

**GENE EXPRESSION PROFILING IN MURINE SPLENIC CELLS
DURING PRION INFECTION**

BY

RHIANNON L.C. HARRIS

**A Thesis
Submitted to the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements for the Degree of**

MASTERS OF SCIENCE

**Department of Medical Microbiology
University of Manitoba
Winnipeg, Manitoba**

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FACULTY OF GRADUATE STUDIES

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**A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of
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Of

MASTER OF SCIENCE

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Abstract

In many experimental rodent scrapie models, as in natural sheep scrapie, the first documented prion disease which was found in sheep, the disease-specific isoform (PrP^{Sc}) of the host prion protein (PrP^{C}) accumulates in the spleen and other lymphoid tissues prior to infecting the central nervous system. PrP^{Sc} builds up in germinal centres and is strongly associated with follicular dendritic cells (FDCs) and possibly circulating dendritic cells and macrophages. In addition, studies have shown that mature FDCs are critical for PrP^{Sc} replication in lymphoid tissues, and dedifferentiation of the cells increases the incubation period of the disease, presumably by lengthening the time for neuroinvasion to take place.

Previously, a small number of changes in expression during scrapie infection were observed when examining spleen tissue as a whole (unpublished observation by Booth lab). I believe that as the majority of cells that make up the spleen are not competent for prion replication, changes in expression in the small proportion of cells in which prions replicate are masked. Therefore, in this project I attempted to isolate a population of splenic cells enriched for FDCs, circulating dendritic cells and macrophages to identify an increased number of gene expression changes. The objective of this study is to use DNA microarrays to profile gene expression in spleen tissue with the aim of identifying changes that accompany prion replication. These may be host factors involved in prion propagation and/or may be useful as preclinical biomarkers for early diagnosis.

To achieve these objectives I developed a cell isolation technique to target a more specific population of cells involved in prion disease, and then used microarray technology to identify differentially expressed genes between scrapie-infected and mock-infected tissues. The microarray platforms used in this project included BMAP 17K cDNA arrays manufactured in our laboratory, Operon mouse AROS v3 32K oligo arrays, and Agilent whole mouse genome 4x44K oligo arrays. Selected differentially expressed genes found upregulated from the microarray studies belonging to the small leucine-rich proteoglycan (SLRP) family were validated at the molecular level using qRT-PCR and at the protein level using both immunohistochemistry and western blot. These SLRPs could be involved in replication and propagation of the prion protein by assisting with the conversion process of PrP^c to PrP^{Sc} and possibly as preclinical biomarkers for prion disease in the future.

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iv List of Abbreviations

aRNA	amplified RNA
BMAP	Brain Molecular Anatomy Project
BSE	Bovine Spongiform Encephalopathy
cDNA	complimentary deoxyribonucleic acid
CJD	Creutzfeldt-Jakob disease
CNS	Central nervous system
CWD	Chronic Wasting disease
DCs	dendritic cells
DNA	deoxyribonucleic acid
dpi	days post infection
EDRF	erythroid differentiation factor
FDCs	follicular dendritic cells
FDR	false discovery rate
FSE	feline spongiform encephalopathy
FFI	fatal familial insomonia
FSI	fatal sporadic insomonia
GAG	glycosaminoglycan
GFAP	glial fibrillary acidic protein
GPI	glycosyl-phosphatidylinositol-anchor
GSS	Gerstmann-Straussler-Scheinker syndrome
i.c.	intracerebral
iCJD	iatrogenic Creutzfeldt-Jakob disease
IHC	immunohistochemistry
i.p.	intraperitoneal
kDa	kilodalton
mRNA	messenger RNA
nt	nucleotide
oligo	oligonucleotide
OR	octapeptide repeat
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PK	Proteinase-K
PMCA	protein misfolding cyclic amplification
PrP	prion protein
PrP ^c	cellular prion protein
PrP ^{Sc}	scrapie prion protein
qRT-PCR	quantitative real-time PCR
RNA	ribonucleic acid
SAM	significance analysis of microarrays
sCJD	sporadic Creutzfeldt-Jakob disease
SLRP	small leucine-rich proteoglycan
TBMs	tingible body macrophages
TBS	tris-buffered saline

Tg
TME
TNF
TSEs
UV
vCJD

transgenic
transmissible mink encephalopathy
tumour necrosis factor
transmissible spongiform encephalopathies
ultraviolet
variant Creutzfeldt-Jakob disease

1 Introduction

1.1 Prion Diseases

Prion diseases or Transmissible Spongiform Encephalopathies (TSEs) are invariably fatal neurodegenerative diseases of both human and animals associated with the conversion of the normal cellular prion protein (PrP^c) into the abnormal isoform (PrP^{Sc}) (Prusiner, 1998).

Prion disease can occur in a wide range of hosts causing a variety of infections. They can be categorized as sporadic, inherited, or infectious. In humans, they include Kuru, Gerstmann-Straussler-Sheinker (GSS) disease, Creutzfeldt-Jakob disease (CJD), and Fatal Insomnia (Prusiner, 1998). In animals they include scrapie in sheep, Bovine Spongiform Encephalitis (BSE) in cows, Chronic Wasting Disease (CWD) in mule, deer, and elk, transmissible mink encephalopathy (TME) in mink, and lastly, feline spongiform encephalopathy (FSE) in cats.

Scrapie was the first documented prion disease initially described in sheep in Iceland during the 18th century (Chakraborty *et al.*, 2005). It was first noticed when sheep began to rub against fences in their pens to stay upright resulting in removal of their wool, hence the name scrapie; This loss of balance and coordination, known as ataxia, is a characteristic clinical symptom of prion diseases. The transmissibility of Scrapie was conclusively demonstrated in 1943 when a flock of Scottish sheep developed scrapie 2 years after they were vaccinated against a common virus using formalin inactivated

extract of lymphoid tissue from an unknowingly infected sheep (Chakraborty *et al.*, 2005).

The first case of BSE was observed in the UK in 1985 leading to a major epidemic throughout the country for the next decade with the infection of about 1 million cows (Johnson, 2005). Exportation of cattle and their feed spread the disease throughout Europe and the world (Johnson, 2005). The source of contamination stemmed from infected meat and bone meal that was being fed to the cattle (Johnson 2005). There were two hypotheses about how this became infected. The first was that there may have been infected sheep carcasses rendered into the bone meal which could have crossed the species barrier and infected the cows, and subsequent rendering of infected cows into the bone meal added to the problem (Johnson, 2005). The second hypothesis is that a sporadic case of BSE in one cow could have initiated the epidemic. It was estimated that about 730, 000 BSE-infected cows entered the human food chain during this time (Glatzel & Aguzzi, 2001). In 1988, ruminant carcasses were banned from the feed in the UK and by the year 2000, it was banned throughout the EU (Johnson, 2005 & Glatzel & Aguzzi, 2001). As a result, the number of cases declined several years later (Johnson 2005 & Glatzel & Aguzzi, 2001).

1.2 Human Prion Diseases

1.2.1 Sporadic forms

These include sporadic CJD (sCJD) and a form of Fatal Sporadic Insomina (FSI) and they constitute most of the cases of CJD (~85%) and possibly a few cases of GSS; there are 1-2 cases per 1 million a year worldwide (Prusiner, 1998 & Johnson,

2005). How disease is caused in these patients is still unknown. Possible explanations are somatic mutation of the *PRNP* gene or spontaneous conversion of PrP^c into PrP^{Sc} (Prusiner, 1998).

Sporadic CJD is the most common form of prion disease and affects men and women equally with the average age of onset being 60 years old (Johnson, 2005). It is rare for people under the age of 40 or over the age of 80 to develop the disease (Johnson, 2005). This disease progresses very rapidly, with the median time to death being 5 months after the initial onset of symptoms, and 90% of people die within 1 year (Johnson, 2005).

1.2.2 Inherited forms

These diseases include familial CJD (fCJD), GSS, and Fatal Familial Insomnia (FFI) and they constitute 10-15% of CJD cases (Johnson, 2005). About fifty different mutations have been found in the human *PRNP* gene which results in non-conservative substitutions that associate with the inherited prion diseases (Johnson, 2005). Specifically, four point mutations at codons 102, 178, 200, and 210, and insertions of five or six octapeptide repeats have been found associated with 95% of familial cases (Johnson, 2005). The P102L mutation was the first to be genetically linked to CNS dysfunction in GSS (Hsiao *et al.*, 1989) and has been subsequently found in many GSS families across the world (Doh-ura *et al.*, 1989 & Goldgaber *et al.*, 1989). In addition, polymorphism at codon 129 can result in the protein containing either a methionine (met) or valine (val) increasing one's susceptibility

to CJD (Johnson, 2005). For example, it has been seen that 80% of sporadic CJD patients are homozygote met or val at position 129 (Johnson, 2005).

1.2.3 Infectious forms

These account for <1% of cases and include the diseases Kuru and variant CJD (vCJD). Kuru was found amongst the Fore people of New Guinea as a consequence of ritualistic cannibalism of their dead (Gajdusek, 1977). The duration of the illness from disease onset was about 3-12 months (Chakraborty *et al.*, 2005). Once cannibalism came to a halt, Kuru began to decline (Prusiner, 1998).

Iatrogenic CJD (iCJD) is not necessarily a different disease than sCJD and its transmissibility is possible by the use of improperly sterilized surgical instruments, transplantation of infected corneas, human growth hormone (HGH), gonadotropin derived from cadaveric pituitaries, as well as cadaveric dura mater grafts (Brown *et al.*, 1992).

The most recently recognized form of CJD, new variant CJD (vCJD) was first observed in 1994 in the UK resulting from the ingestion of meat products contaminated with infectious bovine prion proteins. There have been a number of cases of vCJD linked to the BSE epidemic in Britain (Will *et al.*, 1996) and plenty of supporting evidence for it. Firstly, it was shown that vCJD could be transmitted from cattle to primates when an intracerebral inoculation of BSE-infected brain extract was performed on Macaque monkeys and it produced disease and

pathology similar to vCJD (Lasmezas *et al.*, 1996). In addition, studies have shown that the distribution of neuronal vacuoles and the PrP^{Sc} pattern deposition in the brains of infected mice, a property that is specific to different strains of prion disease, were very similar (Glatzel & Aguzzi, 2001). Secondly, when the proteinase-K digestion patterns were examined from vCJD patients, they were found to be strikingly similar to the patterns seen by BSE-infected animals, distinct from that of sporadic or acquired CJD (Chakraborty *et al.*, 2005). Thirdly, the conformation of the protease resistant form of vCJD prion protein is very similar to that of BSE prions. The different conformations of the prion protein expose different sites in the polypeptide chain to differing actions of proteolytic cleavage (Glatzel & Aguzzi, 2001). The sizes of the resulting fragments can be resolved on a gel and the molecular weight of these fragments, in combination with the different glycosylation patterns of the PrP^{Sc} result in a similar biochemical profile for vCJD and BSE (Glatzel & Aguzzi, 2001). Lastly and most convincingly, the epidemiology of the two diseases displays a strong connection with the highest numbers of vCJD cases being found in the UK where the highest numbers of BSE cases are also found (Glatzel & Aguzzi, 2001). Although the UK has acquired the largest number of cases to date (163 cases), France was also known to have a mini-epidemic with the next highest number of cases seen (23 cases), and isolated cases have been reported spread throughout non-European countries such as Canada (1 case), the US (3 cases), and Japan (1 case) according to The National Creutzfeldt-Jakob Disease Surveillance Unit (NCJDSU) for May 2008 (<http://www.cjd.ed.ac.uk/>).

The characteristics of vCJD include a mean age of onset, 29 years old; course of clinical infection about 14 months, and most clinical cases homozygote met/met at codon 129 (Johnson, 2005). This was the first time that prions were shown to cross the species barrier from animal to human. Of course this raised much public concern and questions about the safety of the human food supply.

Another concern is the risk of transmission through the blood supply. There has not yet been any cases noticed of transmissibility in blood from sCJD and other forms of prion disease, and so far it has been a unique feature of vCJD making it a growing concern. There have been several cases in the UK where recipients of blood donation developed vCJD (Aguzzi & Glatzel, 2006). The first case was a 62-year-old man who developed vCJD 6.5 years after receiving blood from a 24-year-old man donor who subsequently developed vCJD himself 3 years later (Aguzzi & Glatzel, 2006). Another case was an elderly man who died 5 years after the transfusion and the young donor died 2 years following the blood donation (Aguzzi & Glatzel, 2006). The recipient showed no visible signs of neurological disease and no abnormal staining of brain tissue was observed, although he did have an accumulation of abnormal PrP in his lymphoid organs (Aguzzi & Glatzel, 2006). Thus, this is an example of a pre-clinical case and raises concern of blind transmission that could remain a permanent threat to our blood supply. Additionally, there was another case that developed symptoms of vCJD six years after receiving the blood and died about 3 years later. The blood donor

subsequently developed vCJD 20 months after the donation (Aguzzi & Glatzel, 2006). Since the “outbreak” of vCJD in Britain and the recent case of BSE found in Canada in 2003, there has been an increased attention for research in the prion field, especially to develop more sensitive tests that can be used during the long, preclinical stages of disease.

1.3 Prion Protein- Protein-only Hypothesis

In 1982, Dr. Stanley Prusiner suggested the term ‘prion’; the term was derived from evidence that a prion is a small *proteinaceous infectious* particle that is resistant to most of the procedures that inactivate nucleic acids (Prusiner, 1982). Dr. Stanley Prusiner later won a nobel prize in Physiology and Medicine in 1997 for his biochemical enrichment of the activity of the infectious agent and demonstrated its link to a specific protein (Chakraborty *et al.*, 2005). Dr. Prusiner found that there were a number of unique properties of the scrapie agent including i) stability at 90°C for 30 min., (ii) low molecular weight (<50,000 Daltons), (iii) hydrophobic protein required for infectivity, (iv) resistance to nuclease digestion, (v) resistance to UV irradiation at 254 nm, (vi) resistant to psorlean photoadduct formation, (vii) resistant to Zn^{2+} catalyzed hydrolysis, and lastly, (viii) resistant to NH_2OH chemical modification (Prusiner, 1982). Since then, mounting evidence suggests that the causative agent of Prion disease is a protein rather than a virus like particle, although there is still some that believe the ‘virino’ is the infectious agent. The protein-only hypothesis is based on the infectious entity being a proteinaceous material devoid of nucleic acid and the virino hypothesis is that of a small nucleic acid or what has been termed a “slow virus” by Bjorn Sigurdsson in 1954

surrounded by a tightly packed protein coat that protects it from degradation or it could exist as a different kind of structure that makes it resistant (Prusiner, 1982).

There has been overwhelming evidence for the protein-only hypothesis and thus, is the most favoured. Evidence for this hypothesis suggesting it is in fact a protein is based on its sensitivities to the treatments of proteins such as (i) its inactivation by proteinase K digestion, (ii) inactivation by chemical modification with diethyl pyrocarbonate, (iii) inactivation by SDS, (iv) inactivation by chaotropic salts, (v) inactivation by phenol, and lastly its inactivation by urea (Prusiner, 1982). Additional evidence in favour of the protein-only hypothesis is the fact that the infectious agent is resistant to procedures that destroy nucleic acids such as acidity, nucleases, UV irradiation, divalent cation hydrolysis, psoralen photoreaction, and chemical modification by such agents as hydroxylamine (Prusiner, 1982). Many viruses are also resistant to some of the above procedures mentioned, but to date, no virus that is resistant to all these treatments has been identified (Prusiner, 1982). Additional evidence for the protein-only hypothesis comes from the ability of yeast prions to undergo conformational changes and cause alterations of accompanying proteins resulting in aggregate formation (Weissman *et al.*, 2004 & Wickner *et al.* 2002). Strong experimental evidence for the protein-only hypothesis was reported in 2005 when what was named protein misfolding cyclic amplification (PMCA) was used to generate PrP^{Sc} *in vitro*. The procedure not only generated the proteinase resistant form of the protein *in vitro* but this was used to inoculate and ultimately cause *in vivo* disease in Syrian hamsters (Castilla *et al.*, 2005). This evidence is almost definitive confirmation for the protein, however, the original seed

for the PMCA reaction comes from an infected animal leaving a small chance that a further agent enters the PMCA reaction mix. PMCA mimics a PCR reaction and follows the seeding model of replication (will be discussed further). The methods involved are: normal and infectious forms of prion are mixed together in a microsonicator and subject to cycles of incubation and sonication pulses. This provides a template (PrP^{Sc}) for PrP^{C} conformational change and allows for cleavage of built up aggregates to permit access for exponential PrP^{Sc} growth (Castilla *et al.*, 2005). Lastly, evidence against the virino hypothesis, hence favouring the protein-only hypothesis, is that despite decades of effort to isolate a virus or genome, no such entity has yet been found.

1.4 Prion Protein Biochemistry, Structure, and Function

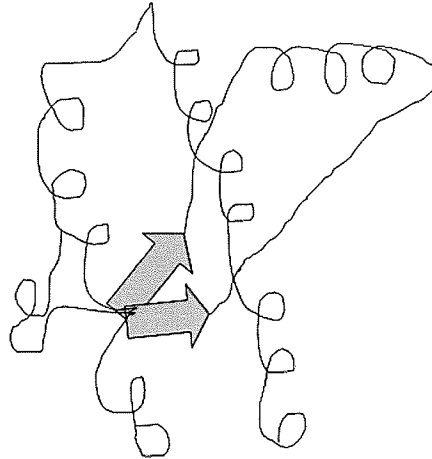
PrP^{C} which is encoded by a single host gene *Prpn*, is highly expressed in neurons in addition to being expressed in skeletal muscle, kidney, heart, secondary lymphoid organs, and immune cells, particularly follicular dendritic cells (FDCs) (Glatzel & Aguzzi, 2001). It contains an N-terminal signal consisting of four or five octapeptide repeats (OR) that are thought to bind copper and an N-linked branched chain carbohydrate attached to asparagine residues (Riesner, 2003). It contains a disulphide bridge linking two internal cysteine residues and a glycosyl phosphatidylinositol (GPI) anchor (Riesner, 2003). PrP^{C} is converted to PrP^{Sc} through an unknown mechanism where portions of its α -helical structure are converted to β -sheets. Studies performed on the prion protein have shown that the secondary structure of PrP^{C} consists of about 40% α -helix and very little β -sheet, whereas PrP^{Sc} , contains about 30% α -helix and 45% β -sheet (Prusiner 1998) and yet, the two proteins share the same amino acid sequence. Examples of the two types of

structures are shown in **Figure 1**. These structural changes result in different physiochemical properties of the PrP, (Prusiner, 1982) but the amino acid sequence remains the same as that encoded by the PrP gene of the host (Prusiner, 1998). One primary amino acid sequence seems to predict two different structures. Thus, considering the two amino acid sequences of the cellular and infectious forms are the same, the scrapie agent is recognized as self antigen by the host and very little to no immune response is mounted against a foreign pathogen (Prusiner, 1998).

The exact function of the prion protein remains speculative. There have been many suggested functions for the prion protein, but no conclusive evidence for any single function as of yet. It has been proposed that it is involved in protection from oxidative stress, apoptosis, cellular signaling, membrane excitability and synaptic transmission, neuritogenesis and copper transport or metabolism (Hijazi *et al.*, 2003).

PrP^c Model

A



PrP^{Sc} Model

B

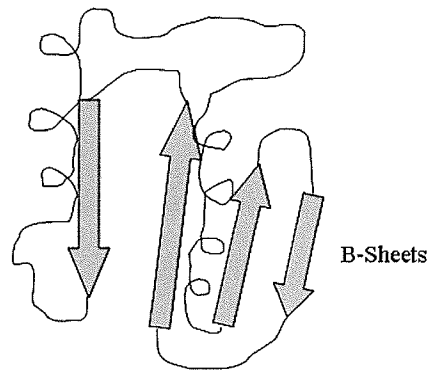


Figure 1: Representative models of the α -helical PrP^c conformer (A) and the β -sheet PrP^{Sc} conformer (B) of the prion protein (Adapted from Prusiner, 2001).

1.5 Cellular Trafficking of the Prion Protein

The biosynthetic pathway of PrP begins with the internalization of the lipid-raft located PrP^c into neurons initiated by OR binding of Cu²⁺ allowing PrP^c movement inside detergent soluble regions of the plasma membrane (Taylor *et al.*, 2005). Subsequently, the N-terminus of the PrP^c interacts with a transmembrane protein activating clathrin mediated endocytosis (Taylor *et al.*, 2005).

PrP^c is synthesized in the rough endoplasmic reticulum (RER) and is transported through the Golgi where it is deposited on the cell surface (Hegde & Rane, 2003). During biosynthesis the PrP undergoes many posttranslational modifications and chaperone-assisted folding events before it is trafficked along the secretory pathway. These modifications include addition of N-linked oligosaccharide chains, disulphide bond formation, signal peptide cleavage and attachment of the GPI anchor (Hegde & Rane, 2003). The cellular trafficking of the prion protein is depicted in **Figure 2**.

Prion Pathway

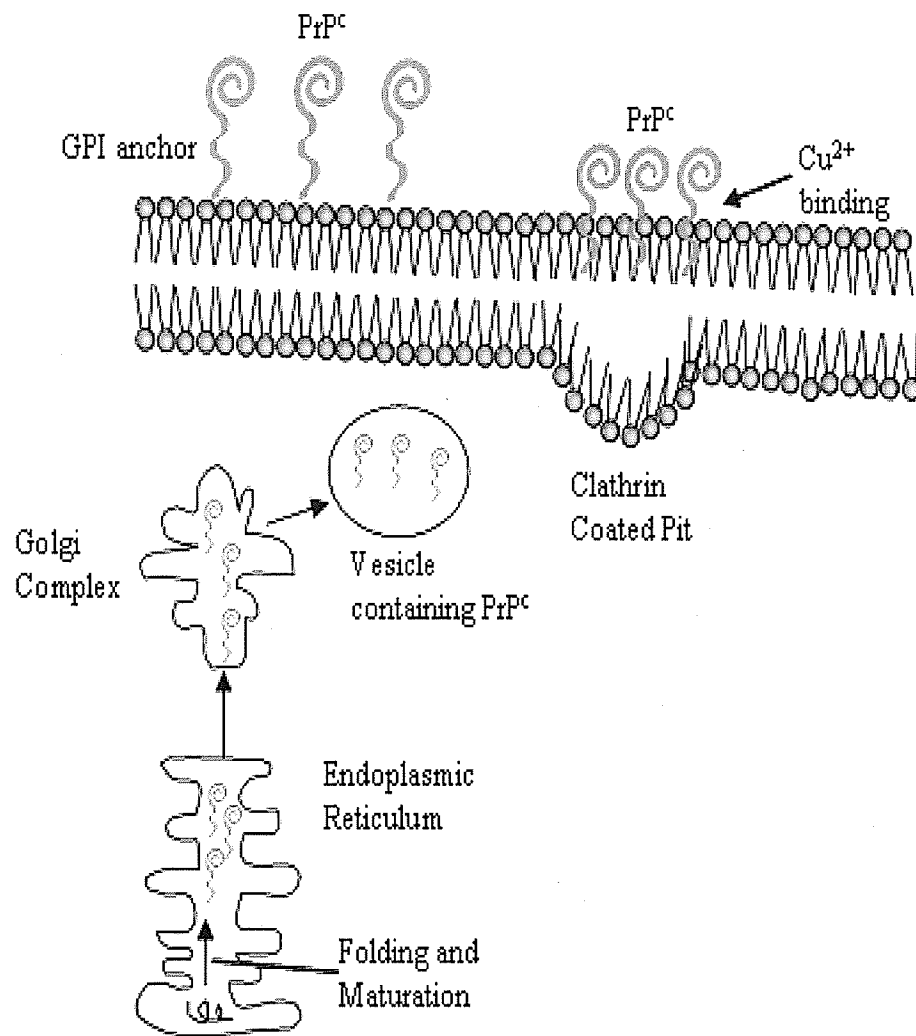


Figure 2: Cellular trafficking of the prion protein (Adapted from Applied Biosystems, www.proteinlounge.com).

1.6 Prion Protein Replication

The exact molecular mechanism is not yet known and remains an area of extensive research. This may perhaps hold the key to prion transmissibility and toxicity. Currently there are two models for the conversion of PrP^c to PrP^{Sc} . The first is the nucleation-polymerization or seeding model. In this model, the PrP^{Sc} acts as a seed to recruit, convert, and stabilize the misfolded PrP^c to create more PrP^{Sc} eventually forming an aggregate or fibril (Soto, 2004). It is proposed that cleavage of the aggregate is needed to access additional template for an exponential increase in PrP^{Sc} (Weissman *et al.*, 2004). If no aggregate preexists, conversion between PrP^c and PrP^{Sc} is reversible where the PrP^c conformation is strongly favoured (Chakraborty *et al.*, 2005). The second model is the template-assisted conversion model. Here, there is a formation of an intermediate step which binds to a molecular chaperone protein X (Soto, 2004). Once bound to the chaperone, the intermediate can then interact with PrP^{Sc} which acts as the template for its conversion (Soto, 2004). For this second model, PrP^c and PrP^{Sc} cannot readily interconvert due to the high activation energy. Common to both models is the fact that PrP^{Sc} acts as a template directing conversion of PrP^c into more infectious PrP^{Sc} .

1.7 Concept of the Prion Strain

Prion strains vary from different incubation periods in a given host, to different clinical manifestations, to various histopathological localizations and expressions in the CNS such as intensity of spongiform degeneration and pattern of amyloid plaques (Aucouturier & Carnaud, 2002). Strains can also display varying sensitivities to proteinase K (PK) digestion (Aucouturier *et al.*, 1999), differing electrophoretic patterns

after PK digestion due to different glycosylation patterns, and lastly, different physiopathogenic features (Aucouturier & Carnaud, 2002). Infra-red spectroscopic studies have shown that the various banding patterns are a result from different N-terminal cleavage sites for proteinase K which suggests the strains represent different PrP^{Sc} conformations (Caughey B *et al.*, 1998).

Unlike conventional pathogens where the nucleic acid genome directs strain-specific properties, the tertiary structure of the prion protein determines different strains (Prusiner, 1998) and the generation of new strains has been found to occur through passaging of prions through animals with different PrP genes (Prusiner, 1998). Evidence from a study with two different inherited human prion diseases that were transmitted to mice expressing a chimeric PrP transgene helps lead to the speculation that strain-specific information is associated with the tertiary structure (Telling *et al.*, 1996). They displayed differences in molecular size that were due to differing sites of proteolytic cleavage at the NH₂ termini of the two human PrP^{Sc} molecules, which would indicate a difference in tertiary structure (Monari *et al.*, 1994).

It has been noticed that passaging of a prion strain from one species to a different species will result in longer, more irregular incubation period or sometimes absence of clinical disease as the incubation period may outlast the lifespan of the individual (Aucouturier & Carnaud, 2002 & Hill & Collinge, 2004). This is what is known as the species barrier. Although, in the latter case, the infectious prion protein was detected in the brain later on in life of the individual, which gives reason to doubt the concept of absolute resistance

(Aucouturier & Carnaud, 2002). PrP primary structure is suspected to influence the conformation of PrP^{Sc} into its different conformers which are more thermodynamically favourable which is another factor that determines species barrier (Hill & Collinge, 2004 & Johnson, 2005). If the PrP primary structure of the host is not conducive to the folding of the PrP into the conformation of the inoculated PrP, then there will be a barrier to transmission (Hill & Collinge, 2004). Transgenic mice that express the prion protein of the natural host can overcome the species barrier (Weissman, 2003). Transgenic mice are mice that have had their genome altered by transgenesis, i.e. insertion of cloned genes or by *in situ* gene replacement using homologous recombination in embryonic stem cells (Weissman, 2003). Once initiation has taken place following intracerebral inoculation of a high dose of the natural isolate, serial passages in mice and hamsters have developed stabilized strains, most of which have originated from sheep scrapie and some of which were cloned (Aucouturier & Carnaud, 2002). Once the strain is stabilized and cloned in a certain species, its characteristics are astoundingly stable throughout subsequent passages (Aucouturier & Carnaud, 2002). The incubation period is shorter and the clinical and histological signs become more consistent (Hill & Collinge, 2004).

Crossing of the species barrier becomes of particular concern when there is possibility of animal to human transmission. Currently, only BSE has been shown to be transmitted to humans in the form of vCJD. It is unpredictable what strains of prions could be a risk to humans next. There has been a particular interest in CWD in North America. It has been seen to spread quite quickly from one animal to another through casual contact and the

environment, and it is of concern that this could be the next TSE to cross the species barrier from animal to human.

1.8 Path of the Infectious Prion Protein

The infectious isoform is thought to follow a path that begins with ingestion and ends in the brain. The natural route of transmission in most infectious prion diseases is by peripheral exposure (Thackray *et al.*, 2003), most likely through the consumption of contaminated meat products. Following ingestion of the prion protein, it must move from the gut lumen through the gastro-intestinal wall. It has been proposed, but not yet established, that uptake from the intestine can occur via the M cells, a population of cells specialized in transferring enteric antigens and pathogens to the gut-associated lymphoid tissues (GALT) (Unterberger *et al.*, 2005), in this case, the Peyer's patches. The PrP^{Sc} rapidly accumulates in the GALT, mainly the mesenteric lymph nodes, and then in the spleen early in the preclinical phase before reaching the central nervous system (CNS) where clinical symptoms become apparent (Mabbott *et al.*, 2000). Abnormal isoforms of the PrP^C are detected on follicular dendritic cells networks (FDCs) and macrophages within germinal centres (GC) (Brown *et al.*, 1999, Lotscher *et al.*, 2003, & Mabbott *et al.*, 2001) of the spleen. How the PrP^{Sc} actually crosses the intestinal barrier to reach the FDCs and subsequently the nerve endings of the peripheral nervous system (first site of neuroinvasion) is still unclear, considering FDCs are known to be immobile cells. M cells, FDCs, and nerve endings need a cell bridge between them in order to propagate the PrP^{Sc}, and it has been suggested that dendritic cells (DCs) can serve this purpose as they are migrating cells that appear to take up PrP^{Sc} and can transport antigen from the GALT

to the mesenteric lymph nodes (Banchereau *et al.*, 2000, Huang *et al.* 2000, & Huang *et al.*, 2002). An example of the path of the PrP^{Sc} from ingestion into the body is shown in **Figure 3.**

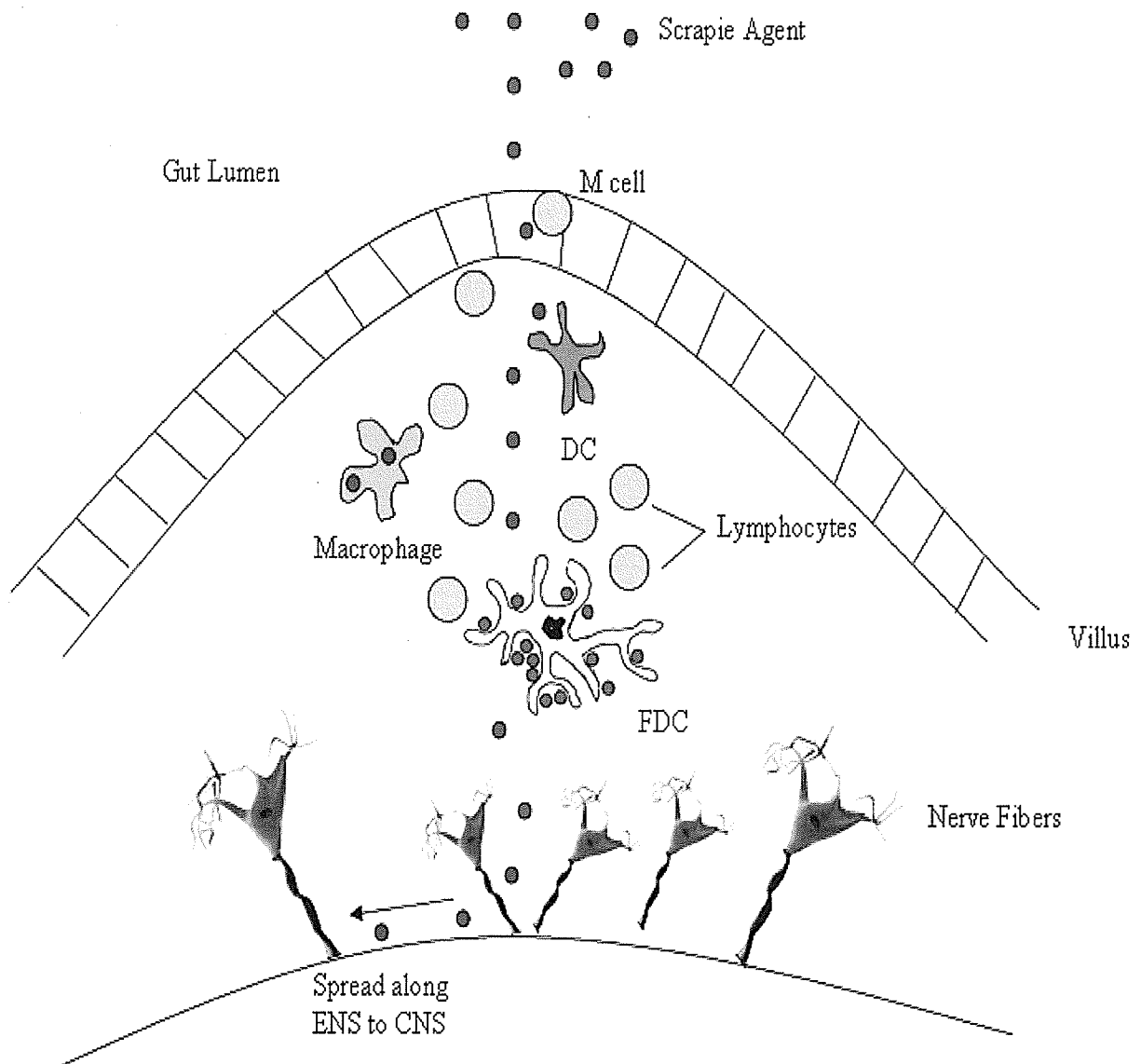


Figure 3: Path of the PrP^{Sc} following ingestion showing its spread from its initial site of accumulation in the GALT to the CNS (Adapted from Mabbott *et al.*, 2001). ENS: enteric nervous system.

1.9 Key Players of the Immune System for Prion Protein Propagation

Several studies have been performed to assess the importance of different cells of the immune system (leukocytes) and the role they play in prion disease (Aucouturier *et al.*, 2002, Bruce *et al.*, 2000, Burthem *et al.*, 2001, Defaweux *et al.*, 2005, Mabbott *et al.*, 2001, & Mabbott *et al.*, 2000). This is an area of interest since prions are associated with lymphoid tissues, such as the spleen, where lymphocytes are produced and where the PrP^{Sc} will accumulate and eventually invade the CNS.

1.9.1 B cells

Previous studies have shown that mice deficient in B lymphocytes have less accumulation of the infectious prion protein in the spleen and subsequently less neuroinvasion (Klein *et al.*, 1997). B cells provide important signals for the maturation and maintenance of other cell types in GCs (Chaplin & Fu, 1998 & Kosco-Vilbois *et al.*, 1997). It has been found if mice are deficient in B cells, they will be indirectly deficient in FDCs, an important player in prion disease, which must have stimulation from the B cells, for example, the release of lymphotoxin β signal to maintain their differentiated state (Brown *et al.*, 2000, Mabbott *et al.*, 2003 & Prinz *et al.*, 2003). Even though B cells have not been found to directly support prion protein replication, they may still carry infectivity (Aucouturier *et al.*, 2001 & Raeber *et al.*, 1999) by being able to passively acquire it through tight association with infected FDCs.

1.9.2 T cells

T cells were previously found not to be important players as studies on athymic (nude mice) have been shown to have similar incubation periods to wild-type control mice (Aucouturier *et al.*, 2002). Although, it should be considered that they might aid other cell types in the infection process. Previous studies have shown that CD4⁺ T lymphocytes that express high levels of cell surface PrP^c could be activated and capture the infectious PrP^{Sc} and transfer it to the sites of neuroinvasion (Aucouturier *et al.*, 2002). Thus, it should not be ruled out that circulating T cells could act as a 'missing link'.

1.9.3 Macrophages

Inside germinal centres, macrophages scavenge apoptotic B lymphocytes, endocytose FDCs immune complexed-coated bodies, and regulate the GC reaction (Smith *et al.*, 1998 & Smith *et al.*, 1991). Accumulations of intralysosomal PrP^{Sc} have been seen within tingible body macrophages (TBMs), an immune cell subset that is specialized in phagocytic activity in the germinal centers of the spleen (Jeffrey *et al.*, 2000 & McGovern *et al.*, 2004). They have been found to contain PrP^{Sc} in the absence of FDCs (Mabott *et al.*, 2000), thus leading to the speculation that they might serve as alternative sites of prion accumulation and replication when there are no functional FDCs (Aucouturier *et al.*, 2002). Macrophages seem to sequester the infection and impair the early stages of replication as well as propagate the infectious particle at the later stages of infection (Beringue *et al.*,

2000). Therefore, their role is rather ambiguous and their involvement with FDCs is currently unclear.

1.9.4 Dendritic Cells (DCs)

Dendritic cells are mobile cells that can retain endocytosed particles without degradation for long periods of time, thus making them ideal candidates for propagating prion proteins throughout the body (Aucouturier & Carnaud, 2002). They are able to transport and transfer PrP^{Sc} to the B and T lymphocytes and FDCs which all help in replication of the protein (Defaweux *et al.*, 2005). They are also essential for transporting prions to the site of neuroinvasion, as nerve fibres are static elements and they need mobile cells to bring them in contact with the infectious protein (Aucouturier & Carnaud, 2002 & Defaweux *et al.*, 2005). However, it is not yet known the exact mechanism by which the infectious prion protein is transported from the lymphoid zones to the enteric nervous system. Dendritic cells have been found in close contact with follicular dendritic cells and exchanges between them have been proposed where DCs have been known to transfer their load of pathogens by secreting specialized vesicles (exosomes) that will bind to the surface of FDCs (Defaweux *et al.*, 2005). This could be a mechanism for the general transfer of pathogenic prion protein between cells.

