix for the prediction of SF2/ASF-specific exonic splicing enhancers. Hum Mol Genet 15, 2490-508

Stephenson DA, Chiotti K, Ebeling C, Groth D, DeArmond SJ, Prusiner SB, Carlson GA (2000) Quantitative trait loci affecting prion incubation time in mice. Genomics 69, 47-53

Stothard P (2000) The sequence manipulation suite: JavaScript programs for analyzing and formatting protein and DNA sequences. Biotechniques 28, 1102-4

Supattapone S, Nguyen HO, Cohen FE, Prusiner SB, Scott MR (1999) Elimination of prions by branched polyamines and implications for therapeutics. Proc Natl Acad Sci U S A 96, 14529-34

Supattapone S, Wille H, Uyechi L, Safar J, Tremblay P, Szoka FC, Cohen FE, Prusiner SB, Scott MR (2001) Branched polyamines cure prion-infected neuroblastoma cells. J Virol 75, 3453-61

Walsh DT, Betmouni S, Perry VH (2001) Absence of detectable IL-1beta production in murine prion disease: a model of chronic neurodegeneration. J Neuropathol Exp Neurol 60, 173-82

Xiang W, Windl O, Wunsch G, Dugas M, Kohlmann A, Dierkes N, Westner IM, Kretzschmar HA (2004) Identification of differentially expressed genes in scrapie-infected mouse brains by using global gene expression technology. J Virol 78, 11051-60

Yadavalli R, Guttmann RP, Seward T, Centers AP, Williamson RA, Telling GC (2004) Calpain-dependent endoproteolytic cleavage of PrPSc modulates scrapie prion propagation. J Biol Chem 279, 21948-56

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