1.9.5 Follicular Dendritic Cells (FDCs)

Follicular dendritic cells differ from dendritic cells as they are found on the follicles of the spleen, they are not haemopoietic in origin, as they are derived from stromal precursor cells in the spleen and lymph nodes, are non-migratory, and non-

phagocytic (Bruce *et al.*, 2000). FDCs are known to trap and retain unprocessed antigens on their surfaces as immune complexes made up of antigen, antibodies, and/or the third part of the complement (C3) (Mabott & Bruce, 2001). Trapping and retaining of antigens for extended periods of time in the form of immune complexes leads to the formation of germinal centres. The stimulated FDCs will also form highly convoluted long labyrinthine like, irregular structures that can contact numerous lymphocytes (Mabott & Bruce, 2001 & McGovern *et al.*, 2004). They have been demonstrated many times as major sites of prion accumulation in the germinal centers of the spleen, lymph nodes, and mucosa-associated lymphoid tissues (Beekes & McBride, 2000, Heggebo *et al.*, 2002, Kitamoto *et al.*, 1991, & McBride *et al.*, 1992). Accumulation occurs on the plasmalella and extracellularly around the FDC dendrites (Aguzzi & Heikenwalder, 2006 & Mabbott *et al.*, 2006). FDC networks in germinal centers of healthy individuals have normal cellular PrP^c all over their surface which could account for their profound ability to capture prions and ultimately become infected (Brown *et al.*, 2000, Brown *et al.*, 1999, & Bruce *et al.*, 2000). It has been found that an absence of mature FDCs can lead to an absence of detectable infectivity and PrP^{Sc} in the spleen and reduced disease susceptibility (Brown *et al.*, 1999 & Mabott *et al.*, 2000). In addition, it has been recognized that when mice are treated with a lymphotoxin- β inhibitor which subsequently inactivates FDCs, splenic accumulation no longer occurs and neuroinvasion is delayed (Mabbott *et al.*, 2003). Therefore, this demonstrates that FDCs contribute to accumulation of infectivity and PrP^{Sc} in the spleen and eventual

neuroinvasion (Brown *et al.*, 2000, Bruce *et al.*, 2000, Kitamoto *et al.*, 1991, Mabott & Bruce, 2001 & McBride *et al.*, 1992).

Complement activation and binding to cellular complement receptors are required to localize antigens on the FDCs (Nielsen *et al.*, 2000 & Van den Berg *et al.*, 1995) where they remain for a significant period of time until they are recognized by the B lymphocytes (Mabott & Bruce, 2001). The complement pathway, in particular activation of C3 via the classical and alternative complement activation pathways, plays an important role in localization and retention of prion protein on FDCs during the first few days after infection (Klein *et al.*, 2001, Lotscher *et al.*, 2003 & Mabott & Bruce, 2001). The exact mechanism through which PrP^{Sc} interacts with the complement components has currently not been found, as antibodies to PrP^{Sc} have not been detected in prion infections, and thus it is unlikely that complement activation of the classical pathway is via an antibody (Mabott & Bruce, 2001).

It should be noted that FDCs form immobile networks with no prominent connection to the nervous system (Aoucoutrier *et al.*, 2002). The topographical location between FDCs and nerve endings plays a key role in determining neuroinvasion (Prinz *et al.*, 2003), the closer together they are, the more accelerated the process. How exactly the prions pass between FDCs and the nerve endings is currently unknown. It has been suggested that prion-infected FDCs may degenerate and liberate prions via exosomes released from FDCs, which then passively diffuse

to the peripheral nerve endings triggering uptake by the nervous system (Bruce *et al.*, 2000 & Prinz *et al.*, 2003). In addition, other cells maybe involved in carrying prions from their sites of exposure or inoculation to germinal centers, and finally to the CNS (Aoucoutrier *et al.*, 2002 & Prinz *et al.*, 2003). Experiments have shown that PrP^{Sc} shed by FDCs can be captured by other cell types (Aoucoutrier *et al.*, 2002). It has been suggested that there is a possible passage to T or B cells, but other cell types such as macrophages or dendritic cell (DCs) have also been implicated (Aoucoutrier *et al.*, 2002).

Lymphoid structures and their associated cells play an important role in the stages of infection that precede neuroinvasion following peripheral inoculation with the infectious prion agent. The invasion process of the infectious prion protein is complex and seems to involve many immune cell types that interact with each other to aid in propagation of the infectious prion protein. Replication and accumulation inside the lymphoreticular system seems to be required for infectious prions to reach their critical mass before invasion of the brain can occur (Aoucoutrier *et al.*, 2002). Lymphoinvasion is a topic that attracts prion researchers because of the fact that it occurs before reaching the CNS where clinical manifestations can be seen, and is thus a target for therapeutic intervention and early diagnosis (Aoucoutrier *et al.*, 2002). The likely candidate lymphocytes that have been identified include FDCs, which seem to play a major role, and DCs and macrophages, which assist in the whole process by helping to disseminate the infectious agent (Aoucoutrier *et al.*, 2002).

1.10 Diagnosis

TSEs are impossible to diagnose and the nature of the TSE agent presents a number of challenges for its early detection via conventional methods. One, there is no detectable/known nucleic acid component, and therefore, common detection methods such as PCR cannot be employed. Two, the infectious agent is just a conformer of the normal host prion protein. Thus, there is no inflammatory or immunological response from the host making standard immunological tests difficult (Kubler *et al.*, 2003). Additionally, finding a specific antibody to the misfolded conformer has proved difficult. To further complicate diagnosis, TSEs are insoluble making purification difficult, have long pre-clinical incubation periods, progress through the body is unclear, the normal conformer is in higher abundance leading to false positives, and lastly its unevenly distributed throughout the body with higher concentrations found in nervous system tissues and lower concentrations found in the more easily accessible bodily fluids that are more commonly used for diagnosis, such as blood and urine (Kubler *et al.*, 2003). Currently, definitive diagnosis can only be made use post-mortem. The search for a diagnostic test that is used ante-mortem or preclinically with easily accessible bodily fluids such as blood or urine continues (Furukawa *et al.*, 2004 & Soto, 2004). Researchers strive to find a test that can be non-invasive, efficient, sensitive, specific, and cost effective.

1.10.1 Clinical Diagnosis

Creutzfeldt - Jakob disease (CJD), in particular sporadic CJD (sCJD) is the most common of the human prion diseases and is characterized by several clinical

features. These include fatigue, insomnia, depression, weight loss, headaches, general malaise, and ill-defined pain sensations (Kubler *et al.*, 2003). Neurological features include extrapyramidal signs, cerebellar ataxia, pyramidal signs, cortical blindness and psychiatric features (Kubler *et al.*, 2003). Familial CJD (fCJD) has very similar clinical manifestations, except the onset of disease is slightly earlier than sCJD (Soto, 2004). Variant CJD (vCJD) initially presents itself as a progressive neuropsychiatric disorder with symptoms of anxiety, depression, apathy, withdrawal and delusions (Henry *et al.*, 2002) followed by ataxia, myoclonus (rapid twitch from a sudden muscular contraction) and dementia (Soto, 2004). VariantCJD has a different clinical presentation from sCJD with a longer duration and a different electroencephalogram (EEG) pattern (Soto, 2004). Gerstmann-Straussler-Scheinker (GSS) is characterized by dementia, Parkinsonian symptoms and its duration is much longer, about 5-8 years (Boellaard *et al.*, 1999). Clinically GSS is similar to Alzheimer's except for the ataxia and seizures that are often found in conjunction with GSS (Soto, 2004). Fatal Familial Insomnia (FFI) is an inherited form of the disease like GSS, except its main clinical feature is insomnia, which then manifests into myoclonus, hallucinations, ataxia, and dementia at the later stages of disease (Cortelli *et al.*, 1999).

1.10.2 Histopathological and Immunohistochemical Diagnosis

Examination of post-mortem brain tissue by immunohistochemistry and histology is the gold standard of TSE diagnosis (Beranger *et al.*, 2001). Histological features include spongiform changes characterized by vacuoles ranging in size from 20-200

microns in diameter, neuronal loss, astrocytic gliosis, and in some cases amyloid plaques (extracellular collections of proteinaceous material) (Kubler *et al.*, 2003). Vacuolation in a specific brain region can be detected via light microscopy (Kubler *et al.*, 2003). Although post-mortem histological analysis is an accurate way of diagnosis (although not possible until after the patient is deceased), it has the disadvantages of being time consuming, labour intensive and carrying it out on a large scale is not feasible (Soto, 2004).

Immunohistochemical staining using antibodies is an extremely accurate procedure for diagnosis, even more so than histological examination (Ingrosso *et al.*, 2002). Antibodies against the astrocytic marker protein glial fibrillary acidic protein (GFAP) can detect the extent of astrocytic gliosis in the brain (Kubler *et al.*, 2003). In addition, immunohistochemistry can be used to detect the PrP^{Sc} infectious protein specific to the disease when examining formalin-fixed or frozen brain tissue (Bolton *et al.*, 1982). Unfortunately, the antibodies currently available are not specific to just the PrP^{Sc} form and thus, pre-treatment of the samples with proteinase K is necessary as it removes the PrP^C form preventing non-specific background staining (Bencsik *et al.*, 2005).

1.10.3 Western blot

Western blotting is another validated method for detection of prion infection (Ingrosso *et al.*, 2002). It has the advantage to potentially examine and identify different forms of PrP^{Sc} by molecular mass or the relative abundance of di-, mono-, and non-glycosylated bands (Ingrosso *et al.*, 2002). After proteinase K (PK)

digestion of an infected sample, a distinct 27-30 kDa PK resistant fragment remains on a SDS-PAGE gel that is not seen in a normal uninfected sample because it has been completely digested by proteinase K. PK resistance of the infectious form is thought to result from the change in tertiary structure exhibited by the diseased conformer of PrP (Chakraborty *et al.*, 2005).

1.10.4 Cerebrospinal Fluid (CSF) Analysis

Identification of the 14-3-3 protein, a normal neuronal protein that is released into the CSF after any amount of neuronal insult, is common practice for the diagnosis of sporadic CJD (Laplanche *et al.*, 1994) and can be detected by a western blot assay. The 14-3-3 protein could be elevated in relation to other neurological diseases and is an example of a surrogate biomarker. This protein is often found in the CSF of patients suffering from other neurodegenerative disorders and thus should only be considered valid if other diseases are ruled out, and different diagnostic tests provide further evidence of prion disease (Blennow *et al.*, 2005 & Soto, 2004).

1.10.5 Electroencephalogram (EEG)

Here, the normal electrical rhythms of the EEG are gradually lost and periodic sharp and slow wave complexes (PSWCs) are a characteristic pattern seen with CJD (Zerr & Poser, 2002). Again this pattern can be associated with other diseases, making this only a supportive test.

Only following clinical suspicion, detection of the 14-3-3 protein, and detection of the characteristic EEG pattern can a diagnosis of probable sCJD be made (Alvarez *et al.*, 2005).

1.10.6 Magnetic Resonance Imaging (MRI)

This test has not been accepted as part of the clinical diagnostic criteria yet, but may still be helpful in a supportive diagnosis. Some cases of sCJD show abnormal signals in the anterior basal ganglia and sometimes in the cortex of the brain (Schroter *et al.*, 2000). Rises in certain signals may represent certain landmarks associated with the disease in its earlier stages such as gliosis or neuronal dysfunction (Vidal *et al.*, 2006). This test has the advantage of being a non-invasive ante-mortem diagnostic technique.

1.10.7 PrP^{Sc} Concentration and/or Amplification

Increasing the concentration or amount of PrP^{Sc} may make it possible to increase the sensitivity of currently used tests for diagnosis. It may also help to make pre-clinical diagnosis in easily accessible bodily fluids possible. Different ligands have been found that can bind specifically to PrP^{Sc} and precipitate out minute quantities, (Fischer *et al.*, 2000 & Weiss *et al.*, 1997) although the specificity to PrP^{Sc} and not to PrP^c has to be further evaluated (Soto, 2004).

Amplifying the PrP^{Sc} by PMCA as mentioned earlier has been attempted *in vitro* and it mimics the production of PrP^{Sc} *in vivo*. This procedure specifically amplifies PrP^{Sc} as PrP^c is converted only in the presence of the infectious form (Saborio *et al.*, 2001). The only hurdle with this test so far is that the sensitivity and specificity in

relevant samples needs to be investigated further, especially for human diagnosis considering it has been found so far only to work with normal brain homogenate as the substrate for the conversion process (Soto, 2004). Although, the use of cellular extracts or finding the factors that catalyse the amplification have been suggested to overcome this hurdle (Soto, 2004). Another strategy that has been tested to amplify PrP^{Sc}, is the cell infectivity assay (Klohn *et al.*, 2003). The strategy involves infecting N2a cells, a neuronal cell population that are highly susceptible to prion infection, and determining which cells are infected and propagating these over several generations to produce high amounts of PrP^{Sc} (Soto, 2004). The advantages of this technique are that it is sensitive, rapid, relatively inexpensive, and is suitable for robotization (Klohn *et al.*, 2003). The only problem with this system to date is, there have been no cellular systems available that can propagate prions from relevant cattle and human origins (Soto, 2004).

1.11 Treatment and Vaccination

Currently there are no effective treatments for prion disease. Development of drugs has been a difficult task as standard antivirals or antibacterials do not work. The problem is that once clinical signs are apparent, the CNS damage is so extensive that any treatment at this point is ineffective. Early diagnosis remains a challenge and until this can be achieved, treatment does not seem feasible, especially considering a drug would have to be made that could cross the blood brain barrier. For the past 25 years, many attempts have been made at a treatment for prion disease. Promising results have come from the use of pentosan polysulphate, a polyanionic sulphated polysaccharide closely related to

cell-derived glycosaminoglycans (Glatzel & Aguzzi, 2001). Studies have shown it can prolong scrapie incubation time and reduce disease susceptibility in mice, although it does not cross the blood brain barrier and must be given intracerebrally (Glatzel & Aguzzi, 2001). Others include cyclic tetrapyrroles like porphyrins and phthalocyanines which have been shown to inhibit formation of PrP^{Sc} *in vitro* and *in vivo* (Glatzel & Aguzzi, 2001). Depletion of FDCs was another approach tried resulting in significant delay of scrapie symptoms (Glatzel & Aguzzi, 2001), but of course there are risks associated with depleting an essential immune cell population in the body. Lastly, the administration of peptides that could break up protein accumulations using a β -sheet breaker peptide has been attempted resulting in significantly reduced titer of infectivity and PrP^{Sc} content (Glatzel & Aguzzi, 2001). Ultimately, any treatments that could reduce expression of PrP^C, the material used to make more PrP^{Sc}, block interactions between PrP^C and PrP^{Sc}, block conversion, refold misfolded prions, block toxic effects of PrP^{Sc}, or prevent invasion of the CNS by PrP^{Sc} could all be helpful (Lam, 2003).

Any attempts at making a vaccine have been ineffective due to the high immune tolerance to PrP^C, but passive transfer of anti-PrP antibodies prior to the onset of clinical symptoms has shown promise in that it delayed the onset of disease in mice (White *et al.*, 2003). Although until a pre-clinical diagnostic test is found, this type of vaccination strategy remains futile.

1.12 Pre-Clinical/Ante-Mortem Diagnosis

There are several diagnostic tests available to test for the presence of a prion infection. Many are time-consuming, expensive, and invasive. So far, the only definitive diagnostic method is post-mortem histological and immunohistochemical analysis of brain tissue. The search for a pre-clinical diagnostic test in easily accessible bodily fluids such as blood or urine is desirable. The only problem is that the concentration of PrP^{Sc} in these fluids is extremely low to non-existent (Kubler *et al.*, 2003). The levels required for detection using current techniques is just not possible to achieve from these fluids (Soto, 2004). Thus several attempts have been made to design an ideal pre-clinical test using easily accessible bodily fluids (Furukawa *et al.*, 2004 & Soto, 2004). Secondary biomarkers of infection are now being screened for using global arrays. The combination of both genomics and proteomics has allowed us to be one step closer to finding different disease-indicating biomarkers in easily accessible bodily fluids indicative of the presence of PrP^{Sc} which will help make detection in the blood more feasible (Kubler *et al.*, 2003). For example, it has been found that a novel transcript coding for erythroid differentiation-related factor (EDRF) progressively decreased in expression over the course of the disease (Miele *et al.*, 2001), although this was later found to be expressed at such variable concentrations between individuals that its use as a biomarker proved impossible, further demonstrating the increased need to find other secondary biomarkers.

1.13 Microarrays

Studying the expression of host genes throughout the course of a disease can aid in our understanding of the disease and lead to the discovery of potential biomarkers in the

blood that would be indicative of the presence of Prion disease. Gene expression or cDNA arrays are a tool that can be used for this purpose to analyze a large numbers of nucleic acid fragments simultaneously through miniaturized hybridization assays.

Expression arrays are based on the Northern blotting principle, where the labeled RNA or cDNA sample of interest is used as the probe to hybridize to the immobilized known target DNA sequence on the solid support, commonly known as a spot. There can be up to tens of thousands of spots which are not visible to the naked eye which can aid in the detection of thousands of genes at once. The relative expression levels of specific transcripts can be determined when equal amounts of each sample labeled with a different fluorescent dye are spotted onto the array and fluorescent signals are measured. Following normalization, the intensity of the two signals can be compared. Subsequently, each spot is analyzed for the intensity of the fluorescent signal which determines the specificity of the sample to that target. This will lead to discovering the functions of genes and their expression. Thus, once the differential expression of host genes is discovered in an infected versus healthy individual these genes can be further examined for their potential as biomarkers for that particular disease. This is an indirect approach to studying the actual causative agent of the disease. If the expression of certain genes are found to be upregulated compared to the control, the subsequent protein produced from these genes could be then possibly be easily detected in the blood via a simple and familiar diagnostic technique such as ELISA or other immunoassays. Thus, expression arrays are possibly the first step towards developing a pre-clinical diagnostic test in the blood for Prion disease when detection of the infectious agent is non-existent.

1.14 Hypothesis

Differential expression of genes expressed by murine splenic cells, specifically involved in infection, will occur that are predominantly involved in prion replication and accumulation in the spleen during the course of prion disease.

1.15 Project Objectives

- 1) identify host factors involved in prion replication to better understand prion pathobiology
- 2) identify novel biomarkers that can be used for early prognostic and diagnostic purposes

Global gene expression analysis, such as the use of microarrays, is a high-throughput efficient method of choice to initially examine the whole host genome for differences between diseased and control individuals to ultimately determine the host factors that could act as markers of disease. Therefore, with limited samples available, three different DNA microarray platforms will be used to increase the statistical confidence to profile gene expression changes in the well established scrapie infected mouse model. Splenic cells that are specifically involved in prion replication (enriched for follicular dendritic cells, dendritic cells, and macrophages) will first be isolated via a bead selection method. By examining this particular cell population, I will enrich for the gene expression changes that are specifically involved with the disease. Next, total RNA will be extracted to obtain information from only those genes that are

expressed, RNA reverse-transcribed to cDNA and amplified, and lastly, a competitive hybridization between infected and control cells to the DNA microarray platforms will be performed. Computational analysis of the data from the microarrays will follow to determine statistically significant genes. These will be further validated at the molecular level by another method to detect differential expression; real-time quantitative PCR. Finally, any potential markers will also be validated at the protein level by immunohistochemistry and western blot with the aim of identifying the host factors involved in prion replication and for the identification of potential early biomarkers of infection.

2 **Materials and Methods**

Note: All work with infectious materials was performed inside a Level 3 containment laboratory.

2.1 **Biological Material**

Different models of mice used with different PrP gene sequences will result in longer or shorter incubation times for different disease strains (Bruce *et al.* 1991). The mouse-adapted scrapie model is well established and the incubation periods in addition to the characteristic neuropathology have been determined and are consistent within days for numerous scrapie strains when brain homogenate from previously-ill mice is injected (Bruce *et al.* 1991). Thus, many studies of the prion protein are based on evidence from experimental mouse models that have been inoculated with Scrapie. PrP Scrapie or PrP^{Sc} is one of a few designations used for the infectious particle and will be the designation of choice for this study considering most of the experiments are performed using a mouse-adapted scrapie model.

For the clinical endpoint studies, intracerebral and intraperitoneal inoculations were performed as previously described (Bruce *et al.* 1991). Intracerebral inoculations were performed using a 26-gauge, 4mm stainless steel needle (New hyde park, N.Y.) on VM mice (*Mus musculus*) with 20 µl of a 10% brain homogenate prepared from clinically ill mice previously infected with the scrapie strain 22A. Intraperitoneal inoculations were performed by injection of 200 µl of a 1% brain homogenate using a 26 gauge, needle/ 1cc syringe. Matching numbers of control mice were ‘mock-

infected' in the same way as above, with brain homogenate of uninfected VM mice. Mice were sacrificed at the onset of clinical symptoms of scrapie (loss of weight, flaccid paralysis of hind limbs, uncoordinated gait, and righting reflex lost) at about 200-300 days post infection along with respective, age-matched, controls. Brains were extracted and stored in 10% formalin for histology/immunohistochemical analysis and fresh spleens were obtained and placed in collagenase D (2 mg/ml) (Roche Applied Science, Penzberg, Germany) in 10mM Hepes (Fisher Biotech, Fair Lawn, New Jersey, USA) solution at 37°C for 50 minutes (mins.) to help disaggregate the spleen for cell isolation. The formalin-fixed brains were imbedded in paraffin and sectioned for further histology and immunohistochemistry work.

For the timecourse study, intracerebral inoculations were performed on C57BL/6 mice using 20 µl of a 1% brain homogenate from clinically ill mice previously infected with the Rocky Mountain Laboratory (RML) scrapie strain. Mice were subsequently sacrificed on days 7, 30, 80, 140 (pre-clinical) and 149 (clinical) days post infection. All the above animal handling techniques were approved by the Canadian Science Centre for Human and Animal Health Animal Care Committee.

2.2 Preparation of a Splenic Cell Suspension Depleted of Leukocytes

2.2.1 Preparation of Splenic Cell Suspension

Spleen was isolated fresh from the mouse and placed in a Petri-dish containing 5 ml of filter-sterilized (0.2 µm filtered) Collagenase D (2mg/ml) (Roche Applied Science, Penzberg, Germany) in 10mM Hepes (Fisher Biotech, Fair Lawn, New

Jersey, USA) solution. The mouse spleen was injected with 500 µl of Collagenase D solution from above using a sterile 1ml syringe and 25-gauge needle and then cut into four or five pieces using autoclaved sterilized scissors. The spleen pieces were incubated in this solution for 50 minutes at 37°C. The whole material (remaining fragments and Collagenase-D released cells) were placed inside a 50 µm Medicon (Dako Cytomation, Carpinteria, CA) that was previously rinsed twice with 2 ml and filled subsequently with 1 ml of PBS AutoMacs Running Buffer (Miltenyi Biotec, Cat. 130-091-221). Subsequently, the lid was placed on the medicon and placed inside the Medimachine™ (Dako Cytomation) to homogenize for 30-60 seconds (secs.). The resulting supernatant containing the disaggregated cells was subsequently removed and placed in a tube on ice containing cold PBS solution from above (PBS inactivated Collagenase D) and fresh PBS solution (total of 15 ml was used for the whole disaggregation) was added to the remaining tissue and disaggregation continued in the same manner until a homogeneous suspension was formed. The cell suspension was then filtered through a 50 µm Filcon (Dako Cytomation) using a sterile syringe to obtain a single cell suspension before magnetic separation and to help remove any extra cellular debris that could clog the column. An aliquot of the cell suspension was obtained for counting using a hemacytometer, and an average of 10^7 - 10^8 total cells were counted depending on the size of the spleen. For the remainder of the procedure it was important to work fast and keep cells cold and use pre-cooled solutions to prevent capping of the antibodies on the cell surface and non-specific cell labeling. Thus, all the remaining procedures were performed on ice and inside a refrigerated (4°C) Beckman Allegra

6KR centrifuge. An aliquot of cells were centrifuged at 300xg, the supernatant pipetted off completely, and the cell pellet resuspended in PBS. This aliquot of cells were taken and fixed in a 2% paraformaldehyde solution in phosphate buffer (Sigma, St. Louis, MO, Cat. P-6148), placed at 4°C and later analysed by flow cytometry to determine the number and types of cells present in the whole spleen homogenate.

2.2.2 Depletion of leukocytes from the whole spleen cell population

Depletion of leukocytes from the whole spleen cell population was performed using reagents from the CD4⁺ Dendritic Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany, Cat. 130-091-262). Volumes for magnetic labeling given below are for up to 10^8 leukocytes. The cell pellet was resuspended in 200 µl of PBS buffer and 50 µl of Biotin-Antibody Cocktail (Miltenyi Biotec) was added. The cell suspension was mixed well with the pipette and incubated for 10 mins. on ice. Afterwards, 150 µl of PBS buffer was added and 100 µl of Anti-Biotin Microbeads (Miltenyi Biotec). The cell suspension was mixed well with the pipette and incubated for 15 minutes on ice. The cells were washed by adding 20x labeling volume (i.e. 10 ml) of PBS buffer and centrifuged at 300xg for 10 minutes at 4°C. The supernatant was pipetted off completely and the cell pellet was resuspended in 500 µl of PBS buffer to prepare for depletion of T, B, NK cells, and granulocytes using the LD separation column (maximum capacity of 5×10^8 total number of leukocytes) (Miltenyi Biotec, Cat. 130-042-901).

2.2.3 Magnetic Separation of Spleen Cell Population

A sterile LD column was placed inside the MidiMACS™ Separation unit (Miltenyi Biotec, Cat. 130-042-302). Preparation of the column was performed by rinsing with 2 ml of PBS buffer by adding 1 ml of buffer successively to the column until the reservoir was empty. Next, the 500 µl cell suspension from above was applied to column and the effluent (desired spleen cell population) was collected. The column was then washed two times with 1 ml of PBS buffer. Washing was performed by adding 1 ml of buffer successively to the column until the reservoir was empty and the effluent of cells was collected in a final volume of 2.5 ml. An aliquot was taken for flow cytometry (see below), fixed in 2% paraformaldehyde (Sigma) and placed at 4°C until flow cytometry could be performed. These cells represented the desired depleted leukocyte splenic cell population where FDCs should be enriched. These cells were centrifuged at 300xg for 10 min. at 4°C and the supernatant was pipetted off completely and the cells were resuspended in 1 ml of RNAlater (Ambion, Austin, TX, Cat. AM7021) and placed at 4°C overnight. RNA extraction was performed on the cells the next day, and if not, the cells were stored in RNAlater (Ambion) at -20°C until RNA extractions could be performed.

2.2.4 Flow Cytometry Analysis

An uninfected spleen was used as a representative sample to test for the presence of FDCs in the splenic cell population. All aliquots fixed from above had the fixative removed before beginning labeling of the cells for flow cytometry. The fixative was washed off by adding 1 ml of PBS solution and centrifuged at 3,000 revolutions per

minute (rpm) for 10 min.* The supernatant was discarded and the pellet was resuspended in its corresponding antibody labeling mixture ready for flow cytometry.

After the spleen was homogenized and before cells were labeled with the biotin antibody (Ab) cocktail, an aliquot of cells were taken and fixed as described above. This cell population represented the whole spleen homogenate. The second aliquot of cells was taken after acquiring the desired spleen cell population containing FDCs and was fixed as described above. Each sample of cells (whole spleen homogenate and FDC enriched population) was aliquoted into five samples. The five samples were labeled with their corresponding Ab mixture as follows: one was left unlabeled and therefore resuspended in PBS only, the second was labeled with secondary polyclonal rabbit anti-goat Ab (to test background fluorescence) conjugated to fluorescein isothiocyanate (FITC) (Dakocytomation) at a 1:20 dilution, the third was labeled with polyclonal goat MD1 specific primary Ab for B cells (Santa Cruz, Cat. MD-1(D14): sc-20615) at a 1:100 dilution, the fourth labeled with just secondary polyclonal rabbit anti-rat Ab conjugated to FITC (DakoCytomation, Cat. F0234) at a 1:20 dilution, and the fifth and last sample was labeled with rat monoclonal FDC-M2 (ImmunoKontakt, Abingdon, Oxon, UK, Code: 212-MK-1FDCM2) specific primary antibody for mouse follicular dendritic cells at a 1:50 dilution. Each Ab was incubated for 30 minutes at room temperature in the dark and the cells were subsequently washed in 1 ml of PBS and centrifuged at 3,000 rpm for 10 min.* The supernatant was removed and the cells were

resuspended in 1 ml of PBS ready for flow cytometry. See **Figure 4** for a summary of the procedures used to test for FDC enriched cells using flow cytometry.

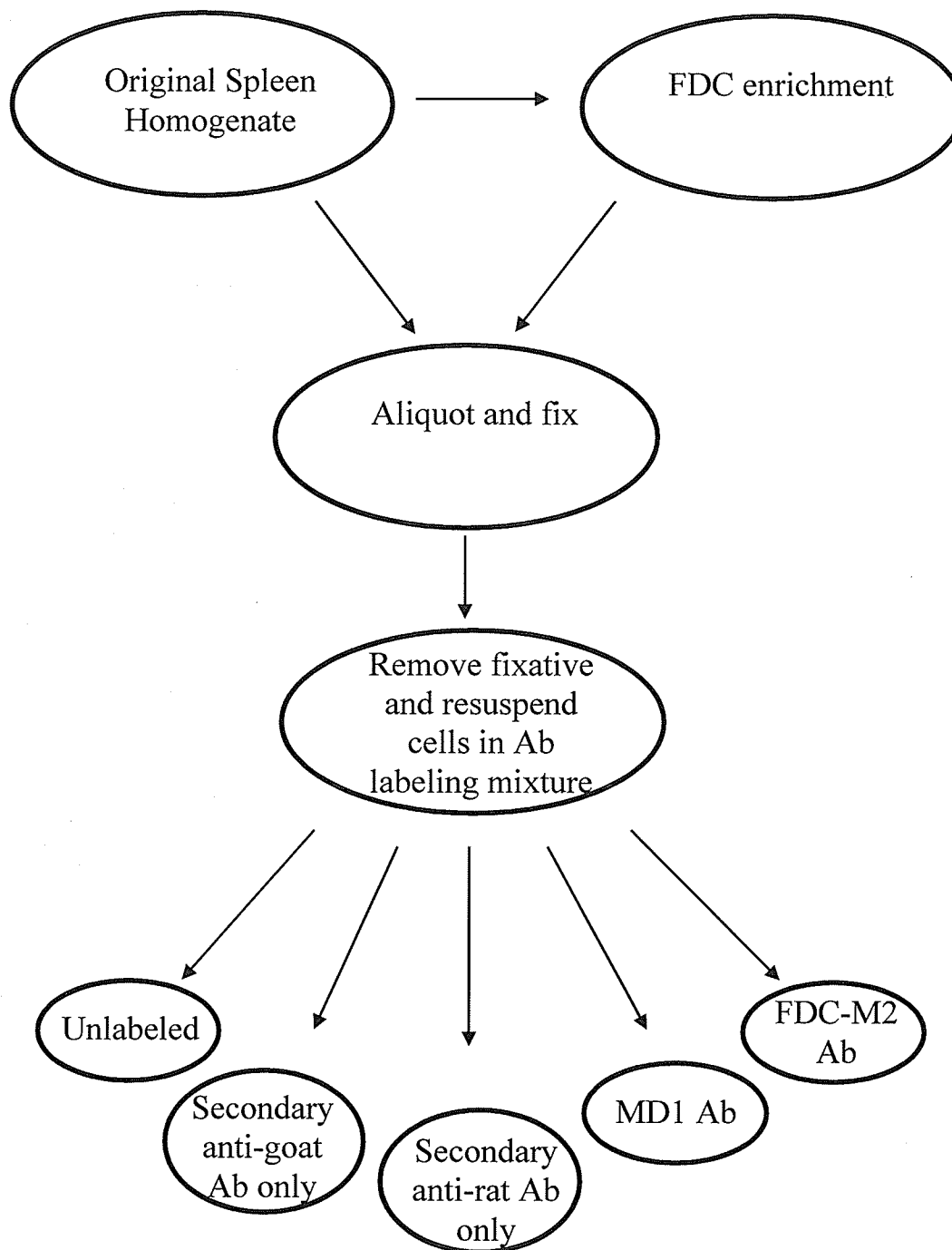


Figure 4: Work flow chart of the analysis of the spleen cell population by flow cytometry.

The FACSCalibur (Becton Dickinson, Oakville, ON) was used to analyze both whole spleen homogenate and enriched splenic cell populations. The instrument was setup and calibrated before each use according to the instrument setup and calibration in the FACSCalibur Handbook

(http://bakerinstitute.vet.cornell.edu/research/cytometry/flow_handbook.html) and a total event rate of 50,000 was used for the analysis. The whole spleen homogenate was analyzed using the side scatter (SSC) and forward scatter plot (FSC) in log scale. This demonstrated the granularity and size of the cells in the spleen respectively. Splenic cell populations were further examined using the fluorescence (FL1 & FL2) plot in log scale. This determined the amount of fluorescence signal generated from the Ab labeled cells and thus, determined which cells were present in the cell populations. The area of the plot with the highest intensity of fluorescence on the FL1 & FL2 plot of the cells labeled with primary Ab were examined with the comparable region on the FL1 & FL2 plot of the cells labeled with secondary antibody only. The percentage labeling of the cells with secondary Ab only (non-specific background binding of the antibody) was subtracted from the percentage labeling of the cells with the primary Ab in these comparable regions to ultimately determine the true calculated percentage of labeling.

2.3 RNA Extractions from FDC Enriched Splenic Cell Population **

The extraction procedure was performed using the Qiagen (Mississauga, On) RNeasy Minikit (Cat. 74104) for isolating total RNA from animal cells ($<5 \times 10^6$ cells) according to the manufacturers instructions. In brief, cells stored in 1 ml of RNAlater (Ambion) overnight were diluted with 0.5 ml of cold PBS and centrifuged in a microfuge* at

10,000 rpm for 10 min. The supernatant containing the RNAlater and PBS was aspirated and the cell pellet was resuspended in 350 µl of lysis buffer (Buffer RLT, Qiagen). During the procedure, cell lysis and homogenization was performed by passing the sample five times through a 20-gauge needle fitted to a sterile RNase-free syringe. To elute, 50 µl of RNase-free water was added to the silica-gel membrane and the samples were microfuged at 10,000 rpm for 1 min*.

* All samples were centrifuged in the eppendorf microfuge (model 5417C).

2.4 DNase Treatment and RNA Clean-up

Spleen tissue contains high amounts of nucleic acids and nucleases, and therefore, removal of all genomic DNA (gDNA) contamination was necessary before RNA could be used for other downstream processes. The TURBO DNA-free™ kit (Ambion, Cat. AM1907) was used according to the manufacturer's instructions. In brief, 0.1 volume of 10x TURBO DNase buffer and 1 µl of TURBO DNase was added to the RNA sample and incubated for the extended incubation time of 1 hr. at 37°C for a more rigorous DNase treatment. Sodium acetate precipitation was further performed according to Ambion's protocol for sodium acetate precipitation of small nucleic acids to remove any excess salts that could interfere with any downstream reactions such as microarrays. The protocol was followed according to the manufacturers instructions except step one was eliminated and the RNA was allowed to precipitate overnight at -80°C overnight and the remainder of the protocol was performed according to the manufacturers protocol. Briefly, the samples were microfuged*** at 10,000 rpm for 45 min. at 4°C and the 80%

ethanol wash was only performed once and afterwards, the RNA was dried in the DNA speed vac (Savant, Global Medical Instrumentation, Ramsey, Minnesota, model DNA 110) to remove excess ethanol used for precipitation. The RNA pellet was then resuspended in 25 µl of RNase-free water, half of what it was eluted in for the extraction above in order to concentrate it. The RNA pellet was allowed to sit at 37°C for 5 min. to ensure it was completely dissolved in the water before resuspension, and then quantitated using the nanodrop (ND-1000 v3.2.1).

*** All samples were microfuged in the Beckman GS-15R centrifuge at 4°C.

2.5 Bioanalyzer

The quality of the extracted RNA including removal of contaminating genomic DNA was tested using the 2100 bioanalyzer (Agilent technologies, Santa Clara, CA). Preparation of the samples and the gel were performed using the Agilent RNA 6000 Nano kit (Agilent, Cat. 5067-1511) according to the manufacturer's instructions.

** All surfaces were decontaminated with RNase-Zap (Ambion, Cat. 9780) prior to beginning RNA work.

2.6 Microarrays

2.6.1 Microarray Construction

There were three types of arrays used for these experiments. The first were arrays that had been prepared in house from two individual libraries, the Brain Molecular

Anatomy Project (BMAP) library and the Mouse Sequence Verified (MSV) Unigene library. There were a total of ~17,000 individual spots on the array including control elements. Inserts from the two libraries was amplified by PCR, and the amplicons were subsequently purified using Millipore multiwell purification plates. The lyophilized PCR products were resuspended in 1x Microspotting Solution Plus (Telechem, Sunnyvale, CA) to a final concentration of 0.25-0.75 $\mu\text{g}/\mu\text{l}$. The DNA amplicons were spotted onto CMT-GAPS-coated glass slides (Corning, NY, Cat. 2549) using the Stealth micro-spotting pins (Telechem).

The second set of arrays used was the mouse genome oligo set from Qiagen's Array-Ready Oligo SetTM (AROS) version 3.0. Each set contained 31,769 arrayable 70mers representing 24,878 genes and 32,829 gene transcripts. The array design is based on the Ensembl (<http://www.ensembl.org/>) Mouse 14.30 Database and Mouse Genome Sequencing Project and contains alternative splicing variants using common, partial common, or individual transcript oligos. The set is provided in 84, 384-well plates containing 31,769 70mers each containing a 5' C6 amino linker. Each well in the plate contained 600 pmol of the specified 70mer (including control elements) lyophilized and were resuspended in ArrayIt buffer (1x, Telechem) for a final concentration of 40 μM . After resuspension, the plates were resealed and shaken on an orbital shaker at 60 rpm overnight at room temp. The 70mers were spotted as per the BMAPs onto CMT-GAPS-coated glass slides (Corning, NY) using the Stealth micro-spotting pins (Telechem).

Lastly, the third set of arrays used was commercially manufactured by Agilent technologies using their 60-mer SurePrint technology. The Agilent whole mouse genome 4x44K version was used (Agilent, Cat G4122F), meaning each slide contained four grids each with ~44,000 individual spots including control elements for a total of ~176,000 spots on one slide.

2.6.2 Amplified (aRNA) Generation, Labeling, and Purification

The production of aRNA from total RNA was obtained using the AminoAllyl MessageAmp^{II} kit (Ambion, Cat. 1753) protocol according to the manufacturer's instructions. The protocol in brief: for each control PBS sample and each infected sample for one microarray experiment, two rounds of amplification were performed from the total RNA in order to generate enough RNA from the enriched splenic cell population to hybridize to the array. For optimal amplification using 2 rounds, 100 ng of input RNA was used to make the first cDNA strand. When the *in vitro* transcription was set-up in the first round, the aminoallyl UTP (aaUTP) was not incorporated into the master mix in order to facilitate 2nd round cDNA synthesis later on, as the enzyme would not be able to convert this to cDNA. When making the cDNA again in the 2nd round of amplification, about 2 µg of aRNA was used to make the 1st strand of cDNA and for the second round of *in vitro* transcription, the aaUTP was now incorporated to facilitate labeling. Once aRNA synthesis was complete, it was purified and split into two samples (~5-20 µg of aRNA per sample) for dye-swapping experiments before vacuum drying in order to account for dye bias in the experiment. Once each sample was dry, they were resuspended in 9 µl of

coupling buffer and one sample was labeled with an Alexa-Fluor 555 carboxylic acid, succinimidyl ester dye (Invitrogen, Molecular Probes, Cat. A20009 Eugene, Oregon, USA) and the other with Alexa-Fluor 647 (Invitrogen, Cat. A20006) dye* each made up in DMSO. Once the aRNA was labeled, it was purified and then quantitated on the nanodrop using the microarray function to check for the dye incorporation into the aRNA samples. Dye incorporation was calculated using Ambion's web-based AminoAllyl Dye Calculator (http://www.ambion.com/techlib/misc/aama_dye_calc.html). Anything ranging from 30-60 dye molecules/1000 nucleotides (nts) was considered good dye incorporation according to Ambion's manufacturers' instructions.

* The Alexa Flour dyes are light sensitive and once the aRNA is labeled with these dyes, all work must be performed in the dark.

2.6.3 Microarray Slide Prehybridization

This procedure was only required for the BMAPs/MSV and the mouse AROS oligonucleotide arrays to prepare them for hybridization. The slides were incubated in a slide rack containing pre-hybridization buffer (see **Appendix 1** for buffer recipe) at 42°C for 45 min. to reduce background signal. Once the pre-hybridization was complete, the slides were washed in nuclease-free water for 5 min. in a slide rack on the shaker at 100 rpm and then dipped once into isopropanol and spin dried in a slide spinner. The 22x60I mm Lifterslips (Erie Scientific Company, Portsmouth, NH, USA) were also dipped once into isopropanol and wiped dry with a kimwipe. The Lifterslip (Erie Scientific Company) was placed on top of the slide silica gel

side down, and the whole assembly was placed at 55°C until it was ready for hybridization. Slides should be and were used within 5 min. of prehybridization.

2.6.4 Microarray Sample Preparation for Hybridization

For the BMAPs/MSV arrays, 5 µg of each labeled aRNA sample was dried down using a DNA speed vac (Savant, model DNA 110)). Each sample was then resuspended in 30 µl of DIG Easy Hyb hybridization buffer (Roche, Cat. 1034946) and the two samples (PBS control and infected) that were being hybridized together onto one slide for a competitive hybridization experiment were mixed together for total of 60 µl, and 1 µl of mouse specific COT1-DNA (20 µg/µl) (Invitrogen, Cat. 18440016) and 1 µl of Poly(A)-DNA (20 µg/µl) (Invitrogen, Cat. polyA.gf) was added to prevent non-specific binding during hybridization. Once the samples were mixed well, they were placed at 95°C for 3 min. to denature any secondary structures and snap cooled on ice for 30 secs.

For the mouse AROS oligonucleotide arrays, 5 µg of each labeled aRNA sample was either dried down to 9 µl using the DNA speed vac (Savant) or was brought up to 9 µl with nuclease-free water to prepare them for fragmentation. The labeled aRNA was then fragmented using Ambion's RNA Fragmentation Reagents (Ambion, Cat. 8740) and procedure for fragmentation was performed according to the manufacturer's protocol. Once fragmentation was complete, 27 µl of Formamide Hybridization buffer (See **Appendix 1** for buffer recipe) was added to each fragmented labeled aRNA sample and each control and infected sample were mixed together for a competitive hybridization.

For the Agilent microarray slides, 1 ug of each labeled aRNA sample was used for an experiment and the samples were prepared, fragmented, and hybed according to the manufacturers instructions from Agilent technologies for two-color microarray-based gene expression analysis protocol version 5.0.1 (August 2006) for 4x44K arrays.

2.6.5 Microarray Slide Hybridization

For the BMAP/MSV and mouse AROS oligonucleotide arrays, the denatured samples from above were added to each prehybridized slide by pipetting the labeled probe mixture (60 µl and 75 µl respectively) underneath the Lifterslip (Erie Scientific Company) slowly until the mixture spread uniformly across the array via capillary action. About 4 ml of DIG Easy Hyb buffer (Roche, Cat. 1034946) was added to the bottom reservoir in the hybridization chamber (Genetix, New Milton, Hampshire, UK) to provide a humid environment during hybridization. The hybridization chambers were sealed and incubated overnight (14-16 hrs.) at 42°C and 55°C for the BMAP/MSV and the mouse AROS oligonucleotide arrays respectively.

For the Agilent whole mouse genome 4x44K arrays, the samples were hybridized to the arrays according to the manufacturers instructions. Briefly, a gasket slide containing four grids was placed face up (Agilent barcode face up) inside the bottom of the hybridization chamber and 100 µl of each labeled sample mixture was added per grid on to the gasket slide (Agilent SureHyb technology, Cat.G2534-

60012). The printed microarray slide (Agilent, Cat. G4122F) was then added printed side down (Agilent barcode down) on top of the gasket slide containing the samples in a sandwich-like manner and the top of the hybridization chamber was placed on top and secured in place with the hybridization chamber's thumbscrews. The chambers (Agilent, Cat.G2534A) were then rotated slowly 180° three times and placed vertically inside the hybridization chamber secured by the hybridization chamber rotator clip. The arrays were incubated overnight (17 hrs.) at 65°C with vertical rotation at a setting of ten on the hybridization chamber rotator.

2.6.6 Microarray Slide Washing and Scanning

Once hybridization was complete, the Lifterslips for the BMAP/MSV were removed by gently dipping the slide into pre-warmed (42°C) low stringency wash buffer (see **Appendix 1**) repeatedly until it dislodged. The BMAP/MSV arrays were subsequently washed once each for 5 min. at 100 rpm in a slide rack on a shaker in low stringency wash buffer (pre-warmed to 42°C), high stringency wash buffer (see **Appendix 1**), and 0.1X SSC buffer (Ambion, 20x SSC buffer, Cat. 9763). After the third and final wash, the slides were dried immediately in a slide spinner and placed in a light tight slide container until they could be scanned within the hour.

The Lifterslips for the mouse AROS oligonucleotide arrays were removed by gently dipping the slide into pre-warmed (42°C) Low Stringency wash buffer (see **Appendix 1**) repeatedly until it dislodged. They were then washed two times for 5 min. at 110 rpm in a slide rack on a shaker each in Low Stringency wash buffer (pre-warmed to 42°C) and High Stringency wash buffer (see **Appendix 1**). For the

third and final wash, the slides were washed once in 0.05x SSC (Ambion, 20x SSC buffer, Cat. 9763) for 5 mins. at 110 rpm in a slide rack on a shaker. Slides were then dried and placed in a light tight slide container as above ready to be scanned. The BMAP/MSV and mouse AROS oligonucleotide slides were scanned using the Agilent Microarray Scanner system with surescan technology (v.6.3).

Briefly, once hybridization was complete, the Agilent microarray slides were washed according to the manufacturer's protocol for 4x44K arrays and scanned with the Agilent Microarray Scanner system using the scanner settings for the 4x44K array format according to Agilent technologies manufacturer's instructions.

2.6.7 Data analysis

For the BMAP/MSV and mouse AROS oligonucleotide arrays, microarray .TIFF images were uploaded into Array-Pro (MediaCybernetics, version 4.5, Bethesda, MD) and spots were identified and grids constructed. To construct grids, the different grid options that were specifically selected for were whole cell area to measure the raw signal intensity of the spot, local corners for the background calculations, trimmed background subtracted from raw intensity for the net intensity measurement, and finally, normalization was not enabled in this program as this was done in subsequent programs. The data obtained was stored in and normalized using the GeneTraffic Microarray Database and Analysis System (version 2.6, Iobion Informatics, La Jolla, CA). Normalization of raw intensities was performed using a linear regression smoothing algorithm (Loess best-fit) over the individual array subgrids. The resulting gene list containing log₂ ratios for the filtered and

normalized intensity values were used for further statistical analysis using the Significance Analysis of Microarrays (SAM) software package (version 3.0, Leland Stanford Junior University). A one-class SAM analysis with a false discovery rate (FDR) of 1% was the criteria used for analysis. Any gene where the spot intensity between the control and the infected mouse differed by a fold change of two was considered significant and was further investigated using the Ingenuity Pathway Analysis Program (IPA) (version 5.0, Ingenuity Systems, Redwood City, CA) This program helped determine the function of genes and placed them into functional networks or biochemical pathways which aided in understanding its role in prion disease.

For the Agilent 4x44K arrays, the .TIFF images were uploaded into Feature Extraction (Agilent technologies, G2567AA FE software, v.9.1), the spots were identified, and the grids constructed using the GE2-v5_91_0806 protocol and the 014868_D_20060807 grid file for the whole mouse genome 4x44K expression arrays which were downloaded from the Agilent technologies website. Background calculations were performed by the feature extraction software using spatial and multiplicative detrending and normalization was performed using linear and lowess. The *p*-value to determine differential expression was 0.01 and the resulting gene list from the feature extraction software provided log₁₀ ratios as the red (AlexaFlour 647)/green (AlexaFlour 555) channel and therefore, not necessarily as infected over control. In order to analyze the fold change for the infected samples, the log₁₀ ratios for some samples had to be inversed. For

example, all the experiments where the infected sample was labeled with AlexaFlour 555, the inverse of the log10 ratio was calculated to determine the true log10 ratio for the infected sample over the control sample. Once all the proper log10 ratios were calculated, the resulting gene list was analyzed in SAM and IPA using the same procedure as above. A process flow chart for feature extraction data analysis is shown in **Figure 5**.

Data Analysis

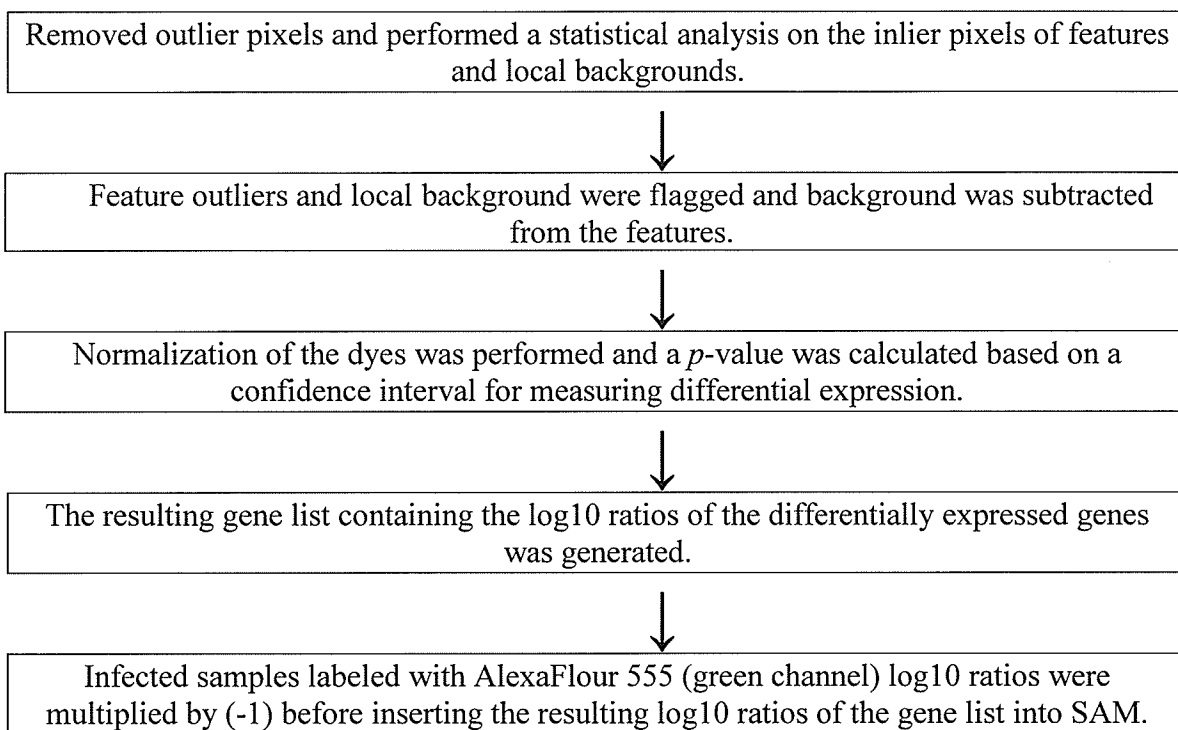


Figure 5: Process flow chart of the feature extraction data analysis (adapted from Agilent G2567AA Feature Extraction Software (v.9.1) User Guide).

2.7 Validation of Microarrays

2.7.1 cDNA generation

Total RNA from the above extractions (section 2.3) used on the microarrays were also used for validation. To begin, total RNA was first converted to cDNA using the High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, Cat. 4322171). An input of 2 ng/ μ l of total RNA was used per cDNA reaction and the protocol was followed according to the manufacturer's instructions. Reactions were performed in 20 μ l volumes where 10 μ l of total RNA (2 ng/ μ l) was added to 10 μ l of the 2X reverse transcription (RT) master mix (See **Table 1** for reaction components and **Table 2** for thermal cycling conditions).

Once cDNA was made, it was purified by adding 1 μ l of RNase cocktail (Ambion, Cat. AM2288) to the 20 μ l of cDNA (1:20 dilution) to remove any remaining RNA from the RT reaction and placed at 37°C for one hour. Lastly, the cDNA reaction was further cleaned up using the QIAquick PCR purification kit (Qiagen, Cat. 28106) according to the manufacturer's instructions. cDNA was eluted by adding 30 μ l of pre-heated (55°C) nuclease-free water to the column and letting it stand for 2 min. before centrifugation. cDNA was then quantitated using the single-stranded cDNA mode on the nanodrop.

Table 1: cDNA reaction preparation for TaqMan™ (Applied Biosystems)

To Prepare 2X RT Master Mix for a 20 µl reaction	
Component	Volume (µl)/reaction
10X RT Buffer	2.0
25X dNTP Mix (100 mM)	0.8
10X RT Random Primers	2.0
MultiScribe™ Reverse Transcriptase	1.0
Nuclease-free H ₂ O	4.2
Total per Reaction	10.0

Table 2: Thermal cycling conditions for the cDNA RT reaction for TaqMan™ (Applied Biosystems)

	Step 1	Step 2	Step 3
Temperature	25 °C	37 °C	85 °C
Time	10 min.	120 min.	5 secs.

2.7.2 Quantitative Real-Time PCR (qRT-PCR)

8-10 ng of cDNA generated from above was used as a template for qRT-PCR. The TaqMan Gene Expression Assay (Applied Biosystems) protocol was followed according to the manufacturer's instructions. Specifically, 20 μ l reaction volumes were used under TaqMan Fast PCR conditions (Optical 96-Well fast thermal cycling plate setup) using the TaqMan Fast Universal PCR Master Mix (2x) without AmpErase UNG (Applied Biosystems, Cat. 4352042) and was run on the 7900 HT Fast Real-Time PCR System. TaqMan MGB probes designed by the manufacturer (Applied Biosystems) that were used varied according to the gene desired to be amplified and a list of these probes can be found in **Table 3** and the cycling conditions can be found in **Table 4**.

Table 3: TaqMan™ MGB probes panel used for qRT-PCR

Name	Gene Symbol	TaqMan™ Probe
Small leucine-rich proteoglycan family		
Fibromodulin	Fmod	Mm00491215_m1
Biglycan	Bgn	Mm00455918_m1
Lumican	LUM	Mm00500510_m1
Chondroadherin	Chad	Mm00483284_m1
Keratocan	Kera	Mm00515230_m1
Proline arginine-rich & leucine-rich repeat (PRELP)	Prelp	Mm00475328_m1
Osteoadherin/Osteomodulin	Omd	Mm_00449589_m1
Epiphykan (Dermatan sulphate proteoglycan 3)	Dspg3	Mm00514611_m1
Mimecan/Osteoglycin	Ogn	Mm00493253_g1
Decorin	DCN	Mm00514535_m1
Large proteoglycan family		
Aggrecan	Agc1	Mm00545794_m1
Versican (Chondroitin Sulfate Proteoglycan 2)	Cspg2	Mm00490179_m1
Perlecan (Heparan sulfate proteoglycan 2)	Hspg2	Mm01181162_m1

Table 4: Real-time cycling conditions for a fast run using the 7900HT System
(Applied Biosystems)

Step	Denature	PCR	
	Hold	Cycle	
		(40)	
		Denature	Anneal/Extend
Time	20 secs.	1 sec.	20 secs.
Temperature	95°C	95°C	60°C

2.8 Immunohistochemistry

2.8.1 Decorin detection in Spleen tissue

Previously prepared paraffin-embedded 4 μ M spleen tissue sections from VM 22A infected and VM PBS control mice were dried overnight. Slides were deparaffinized twice in xylol for 2 min. each soak and then slowly hydrated from alcohol to water with two five min. washes of each 100% alcohol, 90% alcohol, 70% alcohol, and lastly, to running tap water. Antigens were retrieved by boiling slides in citrate buffer pH 6.0 for 5 min. and then washed six times with Tris-Buffered Saline, with Tween 20, pH 8.0 (TBST) (Sigma powder packets, Cat. T9039, prepared according to package instructions). Slides were then blocked initially using a peroxidase block where they were washed two times in 3% hydrogen peroxide for 5 min. and washed six times with TBST. Secondly a serum block was performed where slides were incubated in normal goat serum (1:1000 dilution, DakoCytomation, Cat. X0501) for 20 min. at 45°C. Primary decorin antibody (rabbit polyclonal to decorin, abcam, Cat. ab15052) was added at 5 μ g/ml overnight at 4°C and then washed with TBST six times and incubated with the corresponding secondary antibody (goat anti-rabbit HRP, DakoCytomation, Cat. P0448) at 5 μ g/ml for 20 min. at 45°C. Slides were washed in TBST six times and horse radish peroxidase enzyme was added for 20 min. at 45°C using the StreptABC complexes (DakoCytomation, Cat. K0492). Slides were then washed with TBST six times, and 3,3'-diaminobenzidine tetrahydrochloride (DAB) substrate (DAB tablets, DakoCytomation, Cat. S3000) was added for 5 min. at room temp. and slides were rinsed in water. They were then added to Harris' Hematoxylin (H&E) stain for 2

min., washed in running tap water for 3 mins., then differentiated in 0.5% acid alcohol for 30s, washed in running tap water for 30s, soaked in 1% ammonia water for 3 secs., washed in running tap water for 5 mins., counterstained in 1.0% Eosin for 4 mins., and then dehydrated quickly. Dehydration was carried out by dipping slides two times for 3-4 dips in each 95% alcohol and 100% alcohol. The slides were then cleared in xylol four times for 3-4 dips and mounted on a coverslip using Fisher permount mounting medium (Fisher, Ottawa, ON, Cat. SP15-100) and examined under the light microscope for Brown staining.

2.8.2 Iron Detection in Spleen Tissue

Paraffin-embedded spleen tissue sections (4 μ M) from VM scrapie infected and PBS control mice from 21 d.p.i., 100 d.p.i., and 148 d.p.i. were used to test for the presence of iron using the Perls' Prussian blue reaction for ferric iron. Briefly, slides were deparaffinized and hydrated as above and then soaked in freshly prepared incubating solution (1:1 solution of 2% potassium ferrocyanide and 2% hydrochloric acid) for 10 min. at room temp. Slides were then rinsed in tap water and counterstained in 1% neutral red for 30 secs. The slides were subsequently rinsed in tap water, dehydrated in 95% and 100% alcohol, and mounted with a coverslip as in the above procedure (section 2.8.1). Ferric iron deposits appeared blue and nuclei red upon visualization with the light microscope.

2.9 Western blot

2.9.1 Lysate Preparation

Previously frozen mice spleens were homogenized in NP-40 lysis buffer (0.5% NP-40, 0.5% sodium deoxycholate, and 10mM Tris buffer pH 7.5). Specifically, spleens were homogenized in the medimachine (Dakocytomation) four times using 500 μ l of lysis buffer each time for total of 2 ml of lysis buffer used for the whole spleen tissue. After each round in the medimachine, sample was drawn out and placed in a Petri dish on ice. Petri dish was placed at 4°C on an orbital shaker at 100 rpm for 2-3 hours. Afterwards, homogenate was centrifuged at 4°C in Beckman GS-15R centrifuge for 20 min. at ~14,000 xg (12,000 rpm). The supernatant containing the extracted whole cell proteins or membrane bound proteins was aspirated and quantified using the spectrophotometer (SpectraMax Plus, Molecular Devices, Sunnyvale, CA).

2.9.2 Protein Quantification

A standard curve was prepared by diluting a 2 mg/ml stock solution of bovine serum albumin (BSA, Amersham BioSciences 2-D Quant kit, Uppsala, Sweden, Cat. 80-6483-56) in distilled water to make corresponding 2.0 mg/ml, 1.0 mg/ml, and 0.5 mg/ml solutions for the standard curve. Protein quantities were measured using the Bio-RAD (Mississauga, ON) Bradford protein assay. Briefly, 796 μ l of distilled water, 4 μ l of sample, and 200 μ l of Bradford 1x dye reagent (Bio-RAD, Cat. 500-0205) were added to a cuvette and just 800 μ l of water and 200 μ l of Bradford 1x dye reagent (Bio-RAD) were added for the blank. Absorbance was

measured at 595 nm using the spectrophotometer (SpectraMax Plus) and the protein quantities were determined based on the standard curve measurements.

2.9.3 Decorin Detection

Samples were prepared and the gel was run and transferred according to the manufacturers protocol from Invitrogen (Carlsbad, CA) for NuPAGE Novex Bis-Tris glycine mini gels (1.0mmx10 well) with a few modifications. Briefly, 40 µg of protein sample was mixed with 1x sample buffer (Invitrogen, NuPAGE LDS Sample Buffer 4x, Cat. NP0008) and 1x reducing agent (Invitrogen, NuPAGE Sample Reducing Agent 10x, Cat. NP0009) and boiled at 100°C for 5 min. Afterwards, samples were loaded into the wells (40 µg/lane) including the ladder (BioRad precision plus protein dual colour standards, Cat. 161-0374) of a 10% NuPAGE gel (Invitrogen, Cat. NP0321) inside the buffer tank filled with 1X MOPS SDS Running buffer (20x, Invitrogen, Cat. NP0001) and NuPAGE antioxidant (Invitrogen, Cat. NP0005). Gel ran at 200 volts (V) for 35 min. and once it was finished, it was transferred to a 0.2 µM polyvinylidene difluoride membrane (PVDF) membrane (previously prepared according to invitrogen, Cat. LC2002) in a buffer chamber containing 1x NuPAGE transfer buffer (20x, invitrogen, Cat. NP0006) and NuPAGE antioxidant (Invitrogen) at 30V for one hour. Once transferring was complete, the membrane was inspected visually for signs of proper transfer. Transfer was considered successful if the precision plus protein dual colour standards ladder could be seen on the membrane. The membrane was then blocked to prevent non-specific binding in 10 ml of a 5% skim milk powder solution made

in TBST at room temp. for 30 min. on a rocker. The membrane was then incubated overnight in primary decorin antibody at a 1:250 dilution or 2 µg/ml (rabbit polyclonal to decorin, abcam, Cat. ab15052) overnight at 4°C on a shaker at 50-55 rpm. The next morning, primary antibody was washed off in 5-10 ml of TBST three times at room temp. on a shaker for 5 min. each wash. The membrane was then incubated in secondary goat anti-rabbit horse radish peroxidase (HRP) antibody (1:2000 dilution, DakoCytomation) for 30 min. at room temp. on a shaker. The secondary antibody was subsequently washed off in TBST six times at room temp. on a shaker for 5 min. each wash with 5-10 ml of TBST. Detection was achieved using the Supersignal West Pico Chemiluminescent Substrate (Pierce, Cat. 34080) by incubating the membrane for 5 min. at room temp. in equal amounts of Pierce Supersignal West Pico stable peroxide solution (Cat. 1856135) and Pierce Supersignal West Pico luminol/enhancer solution (Cat. 1856136). After incubation, the membrane was dried off slightly on filter paper and enclosed in a laminating pouch ready for development using the Fluor-STM MultiImager (Bio-RAD). Amount of chemiluminescence was detected and densitometry was performed to determine quantitative differences between samples.

After decorin detection was complete, membranes were stripped and re-probed with monoclonal anti- α -tubulin antibody as a loading control. Membranes that were not stripped immediately were stored in a laminated pouch at 4°C until stripping could be performed. The membranes that had been stored at 4°C for longer periods were wet again with 100% methanol (HPLC grade, Fisher, Cat. A452-4) for 30 secs. to re-

hydrate the membrane. Afterwards, the membranes were washed two times at room temp. in 5-10 ml of TBST on a shaker for 5 min. each wash and 5-10 ml of stripping buffer (Restore Western Blot Stripping Buffer, Pierce/Fisher, Rockford, IL, Cat. 21059) was added and let sit on a shaker for 15 min. All membranes were then washed two times in 5-10 ml of TBST at room temp. for 5 min. each wash. The membranes were blocked with 5-10 ml of 5% skim milk powder in TBST and incubated at room temp. for 30 min. on a shaker. They were then incubated overnight at 4°C on a shaker with mouse monoclonal anti- α -tubulin antibody at a 1:500 dilution (Sigma, Cat T9026). Primary antibody was washed off using the same procedure as for decorin detection above and the membrane was then incubated with goat anti-mouse HRP secondary antibody at a 1:1000 dilution (DakoCytomation, Cat. P0447) for 30 min. at room temp. on a shaker. Secondary antibody washing, development, and imaging was all performed the same way as for the decorin detection above. Lastly, densitometry was performed on the α -tubulin to ensure proper loading of each lane on the western blot.

3 Results

3.1 Scrapie Infection of Mice

3.1.1 Confirmation of prion infectivity in scrapie inoculated mice

For the clinical endpoint studies, 14 VM mice were inoculated with the 22A strain of scrapie either by intracerebral (i.c.) inoculation or intraperitoneal (i.p.) inoculation. Mock-infected VM mice were also included and inoculated with PBS in a similar fashion. Scrapie infected mice were sacrificed after displaying the clinical symptoms associated with disease including, uncoordinated gait, flaccid paralysis of the hind limbs, rigidity, and loss of the righting reflex. The criteria for sacrifice were met if two or more of these symptoms were apparent; tissues were then collected for RNA isolation, histology, and immunohistochemistry. A summary of the mice sacrificed can be found in **Figure 6**. Of these 14 VM mice used for the study, only 4 infected mice and two mock-infected controls (3 biological replicates from each timepoint is statistically valid) were ultimately used for the study due to losses in samples, mice sick with other complications other than prion disease, and non-sufficient amount or quality of RNA obtained for further analysis in these experiments.

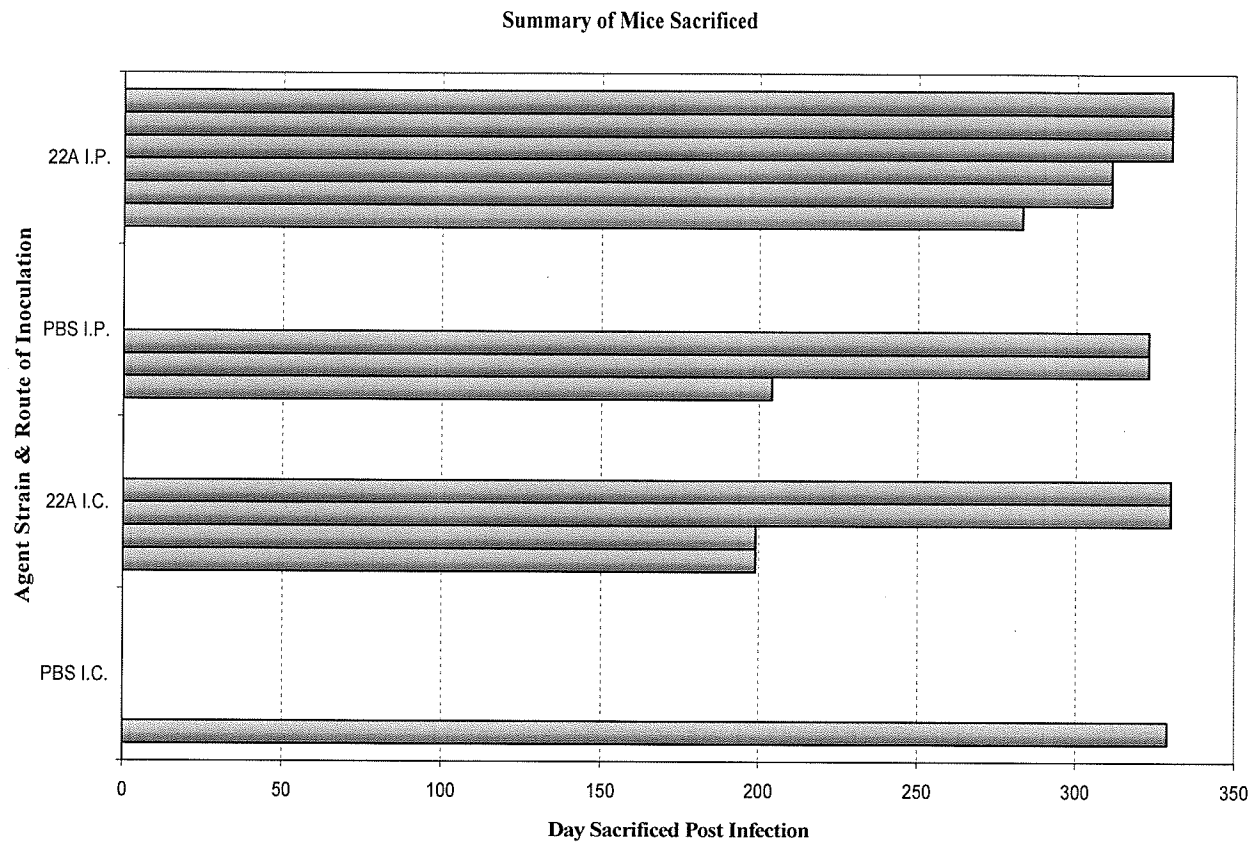
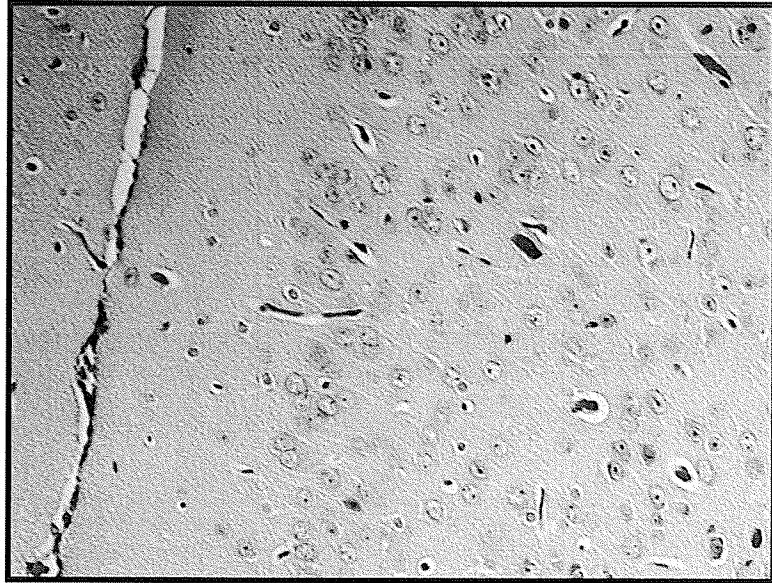
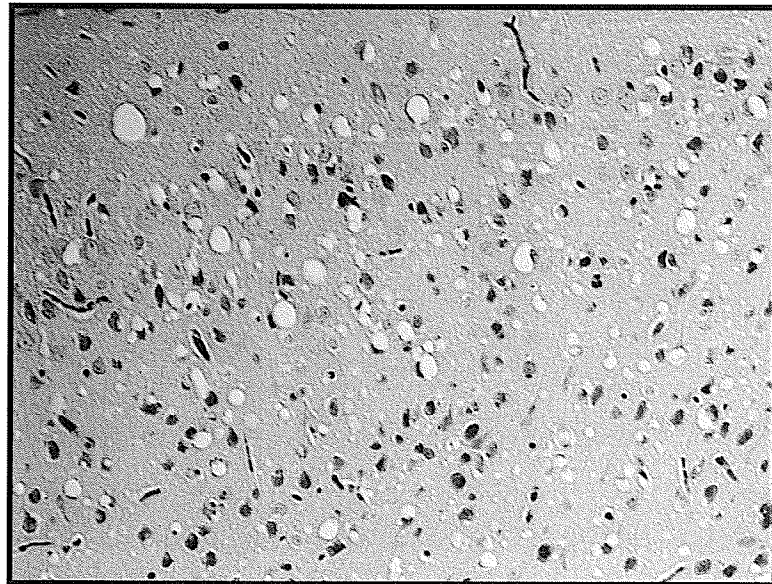


Figure 6: A total of 14 VM mice were sacrificed after advanced signs of clinical disease along with the age-matched control mice. I.p., intraperitoneal, i.c., intracerebral route of inoculation.

Histological and immunological examination of post-mortem brain tissue was employed for confirmation of prion infectivity. As can be seen in **Figure 7**, histological examination of post-mortem brain tissue displays spongiform degeneration of the gray matter evident in the scrapie-infected brain. This is characteristic of prion infection and clusters of vacuoles (holes in the brain) are present representing neuronal loss. **Figure 8** displays immunohistochemistry pictures of scrapie infected and control brains, which also confirm prion infectivity. It is evident by the increased staining of the glial fibrillary acidic protein (marker of astrocytic gliosis) in the scrapie infected mouse that astrocytic gliosis has occurred. This is characteristic of prion disease where proliferation of astrocytes (supporting cells of the brain) occurs to compensate for neuronal loss.

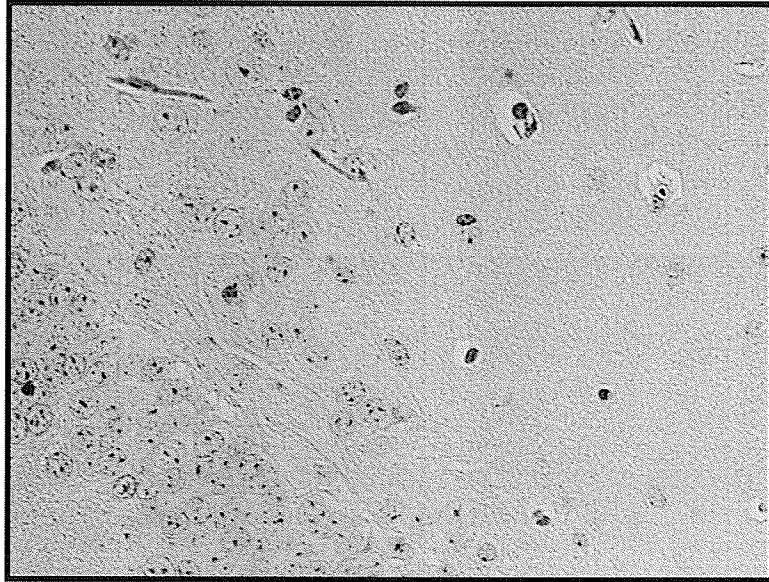


Mock-infected mouse

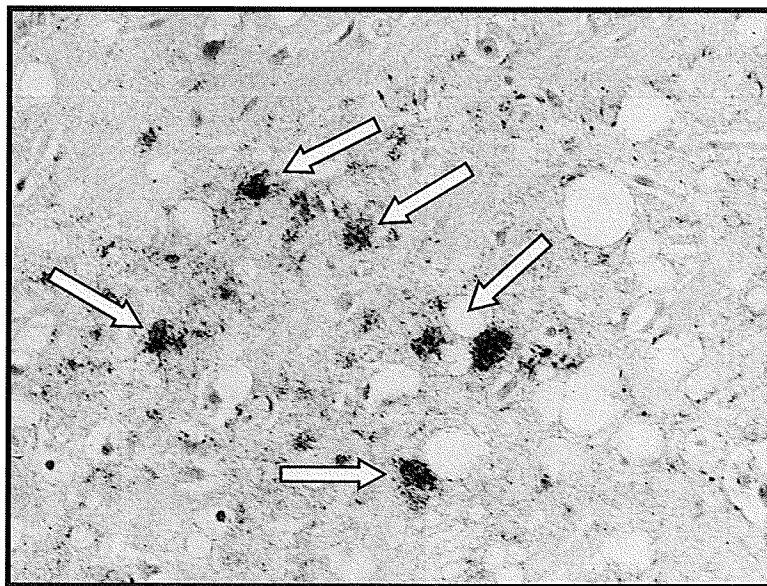


Scrapie-infected mouse

Figure 7: Histological examination of post-mortem brain sections from a mock-infected control and scrapie-infected mouse inoculated i.c. The extensive vacuolation present only in the scrapie-infected mouse are characteristic of prion infection.



Mock-infected mouse



Scrapie-infected mouse

Figure 8: Immunohistochemical examination of post-mortem brain tissue from control and scrapie-infected mouse inoculated i.c. The brown stained deposits present only in the scrapie infected mouse indicative of astrocytic gliosis, confirms extensive astrogliosis which is characteristic of prion disease.

3.2 Spleens of prion infected mice inoculated via both the intracerebral and intraperitoneal route contain PrP^{Sc}

Immunohistochemical examination of spleen tissue infected with scrapie confirmed accumulation of PrP^{Sc} (See **Figure 9**). Spleens were incubated with the 6H4 antibody specific for the prion protein and it is evident after visualization with DAB substrate that PrP^{Sc} accumulation has occurred. See the methods (section 2.8.1) above under immunohistochemistry for decorin detection in spleen tissue. Methods for prion protein detection are the same except mouse 6H4 antibody specific to mouse prion protein (Prionics, Switzerland) was used with a different secondary antibody (Goat anti-mouse HRP, Dakocytomation).

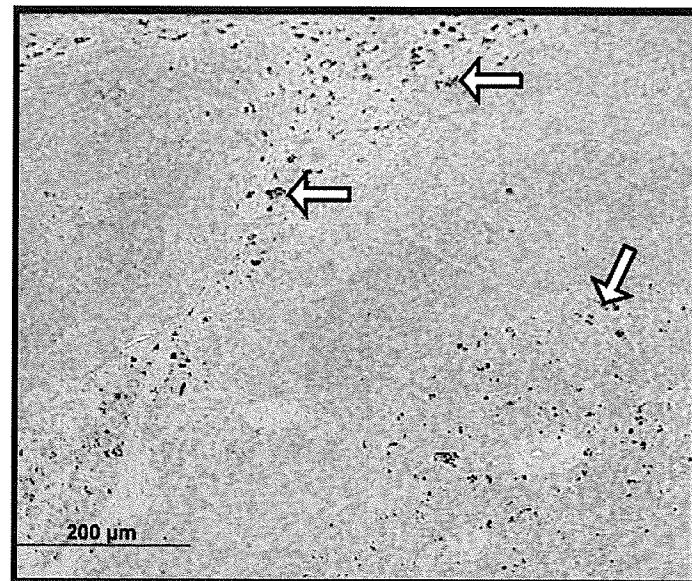
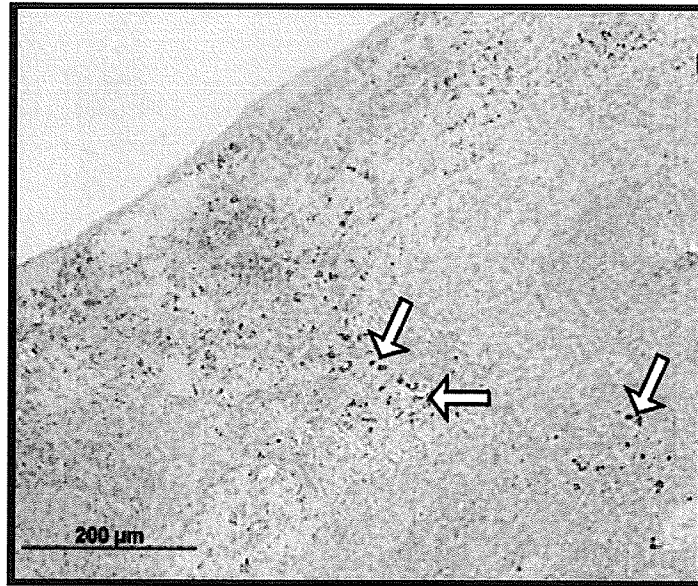


Figure 9: Immunohistochemistry sections of scrapie infected mice spleens at clinical endpoint. 6H4 antibody for the prion protein was used with DAB substrate for visualization of 4μm sections. Arrows indicate sites of PrP^{Sc} accumulation.

3.3 Isolation of a Splenic Cell Population (SCP) enriched for cells associated with prion propagation and pathogenesis

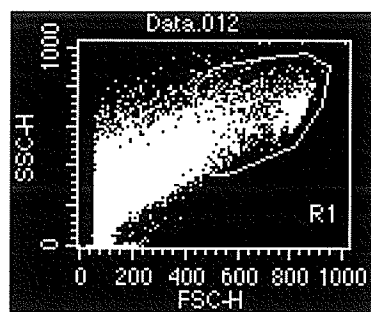
Previous unpublished results from the Booth lab, revealed only a small number of genes that were differentially expressed in the whole spleen tissue during scrapie disease. I hypothesized that gene expression changes in prion disease are associated with the small proportion of splenic cells in which prions have been shown to be propagated i.e. FDCs, dendritic cells and macrophages. These changes in expression are likely diluted, or 'masked' by the preponderance of cell types, such as leukocytes that do not appear to harbour replicating prions. By enriching for the population of cells that are directly involved in prion pathogenesis, it will potentially increase the sensitivity of genomic techniques to identify genes directly involved in the host response to prion replication and infection.

I decided to use a depletion technique to remove leukocytes from a disrupted splenic cell population, thus depleting those cells that have not been shown to be directly involved in prion pathogenesis; These include cells such as T, B, NK cells and granulocytes. Spleens were collected from scrapie infected mice showing signs of prion infection, alongside spleens from age-matched, similarly treated control mice. Tissue was disrupted using enzyme digestion with collagenase D, and mechanical disaggregation using the Dakocytomation Medimachine™ (see Methods section 2.2.1). A suspension of single cells filtered through a 50µm filter was the starting material for preparation of the enriched population. I used reagents from the CD4+ Dendritic Cell Isolation Kit manufactured by Miltenyi Biotec to remove T, B, NK cells and granulocytes. Essentially this is a negative selection procedure in which biotin-conjugated antibodies bind to T, B,

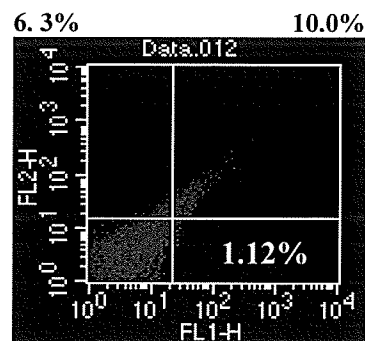
NK cells and granulocytes and are removed using magnetic anti-biotin microbeads. What is left is a cell population enriched for dendritic cells, FDCs and macrophages, referred to as the splenic cell population or SCP.

FACS analysis was used to first analyze the whole spleen homogenate. The unlabelled sample was initially used for gating on the population of interest for further examination. Gating on region 1 in the FSC/SSC plot (**Figure 10A**) gave the highest percentage of FDC cells (**Figure 10D-69.77%**), the population I was interested in, and the highest percentage of B cells (**Figure 10F-71.05%**), a major population in the spleen. Region 1 was also chosen because FDCs are larger cells (further on FSC scale) due to their long dendritic processes and granular (further on SSC scale) along with other cell types present in the spleen (Kitamoto *et al.*, 1991). Additionally, examining another cell type such as the B cells will help to confirm depletion of other cell types present in the spleen and enrichment of FDCs by examining the ratio of FDCs compared with other major cell types present. Once the cell population was gated, fluorescent detection of both FDCs and B cells was further examined. Firstly, the unlabelled gated population was examined, and little to no fluorescent detection (**1.12%**) was found on the FITC fluorescent axis (lower right quadrant) as expected. Secondly, the gated population was labeled with a secondary anti-rat FITC Ab only (**Figure 10C**) to test for background fluorescence when examining the FDCs. Thirdly, the gated population was labeled with a FDC specific Ab (FDC-M2 FITC Ab) (**Figure 10D**) and the fluorescence from the FITC axis from the secondary anti-rat FITC Ab (**Figure 10C-65.83%**) was subtracted from the FITC axis with the FDC specific Ab (**Figure 10D-69.77%**) to give a **3.94%** FDC labeling (**Figure**

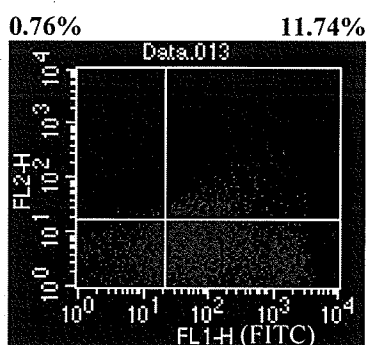
10D- in white) in the whole spleen homogenate. Fourthly, the gated population was labeled with a secondary anti-goat FITC Ab only (**Figure 10E**) to test for background fluorescence when examining the B cells. Lastly, the gated population was labeled with a B cell specific Ab (MD1 FITC Ab) (**Figure 10F**) and the fluorescence from the FITC axis from the secondary anti-goat FITC Ab (**Figure 10E-61.71%**) was subtracted from the FITC axis with the B cell specific Ab (**Figure 10F-71.05%**) to give a **9.34%** B cell labeling (**Figure 10F- in white)** in the whole spleen homogenate.



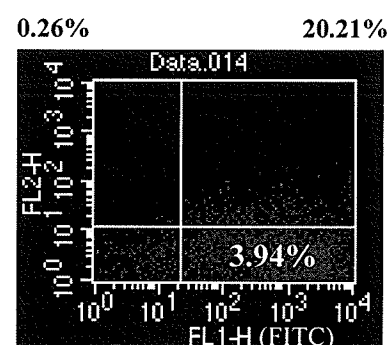
A *Unlabelled*



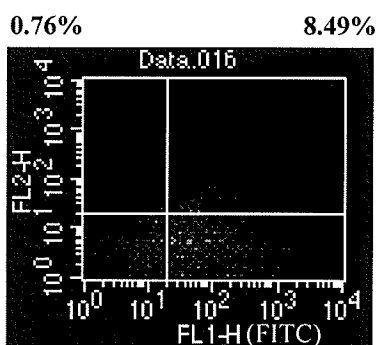
B *Unlabelled*



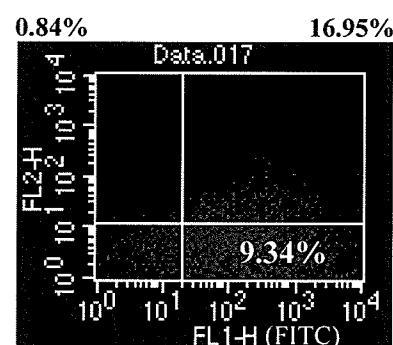
C *Secondary anti-rat FITC Ab*



D *FDC-M2 FITC Ab*



E *Secondary anti-goat FITC Ab*



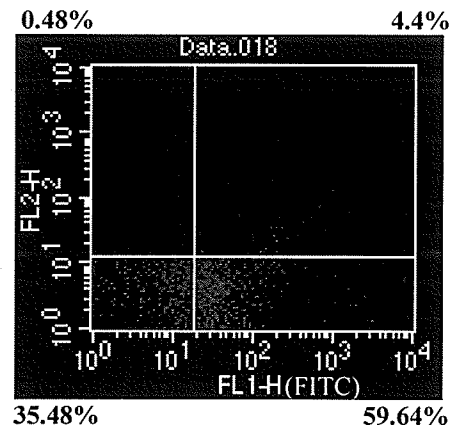
F *MD1 FITC Ab*

Figure 10: FACS analysis of splenic cell populations in whole spleen homogenate. The percentages for each quadrant when gating on region 1 from (A) are shown at the corners of the density plots. Little to no labeling is present on the FITC fluorescence axis (lower right quadrant) (B in white) when gating on region 1 and no antibody label is present. The highest percentage of labeling for both FDCs (FDC-M2 FITC Ab) (69.77%) (D) and B cells (MD1 FITC Ab) (71.05%) (F) can be seen when gating on region 1. (C) and (E) represent background fluorescence for both FDCs and B cells respectively. The percentage of labeled FDCs and B cells are shown in white in the lower right quadrants in (D) and (F) respectively after subtracting the background values from the lower right quadrants in (C) and (E) respectively. Results are representative of at least three separate experiments.

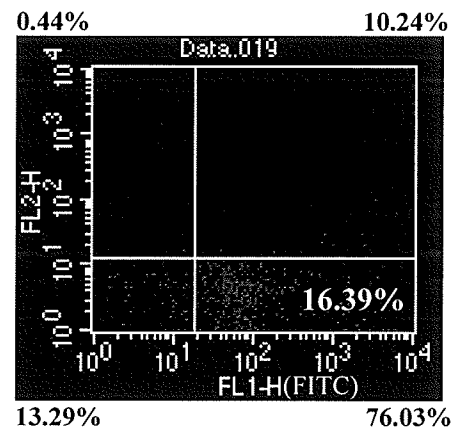
3.3.1 FDCs are present in the leukocyte depleted splenic cell population

I was particularly interested in determining whether FDCs, one of the rarest group of splenic cells, and most strongly implicated in prion pathogenesis, were 'enriched' in this population. I used a mouse FDC specific monoclonal antibody, FDC-M2, to label cells in the whole spleen homogenate, as well as the leukocyte depleted cell population.

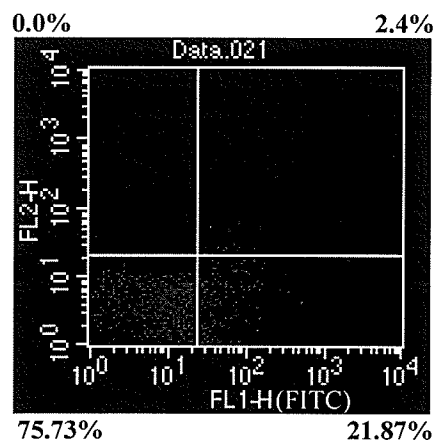
When investigating the leukocyte depleted splenic cell population (enriched population) by flow cytometry, region 1 from the gated population from **Figure 10A** was used to examine the fluorescence. Firstly, the gated population was labeled with the secondary anti-rat FITC Ab only (**Figure 11A**) to test for background fluorescence and the % labeling on this FITC axis (**59.64%**) was subtracted from the % labeling on the FITC axis (**76.03%**) when the gated population was labeled with the FDC-M2 FITC Ab (**Figure 11B**) for FDCs. This resulted in a **16.39%** labeling of FDCs (**Figure 11B-in white**) in the enriched leukocyte depleted splenic cell population. Secondly, the gated population was labeled with the secondary anti-goat FITC Ab only (**Figure 11C**) to test for background fluorescence and the % labeling on this FITC axis (**21.87%**) was subtracted from the % labeling on the FITC axis (**47.83%**) when the gated population was labeled with the MD1 FITC Ab (**Figure 11D**) for B cells. This resulted in a **25.96%** labeling of B cells (**Figure 11D- in white**) in the enriched leukocyte depleted splenic cell population.



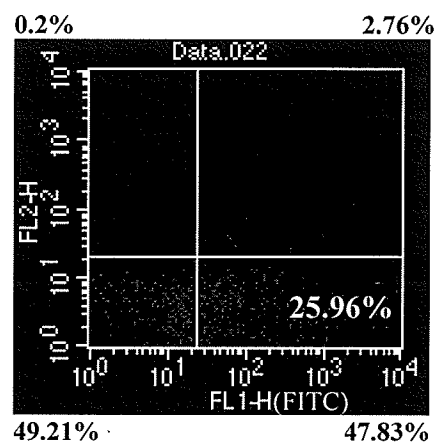
A Secondary anti-rat FITC Ab



B FDC-M2 FITC Ab



C Secondary anti-goat FITC Ab



D MD1 FITC Ab

Figure 11: FACS analysis of splenic cell populations in enriched population. The percentages for each quadrant when gating on region 1 from (**Figure 10A**) are shown at the corners of the density plots. (**A**) and (**C**) represent background fluorescence for both FDCs and B cells respectively. The percentage of labeled FDCs and B cells are shown in white in the lower right quadrants in (**B**) and (**D**) respectively after subtracting the background values from the lower right quadrants in (**A**) and (**C**) respectively. Results are representative of at least three separate experiments.

When comparing the enriched leukocyte depleted population back to the whole spleen homogenate, FDC enrichment has been confirmed as there are 16.39% (**Figure 11B- in white**) FDCs in the enriched population compared with only 3.94% (**Figure 10D- in white**) found in the whole spleen. The ratio of FDCs to B cells is also higher in the enriched population at 0.63% ($16.39\%/25.96\%$) compared with only 0.42% ($3.94\%/9.34\%$) in the whole spleen homogenate.

Unfortunately there also seems to be an increase of B cells in the enriched population as there are 25.96% B cells compared with only 9.34% in the whole spleen homogenate. This could be due to the fact that the column became overloaded and the B cells were not able to remain inside the column and were eluted along with the FDCs. Although there seemed to be a population of B cells in my FDC enriched population, I continued on with my experiments knowing that FDCs had been enriched as this enriched population already contained very few cells. Any further amount of cell separation would reduce cell yields dramatically and would not allow for sufficient amounts of RNA for further experimentation.

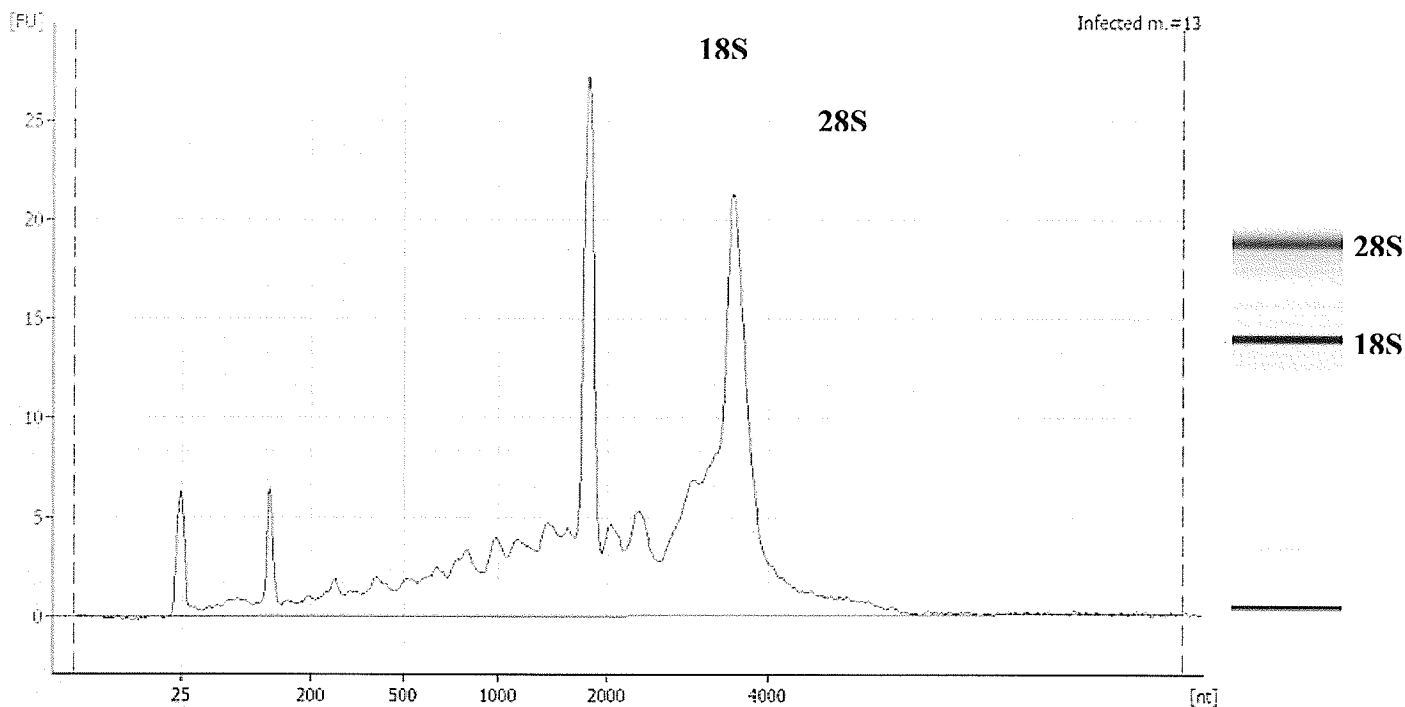
Although now due to advances in RNA amplification technology, future improvements to this experiment could be tried when a very small number of cells are isolated. Firstly, a positive selection technique could be performed where a specific antibody to FDCs is used to extract the FDCs from the leukocyte population. In addition, running the sample numerous times over a column would help to capture and enrich for very rare cell populations.

Future improvements to the flow cytometry analysis could also be done. Firstly, examining the NK/T/B/granulocyte cell population along with the enriched population with antibodies specific to those cell types to determine the amount removed from the enriched population. Secondly, using more than one FDC Ab marker to confirm the presence or enrichment of FDCs. Thirdly, when choosing Abs for detection, I must try and ensure that the background fluorescence from the secondary Ab is minimal as the secondary anti-rat FITC Ab in this study had very high background fluorescence (**Figure 10C & 11A**).

3.4 Isolation of high quality RNA from the leukocyte depleted splenic cell population

The substantial amount of manipulation to isolate the SCP preceding the RNA isolation made the RNA vulnerable to degradation. Therefore, the quality of the RNA isolated from these cell populations and removal of contaminating genomic DNA was carefully monitored using an Agilent Bioanalyzer to ensure that quality was consistent and high enough to use successfully in future manipulations. The Bioanalyzer denotes RNA quality by assigning an RNA integrity number (RIN) for each fluorescently labeled sample. The RIN is calculated by determining the ratio of the 28S/18S ribosomal subunit fluorescent peaks on the electroencephalogram. The ratio should be around two to give the highest RIN of 10. RIN can range from a value of 1.0 (very badly degraded)-10.0 (non-degraded/perfect quality sample). According to Agilent technologies manufacturers' instructions, an RIN of 5.0-10.0 is the acceptable range for RNA of high enough quality for further experimentation. An example of a typical Bioanalyzer result for these

experiments is shown in **Figure 12**. RNA prepared from the isolated splenic cell populations had an average reading of 6.7. A summary of all the RNA isolations performed in this study, including the amounts obtained and the quality of each sample after DNase digestion, are found in **Appendix 2 as Table 5A and 5B**, respectively.



RNA Concentration: 1 ng/ μ l
rRNA Ratio (28S/18S): 2.3
RNA Integrity Number (RIN): 7.3

Figure 12: An example electroencephalogram alongside the gel image from the bioanalyzer of a clinical endpoint VM 22A infected mouse RNA sample displaying the two intact 18S and 28S ribosomal subunits. The RIN value of 7.3 is within the acceptable range after manipulation. Removal of genomic DNA can be confirmed by the non-existence of a larger band higher up on the gel and degradation products of RNA can be visualized as smaller bands towards the bottom of the gel.

3.5 Quality Control Check of Microarray Experiments

Quality control of the microarray experiments was guaranteed by self/self hybridization on the BMAP/MSV arrays using the isolated splenic cell population. This was done by splitting the isolated RNA from the enriched population into two equal fractions and labeling them with each the red and the green dye. Ideally, when performing a self/self hybridization, the correlation coefficient of the raw intensities of the red and green dye should be very close to a value of 1.00 considering it is the same sample competing for the same spot on the array. Thus, there should be a similar intensity for both the red and green dye and they should hybridize equally. This quality control check ensures that each sample is incorporating the same amount of dye, degradation of the dye has not occurred by ozone or photobleaching, and hybridization temperatures and washing conditions have remained constant. In **Figure 13**, little to no difference in intensity was observed for the red and the green channel, thus showing that the sample binds in a similar manner despite the label that it possesses. The R^2 value of 0.906 is close to one meaning there is little differential binding between the differently labeled samples.

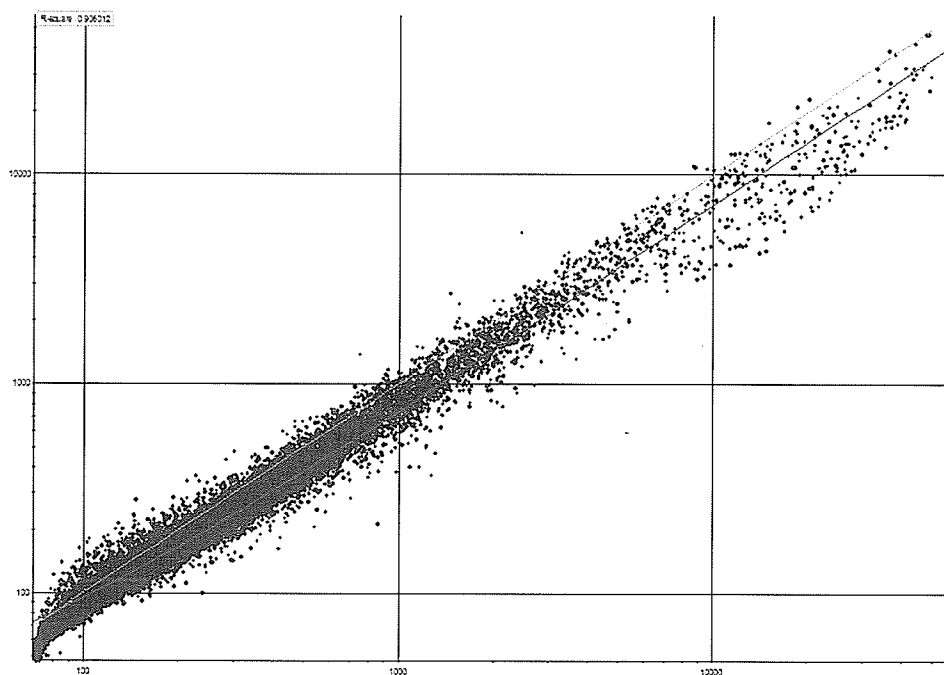


Figure 13: Scatterplot of the raw intensity values for each spot in log scale for the Alexa Fluor 555 (green) channel (y-axis) against the Alexa Fluor (red) channel (x-axis). The correlation or R^2 value is very close to one (0.91) concluding that there is no differential expression in this self-self hybridization.

3.6 Microarray analysis of the lymphocyte depleted cell population revealed a unique profile, markedly distinct from that of whole spleen

A microarray analysis to determine gene expression in the leukocyte-depleted cell population revealed that this was markedly different to that found in whole spleen tissue. RNA isolated from normal mouse whole spleen tissue was hybridized to microarrays along with RNA extracted from leukocyte-depleted, age-matched splenic cells using competitive hybridization. Statistical analysis revealed that at least 3422 genes were significantly differentially represented in the SCP in comparison with whole spleen tissue using a conservative 1% False Discovery Rate as criteria for a one-class modified t-test; the software Significance Analysis for Microarrays, or SAM, was used for analysis, for more details see the Methods section under data analysis (section 2.6.7). A graphical view of the analysis which shows the expected false discovery rate versus the actual false discovery rate is provided as **Figure 14**; over represented genes in comparison to whole spleen are shown in red and under-represented, green. The list of 3422 significant genes was further narrowed down to a total of 937 genes (216-overrepresented and 721-underrepresented) based on a further criterion of ≥ 2 fold change in gene expression between the two populations. Specifically, **Table 6A** displays the top 25 genes that are overrepresented and **Table 6B**, the top 25 genes that are underrepresented in the FDC enriched population compared with the whole spleen tissue. For the complete list of 929 genes, see **Appendix 3, Table 7**.

Significant: 3422
Median number of false positives: 31.35
False Discovery Rate (%): 0.92

SAM Plotsheet

Tail strength (%): 58.7
se (%): 68

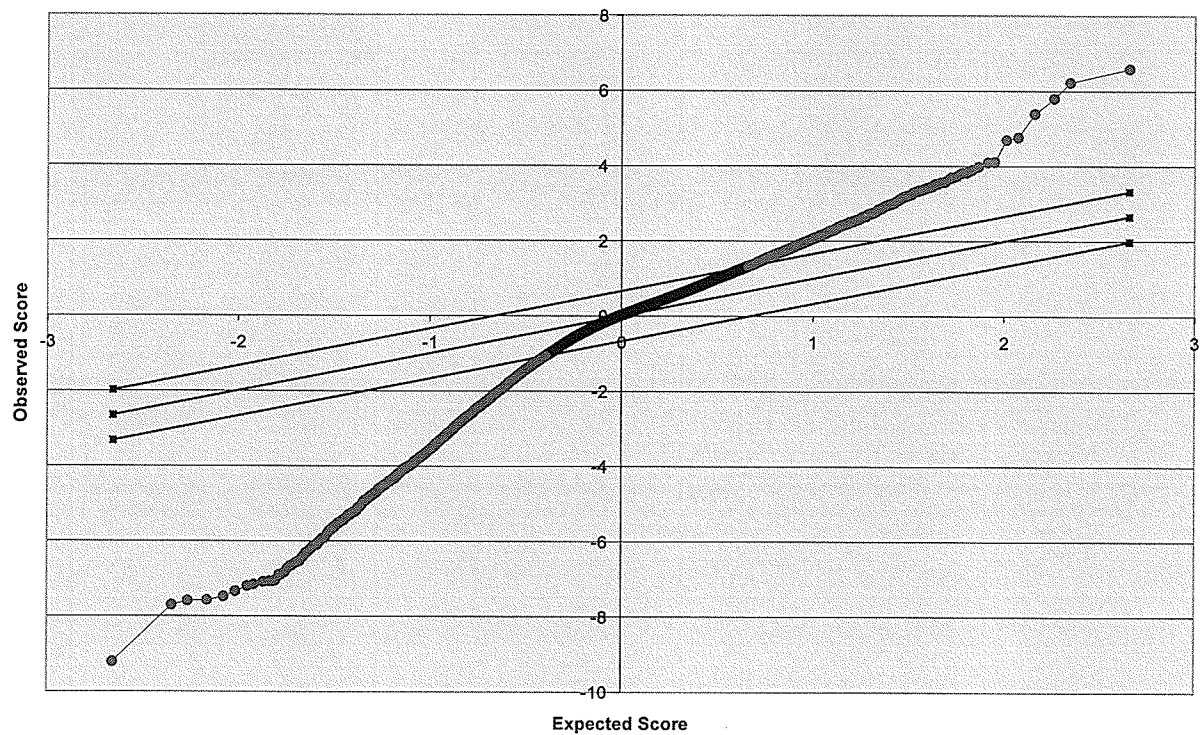


Figure 14: One-class SAM analysis displaying the number of significantly different genes represented by the enriched splenic cell population compared with whole spleen tissue. Red indicates over-represented genes, green indicates under-represented genes, and black indicates genes that are similarly expressed between the two populations.

Table 6A: Top 25 Genes Overrepresented in the Splenic Cell Population

ID	Genes	Description	Fold Change
AI835777	SDF4	stromal cell derived factor 4	8.057
AI429738	NACAD	NAC alpha domain containing	7.226
AI426455	SAP30	Sin3A-associated protein, 30kDa	6.089
AI426448	TFRC	transferrin receptor (p90, CD71)	5.745
AI451692	TAL1	T-cell acute lymphocytic leukemia 1	5.049
AI428454	WDR48	WD repeat domain 48	4.92
AI853499	HMGB3	high-mobility group box 3	4.433
AI849269	EG668636	predicted gene, EG668636	4.22
AI661153	HARS	histidyl-tRNA synthetase	3.977
AI451697	SPTA1	spectrin, alpha, erythrocytic 1 (elliptocytosis 2)	3.732
AI845477	NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	3.679
AI425937	PTOV1	prostate tumor overexpressed gene 1	3.603
AI851142	DDI2	DDI1, DNA-damage inducible 1, homolog 2 (S. cerevisiae)	3.6
AI425940	ANK1	ankyrin 1, erythrocytic	3.568
AI845839	CPEB4	cytoplasmic polyadenylation element binding protein 4	3.506
AI661037	NEIL3	nei endonuclease VIII-like 3 (E. coli)	3.452
AI854532	SLC16A1	solute carrier family 16, member 1 (monocarboxylic acid transporter 1)	3.431
AI426367	AW215868	expressed sequence AW215868	3.396
AI449243	LANCL2	LanC lantibiotic synthetase component C-like 2 (bacterial)	3.373
AI840625	CTTNBP2	cortactin binding protein 2	3.332
AI451300	TFDP2	transcription factor Dp-2 (E2F dimerization partner 2)	3.312
AI849423	RNF11	ring finger protein 11	3.308
AI415666	MDK	midkine (neurite growth-promoting factor 2)	3.231
AI323435	KIF4A	kinesin family member 4A	3.217
AI843623	RAD21	RAD21 homolog (S. pombe)	3.131
AI845688	PRO1073	PRO1073 protein	3.112

Table 6B: Top 25 Genes Underrepresented in the Splenic Cell Population

ID	Genes	Description	Fold Change
AI849002	EDG1	endothelial differentiation, sphingolipid G-protein-coupled receptor, 1	-24.579
AI326372	PNLIP	pancreatic lipase	-19.674
AI839103	HPGD	hydroxyprostaglandin dehydrogenase 15-(NAD)	-18.487
AI447342	9230111E07RIK	RIKEN cDNA 9230111E07 gene	-16.694
AI450813	GIMAP4	GTPase, IMAP family member 4	-13.47
AI449362	JMJD1C	jumonji domain containing 1C	-12.728
AI838693	SEPP1	selenoprotein P, plasma, 1	-11.527
AI846778	DCN	decorin	-11.479
AI451311	SPIC	Spi-C transcription factor (Spi-1/PU.1 related)	-11.247
AI842572	ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta	-10.772
AI842703	COL3A1	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	-10.392
AI839214	ATP2B1	ATPase, Ca ⁺⁺ transporting, plasma membrane 1	-10.213
AI851778	CTGF	connective tissue growth factor	-9.977
AI447928	ANKRD12	ankyrin repeat domain 12	-9.848
AI844675	BCL2	B-cell CLL/lymphoma 2	-9.363
AI853088	FSTL1	folliculin-like 1	-9.254
AI848248	APOE	apolipoprotein E	-8.9
AI853415	GNB4	guanine nucleotide binding protein (G protein), beta polypeptide 4	-8.561
AI326367	PRSS2 (includes EG:5645)	protease, serine, 2 (trypsin 2)	-8.441
AI528519	C3	complement component 3	-8.257
AI850349	RAB1A	RAB1A, member RAS oncogene family	-8.086
AI451642	BANK1 (includes EG:242248)	B-cell scaffold protein with ankyrin repeats 1	-8.083
AI448110	GIMAP3	GTPase, IMAP family member 3	-8.059
AI528734	CXCL12	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	-7.962
AI842153	FAM19A5	family with sequence similarity 19 (chemokine (C-C motif)-like), member A5	-7.941
AI836624	CLU	clusterin	-7.852

3.6.1 Gene expression profiling of the SCP revealed depletion expression of lymphocyte-specific markers and enrichment of dendritic cell-specific gene expression

Using this list I identified a number of lymphocyte marker genes to be depleted from the population and a number of dendritic-specific genes to be enriched, thus providing further confirmation that the cell population isolated was depleted for lymphocytes and contained FDCs and dendritic cells. Depleted lymphocyte specific markers included CD83, CD86, CD9, HLA-DOB, LY86, LY6E, LCK, NFAT5, and THY1. Dendritic cell markers enriched in the isolated population include CD209, ICAM4, and IER5. Contrary to my expectations I found that macrophage associated genes were also depleted in the splenic cell population suggesting that macrophages were depleted, rather than enriched, in a similar fashion to lymphocytes. The under-represented genes in the splenic population included PGRMC1, GFRA2, SCARB2, PTPNS1/SIRPA, CSTB, CTSS, SPP1, MPEG1, PTGS1, and LPL. These genes and the associated fold changes in terms of enrichment or depletion in comparison with whole spleen tissue are provided in **Table 7 in Appendix 3.**

Gene expression in an enriched follicular dendritic cell population has recently been published by Huber *et al.* (2005). In this study two different methods were employed to enrich populations of FDCs for gene expression analysis. In one method, FDCs were removed from mesenteric lymph nodes and spleens by treatment with an antibody targeted to the lymphotoxin-beta receptor; in this case

maturation of FDCs was blocked as lymphotoxin-beta signaling is required. Gene transcripts depleted following treatment were identified. In the other method FDC cell clusters were directly purified from mouse spleen and transcriptional profiles also characterized relative to FDC-depleted spleen preparations. A core group of 12 genes were identified in common to both of these procedures; PRNP, GDA, LYZS, GLYCAM1, GPM6B, C030033F14ik, CLU, ENPP2, MFGE8, SERPIN1A, COCH and CXCL13a. Interestingly, these genes (except for PRNP) were under-represented in my splenic cell population. This could be explained in a number of ways, either my splenic population was depleted for FDCs, which I deemed unlikely as my FACS analysis results showed FDCs were present in my splenic cell population, and at a higher concentration than in a disrupted whole spleen cell population. The most likely explanation for this discrepancy was that these genes were not specifically expressed in FDCs but also present in other populations depleted during the purification procedure. For example LYZS is highly expressed in mature macrophages which appear to be under-represented in my isolated splenic cell population and CXCL13a is expressed in a number of cell types including B and T lymphocytes as well as FDCs. Strikingly, one of the genes expressed most highly in the splenic cell population in comparison with whole spleen was the PRNP gene itself, coding for cellular prion protein, which was enriched over 3 fold. As expression of PRNP it-self is required for replication of infectious prions, this result indicated that a population of cells had been selected in which prion replication was possible. Prion protein is highly expressed in FDCs and myeloid dendritic cells in the spleen and so this result is highly suggestive that enrichment

for these cell types contributed to the observed enrichment for PRNP RNA. Interestingly, when the list of genes enriched in the splenic cell population was compared with ~100 genes previously found to be de-regulated in whole spleen of mice infected with scrapie using the same microarray platform in our lab, we found 25 in common to both lists (**See Table 8**). This provided further evidence that scrapie responsive genes were likely to be found in my isolated splenic cell population.

Table 8: Comparison of the Genes Found in the Enriched Splenic Cell Population with Genes found Previously in the Whole Spleen

Accession number	Gene Name	Description	Fold Change from FDC vs. whole spleen data	Fold Change found previously by Booth Lab in whole spleen
AI846014	45S preRNA	Homology with <i>Mus musculus</i> 45s preRNA (by BLAST)	10.31	2.46
AI854627	2310034G01RIK	RIKEN cDNA 2310034G01 gene	2.63	1.6
AI851523	AI851523	expressed sequence AI851523	2.16	1.59
AI448873	ZC3H8	zinc finger CCCH-type containing 8	-2.46	-1.46
AI839176	GLUL	glutamate-ammonia ligase (glutamine synthetase)	-2.64	-1.47
AI839342	XAB1	XPA binding protein 1, GTPase	-4.66	-1.41
AI843911	TSC22D3	TSC22 domain family, member 3	-3.67	-1.59
AI844687	PPP3CB	protein phosphatase 3 (formerly 2B), catalytic subunit, beta isoform	-3.24	-1.57
AI844703	TSPAN17	tetraspanin 17	-2.26	-1.74
AI844893	PLEC1	plectin 1, intermediate filament binding protein 500kDa	-3.72	-1.55
AI844927	YKT6	YKT6 v-SNARE homolog (<i>S. cerevisiae</i>)	-3.51	-1.45
AI844948	2010001A14RIK	RIKEN cDNA 2010001A14 gene	-3.11	-1.47
AI845192	PRPH	peripherin	-3.43	-1.47
AI845325	TEX9	testis expressed 9	-3.08	-1.5
AI848258	AI848258	expressed sequence AI848258	-2.71	-1.44
AI848816	GAS1	growth arrest-specific 1	-4.14	-1.43
AI850079	PIB5PA	phosphatidylinositol (4,5) biphosphate 5-phosphatase, A	-2.76	-1.61
AI852222	TMCC2	transmembrane and coiled-coil domain family 2	2.18	-2.48
AI449992	Unknown	EST	-5.00	-1.5
AI836229	Hbxap	Neurofilament triplet H protein	-2.96	-1.41
AI843288	Unknown	EST	-4.04	-1.43
AI844137	Brca2	Breast cancer 2	-3.33	-1.43
AI844960	Unknown	EST	-3.15	-1.44
AI845238	RIKEN cDNA 1700030G0 6 gene	Probable S-adenosylmethionine synthetase	-2.90	-1.4
AI850097	Nrbp2	Nuclear receptor binding protein 2	-3.13	-1.43

3.7 Identification of scrapie responsive genes in the SCP

Prior to experiments to identify scrapie responsive genes I performed some quality control experiments to ensure that cell populations isolated at different times contained a similar representation of RNA content (**See Appendix 2**). Hybridization of RNA from age-matched uninfected mice at different times was hybridized to microarrays and I determined that similar gene expression profiles, within a 95% confidence level, were obtained confirming the consistency of my methodology.

The following experiment was performed to identify potential scrapie responsive-genes within the splenic cell population. I isolated populations of cells from four VM mice inoculated with the 22A strain of scrapie showing clinical symptoms of infection, plus an equal number of age-matched control mice, using the methodology previously described. Three different array platforms were used (BMAP/MSV spotted cDNAs, AROS spotted oligos, and Agilent whole mouse genome arrays) to provide extensive analysis of as many genes as possible, and also for cross-platform validation of gene expression. Considering the number of biological replicates was low, this strategy was important in identification of biologically relevant genes rather than false positives generated from technical effects.

Data was then analyzed using a number of statistical methods. Raw intensities for each spot were determined using the ArrayPro software and background values were subtracted. These values were then normalized across the array, using Lowess sub-grid

normalization, to remove any systematic variation in the experiment. This is required to adjust for the differences in the labeling and detection efficiencies for the fluorescent labels (dye bias) and differences such as quantity of the starting RNA from the two samples in the experiment so that only differences in expression levels of genes between the two samples is measured. The effect of data normalization is provided in **Figure 15**.

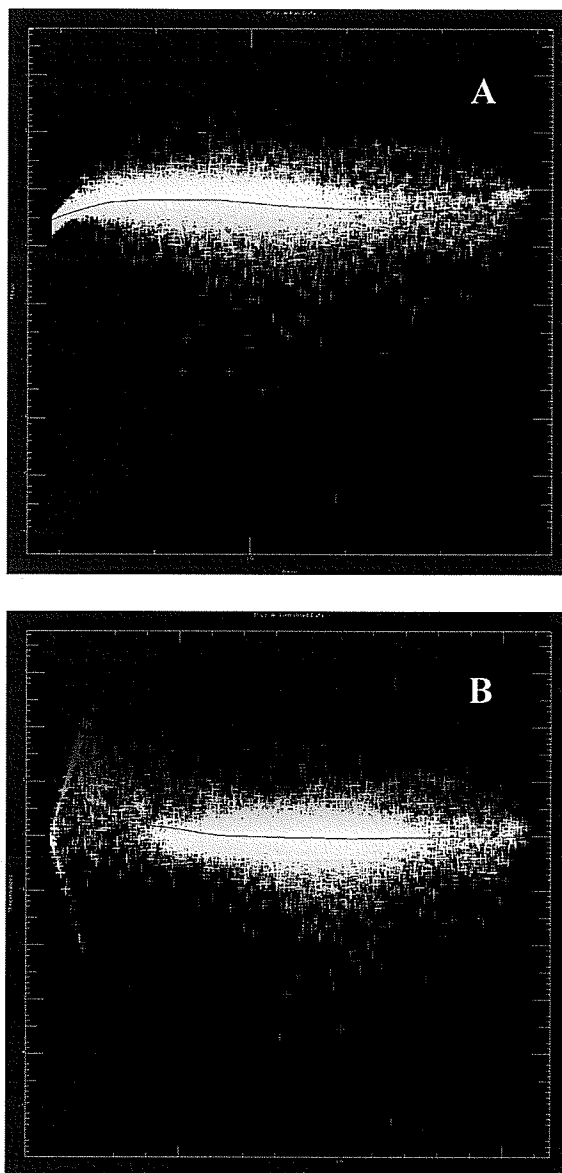


Figure 15: Example scatterplot displaying normalization of microarray data using RNA from clinical VM 22A mice versus VM control mice. A) Represents the un-normalized raw data and B) Represents the data after it has been normalized in Genetraffic. The X-axis represents the average log intensity from the two dyes while the Y-axis represents the log-ratios of infected over control or fold change. The infected sample is labeled with Alexa Fluor (green) and the control sample is labeled with Alexa Fluor 647 (red). Yellow represents no change in expression. The tailing affect seen only in graph B is due to increased sensitivity of the normalized data to identify lower intensity spots.

SAM or the statistical analysis of microarrays software package was used to determine differential expression on the resulting filtered gene list. SAM determines differential expression by selecting statistically significant gene changes through multiple t tests. Ultimately, each gene will be assigned a t statistic or SAM score based on its change in gene expression relative to the standard deviations of the repeated measurements of that gene across different arrays. Thus, by increasing the number of biological replicates used, it will increase the statistical power of an experiment. SAM scores (q-values) are then assigned for each gene and the significant genes found in the output list are determined by the user cutoff. The percentage of these genes that could be significant by chance is defined as the false discovery rate (FDR). The FDR is calculated by analyzing permutations of the measurements for each gene. The user can then adjust the threshold cutoff value to include more or less genes and the FDR is recalculated accordingly. In these experiments, a false discovery rate of 1% was chosen; given the smaller sample size of only four infected mice, this provided greater statistical confidence. The resulting SAM plots are given for each type of array in **Figure 16, 17 and 18.**

Significant: 3083
Median number of false positives: 27.19
False Discovery Rate (%): 0.89

SAM Plotsheet

Tail strength (%): 56.3
se (%): 21.1

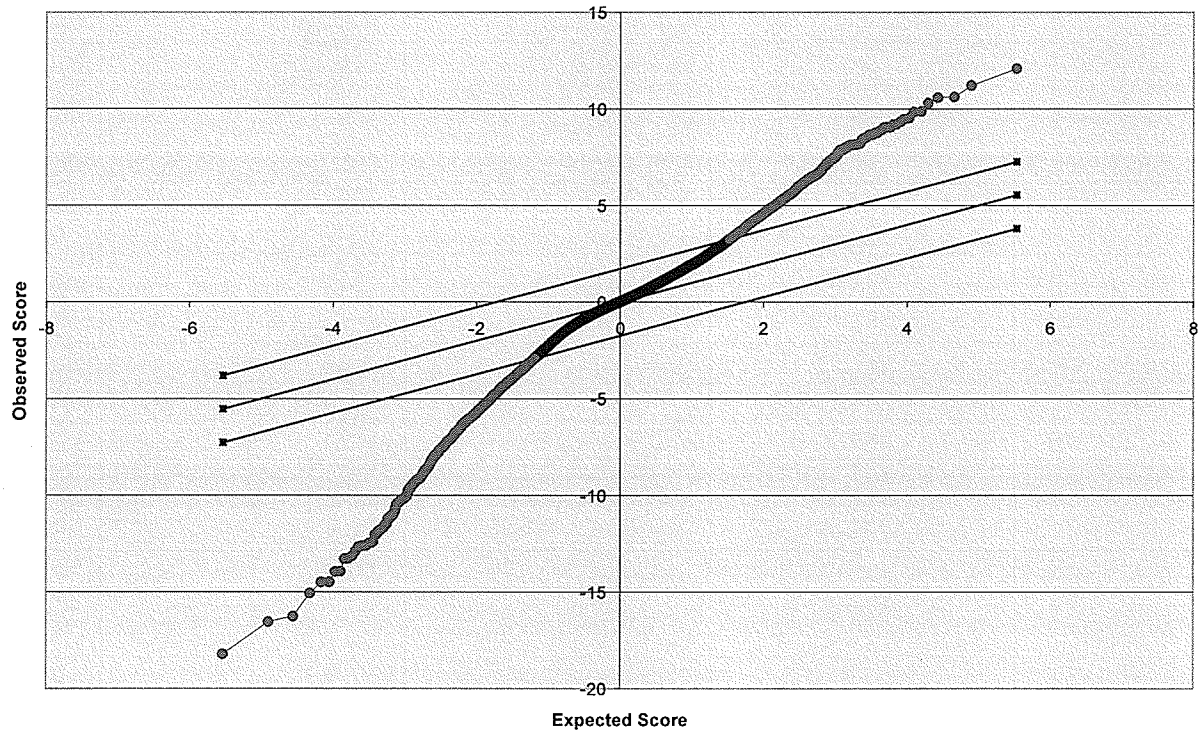


Figure 16: One-class SAM analysis displaying the number of significantly differentially expressed genes on the BMAP/MSV array by the prior infected enriched splenic cell population. Red indicates upregulated genes, green indicates downregulated genes, and black represents no differential expression.

Significant: 4060

Median number of false positives: 35.17

False Discovery Rate (%): 0.87

SAM Plotsheet

Tail strength (%): 51.2

se (%): 46.7

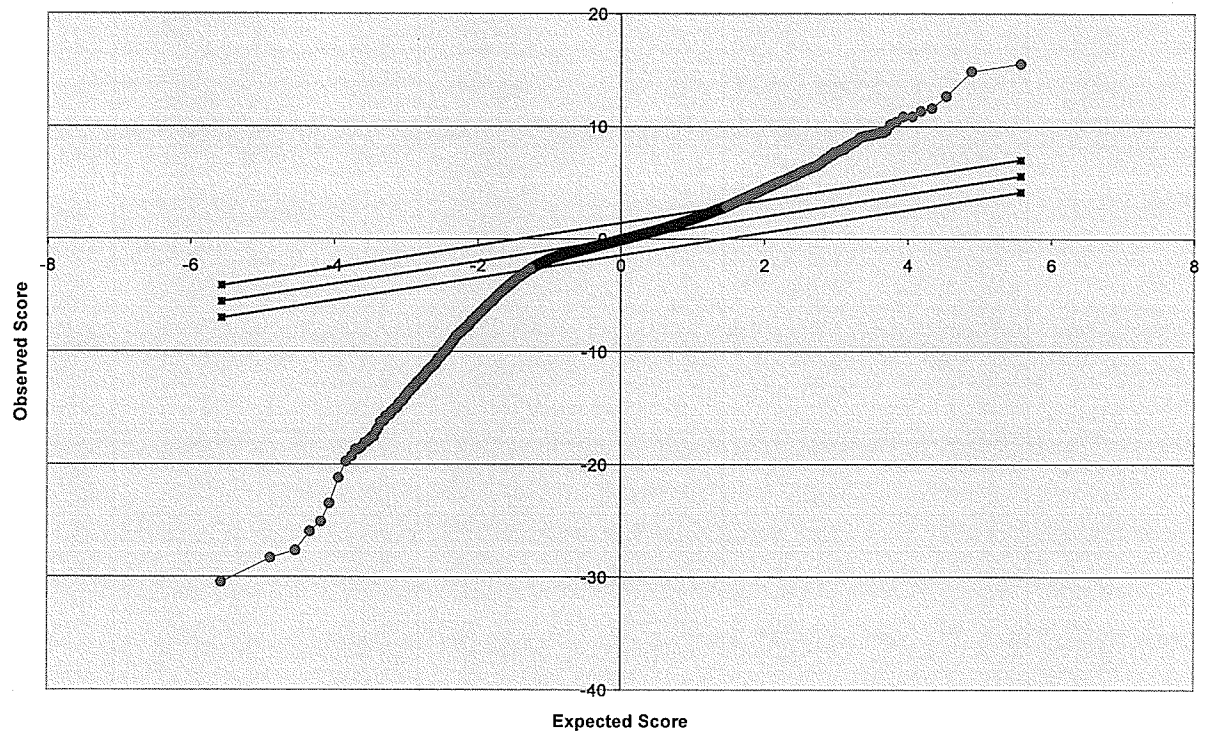


Figure 17: One-class SAM analysis displaying the number of significantly differentially expressed genes on the oligo array by the prion infected enriched splenic cell population. Red indicates upregulated genes, green indicates downregulated genes, and black represents no differential expression.

Significant: 21192
Median number of false positives: 187.04
False Discovery Rate (%): 0.88

SAM Plotsheet

Tail strength (%): 67.2
se (%): 58.6

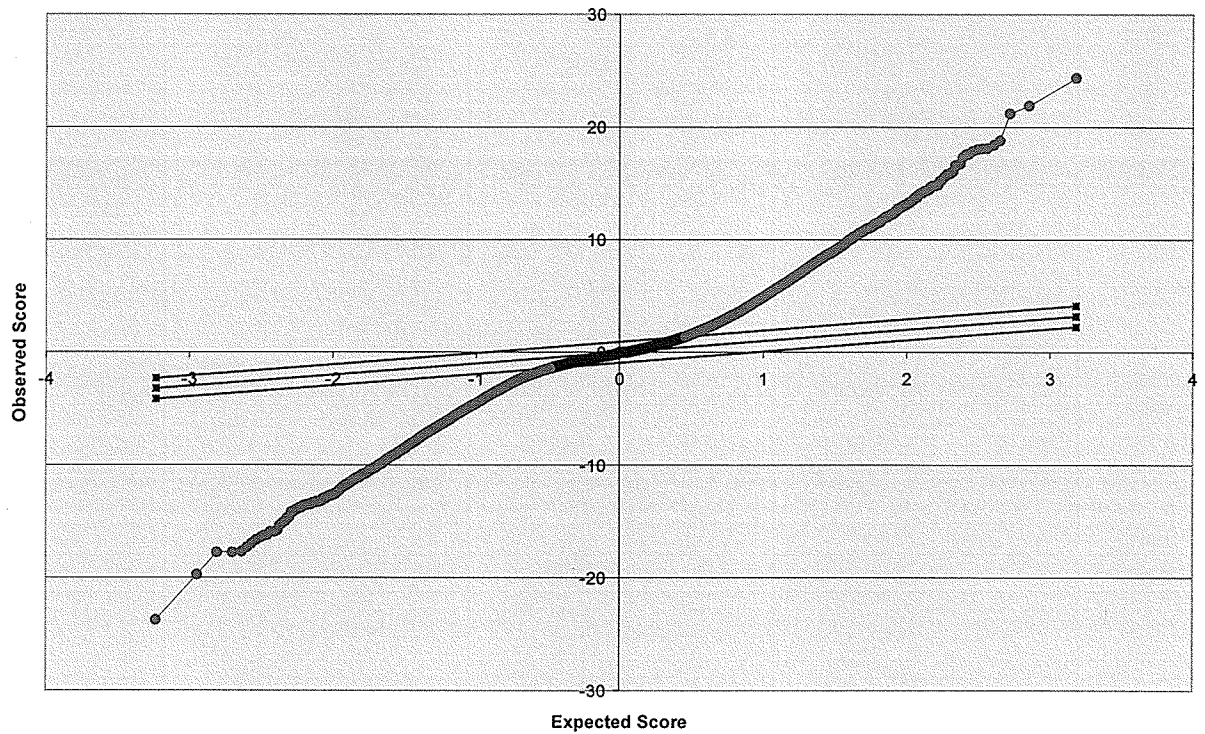


Figure 18: One-class SAM analysis displaying the number of significantly differentially expressed genes on the agilent array by the prion infected enriched splenic cell population. Red indicates upregulated genes, green indicates downregulated genes, and black represents no differential expression.

Genes from all three types of arrays that were significant at the 1% FDR (q-value of 0.01 in SAM) and had a fold change greater than two (either upregulated or downregulated) were used for further analysis in Ingenuity Pathway Analysis (IPA), a web-based bioinformatics tool that helps researchers to analyze and understand complex biological systems. After the dataset was uploaded into IPA, duplicate genes (i.e. genes found on two or more arrays) from all three were selected for and used for further analysis. The list of genes was reduced to ~1000 genes that were found significant at the 1% FDR and greater than 2 fold change for all three types of arrays. The top up-regulated and down-regulated genes across all arrays can be found in **Tables 9 & 10**. A full list of all the genes found is provided in **Table 11 of Appendix 4**.

Table 9: Top upregulated genes consistently found across all three array platforms

Genes	Description	Fold Change
DCN	decorin	27.619
PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	12.925
IGHM	immunoglobulin heavy constant mu	12.605
IGFBP7	insulin-like growth factor binding protein 7	11.345
CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)	11.164
ISL1	ISL1 transcription factor, LIM/homeodomain, (islet-1)	10.463
CHRD1	chordin-like 1	10.224

Table 10: Top downregulated genes consistently found across all three array platforms

Genes	Description	Fold Change
GFAP	glial fibrillary acidic protein	-44.07
HEMGN	hemogen	-32.651
SLC2A4	solute carrier family 2 (facilitated glucose transporter), member 4	-29.637
RHD	Rh blood group, D antigen	-25.807
SOX6	SRY (sex determining region Y)-box 6	-24.699
ABCB10	ATP-binding cassette, sub-family B (MDR/TAP), member 10	-24.07
KIF23	kinesin family member 23	-19.985
ASPM	asp (abnormal spindle) homolog, microcephaly associated (Drosophila)	-18.486
FN3K	fructosamine 3 kinase	-16.405
SLC16A1	solute carrier family 16, member 1 (monocarboxylic acid transporter 1)	-16.276

3.8 Scrapie responsive genes include a number of significant groups of functionally related genes based on gene ontology

I used an online tool based on a curated database, the Ingenuity Pathways Knowledge Base (IPKB), to annotate genes and to determine potential regulatory networks and pathways. The IPKB contains information on human, mouse, and rat genes including annotations, synonyms and over 1.4 million published biological interactions between genes, proteins and drugs. This database is continually updated and supplemented with curated relationships taken from MEDLINE abstracts; i.e. each gene interaction held in the IPKB is supported by published information. Thus, the IPKB provides a framework by which lists of genes identified by large-scale microarray studies can be annotated in terms of their functional relationships, and those that have been shown to interact.

I first identified key biological functions, including diseases and disorders, molecular and cellular functions, and physiological system development and functions that contain disproportionately high number of genes from the scrapie responsive gene-list in relation to the assayed gene population as a whole; the assayed gene group is the total gene-list from the microarrays used. Representative categories of biological functions and/or diseases which are over-represented (p values <0.01) are provided in **Figure 19**. This list of genes includes genes involved in regulation of the cell cycle, gene expression and cell death. In addition to grouping the significant genes into biological functional groups, they were also grouped into canonical pathways (well-known, widely recognized pathways). Pathways in which a significant number of scrapie responsive genes are involved are provided in **Figure 20**. These pathways are mainly involved in cell signaling and include p53- and Huntington's disease-signaling as well as cell cycle regulation in response to insult. In addition to canonical pathways, the genes can be grouped according to data on

toxicological pathways identified mainly as reactions to pharmacological products. **Figure 21**, displays the top five toxicological groups in the cell displaying similar threshold and ratio values used as cut-offs for canonical pathways. This also includes p53 and cell cycle regulatory pathways as well as NF-kB signaling.

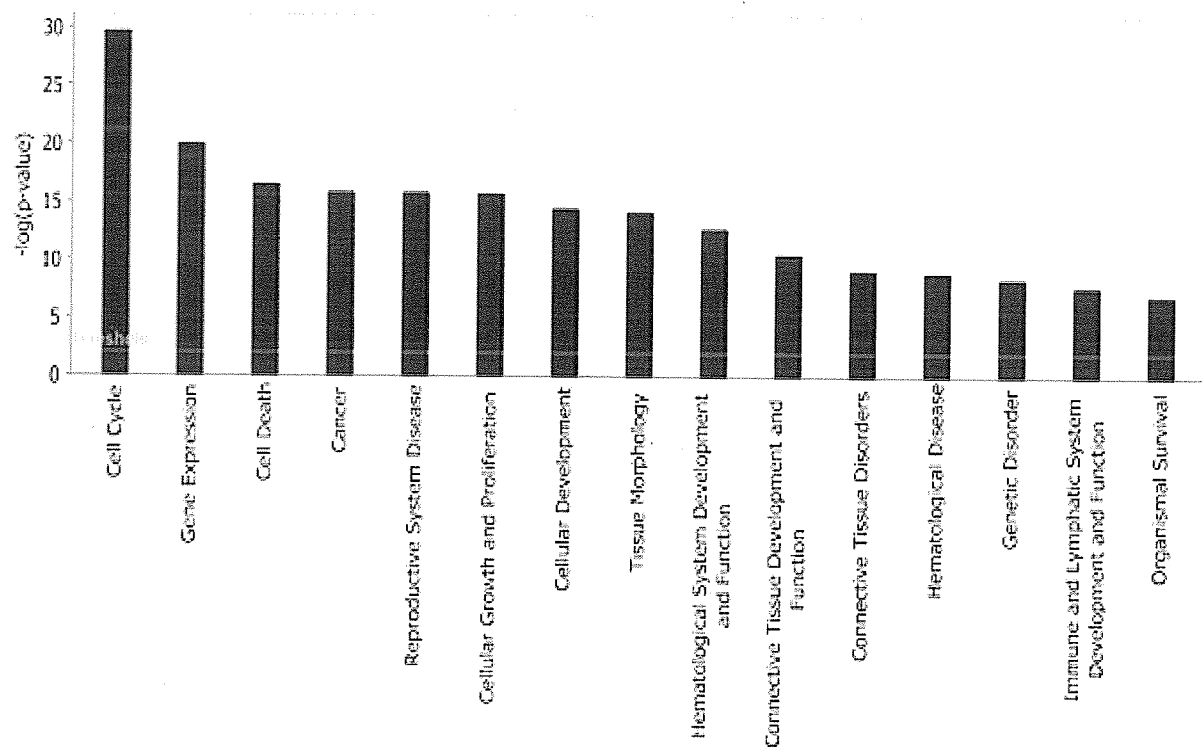


Figure 19: Top biological functional categories from significant genes found on all three types of arrays. (Ingenuity Systems, Inc.)

Analysis: All_three_up_and_down_duplic - 2007-08-28 11:25 PM

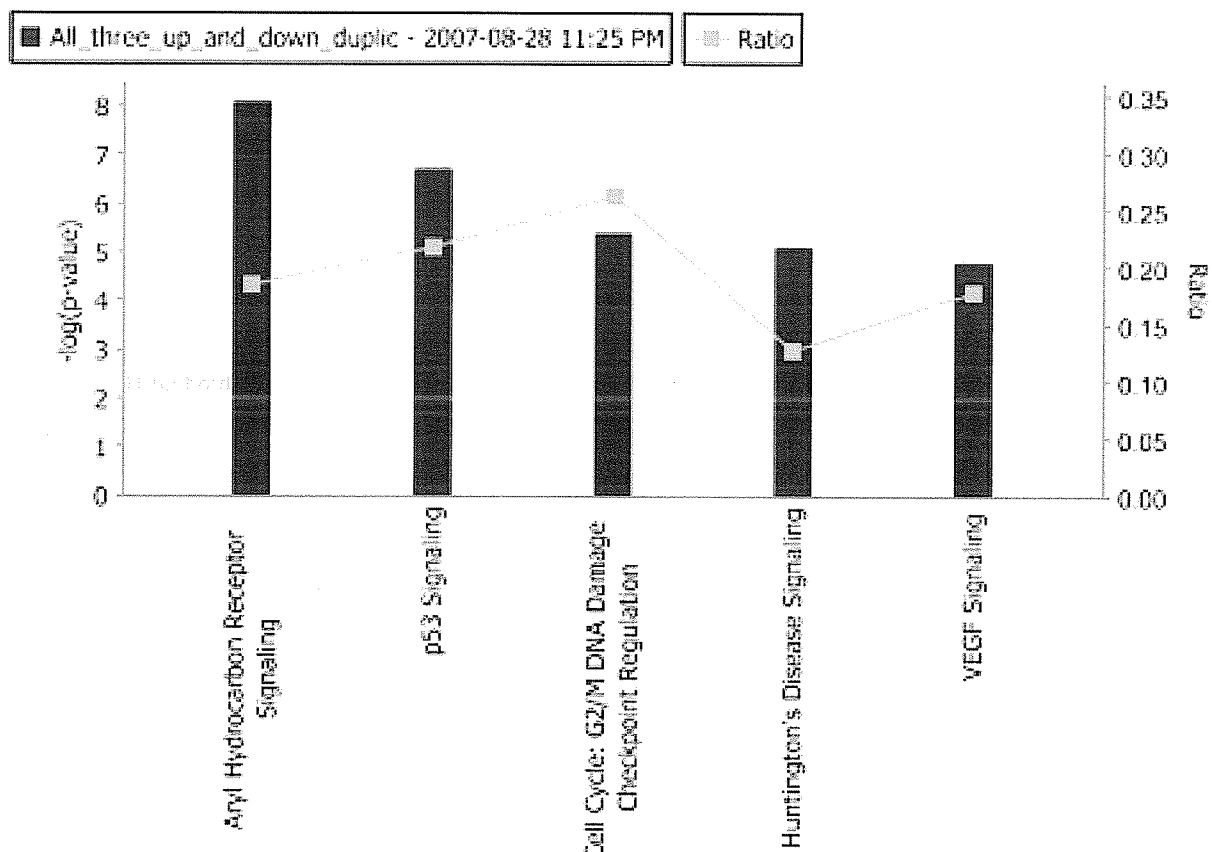


Figure 20: The top five canonical pathways where all the significant genes were found on the three types of arrays. (Ingenuity Systems, Inc.)

Analysis: All_three_up_and_down_duplic - 2007-08-28 11:25 PM

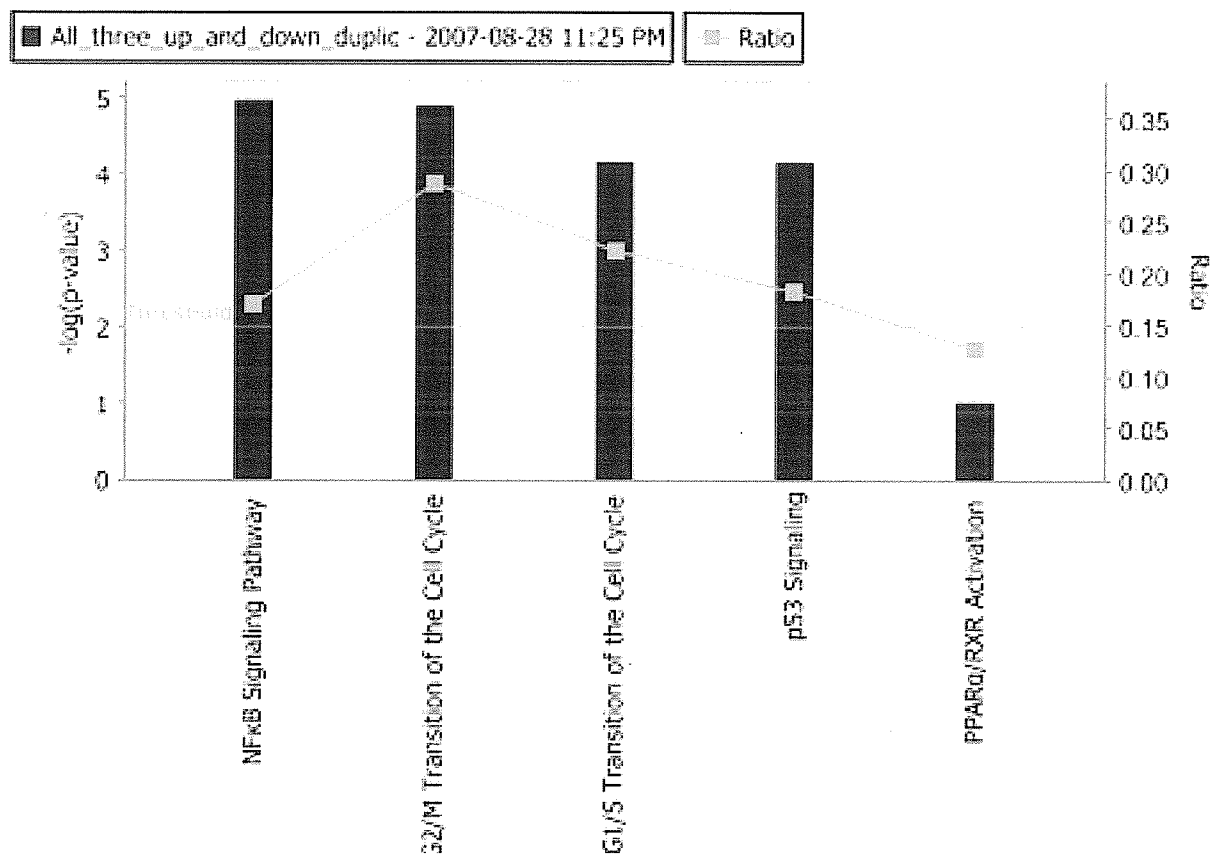


Figure 21: The top five toxicological pathways where all the significant genes were found on the three types of arrays. (Ingenuity Systems, Inc.)

3.8.1.1 Scrapie responsive genes include networks of potentially interacting genes

Individual regulated genes may interact to have a coordinated role in specific pathways; I identified potential networks of interacting PRG's using the Ingenuity Pathway Analysis tool. The methodology integrates genomic data with mining techniques to predict protein networks that comprise protein-protein interactions and other functional linkages. Each potential network is given a score, which is a probabilistic fit between the networks and a list of biological functions stored in the IPKB. The score takes into account the number of focus genes (from the scrapie responsive gene-list) in the network, and the size of the network, to approximate how relevant it is to the original list of genes; these scores are used to rank the networks. As the list was extensive, the analysis was restricted to those networks that had scores >9 (a score of 3 or greater was considered significant, the score being the negative exponent of the p -value ($p < 0.001$)). The top five networks, their significance, and the number of input genes found in each network can be found in **Table 12**. Each gene listed in bold represents user input genes, the genes in regular font are genes used by IPA to complete the network, and an asterisk beside any gene indicates a duplicate identifier.

Table 12: Top five networks the significant genes can be grouped into in IPA from all three types of arrays

ID	Molecules in Network	Score	Focus Molecules	Top Functions
1	↓ANLN, ↑APBB2, ↓APLP2, ↓CASP3, ↓CAST, ↓CD24, ↓CENPE, ↑CEP250, ↓DNAJB6, ↓E2F4, ↑FEM1B, ↓GINS1, ↓HMGB3, ↓KIF22, ↓KIF4A, ↓MAD2L1, MHC Class I, ↓NAP1L4, ↓NCAPD2, ↓NCAPG2, ↓NDC80, ↓NEK2, ↓PTMA, ↑RBM14B, ↓SFRS1, ↓SLK, ↓SMC2, ↓SMC4, ↑SNRP70, ↓SPC25, ↑SPON1, ↓SSB, ↓SSX2IP, ↓TRA2A, ↓TRPC2	44	34	Cell Cycle, Cellular Assembly and Organization, DNA Replication, Recombination, and Repair
2	↓ABCB10, ↓ANK1, ↑ANK3 [†] , ↑CAMK2D, CD3, ↑CITED2, ↓DARC, ↓EPB42, ↓GATA1, ↑GORASP2, ↓GRB10 [†] , ↓HBA2, ↓HBD, ↓HBG1, ↓HMBS, ↓IL1RL1, ↑KITLG (includes EG:4254), ↓KLF1, ↓LDB1, ↓LRRN3, ↓MAFG, Msp, ↑MYO5A, ↑NCAM1, ↓NFE2, ↑PTPRC, ↑PTPRE, ↑PTPRF, ↑RASA2, ↓RHO, ↓SPTB, ↓STMN1, ↓TAL1, ↑TMED10, ↓TNFAIP2	41	33	Hematological Disease, Organismal Injury and Abnormalities, Cellular Development
3	↓ADAM10, ↓BLM, ↑CAV2, ↑COL18A1, ↑COL1A1, ↑COL2A1, Cyclin A, ↑DAG1, ↑DARS, ↑DCN, ↑EGFR, ↓FANCD2, ↓FEN1, ↓FKBP4, ↑FUT8, ↓HIST1H1B, ↑IGFBP7, ↑ITGB4BP, ↑LAMA2, ↓LIG1, ↑NRXN1, ↓PCNA, ↓PDCD5, ↑PLA2R1, ↓POLH, SPA, ↓RPA1, ↓RPA2, ↓RPA3, ↑SGCB, ↑SGCE, ↓SOX6, ↓TIMELESS, ↓TIPIN, ↓WRN	41	33	DNA Replication, Recombination, and Repair, Cell Cycle, Cancer
4	↓ARHGAP11A, ↓C10RF96, ↓CD59, ↑CD52, ↓CENPA, ↓EXOSC8, ↓EXOSC9, ↓EZH2, ↑FABP4, ↑GAPDH (includes EG:14433), ↓GTF2A2, ↓H3F3A, ↓H3F3B, ↓HDAC2, ↑HEL2, Histone H3, ↑KCTD1, ↑KIAA1276, ↓KIAA1524, ↓MARCH5, ↑MED12, ↑MEIS1, ↓MYH10, ↓NFYB, ↑OGT, ↓PIGA, ↓PIGQ, ↓RBBP4, ↑RBM51, RNA polymerase II, ↓RNMT [†] , ↓SLC39A8, ↓SURB7, ↓SUZ12 [†] , ↓TRAK2	41	33	Post-Translational Modification, Cardiovascular System Development and Function, Tissue Morphology
5	↑AKT1, ↓ALAD, ↓ANP32A, ↓ARL4A, ↓BIRC5, ↑CBX4, CD8, ↑CD79B, ↓CDC45L, ↓COCA8, ↓CDKN3, ↓CSTF2, ↓CSTF3, ↓DNMT1, ↑FBLN1, ↑FCGR2B, ↑IGF2, Igfb, ↑IGH-1A, ↑IGHG1, ↑IGHM, LEF/TCF, ↑LVZ, ↑NCBP2 [†] , ↓NCBP1 (includes EG:4686), ↓RELN, ↓SIVA1, ↓SLC30A1, ↓SUB1 [†] , ↓SUV39H1, ↑TCF4, ↑TCF7L1, ↓UHRF1, ↑VCAN [†] , ↑ZNF91	39	32	RNA Post-Transcriptional Modification, Cell Death, Embryonic Development

Ingenuity Systems, Inc.

Each top network contains genes that appear central to the pathway with many genes that interact with them and thus are worth investigating. The number one top network is provided in **Figure 22** and contains two down-regulated genes that are central to the pathway. These two genes are Caspase 3 and E2F4, which are involved mostly in apoptosis and cell cycle regulation respectively. Specifically, Caspase 3 is the last important gene in controlling cellular demise (Culmsee & Landshamer, 2006) and E2F4 is a gene involved in the transcription repression complex in p53 signalling controlling the progression of the cell cycle. It is interesting to point out that both these genes are downregulated leading to the speculation that there is a promotion of cellular survival. **Figure 23** displays network number three containing the central up-regulated gene EGFR which is also involved in apoptosis signaling, axonal guidance signaling and N-glycan biosynthesis. PCNA is the central down-regulated gene in network number three and is also involved in apoptosis and p53 signaling. Lastly, **Figure 24** displays network number five containing the central up-regulated gene Akt1 which is heavily involved in immune cell signaling and of interest, axonal guidance signaling, p53 signaling, vascular endothelial growth factor (VEGF) signaling, amyloid processing, and Wnt/ β -catenin signaling. The legend for the networks can be found in **Appendix 5**, including what each node type and edge type means. These networks are discussed in the context of prion disease in the Discussion

Section of this thesis.

Network 1: All_three_up_and_down_duplic - 2007-08-28 11:25 PM: All_three_up_and_down_duplicates_only.xls

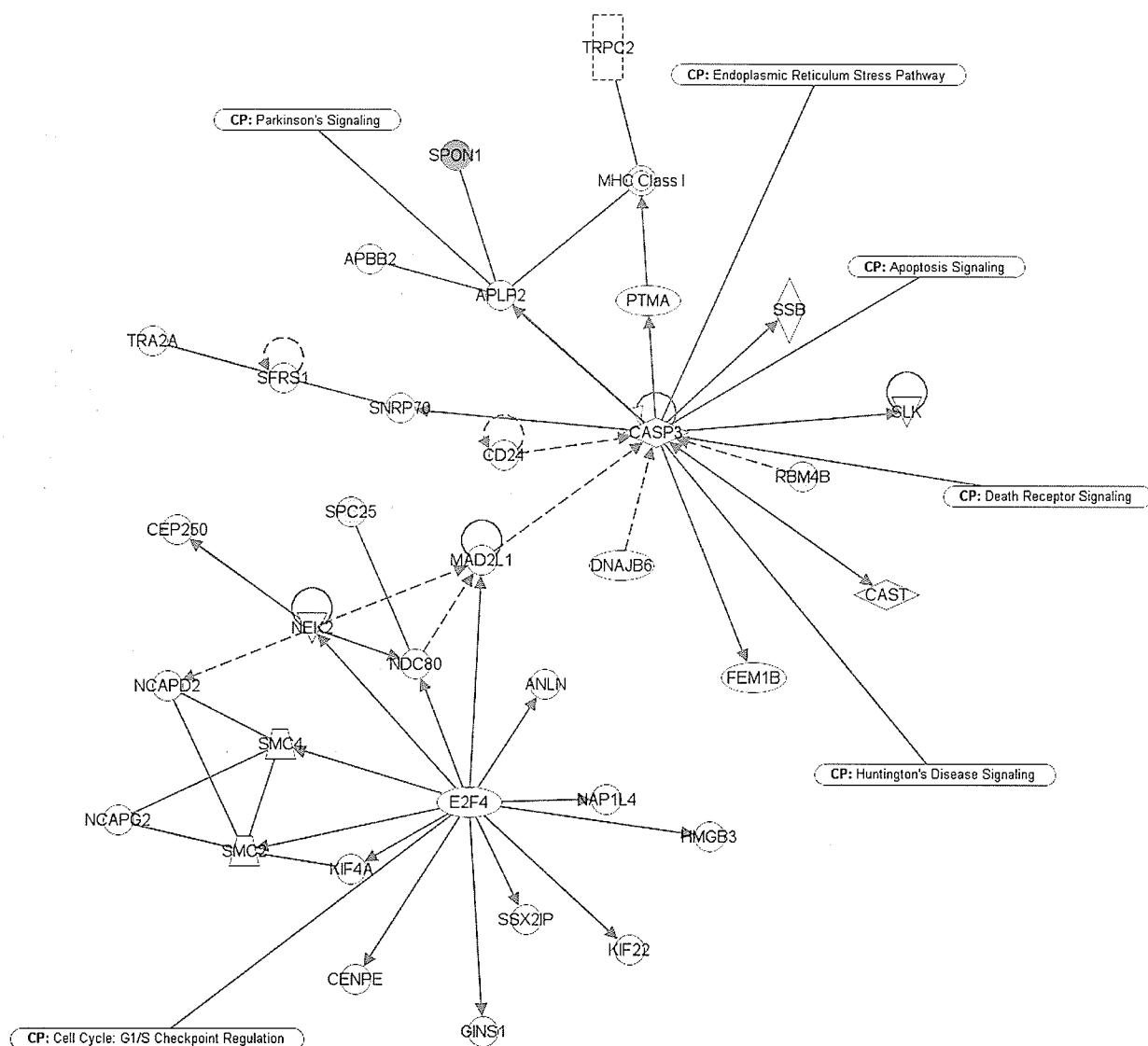


Figure 22: Network number one displaying the central downregulated genes Caspase 3 and E2F4 and their corresponding canonical pathways (CP) to which they are a member. Upregulated genes are shown in red and downregulated genes are shown in green. (Ingenuity Systems, Inc.)

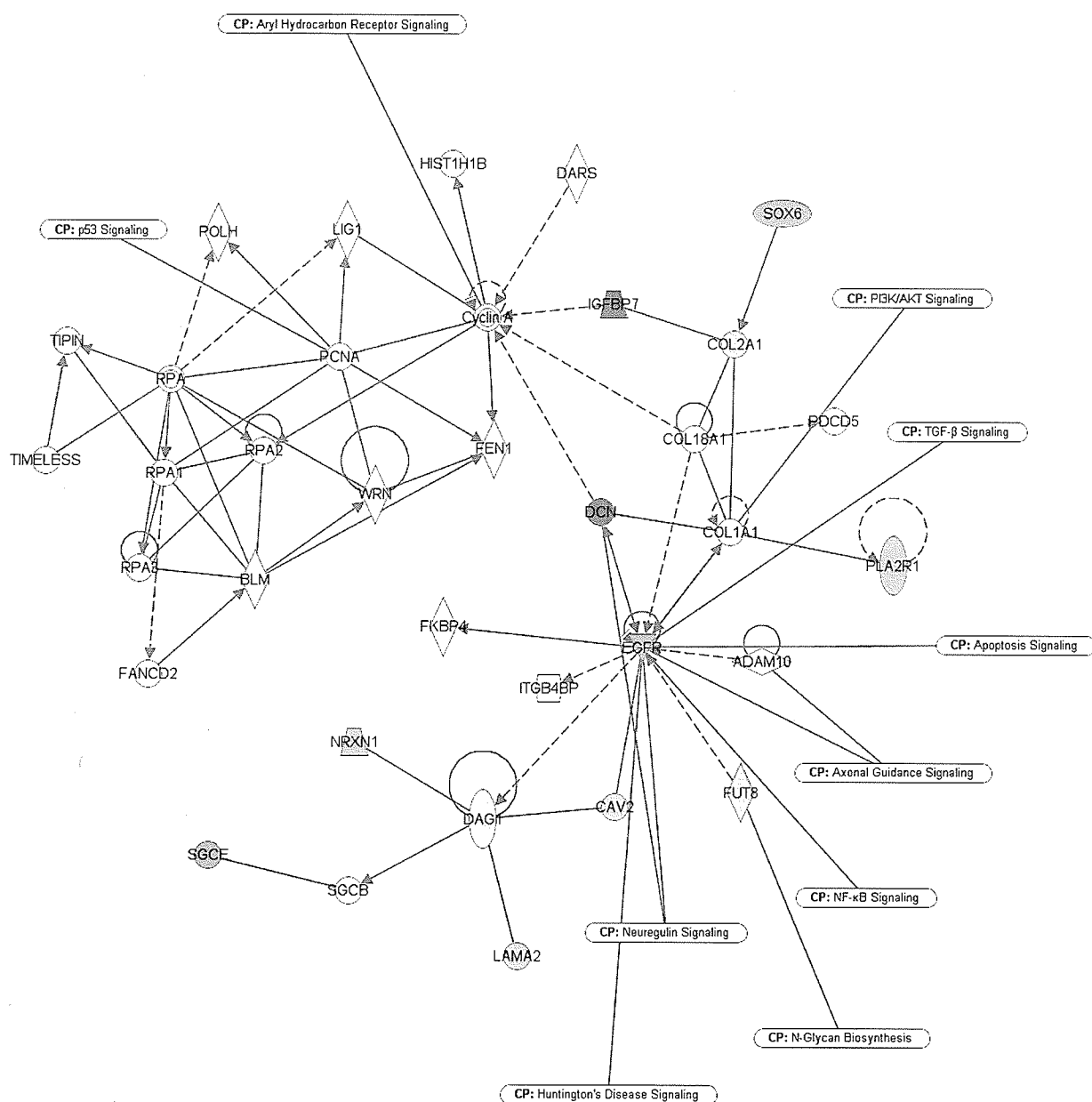
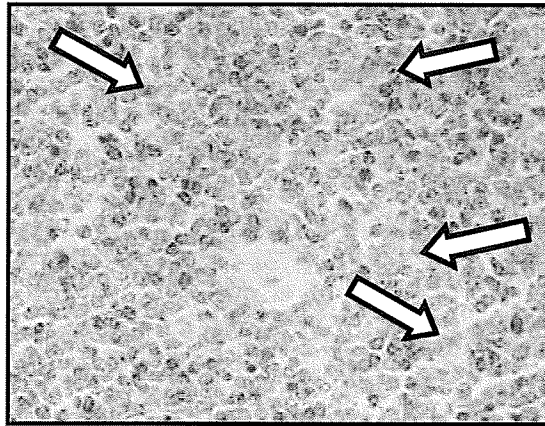


Figure 23: Network number three showing the central upregulated gene EGFR and the central downregulated gene PCNA with their corresponding canonical pathways (CP) to which they are member. Upregulated genes are shown in red and downregulated genes are shown in green. (Ingenuity Systems, Inc.)

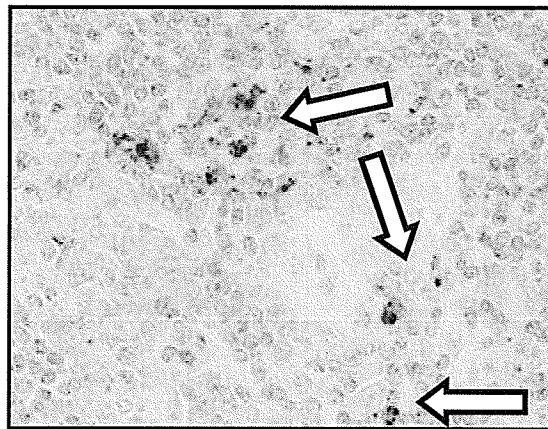
130

I also used a second Bioinformatics strategy to look for de-regulated pathways potentially involving scrapie responsive genes. This is hosted by the National Institute of Allergy and Infectious Diseases (NIAID), NIH and is called DAVID: Database for Annotation, Visualization, and Integrated Discovery (<http://david.abcc.ncifcrf.gov/>) (Dennis *et al.* 2003). Using this tool I found that a number of genes involved in the porphyrin metabolism pathway were significantly downregulated in the scrapie infected SCP in comparison to uninfected SCP. Porphyrins are large aromatic ring compounds that can readily bind cation metals such as iron, copper, and aluminum inside their hydrophobic ring (Caughey WS *et al.*, 1998) and thus may have the potential to sequester or accumulate these metal cations inside of tissues. Interestingly, a significant number of these genes code for products which directly interact in this pathway, shown in **Figure 25**. These four genes are: aminolevulinate, delta-dehydratase (-8.0), hydroxymethylbilane synthase (-8.6), uroporphyrinogen III synthase (-15.0), and coproporphyrinogen oxidase (-4.0).

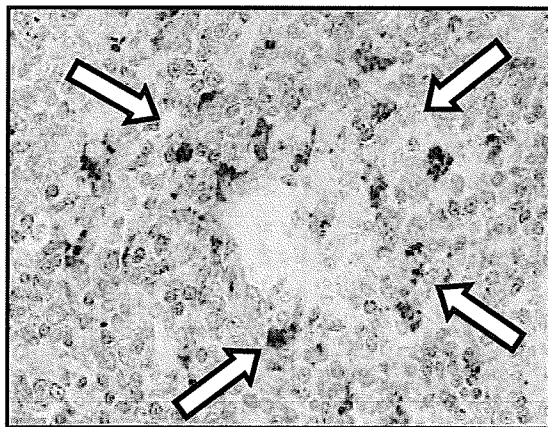
Notably, iron dysregulation has been shown previously to occur in the brains of prion infected animals and has also been shown to be associated with other neurodegenerative diseases (Lee *et al.* 2006). Indeed, our lab has previously shown HMBS and coproporphyrinogen oxidase to be de-regulated in the brains of scrapie infected mice (Booth *et al.* 2004). I hypothesized that a decrease in metabolism of porphyrins in the spleen of infected mice may lead to the accumulation of metal cations such as iron as these are ultimately bound by porphyrins. To test this I stained Parrafin-embedded spleen tissues from scrapie infected mice throughout the course of disease, at 21 dpi, 100 dpi, and 148 dpi, with Perls' Prussian blue which reacts with ferric iron to produce a blue colour. Consistent with my hypothesis I found an accumulation of iron deposits in the spleens over the course of the disease (**See Figure 26**).



21 dpi



100 dpi



148 dpi

Figure 26: Representative images of scrapie infected follicles of mouse spleen showing iron accumulation overtime. The Perls' Prussian Blue reaction for ferric iron was used to obtain blue staining representing iron deposits in the spleen.

In addition to the downregulated genes found in the porphyrin metabolism pathway, there was a gene, GFAP, which was found to be one of the top downregulated genes in the SCP (**Table 10**). Interestingly, this is a gene that is normally found upregulated in the brains of prion infected individuals (Kubler *et al.*, 2003) indicative of astrocytic gliosis, a characteristic marker of prion disease. This difference in expression displays tissue selective expression observed throughout the course of prion disease and emphasizes the importance of studying the tissue/cell specific population of interest when searching for potential biomarkers of infection.

3.9 Scrapie responsive genes are generally expressed in a number of different tissues

Gene expression in spleen tissue is poorly characterized in comparison to many other tissues. To gain more information on the list of 1000 scrapie responsive genes I compared them to a list of genes found to be tissue selective by Limvipuvadh *et al.* (2007). In this study a number of genes in the HugaIndex database, a repository for gene expression data using oligonucleotide arrays on normal human tissues, were grouped by tissue selective expression, including brain, kidney, liver, lung, muscle, prostate, and vulva selective genes as well as housekeeping genes. When comparing the comprehensive differentially regulated gene list from the splenic cell population above with each of these tissue selective lists, the list was narrowed down to 843 genes that could be considered spleen selective genes by process of elimination by eliminating gene expression associated with other tissues of the body. **Figure 27**, displays the breakdown of tissue selective expression found in the splenic cell population. As can be seen

highlighted in purple in **Figure 27**, the brain, muscle, and liver are the top tissues that the splenic cell population expression was related to, including general housekeeping genes.

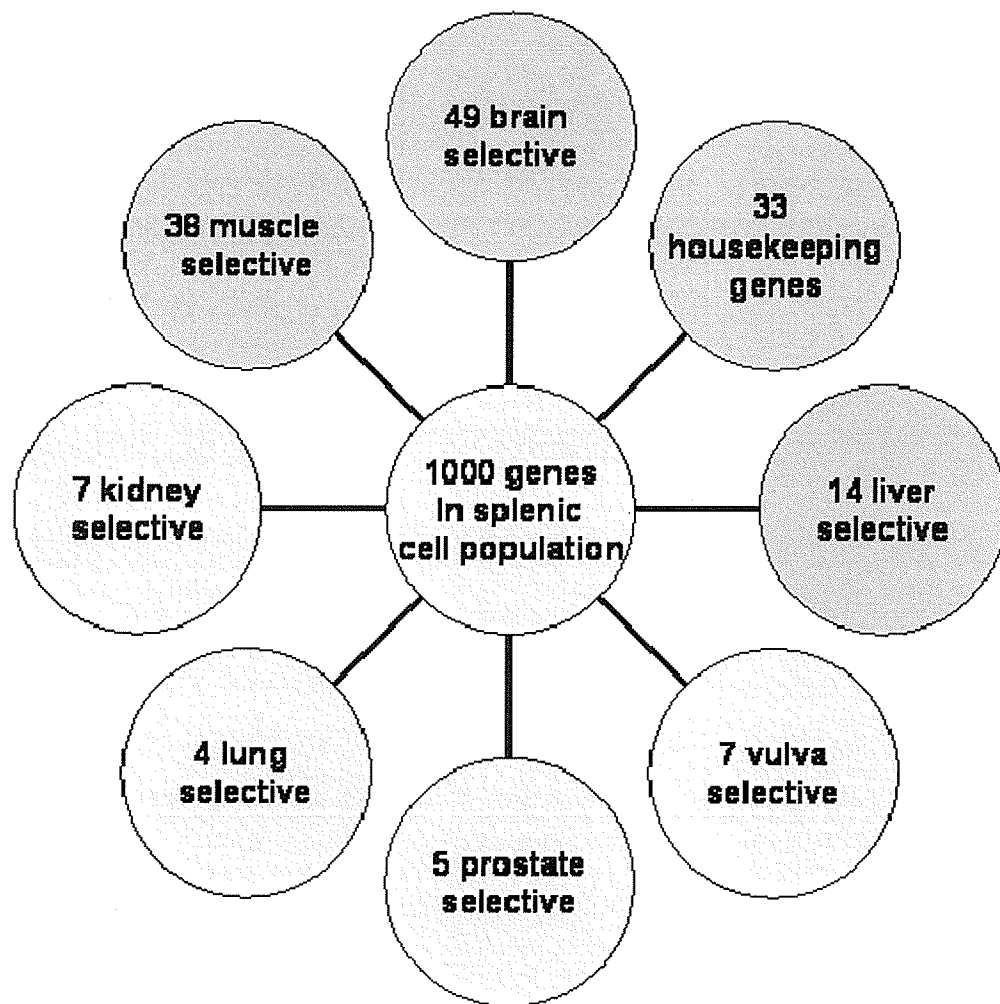


Figure 27: Tissue selective expression associated with the 1000 differentially expressed genes by the splenic cell population in the VM 22A clinical endpoint mice.

Of the 843 spleen selective genes, 48 genes could be considered specific to the splenic cell population isolated by comparing the 843 spleen selective gene list with the whole spleen versus splenic cell population data from the microarray experiment performed earlier. The genes that were overrepresented by the splenic cell population were compared with the spleen selective genes and the resulting splenic cell population specific gene list can be seen in **Table 13**.

Table 13: Splenic cell population specific genes differentially regulated at clinical endpoint of scrapie

ID	Genes	Description	Fold Change
A_52_P589735	ACP1	acid phosphatase 1, soluble	-5.55
AI661341	ADAM10	ADAM metallopeptidase domain 10	-2.541
A_52_P145349	ALS2CR2	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 2	-3.044
A_52_P512955	ANLN	anillin, actin binding protein	-7.197
A_51_P111757	ASB1	ankyrin repeat and SOCS box-containing 1	-3.044
A_51_P518488	ATPIF1	ATPase inhibitory factor 1	-3.062
A_51_P455647	CA2	carbonic anhydrase II	-4.317
A_51_P163444	CARHSP1	calcium regulated heat stable protein 1, 24kDa	-2.805
A_51_P361022	CDC20	cell division cycle 20 homolog (S. cerevisiae)	-3.135
A_51_P164021	CENPE	centromere protein E, 312kDa	-9.739
A_52_P227880	CENPF	centromere protein F, 350/400ka (mitosin)	-13.812
A_52_P4666	CENPK	centromere protein K	-10.336
A_52_P462049	CLCN3	chloride channel 3	-12.518
A_52_P323415	CPEB4	cytoplasmic polyadenylation element binding protein 4	-7.489
A_51_P500718	DCK	deoxycytidine kinase	-12.547
A_51_P248067	EZH2	enhancer of zeste homolog 2 (Drosophila)	-5.289
A_51_P398525	FN3K	fructosamine 3 kinase	-16.405
A_51_P112223	GSTA4	glutathione S-transferase A4	-4.736
A_51_P260169	GSTM3 (includes EG:2947)	glutathione S-transferase M3 (brain)	-3.591
A_51_P245275	H2AFX	H2A histone family, member X	-3.288
A_51_P125368	HARS	histidyl-tRNA synthetase	-3.815
A_51_P179672	HBG1	hemoglobin, gamma A	-2.064
A_51_P138548	HMGB3	high-mobility group box 3	-4.716
AF296283	ICAM4	intercellular adhesion molecule 4 (Landsteiner-Wiener blood group)	-2.832

A_52_P342880	ITSN1	intersectin 1 (SH3 domain protein)	-6.334
A_51_P167551	KIAA1370	KIAA1370	5.195
A_52_P670275	KIF14	kinesin family member 14	-6.105
A_51_P493467	KIF22	kinesin family member 22	-4.161
A_51_P254805	KIF4A	kinesin family member 4A	-7.014
A_52_P210011	MARCH2	membrane-associated ring finger (C3HC4) 2	-8.036
A_51_P424810	NCAPG2	non-SMC condensin II complex, subunit G2	-3.672
A_52_P305246	NUDT4	nudix (nucleoside diphosphate linked moiety X)-type motif 4	-8.566
A_51_P491329	PDZK1IP1	PDZK1 interacting protein 1	-7.182
A_51_P183400	PICALM	phosphatidylinositol binding clathrin assembly protein	-2.057
A_51_P458839	PIGA	phosphatidylinositol glycan anchor biosynthesis, class A (paroxysmal nocturnal hemoglobinuria)	-4.35
A_51_P207550	PLA2G12A	phospholipase A2, group XIIA	-3.082
A_51_P492087	POLE3	polymerase (DNA directed), epsilon 3 (p17 subunit)	-3.13
A_51_P302503	PPP1R15A (includes EG:23645)	protein phosphatase 1, regulatory (inhibitor) subunit 15A	-5.476
A_51_P221062	PRKAR2B	protein kinase, cAMP-dependent, regulatory, type II, beta	-2.671
A_51_P488928	PSCD3	pleckstrin homology, Sec7 and coiled-coil domains 3	-2.831
A_52_P39237	RAD21	RAD21 homolog (S. pombe)	-3.794
A_52_P419873	RB1	retinoblastoma 1 (including osteosarcoma)	-4.662
A_51_P251639	RNF123	ring finger protein 123	-4.053
A_51_P513530	SPAG5	sperm associated antigen 5	-8.036
A_51_P510441	SUV39H1	suppressor of variegation 3-9 homolog 1 (Drosophila)	-3.109
A_51_P255853	TAL1	T-cell acute lymphocytic leukemia 1	-12.116
A_51_P136014	TFDP2	transcription factor Dp-2 (E2F dimerization partner 2)	-3.266
A_52_P202029	TRAK2	trafficking protein, kinesin binding 2	-9.304

3.10 Validation of Decorin expression using qRT-PCR

Alternative methods are available to confirm microarray results due to their inherent variable nature, including qRT-PCR. As can be seen in **Table 9**, the most upregulated gene in the isolated SCP from scrapie infected animals was decorin (DCN), a gene belonging to the small leucine-rich proteoglycan family. This gene was one of the few identified in this study that has previously been associated with neurodegenerative diseases such as prion disease, characterized by the production of amyloid. In addition it has been associated with the splenic amyloid production in animal models of AA amyloid, and with the growth factor TGF β 1 which has been shown to be associated with prion disease, playing a role both in the brain and the spleen (Sorensen *et al.*, 2008; Cunningham *et al.* 2005). Upregulation of decorin was first confirmed in the four clinical endpoint VM 22A infected mice used in the microarray study. Results of this study are provided in **Figure 28**. RT-PCR confirmed decorin up-regulation in the SCP from infected spleen, the level of expression being approximately 60-fold higher than that seen in the SCP from age-matched mock-infected mice.

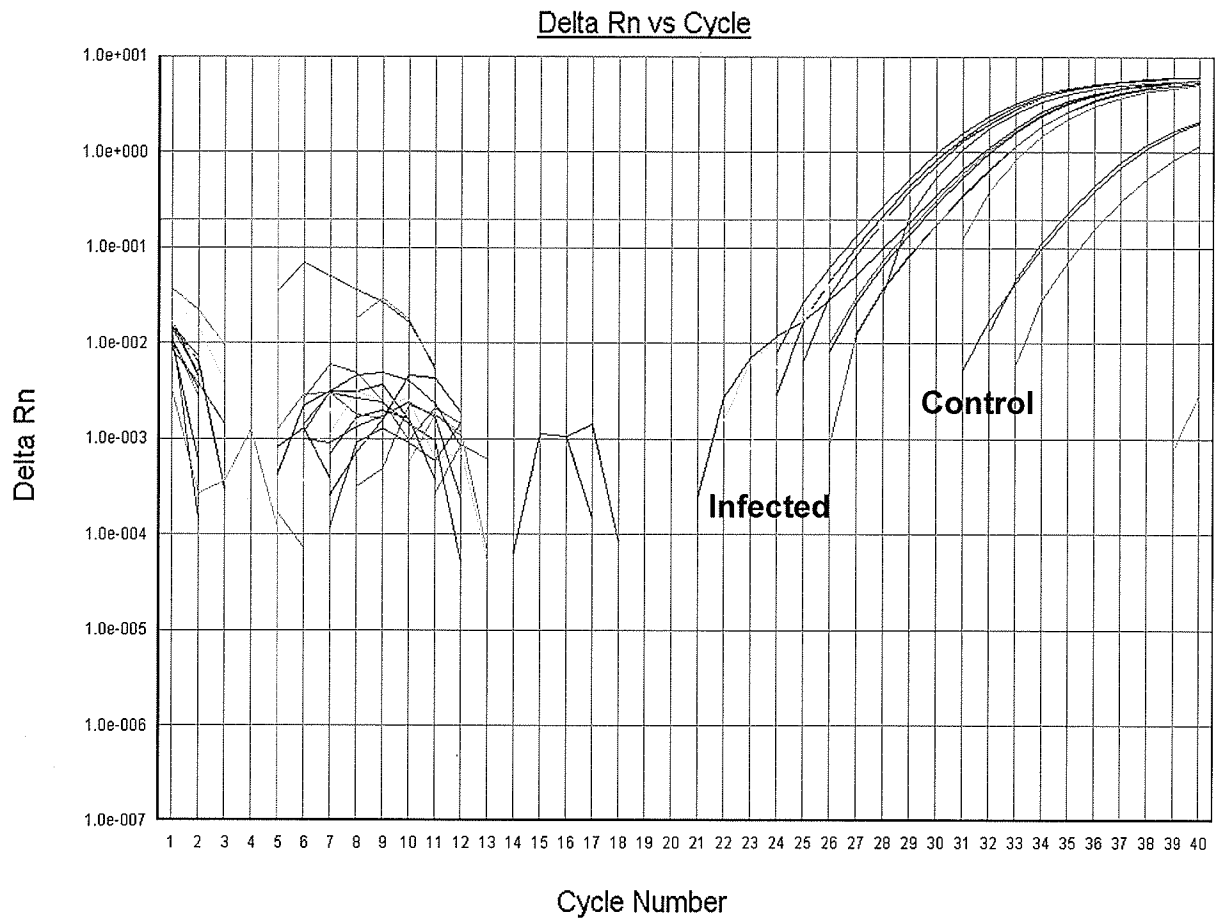


Figure 28: DCN upregulation confirmed by qRT-PCR from the four infected clinical VM 22A mice and one mock-infected control used in the microarray study. Experiment was performed in triplicate.

Next, decorin expression was determined in another mouse model of scrapie, C57BL/6 mice infected with the RML strain of scrapie. This model (RML strain) was chosen for the timecourse study due to its reduced incubation period (Bruce *et al.* 1991). In this experiment, the SCP was isolated at time-points through-out infection. The results are provided in **Figure 29**. The resulting expression values are after normalization to the endogenous GAPDH reference gene and are expressed as the mean of $2^{-\Delta\Delta Ct} \pm$ the standard error of the mean for three infected mice at each time-point compared with three mock-infected control mice. Interestingly, although I still found a statistically significant over-expression of decorin (4-fold was highest level), the levels were much lower than that seen in the VM model (60-fold change). It is possible that these differing results are indicative of genetic differences between the two different hosts and difference between strains used.

C57Bl/6 RML Timecourse

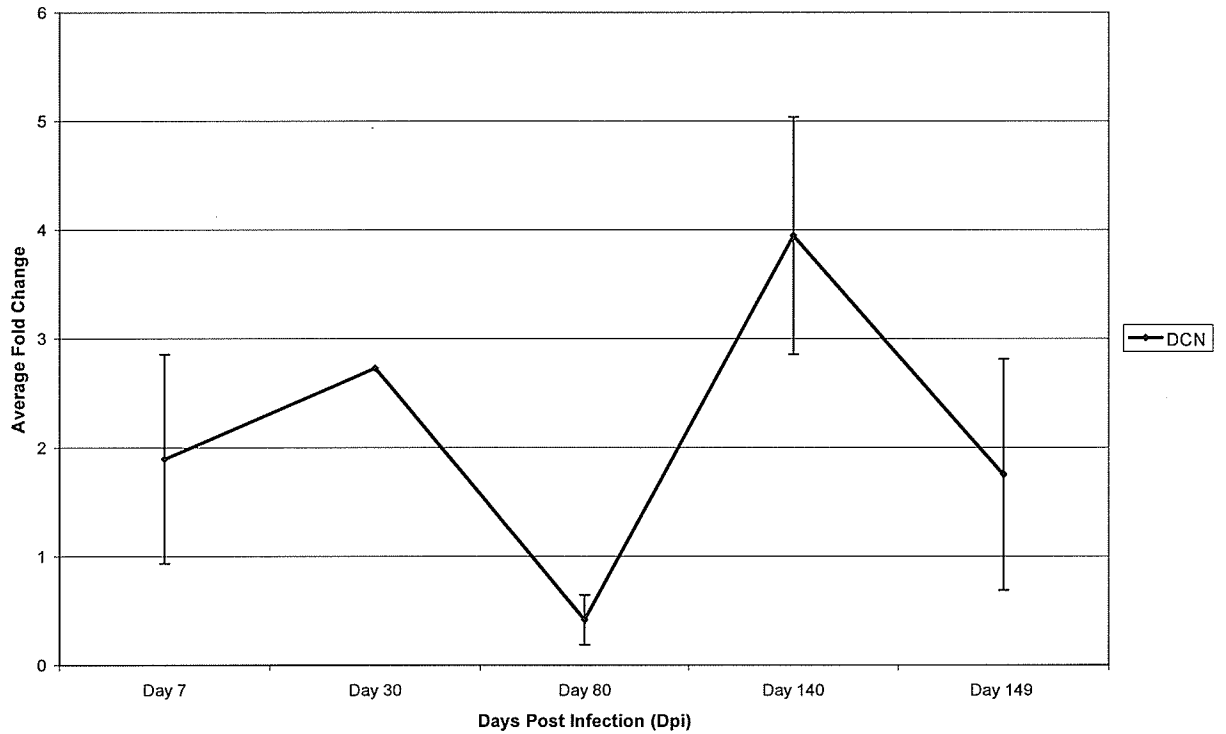
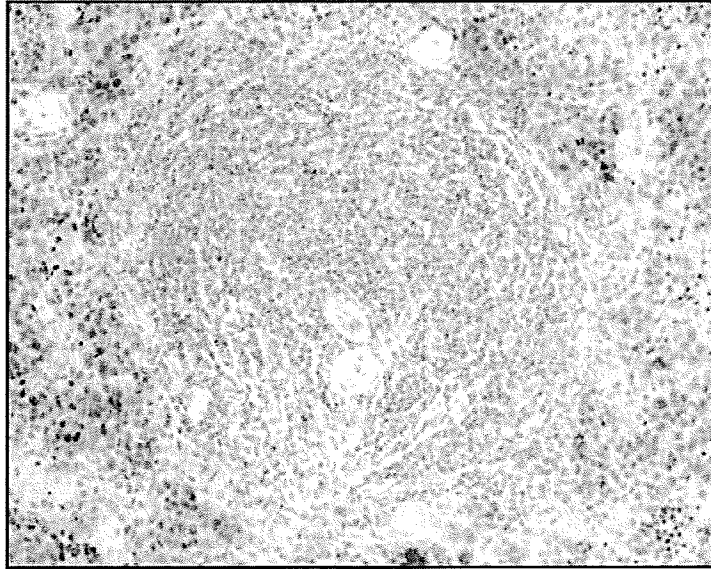


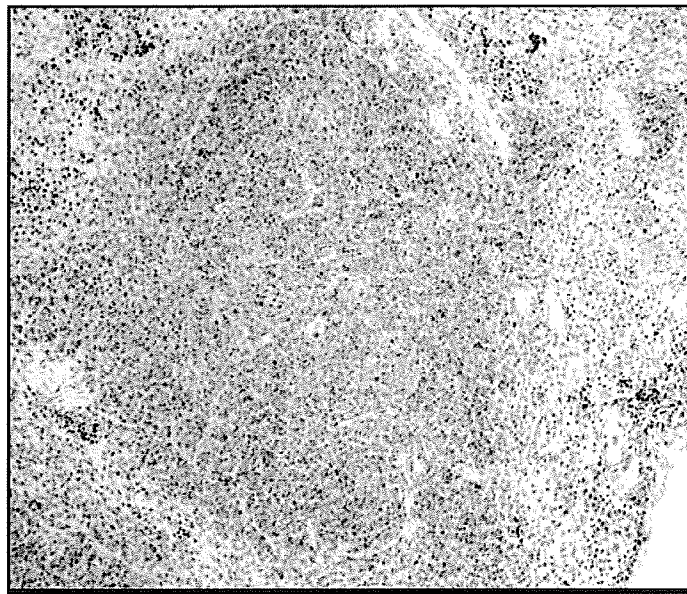
Figure 29: Timecourse expression of Decorin in C57Bl/6 RML infected mice displaying the average fold change between three infected mice at each timepoint relative to three mock-infected control mice. Error bars shown are representative of the standard error of the mean (SEM) between the mice. **Note:** Due to sample losses, no SEM shown for Day 30 as only one sample was used.

3.11 Immunohistochemistry showed an accumulation of decorin in the spleens of scrapie infected mice

Considering that the DCN gene was found to be so highly upregulated in my SCP during scrapie (**Table 9**), immunohistochemistry was used to determine whether accumulation of decorin could be seen at the protein level in mouse spleen. In previous reports of decorin upregulation in AA amyloidosis, decorin was clearly found to be associated with amyloid deposits in spleen follicles (Wien *et al.* 2004). As PrP^{SC} is also known to accumulate in spleen follicles (Bruce *et al.* 2000) I expected to see a similar pattern of staining. Formalin fixed, paraffin-embedded spleen tissues from VM 22A mice and age-matched, mock-infected control mice were stained with a decorin specific primary Ab. The results are provided in **Figure 30** where decorin staining can be clearly seen. The spleens from mock-infected mice showed staining throughout the spleen; however although a similar staining pattern is visualized in the spleens of infected mice, a concentration of staining is found within the follicles. This staining pattern appears to confirm the up-regulation of decorin expression at the protein level and also is consistent with its up-regulation being particularly marked in the follicles where PrP^{SC} replication and accumulation is known to occur.



Mock-infected

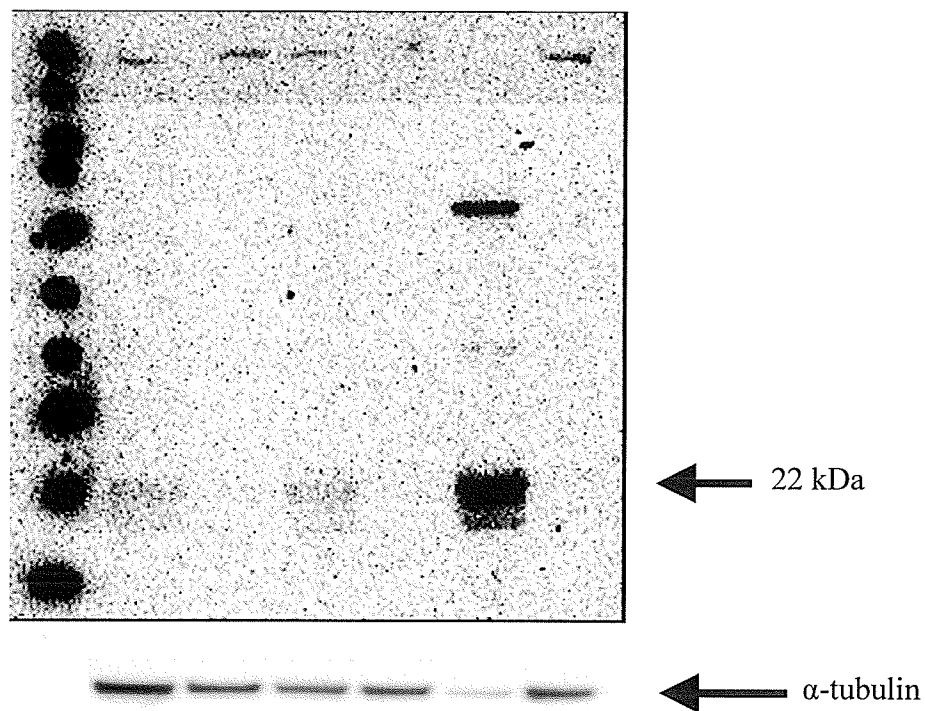


22A infected

Figure 30: Spleen follicles from VM mice stained with a decorin specific Ab. Brown staining represents the presence of decorin using immunohistochemistry.

3.12 Western Blotting confirmed decorin accumulation in the spleens of scrapie infected mice

Western blot was also performed to investigate whether western blot was sensitive enough to detect decorin up-regulation in whole spleen tissue homogenate. Spleen homogenates from VM mice infected with the 22A scrapie strain, brain tissue from a BSE infected cow, and a vCJD infected human were used. The corresponding age-matched spleen homogenate from mock-infected mice was run alongside. The resulting decorin labeled Western blot is shown in **Figure 31**. The blots were stripped and probed with an α -tubulin Ab as a control to ensure equal loading in each lane. Significant expression of decorin was evident in the spleen homogenate of all the infected mice whereas decorin was at a level that was undetectable in the amount of homogenate loaded for the mock-infected controls.



Ladder BSE PBS 22A PBS vCJD PBS

Figure 31: Western blot of VM mice spleen homogenates from BSE, 22A, and vCJD infected mice. Decorin is represented by the 22 kDa band present in each of the infected samples.

Given the extent of decorin upregulation in these mouse models I decided to investigate whether decorin was similarly up-regulated in spleens of naturally infected human vCJD patients. I obtained spleen homogenates for two human vCJD cases and a matched control sample from the CJD resource centre (Potters Bar, UK) (www.nibsc.ac.uk/cjd/). Western blotting procedures were performed in the same way as for the above procedures for detection of mouse decorin. The resulting blot is provided in **Figure 32**. Although the difference in decorin levels between normal and infected patients is not as marked as found in the mouse models, there is a 2-3 fold increase in expression of the top 22 kDa decorin band in the two vCJD cases versus the control un-infected individual.

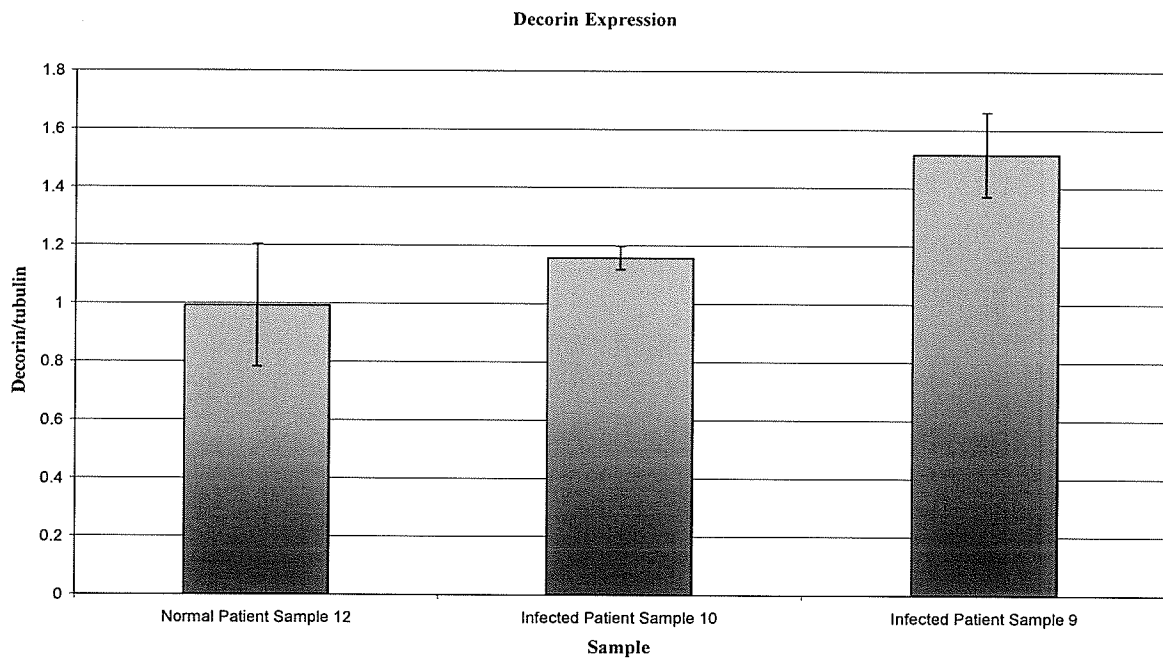
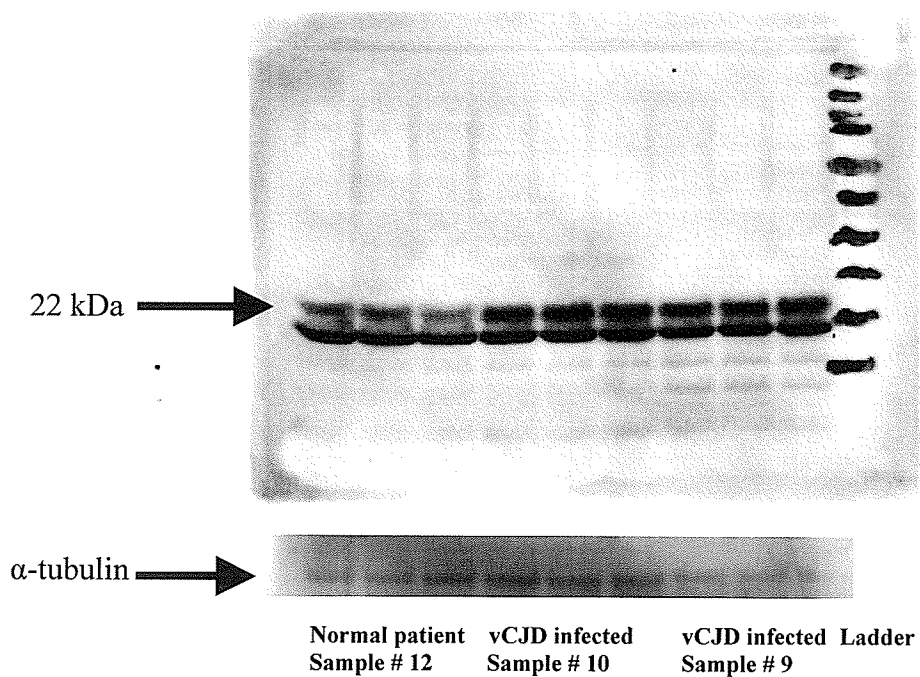


Figure 32: Western blot analysis of human vCJD spleen homogenate samples from the CJD resource centre. Densitometry was performed and DCN expression values were normalized to tubulin expression for each sample. The relative quantification was calculated as the ratio of DCN expression to tubulin expression as shown in the chart above. Each sample was run in triplicate.

3.13 Analysis of expression levels of proteoglycan family members

Decorin is a member of the small leucine-rich proteoglycan family, a number of family members have been documented as potentially associated with diseases in which amyloid is deposited, for example Alzheimers disease (Snow *et al.*1992). Indeed the related protein heparan sulphate proteoglycan has previously been shown to be associated with amyloid plaques in scrapie (McBride *et al.* 1998). I decided to use RT-PCR to determine whether other members of this protein family are also up-regulated. In addition to decorin, only the Agilent microarray platform was also able to pick up chondroadherin, proline arginine-rich & leucine-rich repeat, lumican, and osteoglycin to be up-regulated. In total I performed RT-PCR to measure the expression of 12 additional proteoglycan genes (provided in **Table 3**) on the same four clinical endpoint VM 22A mice samples that were used in the microarray experiment. All gene expression values were normalized to the endogenous GAPDH reference gene and each gene is expressed as the mean of $2^{-\Delta\Delta Ct}$ (fold change) $\pm$ the standard error of the mean in **Figure 33** between four infected mice where $\Delta Ct = Ct \text{ of target gene} - Ct \text{ of GAPDH}$. Decorin (DCN), Chondroadherin (Chad), and Proline arginine-rich & leucine-rich repeat (PRELP) were upregulated genes validated on both the microarray and with qRT-PCR. In addition to these three genes, Biglycan (Bgn), also a member of the small leucine-rich proteoglycan family, was found using qRT-PCR, but was originally not picked up from the analysis of the microarrays as its fold change of 1.79 was found only on the AROS oligo microarray, being just below the cutoff criterion of a ≥ 2 fold change.

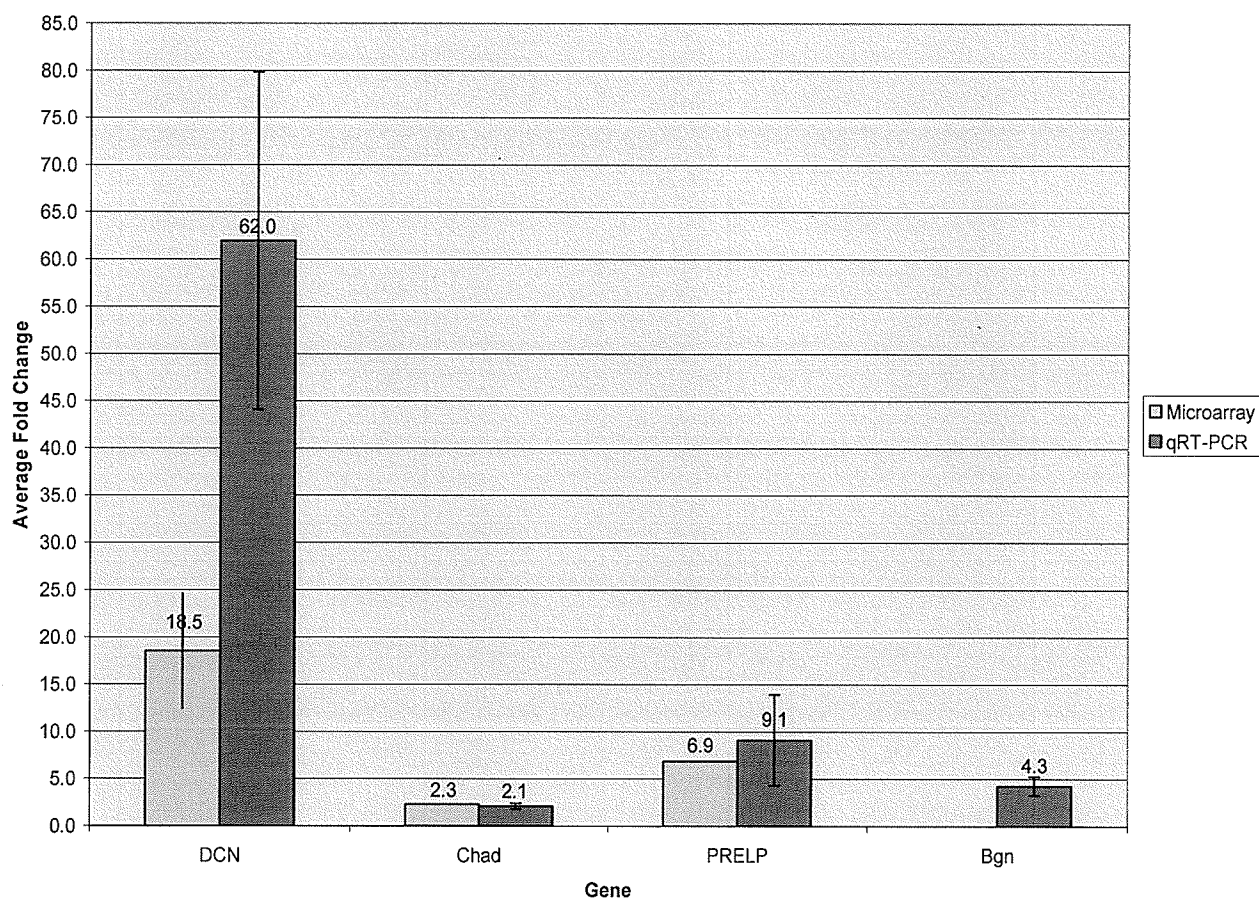


Figure 33: A comparison of the genes found upregulated during prion infection in clinical endpoint VM 22A infected mice by microarrays and qRT-PCR. Average fold change is given $\pm$ standard error of the mean between the four biological replicates via qRT-PCR and between the three array platforms within the 1% FDR.

Next I used RT-PCR to determine the expression of the other proteoglycan members in another mouse model of scrapie, C57BL/6 mice infected with the RML strain of scrapie. I isolated the SCP at different timepoints of infection and determined the expression values of each gene as discussed above. In addition to DCN, PRELP and Bgn also showed significant over-expression in this timecourse experiment (**See Figure 34**).

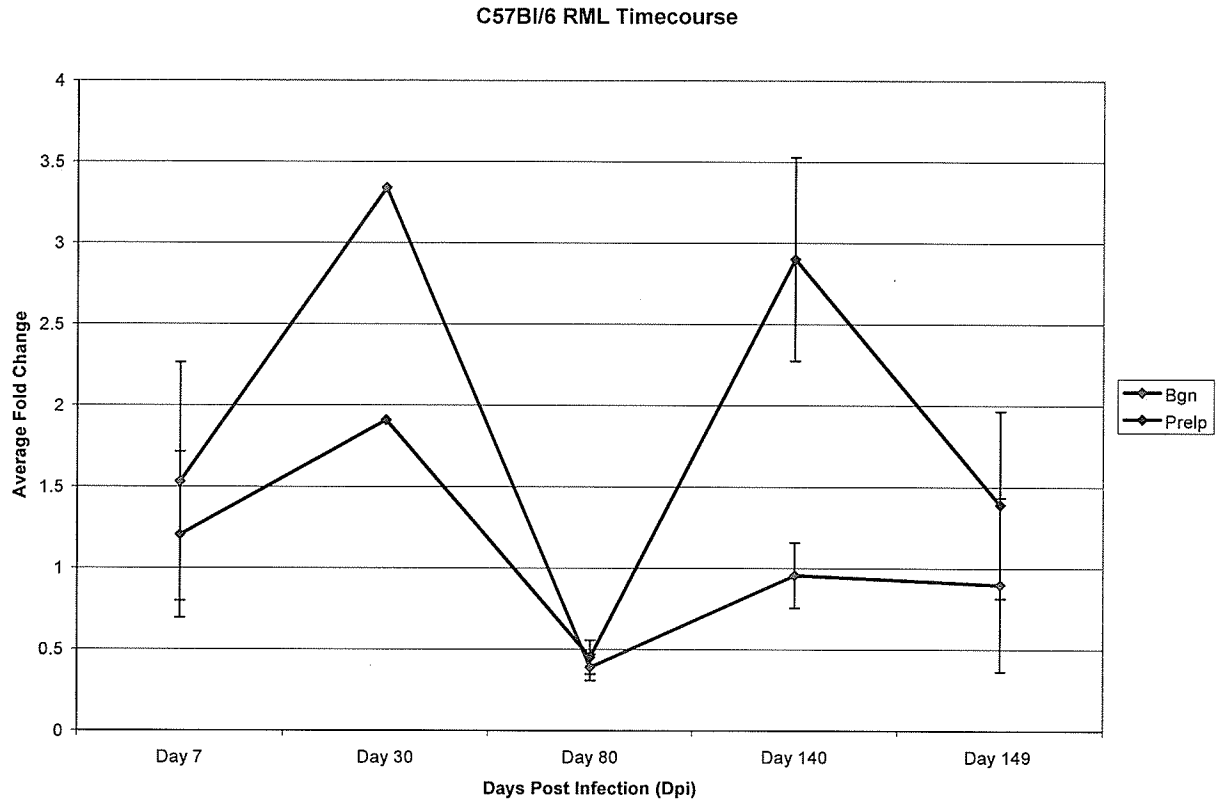


Figure 34: Timecourse expression of Bgn and PRELP in C57Bl/6 RML infected mice displaying the average fold change between three infected mice at each timepoint relative to three mock-infected control mice. Error bars shown are representative of the standard error of the mean (SEM) between the mice.

4 Discussion

When examining the differentially expressed genes generated from the isolated splenic cell population of the VM 22A clinical endpoint mice, I used an online tool based on a curated database, the IPKB, to annotate genes and to determine potential regulatory networks and pathways. The IPKB helps provide a framework by which lists of genes identified by large-scale microarray studies can be annotated in terms of their functional relationships, and those that have been shown to interact. Individual regulated genes may interact to have a coordinated role in specific pathways; I identified potential networks of interacting PRG's using the Ingenuity Pathway Analysis tool. The methodology integrates genomic data with mining techniques to predict protein networks that comprise protein-protein interactions and other functional linkages. Each potential network is given a score, which is a probabilistic fit between the networks and a list of biological functions stored in the IPKB. The score takes into account the number of focus genes (from the scrapie responsive gene-list) in the network, and the size of the network, to approximate how relevant it is to the original list of genes; these scores are used to rank the networks. As the list was extensive I restricted the analysis to those networks that had scores >9 (a score of 3 or greater was considered significant, the score being the negative exponent of the p -value ($p < 0.001$)). Of the top five networks listed in **Table 12**, three were of particular interest in the context of prion disease and are discussed below.

4.1 Network One- Downregulation of Caspase 3 and E2F4

Beginning with the examination of network number one in **Figure 22**, there are two central downregulated genes present that are worth mentioning. Firstly, there is Caspase 3 which is a key gene involved in apoptosis where it is activated in a p53 dependent manner. Caspase 3 is one of the last key genes in a cell to control the cell's demise (Culmsee & Landshamer, 2006). In fact, activation of caspases in general, is considered to be the trademark of apoptosis (Culmsee & Landshamer, 2006). Also related to apoptosis signaling is the induction of NF-K β signaling, and as can be seen in **Figure 21**, NF-K β signaling is one of the top toxicological pathways and can lead to increased expression of Bcl2, a member of a family of proteins that is important for maintenance of mitochondrial integrity and thus protect the cell after severe stress if upregulated (Culmsee & Landshamer, 2006). As can be seen below in **Figure 35**, Caspase 3 and Bcl2 are both involved together in apoptosis signaling amongst other genes found differentially regulated in the VM 22A clinically infected mice. Downregulation of Caspase 3 expression, the pro-apoptotic protein and upregulation of Bcl2, the anti-apoptotic protein, would suggest there is a host cellular response for cellular survival inside the spleen, and in particular the splenic cell population that was isolated.

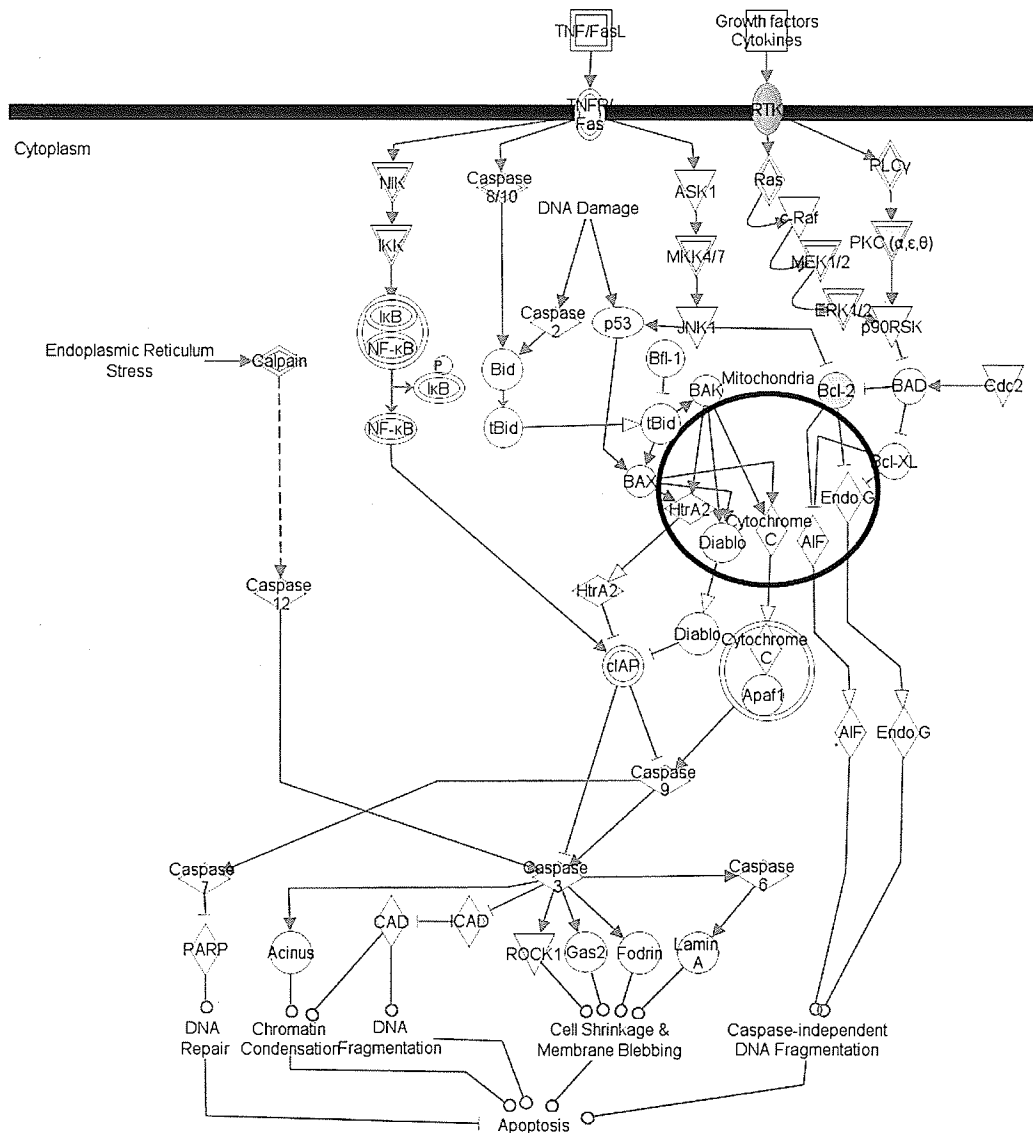


Figure 35: Apoptosis signaling pathway within the cell displaying the upregulation of Bcl2 in red and downregulation of Caspase 3 in green. (Ingenuity Systems, Inc.)

The second central downregulated gene in network number one in **Figure 22**, is E2F4. E2F4 is a transcription factor involved in the G1/S checkpoint regulation in the cell cycle. As can be seen in **Figure 36**, it is part of the transcription repression complex involved with p53 signaling. Also displayed are a number of other genes in this complex that are downregulated (genes shown in green). This complex controls the progression through the cell cycle from the G1 to the S phase. If these genes are all downregulated, then transcription of the S phase genes should be turned on (Nebert *et al.*, 2000). Aryl hydrocarbon (Ahr) signaling, the top canonical pathway found (**Figure 20**), is involved in the cross-talk between these transcription factors (Nebert *et al.*, 2000).

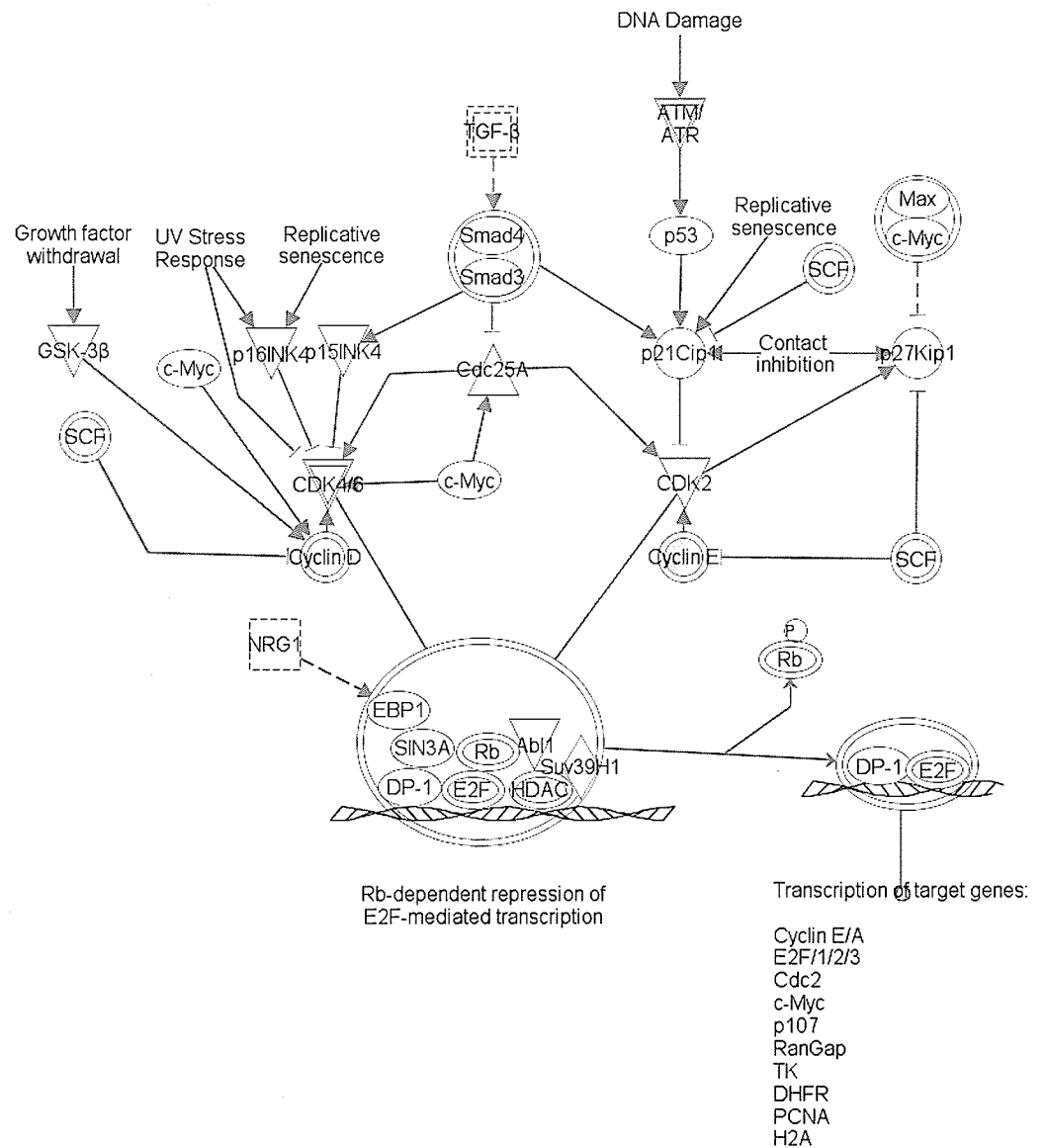


Figure 36: P53 cell signaling pathway involved in the cell cycle G1/S checkpoint regulation displaying the downregulation of the E2F transcription factors in green. (Ingenuity Systems, Inc.)

The role of these apoptotic signaling proteins in the spleen is interesting as it is opposite to what is seen in the brain. For example, in Alzheimer's disease, amyloid beta has been seen to reduce Bcl2 expression and enhance Caspase 3 activation increasing apoptosis of neurons and increasing their vulnerability to oxidative stress (Culmsee & Landshamer, 2006).

4.2 Network Five- Upregulation of Akt1

In relation to Bcl2, is another gene, Akt1 that was a central upregulated gene found in network number five in **Figure 24**. Akt1 and Bcl2 are part of the VEGF signaling pathway that was one of the top five canonical pathways found in **Figure 20**. VEGF is a homodimeric growth factor that is constitutively expressed in the brain (Kilic *et al.*, 2006). It has been shown that VEGF can protect neurons from death during neurodegeneration (Kilic *et al.*, 2006). VEGF has been demonstrated in mice that when administered after a stroke, it could prevent neuronal injury by inhibiting Caspase-3 (Kilic *et al.*, 2006). As can be seen in **Figure 37** below, Akt1 and Bcl2 work together in the VEGF signaling pathway to promote cellular survival, again demonstrating the preservation of cell integrity inside the spleen.

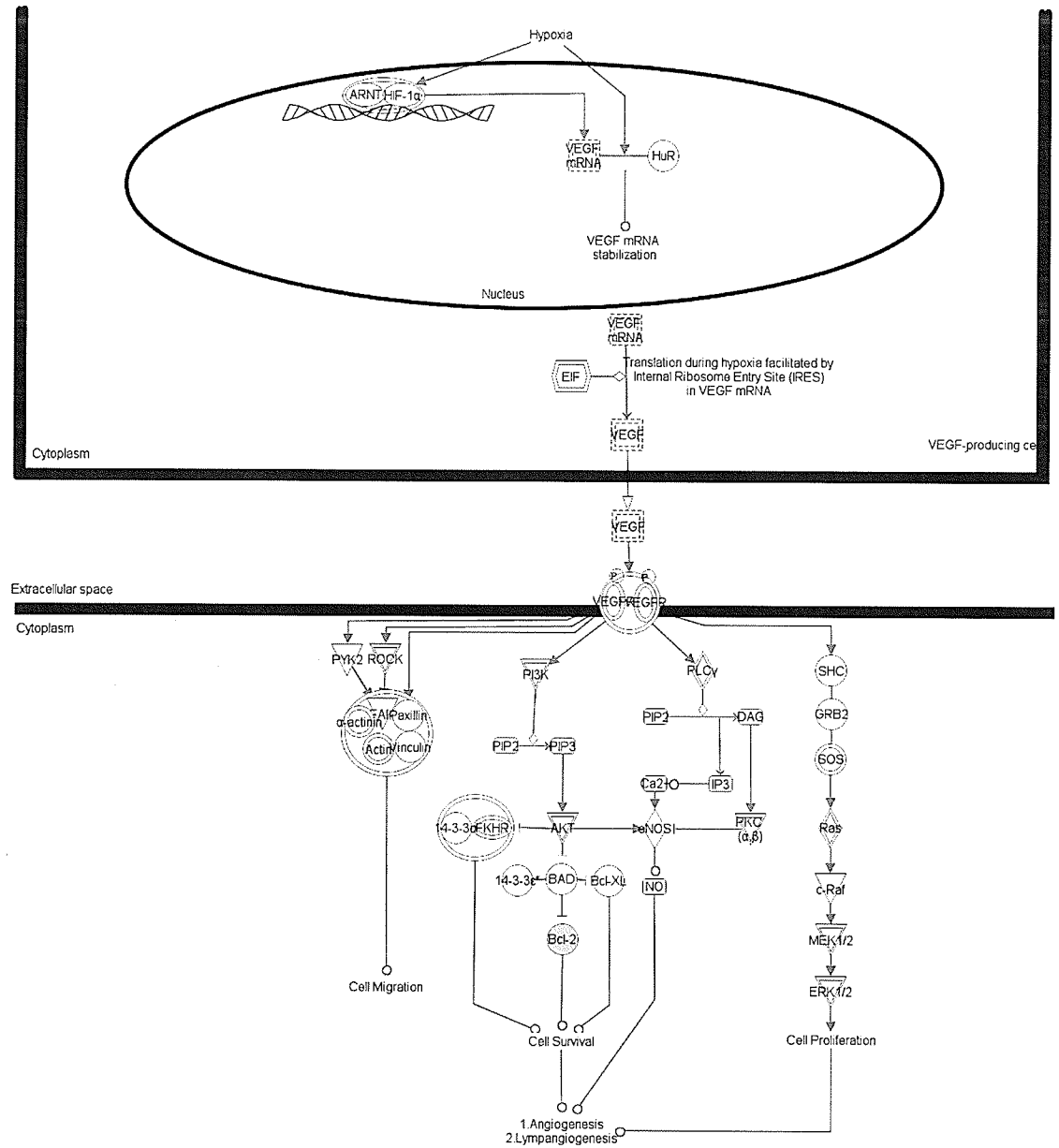


Figure 37: VEGF signaling inside the cell displaying the upregulation of Akt1 in red in response to the binding of VEGF to its receptor and the upregulation of Bcl2 which both stimulate the cell signaling transduction pathway to promote cellular survival. (Ingenuity Systems, Inc.)

In addition to cell cycle regulation, it is of interest to note that Akt1 also has involvement in axonal guidance, amyloid processing, and immune cell signaling including B cell receptor signaling, natural killer cell signaling, and GM-CSF signaling. As can be seen in **Figure 38**, amyloid processing ultimately results in neuronal death characteristic of prion disease. In this pathway Akt1 inhibits GSK-3 β which ultimately promotes neuronal death. Additionally, it is of no surprise that immune cell signaling would be present in the spleen (the organ associated with immune response in the body) and especially since no immune response is found associated with prion disease (Kubler *et al.*, 2003), there has to be some sort of suppression or regulation of the immune response during infection. In fact, it has been found that decorin, the top upregulated gene in the splenic cell population inhibits the macrophage colony-stimulating factor (M-CSF) and thus inhibits proliferation of macrophages (Xaus *et al.*, 2001), another factor that may play a role in suppression of the immune system during prion disease.

Amyloid Processing

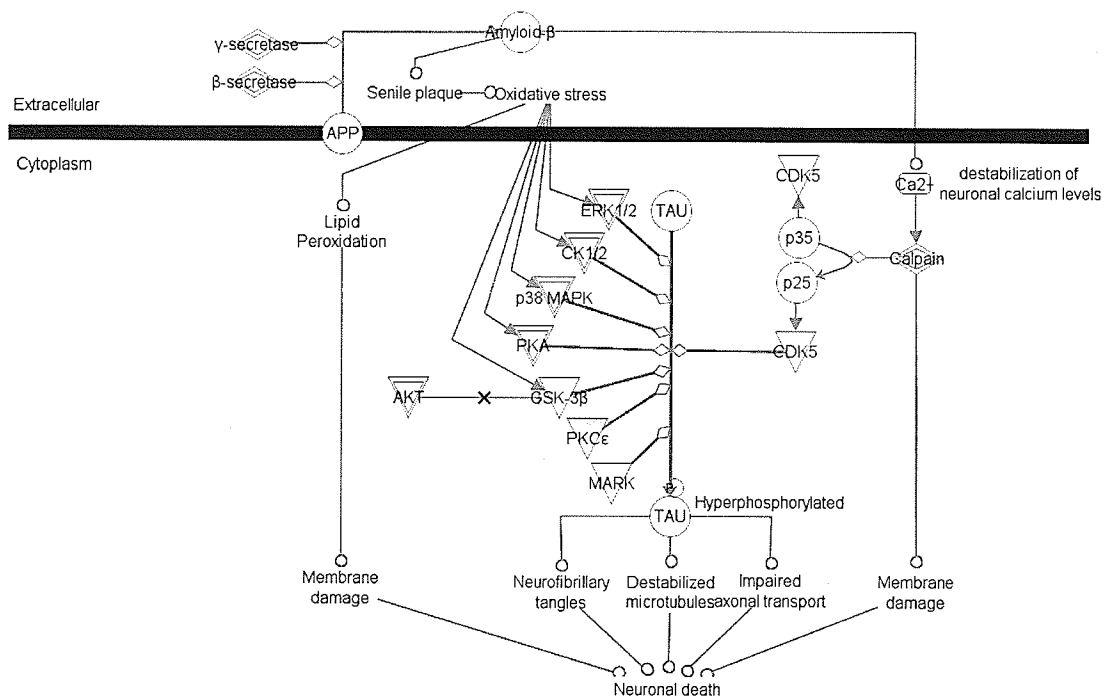


Figure 38: Amyloid processing inside the cell is associated with Akt1 where if upregulated as shown in red will inhibit the GSK-3 β which normally would lead to neuronal death. (Ingenuity Systems, Inc.)

4.3 Aryl Hydrocarbon receptor (Ahr) signaling pathway

The top canonical pathway found in **Figure 20** after analyzing all the differentially expressed genes in IPA from the splenic cell population was the aryl hydrocarbon receptor (Ahr) signaling pathway. Ah genes and their receptors are a group of genes that are interrelated in terms of their expression in response to certain endogenous or exogenous stimuli (Nebert *et al.*, 2000). The Ah genes are endogenously expressed in most vertebrates and play an important role in cell defense against oxidative stress and in cell cycle control (Nebert *et al.*, 2000) in particular, being gatekeepers at the different checkpoints in the cell cycle where the cell will choose between either apoptosis or continuation through the cell cycle (Nebert *et al.*, 2000). There are two fundamental control checkpoints in the eukaryote cell cycle, the G1/S boundary and the G2/M boundary. Both these cell checkpoints are amongst the top canonical pathways in **Figure 20** and amongst the top toxicological pathways in **Figure 21**. At both of these checkpoints, the cell has the decision between apoptosis or continuation through the cell cycle which is mainly controlled by the p53 protein, hence why p53 signaling is also found amongst the top canonical and toxicological pathways above.

4.4 G1/S checkpoint cell cycle regulation

The downregulation of Caspase 3 and E2F4 and the upregulation of Akt1 and Bcl2 in the splenic cell population are all related to cell cycle regulation at the G1/S checkpoint and all promote the cellular survival and progression of the cell towards the S phase. These results are not surprising considering there are no pathological changes associated with the spleen during prion infection in comparison to the brain where an immense amount of apoptosis of neurons is visible (Aucouturier & Carnaud, 2002). **Figure 39** below gives a

pictorial summary of how I think these genes may be acting together to promote cellular survival inside the spleen.

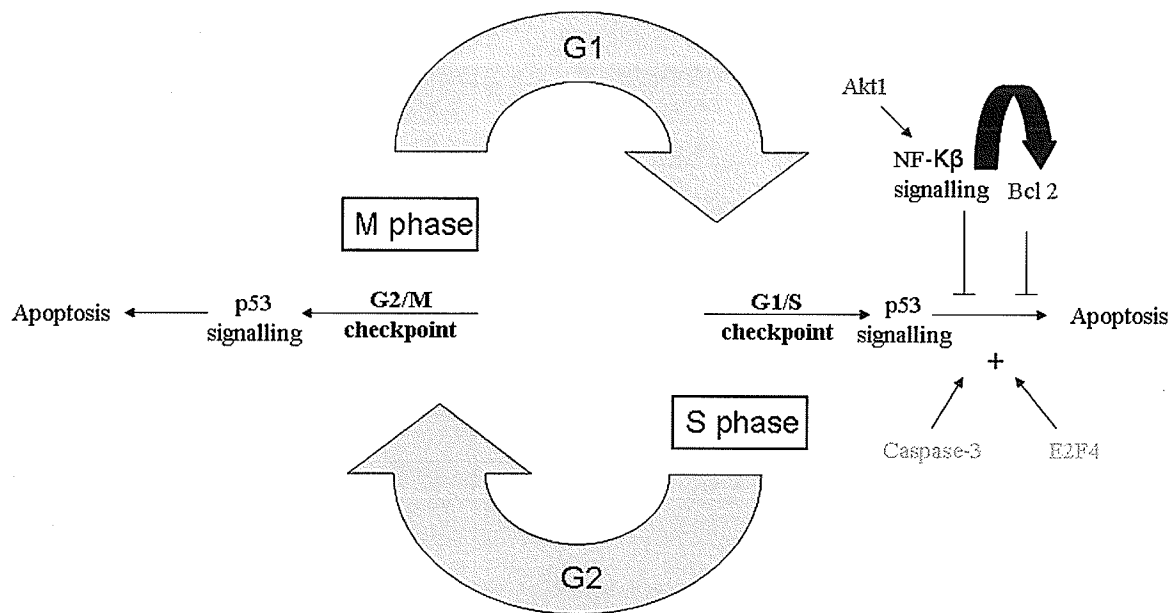


Figure 39: Interaction of the genes differentially expressed (upregulated in red and downregulated in green) by the splenic cell population in cell cycle regulation and progression through to the S phase to promote cellular survival.

4.5 Network three- Upregulation of EGFR

Lastly, another gene of interest found amongst the top networks was EGFR, a central upregulated gene in network number three. EGFR or epidermal growth factor receptor is involved in axonal guidance like Akt1, but it is also involved in N-glycan biosynthesis. The top upregulated gene decorin (DCN) found amongst the differentially expressed splenic cell population and the remainder of the focus for this study interacts directly with EGFR in this network and interestingly contains potential sites for N-glycosylation (Kalamajski *et al.*, 2007).

4.6 Porphyrin Metabolism Pathway- Accumulation of Iron

Lastly, in addition to finding genes associated with cellular survival, there were a number of genes found on the microarray that were part of the porphyrin metabolism pathway as can be seen in **Figure 25**. Porphyrins are tetrapyrrole compounds containing hydrophobic aromatic rings that can bind strongly and selectively to proteins and affect changes in their conformation (Caughey WS *et al.*, 1998). They can especially bind metal cations inside their hydrophobic ring core including such metals as iron, copper, and aluminum to name a few (Caughey WS *et al.*, 1998). Interestingly, the prion protein contains an N-terminal signal consisting of four or five octapeptide repeats that are thought to bind copper (Riesner, 2003) and thus, these porphyrin rings may be involved in the binding of the prion protein and possibly involved in the conversion of PrP^c to PrP^{Sc}. In fact, it has been postulated that porphyrins inhibit the formation of PrP^{Sc} (Caughey WS *et al.*, 1998). Furthermore, considering porphyrin rings can bind cations, they could be considered as metal chelators that sequester the free metal cations. Thus, considering that the genes

associated with porphyrin metabolism pathway were downregulated, I predicted that there would be less sequestering of the metal cations. I chose to investigate the metal cation iron in particular, as it has been found to be dysregulated in many neurodegenerative disorders including Alzheimer's, Parkinson's, and of course Prion disease (Molina-Holgado *et al.*, 2007), and thus an accumulation of iron might be observed over the course of the disease. This was in fact true when iron deposits were stained for in the spleen using the Perls' Prussian blue reaction for ferrous iron seen in **Figure 26** in the VM scrapie infected mice over the course of infection. This result is interesting, as iron has also been found to be essential for maintaining homeostatic function in the development of the brain, but too much iron is associated with neuroinflammation, neurodegeneration, and cell death (Molina-Holgado *et al.*, 2007) and this has been considered possibly a primary cause of and not just a consequence of neurodegeneration (Lee *et al.*, 2006).

Iron has also been reported to play an important role in protein aggregation (Molina-Holgado *et al.*, 2007) and of course prion disease is associated with the formation of amyloid-like fibrils which are aggregated proteins. It has been proposed that PrP^{Sc} can interact with free radicals and metal ions generating redox activity which consequently causes oxidative damage. Notably, this has been implicated in prion disease as either a primary cause of disease or as a consequence of disease progression (Molina-Holgado *et al.*, 2007).

4.7 Decorin

Considering decorin was so highly upregulated across all arrays (**Table 9**) and its upregulation was confirmed at the molecular level using qRT-PCR in **Figure 28**, this gene was considered an important gene to investigate further. Thus, its upregulation was predicted to also be detected at the protein level by immunohistochemistry and western blot on whole spleen tissue. When immunohistochemistry was performed in **Figure 30**, it could be seen that there was indeed a distinct difference between the mock-infected control and scrapie infected mouse. Thus, when investigating it via western blot it was decided to investigate different strains of prion including BSE, 22A (scrapie), and vCJD to test its overall expression in prion diseases. It was found to be upregulated in all three strains, with it being particularly high in the vCJD sample. Although this intense band at 22 kDa seen in the vCJD sample may be due to the fact that the antibody used for decorin more specifically recognizes the human decorin peptide sequence and thus would have an increased binding for the vCJD sample. In addition to the 22 kDa band present, there is also a distinct band shown at about 51 kDa in **Figure 31** in the vCJD sample. This band could be representative of the protein form ready for internalization and recycling. The metabolic turnover of decorin involves the binding of its protein core to a 51 kDa membrane-protein where this complex is then internalized and transported to the perinuclear compartment (Fransson *et al.*, 2000). Again, this may only be visible in the vCJD sample due to the specificity of the antibody to human decorin. In order to investigate this phenomenon with the vCJD sample further, human vCJD spleen homogenates were acquired through the CJD resource centre (Potters Bar, UK) (www.nibsc.ac.uk/cjd/) from patients confirmed positive for vCJD to relate decorin's expression from the mouse model to human samples. In **Figure 32** it can be seen that

there is an upregulation of decorin in the infected patient spleen homogenate samples compared to the normal patient when comparing the 22 kDa bands. The other more intense smaller bands may be representative of the different phosphorylation states of decorin. The activated form of decorin containing its glycosaminoglycan (GAG) chain is more phosphorylated as the GAG chain is synthesized resulting in a bigger protein (Fransson *et al.*, 2000). Thus, the top 22 kDa band would be more representative of the activated form of decorin containing its GAG chain and therefore, would be the band to compare back to the normal patient sample. This result correlates well with what was found in the mouse model in **Figure 31** showing promise that decorin is in fact upregulated at the endpoint of disease and could be used as a potential biomarker of disease.

Decorin is a member of class I of the small leucine-rich secreted proteoglycans (SLRPs) in the extracellular matrix (ECM) containing one GAG chain and GAGs have been implicated to be important in prion pathogenesis (Ben-Zaken *et al.*, 2003). ECM associated proteoglycans play essential roles in fibrillogenesis, regulating matrix assembly, recruitment, storage, and modulation of growth factors and cytokines (Desnoyers *et al.*, 2001). For example, it has been shown that DCN can control and suppress cancer growth and invasion by influencing the biological activity of growth factors such as TGF- β , platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF) (Koninger *et al.*, 2004). Interestingly, all three of these growth factor receptors were upregulated in the splenic cell population. Considering there was such a strong signal for cell proliferation and

survival shown above by the different genes in the networks, perhaps the upregulation of DCN is a compensatory, regulatory mechanism by the cell to increase cell survival while preventing detrimental cancerous cell growth.

In **Figure 27**, there are 49 brain selective genes that are associated with the splenic cell population expression. DCN is part of this list of 49 genes that are specific to the brain. This is not surprising since it has been found to accumulate in cerebral prion amyloid plaques (Ben-Zaken *et al.*, 2003) in addition to amyloid plaques and neurofibrillary tangles in other neurodegenerative disorders such as Alzheimer's disease (Snow *et al.*, 1992). Also, some studies have shown that decorin can induce plasminogen and plasmin in the injured CNS by microglia *in vitro* which play roles in suppressing axon growth inhibitory components (Davies *et al.*, 2006) promoting both neuronal and glial cell survival, thus possibly why axonal guidance is revealed as one of the canonical pathways for EGFR which interacts with decorin. Additionally, it has been found to associate with the infectious PrP^{Sc} isoform increasing the infectivity of less aggregated PrP^{Sc}, (Ben-Zaken *et al.*, 2003) as well as increase the endocytosis of PrP, suggesting a role for subcellular trafficking of PrP (Ben-Zaken *et al.*, 2003).

Proteoglycans in general can be synthesized to contain chondroitin or dermatan sulfate or keratin sulfate chains (Iozzo, 1999). Decorin is an example of a dermatan sulfate proteoglycan and is an extracellular secreted proteoglycan found in most tissues, including the CNS (Logan *et al.*, 1999) which contains a pro-peptide which may serve as a recognition signal for the first enzyme involved in the production of GAG chains.

GAGs have been postulated to be involved in PrP isoform metabolism (Ben-Zaken *et al.*, 2003). GAGs have been suggested as cellular receptors for prion rods due to the fact that one, there are several heparin-binding sites (heparan sulfate is an example GAG) on the cellular prion protein as well as in the PrP 27-30 core (Horonchik *et al.*, 2005), two, cell line experiments have shown that cellular heparan sulfate (HS) is crucial for PrP^{Sc} formation (Horonchik *et al.*, 2005), and three, certain soluble heparan mimetics have been found to bind prion rods and prevent their internalization (Horonchik *et al.*, 2005). Possible mechanisms of GAG involvement in PrP^{Sc} metabolism that have been suggested are assisting in the conversion of PrP^c to PrP^{Sc} by helping to bring the PrP^{Sc} template or seed into close proximity with PrP^c prompting its conversion to the infectious isoform (Ben-Zaken *et al.*, 2003).

In addition to decorin, there were other genes also part of the proteoglycan family that were differentially regulated over the course of the disease including Bgn and PRELP which are shown in **Figure 34**. Proteoglycans can be classified into three classes. Class I includes decorin and biglycan which demonstrate the highest homology (~57%) with both containing a pro-peptide (Iozzo, 1999). PRELP is a member of class II in its own subfamily which binds and interacts with heparan sulfate like decorin which may be involved in prion pathogenesis as described above (Iozzo, 1999). Decorin and biglycan each contain an N-terminal domain that is substituted with either one (decorin) or two (biglycan) chondroitin/dermatan sulfate side chains (Iozzo, 1999). The pro-peptide found within decorin and biglycan is believed to be the recognition signal for the first enzyme (xylosyltransferase, Xyl) involved in the synthesis of GAG chains (Iozzo, 1999).

As can be observed in **Figure 29 & 34** there is a similar pattern that the proteoglycans follow throughout the course of the disease. They all contain an initial spike at 30 d.p.i., all drop off at 80 d.p.i., and are then upregulated again at 140 d.p.i. when clinical signs start to become apparent, and lastly, level off near the end of infection. Considering GAG chains have been found to be associated with PrP^{Sc} binding by acting as cellular like receptors and may be involved in the conversion of PrP^c to PrP^{Sc}, the initial spike at 30 d.p.i. may be representative of the initial accumulation inside the spleen, and then at 80 d.p.i. there may be a downregulation due to the fact that PrP^{Sc} has vacated the spleen and been transported to the brain, and lastly, it could be possible that nearer to the endpoint of infection, prion infectivity could travel back down the nerves into the spleen again.

5 Conclusions

Secondary lymphoid organs have been shown to be critical in the pathogenesis of prion disease (Mabbott *et al.*, 2000) and in particular, FDCs have been shown to be essential for PrP^{Sc} replication in lymphoid tissues (Mabbott *et al.*, 2001). In this study, isolation of a distinct splenic cell population containing FDCs enabled the discovery of differentially expressed genes in the spleen during the course of prion infection which was otherwise not possible when examining the whole spleen tissue. A majority of the genes found differentially expressed were related to cell cycle regulation and cellular survival possibly due to the fact that there are no pathological changes associated with the spleen during infection.

Downregulated genes were found within a series in the porphyrin metabolism pathway suggesting a role for porphyrins in prion pathogenesis. Porphyrins bind metal cations and their downregulation would likely result in an accumulation of metals in the body. The metal cation iron was investigated due to the fact that it has been seen to accumulate in the brain in other neurodegenerative disorders like Alzheimer's and Parkinson's disease and has been found to play a role in protein aggregation and oxidative damage. Interestingly, iron was found accumulated in the spleen via immunohistochemistry during scrapie infection confirming the microarray results.

The top upregulated genes found, the SLRPs, and in particular decorin (highest upregulated gene validated at both the molecular and protein level) seemed to be important in prion pathogenesis. SLRPs contain GAG chains which have been postulated

as infectious prion rod receptors and may be involved in the conversion of PrP^c into PrP^{Sc} by bringing the PrP^{Sc} template in close contact with PrP^c prompting its conversion. In addition, decorin has also been found associated with amyloid-like fibrils in the brain. Thus, my hypothesis has proved true as the upregulation of SLRPs, in particular decorin, found during prion disease in this study have been speculated to aid in the conversion process of PrP^c to PrP^{Sc} , hence differential expression by the murine splenic cells in response to infection seems to be involved in prion replication and accumulation.

6 Future Directions

In this study, a distinct splenic cell population of FDCs, dendritic cells, and macrophages was isolated using an affinity bead technique. FDCs have been shown to be one of the most important cell types for PrP^{Sc} replication inside lymphoid tissues (Mabbott *et al.*, 2001), and thus it would be ideal to isolate this particular cell type only. Future work such as laser capture microdissection could be employed in order to isolate only FDCs from the spleen. Subsequently, isolate the RNA, and perform microarray experiments to look for differential expression that would be more specific to FDCs. This would preferentially enrich for expression that would be more specific to prion accumulation and replication. These genes could then be confirmed at the protein level using western blot and immunohistochemistry where double labeling could be performed to view the genes that colocalize with FDCs. In addition, serum samples infected with a variety of different prion strains (to confirm the consistency) could be tested using an ELISA assay to detect for potential biomarkers of prion disease found by the microarray.

7 Appendices

7.1 Appendix 1

Buffer compositions

1. Pre-Hybridization Solution

For a 250 ml solution:

62.5 ml 20X SSC
2.5 ml 10% SDS
2.5 g BSA
185 ml dH₂O

Filter sterilize and pre-warm to 42°C.

2. Formamide Hybridization Buffer Mix

For one 75 µl hybridization volume per array:

22.5 µl Deionized formamide
18.75 µl 20X SSC
0.75 µl 10% SDS
1.0 µl COT1-DNA
1.0 µl Poly(A)-DNA
10 µl nuclease-free H₂O
11 µl of each labeled aRNA sample

3. Low Stringency Wash Buffer (1X SSC and 0.2% SDS) (For the BMAPs/MSVs)

For a 2L solution:

100 ml 20X SSC
40 ml 10% SDS
1860 ml dH₂O

4. Low Stringency Wash Buffer (2X SSC and 0.5% SDS) (For the mouse oligo arrays)

For a 2L solution:

200 ml 20X SSC
100 ml 10% SDS
1700 ml dH₂O

5. High Stringency Wash Buffer (0.1X SSC and 0.2% SDS) (For the BMAPs/MSVs)

For a 2L solution:

10 ml 20X SSC
40 ml 10% SDS
1950 ml dH₂O

6. High Stringency Wash Buffer (0.5X SSC and 0.2% SDS) (For the mouse oligo arrays)

For a 2L solution:

50 ml 20X SSC
40 ml 10% SDS
1910 ml dH₂O

7.2 Appendix 2

Table 5A: Summary of RNA isolations for VM 22A clinical endpoint studies

Mouse	Total Amount of RNA (ng)	Quality of RNA (Abs 260/280)	Quality of RNA (RIN #)
VM PBS control #2	4600.00	2.08	4.5
VM PBS control #3	2300.00	2.13	6.3
VM 22A infected #8	400.00	1.95	6.5
VM 22A infected #9	1575.00	2.04	7.6
VM 22A infected #10	825.00	2.07	7.8
VM 22A infected #13	750.00	2.12	7.3

Table 5B: Summary of RNA isolations for C57BL/6 RML timecourse studies

Mouse	Total Amount of RNA (ng)	Quality of RNA (Abs 260/280)	Quality of RNA (RIN #)
7 dpi			
C57BL/6 PBS control #1	4462.00	2.07	8.2
C57BL/6 PBS control #2	7933.50	2.08	8.0
C57BL/6 PBS control #3	4988.75	2.03	8.5
C57BL/6 RML infected #1	2248.25	2.03	9.0
C57BL/6 RML infected #2	2289.75	2.00	8.3
C57BL/6 RML infected #3	2100.75	2.25	9.2
30 dpi			

C57BL/6 PBS control #1	4768.75	2.06	9.7
C57BL/6 PBS control #3	1995.00	2.07	9.2
C57BL/6 RML infected #3	282.50	2.16	Too low of concentration
80 dpi			
C57BL/6 PBS control #1	2411.75	2.05	9.0
C57BL/6 PBS control #2	4297.25	2.03	8.9
C57BL/6 PBS control #3	1527.00	2.03	8.1
C57BL/6 RML infected #1	4576.75	2.04	8.8
C57BL/6 RML infected #2	4739.00	2.03	5.3
C57BL/6 RML infected #3	2642.50	2.01	8.4
140 dpi			
C57BL/6 PBS control #1	4286.50	2.04	7.3
C57BL/6 PBS control #2	3999.50	2.01	7.9
C57BL/6 PBS control #3	8904.50	2.00	8.2
C57BL/6 RML infected #1	1878.75	2.00	6.1
C57BL/6 RML infected #2	2319.75	1.98	7.5
C57BL/6 RML infected #3	1965.50	1.99	7.1
149 dpi (clinical)			
C57BL/6 PBS control #1	316.00	1.93	Too low of concentration
C57BL/6 PBS control #2	6023.75	2.05	5.9

C57BL/6 PBS control #3	6407.75	2.04	7.5
C57BL/6 RML infected #1	6460.75	2.06	6.5
C57BL/6 RML infected #2	2031.00	1.95	6.3
C57BL/6 RML infected #3	10709.50	2.03	3.7

7.3 Appendix 3

Table 7: Complete List of Overrepresented and Underrepresented Genes in the Splenic Cell Population when Compared with the Whole Spleen

ID	Genes	Description	Fold Change
AI323320	4-Sep	septin 4	-2.084
AI465345	8-Sep	septin 8	2.038
AI413394	0610005C13RIK	RIKEN cDNA 0610005C13 gene	2.166
AI851333	1110020G09RIK	RIKEN cDNA 1110020G09 gene	2.157
AI844948	2010001A14RIK	RIKEN cDNA 2010001A14 gene	-3.105
AI854627	2310034G01RIK	RIKEN cDNA 2310034G01 gene	2.631
AI850450	2310047K21RIK	RIKEN cDNA 2310047K21 gene	-2.148
AI848448	2610203C20RIK	RIKEN cDNA 2610203C20 gene	-2.658
AI846776	4833420G11RIK	RIKEN cDNA 4833420G11 gene	2.592
AI451474	4833420G11RIK	RIKEN cDNA 4833420G11 gene	2.533
AI846433	4933409K07RIK	RIKEN cDNA 4933409K07 gene	2.162
AI450287	6330509M05RIK	RIKEN cDNA 6330509M05 gene	-3.544
AI845092	6330509M05RIK	RIKEN cDNA 6330509M05 gene	-3.193
AI852607	6330564D18RIK	RIKEN cDNA 6330564D18 gene	2.219
AI848129	6430520M22RIK	RIKEN cDNA 6430520M22 gene	-3.32
AI843657	6430520M22RIK	RIKEN cDNA 6430520M22 gene	-2.075
AI843542	6720469N11RIK	RIKEN cDNA 6720469N11 gene	-3.119
AI447342	9230111E07RIK	RIKEN cDNA 9230111E07 gene	-16.694
AI449141	9530018I07RIK	RIKEN cDNA 9530018I07 gene	-3.881
AI447811	9630023C09RIK	RIKEN cDNA 9630023C09 gene	-2.316
AI845034	A530058N18RIK	RIKEN cDNA A530058N18 gene	-2.405
AI849446	AADACL1	arylacetamide deacetylase-like 1	-3.112
AI450792	AADACL1	arylacetamide deacetylase-like 1	-2.002
AI844088	AARS2	alanyl-tRNA synthetase 2, mitochondrial (putative)	-5.925
AI837050	AATK	apoptosis-associated tyrosine kinase	-4.897
AI853123	ABAT	4-aminobutyrate aminotransferase	2.217

AI845514	ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1	-4.093
AI836521	ABCA2	ATP-binding cassette, sub-family A (ABC1), member 2	-3.134
AI849213	ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1	2.659
AI853507	ABCB10	ATP-binding cassette, sub-family B (MDR/TAP), member 10	2.365
AI323653	ABCG1	ATP-binding cassette, sub-family G (WHITE), member 1	-2.99
AI840283	ABHD14B	abhydrolase domain containing 14B	-2.448
AI850161	ABHD4	abhydrolase domain containing 4	-3.705
AI843572	ABI3BP	ABI gene family, member 3 (NESH) binding protein	-3.601
AI847791	ACHE	acetylcholinesterase (Yt blood group)	2.032
AI427919	ACOT9	acyl-CoA thioesterase 9	-2.602
AI852457	ACP1	acid phosphatase 1, soluble	2.099
AI842933	ACP2	acid phosphatase 2, lysosomal	-3.546
AI838959	ACTA2 (includes EG:59)	actin, alpha 2, smooth muscle, aorta	-2.106
AI836381	ACTG1	actin, gamma 1	-2.237
AI837829	ADAM10	ADAM metallopeptidase domain 10	2.424
AI428523	ADAMTS10	ADAM metallopeptidase with thrombospondin type 1 motif, 10	-2.627
AI847423	ADCY3	adenylate cyclase 3	-2.31
AI852237	ADD3	adducin 3 (gamma)	-3.092
AI848880	ADFP	adipose differentiation-related protein	-2.226
AI843891	ADPGK	ADP-dependent glucokinase	-2.816
AI836109	ADPRHL2	ADP-ribosylhydrolase like 2	2.016
AI413331	AI413331	expressed sequence AI413331	2.875
AI450236	AI450236	expressed sequence AI450236	-3.011
AI842902	AI450236	expressed sequence AI450236	-2.76
AI447871	AI506767	expressed sequence AI506767	-7.096
AI848258	AI848258	expressed sequence AI848258	-2.714
AI848615	AI848615	expressed sequence AI848615	-2.068
AI851523	AI851523	expressed sequence AI851523	2.165
AI838420	AKT1S1	AKT1 substrate 1 (proline-rich)	-2.317
AI850327	AKTIP	AKT interacting protein	-2.013
AI836320	ALAS1	aminolevulinate, delta-, synthase 1	-2.864
AI323970	ALDOA	aldolase A, fructose-bisphosphate	-2.53
AI839550	ALKBH6	alkB, alkylation repair homolog 6 (E. coli)	-3.549
AI429583	ALS2CR2	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 2	2.269
AI450899	AMPD2	adenosine monophosphate deaminase 2 (isoform L)	-5.375
AI325237	AMY2A	amylase, alpha 2A (pancreatic)	-49.995

AI851173	ANKIB1	ankyrin repeat and IBR domain containing 1	-2.412
AI451247	ANKRD43	ankyrin repeat domain 43	2.458
AI837373	ANKRD43	ankyrin repeat domain 43	2.411
AI429324	ANLN	anillin, actin binding protein	2.552
AI843178	ANP32E	acidic (leucine-rich) nuclear phosphoprotein 32 family, member E	2.025
AI838487	ANXA3	annexin A3	-2.37
AI845042	ANXA5	annexin A5	-2.976
AI839301	APBB1	amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65)	-2.496
AI415230	APLN	apelin, AGTRL1 ligand	-3.701
AI893652	APOC1	apolipoprotein C-I	-3.842
AI848248	APOE	apolipoprotein E	-8.9
AI838965	AQP1	aquaporin 1 (Colton blood group)	2.745
AI451504	AQP11	aquaporin 11	-2.735
AI850331	ARF1	ADP-ribosylation factor 1	-2.307
AI851136	ARFGEF1	ADP-ribosylation factor guanine nucleotide-exchange factor 1(brefeldin A-inhibited)	-3.089
AI840874	ARHGAP15	Rho GTPase activating protein 15	-2.254
AI842572	ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta	-10.772
AI853706	ARHGEF3	Rho guanine nucleotide exchange factor (GEF) 3	-5.21
AI840147	ARID4B	AT rich interactive domain 4B (RBP1-like)	-2.884
AI845295	ARID5A	AT rich interactive domain 5A (MRF1-like)	-4.182
AI846227	ARMCX1	armadillo repeat containing, X-linked 1	-2.2
AI448196	ARMCX4	armadillo repeat containing, X-linked 4	-2.424
AI413346	ASAH1	N-acylsphingosine amidohydrolase (acid ceramidase) 1	-4.732
AI849776	ASAH1	N-acylsphingosine amidohydrolase (acid ceramidase)-like	-4.001
AI851378	ASB1	ankyrin repeat and SOCS box-containing 1	2.016
AI464444	ATG4B	ATG4 autophagy related 4 homolog B (S. cerevisiae)	-2.283
AI843486	ATP11B	ATPase, class VI, type 11B	-3.105
AI854213	ATP13A2	ATPase type 13A2	-2.078
AI853492	ATP2A2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	-2.132
AI839214	ATP2B1	ATPase, Ca++ transporting, plasma membrane 1	-10.213
AI841797	ATP6V0A1	ATPase, H+ transporting, lysosomal V0 subunit a1	-2.869
AI849250	ATP6V0D1	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d1	-2.115
AI323712	ATP6V1B2	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B2	-2.969
AI464431	ATP6V1G3	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G3	2.849
AI853890	ATP7A	ATPase, Cu++ transporting, alpha polypeptide (Menkes syndrome)	-5.373
AI853679	ATPIF1	ATPase inhibitory factor 1	2.925

AI662482	AU019833	expressed sequence AU019833	-2.334
AI448377	AU020206	expressed sequence AU020206	-3.03
AI854375	AW047464	expressed sequence AW047464	-3.577
AI426367	AW215868	expressed sequence AW215868	3.396
AI836482	B3GAT3	beta-1,3-glucuronyltransferase 3 (glucuronosyltransferase I)	-4.225
AI450126	B3GNT2	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 2	-2.438
AI848088	B4GALT4	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 4	-2.337
AI853785	B830032F12	hypothetical protein B830032F12	-6.95
AI850293	BACE1	beta-site APP-cleaving enzyme 1	-3.187
AI666530	BACH2	BTB and CNC homology 1, basic leucine zipper transcription factor 2	-4.105
AI451642	BANK1	B-cell scaffold protein with ankyrin repeats 1	-8.083
AI844215	BAT2D1	BAT2 domain containing 1	-4.807
AI447892	BB163080	expressed sequence BB163080	-2.282
AI854682	BC005561	cDNA sequence BC005561	-2.577
AI662437	BC094916	cDNA sequence BC094916	-2.06
AI846419	BCL11B	B-cell CLL/lymphoma 11B (zinc finger protein)	-2.518
AI844675	BCL2	B-cell CLL/lymphoma 2	-9.363
AI851591	BCLP2	chitinase like protein 2	-2.283
AI852358	BICC1	bicaudal C homolog 1 (Drosophila)	-5.193
AI848746	BIN1	bridging integrator 1	-2.863
AI451876	BLK	B lymphoid tyrosine kinase	-3.902
AI852694	BLZF1	basic leucine zipper nuclear factor 1 (JEM-1)	2.639
AI842861	BMPR2	bone morphogenetic protein receptor, type II (serine/threonine kinase)	-3.109
AI847702	BOK	BCL2-related ovarian killer	-2.051
AI426366	BPTF	bromodomain PHD finger transcription factor	-2.917
AI325194	BRCA1	breast cancer 1, early onset	2.708
AI450617	BRD2	bromodomain containing 2	-2.268
AI845655	BRPF3	bromodomain and PHD finger containing, 3	2.314
AI841611	BST2	bone marrow stromal cell antigen 2	2.538
AI848411	BTG1	B-cell translocation gene 1, anti-proliferative	-3.04
AI853230	C10ORF132	chromosome 10 open reading frame 132	2.366
AI851681	C10ORF56	chromosome 10 open reading frame 56	-2.158
AI850090	C10ORF58	chromosome 10 open reading frame 58	-4.987
AI844061	C12ORF34	chromosome 12 open reading frame 34	-3.413
AI451667	C12ORF41	chromosome 12 open reading frame 41	-2.59
AI841498	C12ORF57	chromosome 12 open reading frame 57	-2.716

AI425989	C130050O18RIK	RIKEN cDNA C130050O18 gene	5.487
AI451544	C13ORF7	chromosome 13 open reading frame 7	-2.117
AI845606	C16ORF77	chromosome 16 open reading frame 77	-2.106
AI842148	C17ORF63	chromosome 17 open reading frame 63	-2.463
AI851663	C19ORF12	chromosome 19 open reading frame 12	-2.958
AI842324	C19ORF6	chromosome 19 open reading frame 6	2.226
AI850221	C1ORF102	chromosome 1 open reading frame 102	-2.614
AI837233	C1ORF128	chromosome 1 open reading frame 128	2.772
AI840384	C1ORF164	chromosome 1 open reading frame 164	-4.596
AI849033	C1ORF216	chromosome 1 open reading frame 216	-2.748
AI850837	C1ORF26	chromosome 1 open reading frame 26	2.144
AI447276	C1ORF96	chromosome 1 open reading frame 96	-2.718
AI428772	C20ORF4	chromosome 20 open reading frame 4	-2.205
AI464450	C21ORF57	chromosome 21 open reading frame 57	2.093
AI450119	C22ORF13	chromosome 22 open reading frame 13	2.282
AI852652	C2ORF18	chromosome 2 open reading frame 18	-2.801
AI844960	C2ORF39	chromosome 2 open reading frame 39	-3.149
AI428934	C3ORF34	chromosome 3 open reading frame 34	-2.239
AI448250	C4ORF32	chromosome 4 open reading frame 32	2.564
AI451137	C6ORF113	chromosome 6 open reading frame 113	-2.085
AI414295	C6ORF25	chromosome 6 open reading frame 25	-2.289
AI844713	C6ORF70	chromosome 6 open reading frame 70	-2.679
AI841511	CA2	carbonic anhydrase II	2.759
AI847157	CACHD1	cache domain containing 1	2.21
AI848016	CADM1	cell adhesion molecule 1	-5.163
AI845409	CALD1	caldesmon 1	-5.305
AI841978	CAPNS1	calpain, small subunit 1	-2.05
AI847627	CARHSP1	calcium regulated heat stable protein 1, 24kDa	2.43
AI835012	CARS2	cysteinyl-tRNA synthetase 2, mitochondrial (putative)	2.005
AI839368	CBR1	carbonyl reductase 1	2.13
AI851678	CBX7	chromobox homolog 7	-2.643
AI838606	CCDC24 (includes EG:381546)	coiled-coil domain containing 24	2.029
AI464397	CCDC46	coiled-coil domain containing 46	-2.095
AI838653	CCDC80	coiled-coil domain containing 80	-5.63
AI848406	CCDC88A	coiled-coil domain containing 88A	-2.582
AI450288	CCDC88A	coiled-coil domain containing 88A	-2.411

AI838322	CCL21	chemokine (C-C motif) ligand 21	-6.052
AI323828	CCNB1	cyclin B1	2.218
AI854520	CCND2	cyclin D2	-2.692
AI854364	CCNYL1	cyclin Y-like 1	-2.719
AI326802	CCRL1	chemokine (C-C motif) receptor-like 1	2.129
AI842998	CCT6A	chaperonin containing TCP1, subunit 6A (zeta 1)	-4.599
AI850351	CD109	CD109 molecule	-2.403
AI451898	CD163	CD163 molecule	-2.12
AI451623	CD209	CD209 molecule	2.974
AI847784	CD34	CD34 molecule	-2.428
AI449234	CD48	CD48 molecule	-2.261
AI840607	CD53	CD53 molecule	-4.762
AI449164	CD79B	CD79b molecule, immunoglobulin-associated beta	-4.7
AI840865	CD81	CD81 molecule	-2.392
AI837100	CD83	CD83 molecule	-3.836
AI854515	CD9	CD9 molecule	-2.8
AI844342	CD97	CD97 molecule	-2.129
AI327152	CDC20	cell division cycle 20 homolog (S. cerevisiae)	2.439
AI852072	CDC2L5	cell division cycle 2-like 5 (cholinesterase-related cell division controller)	-2.069
AI323806	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	-2.127
AI451502	CDT1	chromatin licensing and DNA replication factor 1	2.524
AI450766	CENPK	centromere protein K	2.453
AI426416	CENPN (includes EG:72155)	centromere protein N	2.022
AI843596	CENPP	centromere protein P	2.008
AI842563	CENTD1	centaurin, delta 1	-2.621
AI450829	CEPT1	choline/ethanolamine phosphotransferase 1	-2.333
AI848610	CERK (includes EG:64781)	ceramide kinase	-3.221
AI851511	CIAPIN1	cytokine induced apoptosis inhibitor 1	2.131
AI849432	CLCN3	chloride channel 3	2.06
AI845037	CLDN12	claudin 12	-4.359
AI465458	CLEC1B	C-type lectin domain family 1, member B	-3.449
AI450157	CLIC1	chloride intracellular channel 1	-3.671
AI841572	CLK3	CDC-like kinase 3	2.089
AI845270	CLOCK	clock homolog (mouse)	-2.168
AI447413	CLSPN	claspin homolog (Xenopus laevis)	2.021
AI836624	CLU	clusterin	-7.852

AI841689	CMTM3	CKLF-like MARVEL transmembrane domain containing 3	-2.644
AI854839	CNDP2	CNDP dipeptidase 2 (metallopeptidase M20 family)	-2.09
AI846215	CNOT6L	CCR4-NOT transcription complex, subunit 6-like	-3.329
AI851633	CNOT7	CCR4-NOT transcription complex, subunit 7	-2.513
AI844874	COL1A1	collagen, type I, alpha 1	-4.795
AI838652	COL1A2	collagen, type I, alpha 2	-4.596
AI842703	COL3A1	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	-10.392
AI839275	COL4A1	collagen, type IV, alpha 1	-7.238
AI843282	COL4A2	collagen, type IV, alpha 2	-2.163
AI666407	COPS2	COP9 constitutive photomorphogenic homolog subunit 2 (Arabidopsis)	2.078
AI837962	CORO1A	coronin, actin binding protein, 1A	-3.616
AI851168	COX5B	cytochrome c oxidase subunit Vb	-2.315
AI854115	CPA2	carboxypeptidase A2 (pancreatic)	-2.631
AI845839	CPEB4	cytoplasmic polyadenylation element binding protein 4	3.506
AI842820	CPM	carboxypeptidase M	-2.034
AI854421	CPNE6	copine VI (neuronal)	-2.124
AI851449	CRAT	carnitine acetyltransferase	2.02
AI840752	CREB3	cAMP responsive element binding protein 3	-2.424
AI426567	CRKRS	Cdc2-related kinase, arginine/serine-rich	-2.243
AI843500	CRMP1	collapsin response mediator protein 1	-2.022
AI528760	CSDA	cold shock domain protein A	2.469
AI323836	CSF1	colony stimulating factor 1 (macrophage)	-5.633
AI848964	CSF2RB	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)	-3.042
AI836532	CSRP1	cysteine and glycine-rich protein 1	-2.271
AI845353	CTA-126B4.3	CGI-96 protein	-2.738
AI851778	CTGF	connective tissue growth factor	-9.977
AI845860	CTLA2A	cytotoxic T lymphocyte-associated protein 2 alpha	-3.198
AI414010	CTSB	cathepsin B	-2.53
AI450135	CTSC	cathepsin C	-4.033
AI840178	CTSH	cathepsin H	-3.001
AI844398	CTSL2	cathepsin L2	-2.163
AI845967	CTSS	cathepsin S	-2.576
AI854281	CUL2	cullin 2	2.227
AI853265	CUL4A	cullin 4A	2.639
AI845691	CX3CR1	chemokine (C-X3-C motif) receptor 1	-3.4
AI528734	CXCL12	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	-7.962

AI843749	CXXC5	CXXC finger 5	-2.537
AI850017	CYB5B	cytochrome b5 type B (outer mitochondrial membrane)	-3.663
AI447283	CYBB	cytochrome b-245, beta polypeptide (chronic granulomatous disease)	-2.5
AI852010	D11BWG0517E	DNA segment, Chr 11, Brigham & Women's Genetics 0517 expressed	-2.616
AI451219	D16BWG1543E	DNA segment, Chr 16, Brigham & Women's Genetics 1543 expressed	-2.027
AI848328	DCK	deoxycytidine kinase	2.139
AI854218	DCLK1	doublecortin-like kinase 1	-2.252
AI846778	DCN	decorin	-11.479
AI851142	DDI2	DDI1, DNA-damage inducible 1, homolog 2 (<i>S. cerevisiae</i>)	3.6
AI452243	DDX46	DEAD (Asp-Glu-Ala-Asp) box polypeptide 46	-6.054
AI844007	DDX54	DEAD (Asp-Glu-Ala-Asp) box polypeptide 54	-5.364
AI451187	DENND3	DENN/MADD domain containing 3	-3.058
AI851943	DENND4A	DENN/MADD domain containing 4A	2.265
AI850806	DGKA	diacylglycerol kinase, alpha 80kDa	-3.555
AI845580	DHRS1	dehydrogenase/reductase (SDR family) member 1	-2.34
AI847502	DLD	dihydrolipoamide dehydrogenase	-2.001
AI851519	DMXL2	Dmx-like 2	-2.837
AI324230	DNAJB2	DnaJ (Hsp40) homolog, subfamily B, member 2	2.245
AI844307	DNAJC1	DnaJ (Hsp40) homolog, subfamily C, member 1	-2.553
AI842007	DNTTIP1	deoxynucleotidyltransferase, terminal, interacting protein 1	2.151
AI426672	DNTTIP2	deoxynucleotidyltransferase, terminal, interacting protein 2	-3.741
AI843296	DOCK2	dedicator of cytokinesis 2	-2.66
AI451373	DOCK9	dedicator of cytokinesis 9	-2.233
AI450713	DOK3	docking protein 3	-3.042
AI852882	DOPEY2	dopey family member 2	2.191
AI853171	DPYD	dihydropyrimidine dehydrogenase	-2.904
AI465497	DPYSL2	dihydropyrimidinase-like 2	-2.076
AI325494	DSTN	destrin (actin depolymerizing factor)	-4.484
AI847873	DTX1	deltex homolog 1 (<i>Drosophila</i>)	-2.202
AI449438	DTX4	deltex 4 homolog (<i>Drosophila</i>)	-2.337
AI845584	DUSP6	dual specificity phosphatase 6	-2.149
AI838217	DYM	dymeclin	2.019
AI848183	DYNLT3	dynein, light chain, Tctex-type 3	-4.16
AI447263	DYX1C1	dyslexia susceptibility 1 candidate 1	-3.953
AI837948	E130012A19RIK	RIKEN cDNA E130012A19 gene	-2.778
AI425975	E130309D14RIK	RIKEN cDNA E130309D14 gene	2.576

AI452102	E230029C05RIK	RIKEN cDNA E230029C05 gene	-2.829
AI323636	EAR2	eosinophil-associated, ribonuclease A family, member 2	-2.378
AI845842	EDC4	enhancer of mRNA decapping 4	-2.087
AI323610	EDG2	endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2	-2.09
AI852050	EDNRA	endothelin receptor type A	-3.054
AI848228	EFR3A	EFR3 homolog A (S. cerevisiae)	-2.565
AI327292	EG666451	predicted gene, EG666451	-2.135
AI427644	EGFR	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)	-2.529
AI854636	EGR1	early growth response 1	2.714
AI843660	ENC1	ectodermal-neural cortex (with BTB-like domain)	-3.253
AI854297	ENDOD1	endonuclease domain containing 1	-2.102
AI839722	ENO1	enolase 1, (alpha)	-2.976
AI839129	ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	-2.324
AI662244	ENSMUSG00000065522	predicted gene, ENSMUSG00000065522	-9.673
AI452330	ENSMUSG00000073019	predicted gene, ENSMUSG00000073019	-2.735
AI852290	ENSMUSG00000074335	predicted gene, ENSMUSG00000074335	-2.534
AI325486	EPB49	erythrocyte membrane protein band 4.9 (dematin)	2.374
AI847976	ERH	enhancer of rudimentary homolog (Drosophila)	-7.443
AI851365	ERMP1	endoplasmic reticulum metalloproteinase 1	-4.831
AI841880	ESAM	endothelial cell adhesion molecule	-2.871
AI851815	ETHE1	ethylmalonic encephalopathy 1	-2.4
AI852037	ETS2	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	-3.064
AI323840	EZH2	enhancer of zeste homolog 2 (Drosophila)	2.418
AI413964	F2RL3	coagulation factor II (thrombin) receptor-like 3	-2.31
AI840354	FADS1	fatty acid desaturase 1	-5.358
AI845618	FAM100B	family with sequence similarity 100, member B	-3.889
AI413094	FAM101B	family with sequence similarity 101, member B	-4.845
AI853578	FAM108B1	family with sequence similarity 108, member B1	-2.69
AI850234	FAM113A	family with sequence similarity 113, member A	-2.251
AI847810	FAM13C1	family with sequence similarity 13, member C1	-2.169
AI839888	FAM149A	family with sequence similarity 149, member A	-2.626
AI842153	FAM19A5	family with sequence similarity 19 (chemokine (C-C motif)-like), member A5	-7.941
AI450214	FAM26F	family with sequence similarity 26, member F	-2.824
AI852013	FAM38A	family with sequence similarity 38, member A	-2.154
AI841826	FAM3C	family with sequence similarity 3, member C	-2.476
AI447893	FAM46A	family with sequence similarity 46, member A	-2.107

AI854204	FAM76A	family with sequence similarity 76, member A	2.162
AI853278	FAM98B	family with sequence similarity 98, member B	2.211
AI851495	FBLN5	fibulin 5	-3.179
AI846617	FBXL5	F-box and leucine-rich repeat protein 5	-2.224
AI848876	FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	-4.458
AI839252	FCGRT	Fc fragment of IgG, receptor, transporter, alpha	-2.116
AI449202	FCRL1	Fc receptor-like 1	-2.291
AI662509	FCRLA	Fc receptor-like A	-4.226
AI426259	FCRLA	Fc receptor-like A	-2.45
AI846476	FERMT2	fermitin family homolog 2 (Drosophila)	-4.097
AI849859	FHL1	four and a half LIM domains 1	-3.347
AI464359	FILIP1L	filamin A interacting protein 1-like	-2.399
AI840931	FKBP1A	FK506 binding protein 1A, 12kDa	-2.711
AI846411	FLJ20699	hypothetical protein FLJ20699	-7.606
AI838747	FLNA (includes EG:2316)	filamin A, alpha (actin binding protein 280)	-3.068
AI854778	FMNL2	formin-like 2	-5.105
AI852370	FN3K	fructosamine 3 kinase	2.345
AI844747	FOXC1	forkhead box C1	-2.02
AI851964	FOXP1	forkhead box P1	-3.329
AI854804	FRG1	FSHD region gene 1	2.417
AI853088	FSTL1	follicle-stimulating hormone-like 1	-9.254
AI844976	FURIN	furin (paired basic amino acid cleaving enzyme)	-4.169
AI846924	FXN	frataxin	2.104
AI841690	FXVD5	FXVD domain containing ion transport regulator 5	-4.092
AI839015	FYB	FYN binding protein (FYB-120/130)	-2.995
AI842958	FYCO1	FYVE and coiled-coil domain containing 1	-3.559
AI849675	FYN	FYN oncogene related to SRC, FGR, YES	-5.439
AI449279	FZD1	frizzled homolog 1 (Drosophila)	-2.512
AI840074	G3BP2	GTPase activating protein (SH3 domain) binding protein 2	-3.063
AI841884	GALNT2	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2 (GalNAc-T2)	2.117
AI848816	GAS1	growth arrest-specific 1	-4.14
AI844626	GATM	glycine amidinotransferase (L-arginine:glycine amidinotransferase)	-3.297
AI326106	GBP2	guanylate binding protein 2, interferon-inducible	-2.114
AI840028	GDAP2	ganglioside induced differentiation associated protein 2	-2.056
AI853332	GDF10	growth differentiation factor 10	-2.101
AI850995	GFOD1	glucose-fructose oxidoreductase domain containing 1	-3.569

AI854706	GFOD1	glucose-fructose oxidoreductase domain containing 1	-2.1
AI324119	GFPT1	glutamine-fructose-6-phosphate transaminase 1	2.285
AI846599	GFRA2	GDNF family receptor alpha 2	-2.044
AI846732	GGH (includes EG:14590)	gamma-glutamyl hydrolase	-2.412
AI448110	GIMAP3	GTPase, IMAP family member 3	-8.059
AI662191	GIMAP5	GTPase, IMAP family member 5	-3.756
AI451119	GIMAP7	GTPase, IMAP family member 7	-5.33
AI854638	GINS2	GINS complex subunit 2 (Psf2 homolog)	2.618
AI852313	GJA1	gap junction protein, alpha 1, 43kDa	-3.731
AI844585	GLB1	galactosidase, beta 1	-2.093
AI450005	GLT8D1	glycosyltransferase 8 domain containing 1	2.437
AI839176	GLUL	glutamate-ammonia ligase (glutamine synthetase)	-2.635
AI427643	GMFG	glia maturation factor, gamma	-2.59
AI837611	GMPR	guanosine monophosphate reductase	-2.45
AI835098	GNA11	guanine nucleotide binding protein (G protein), alpha 11 (Gq class)	-2.644
AI853415	GNB4	guanine nucleotide binding protein (G protein), beta polypeptide 4	-8.561
AI413962	GNG11	guanine nucleotide binding protein (G protein), gamma 11	-2.186
AI842738	GNG12	guanine nucleotide binding protein (G protein), gamma 12	-2.414
AI844249	GNG2	guanine nucleotide binding protein (G protein), gamma 2	-3.214
AI835435	GPI	glucose phosphate isomerase	-2.251
AI447234	GPR174	G protein-coupled receptor 174	-2.092
AI852526	GPR88	G protein-coupled receptor 88	-2.516
AI848722	GPRIN1	G protein regulated inducer of neurite outgrowth 1	3.224
AI846605	GRN	granulin	-2.971
AI449548	GSDMDC1	gasdermin domain containing 1	-2.154
AI849200	GSTA4	glutathione S-transferase A4	2.519
AI842165	GSTM3 (includes EG:2947)	glutathione S-transferase M3 (brain)	2.492
AI450198	GTPBP2	GTP binding protein 2	-2.343
AI451062	GUCY1A3	guanylate cyclase 1, soluble, alpha 3	-2.869
AI852538	GYG1	glycogenin 1	-2.274
AI844648	H2AFX	H2A histone family, member X	2.812
AI844100	HAGH	hydroxyacylglutathione hydrolase	2.187
AI661153	HARS	histidyl-tRNA synthetase	3.977
AI385765	HBG1	hemoglobin, gamma A	2.163
AI851184	HCFC2	host cell factor C2	-3.552
AI849346	HCN4	hyperpolarization activated cyclic nucleotide-gated potassium channel 4	2.219

AI847994	HDGFRP3	hepatoma-derived growth factor, related protein 3	-2.505
AI842949	HEXA	hexosaminidase A (alpha polypeptide)	-4.244
AI854206	HEXB	hexosaminidase B (beta polypeptide)	-3.323
AI850020	HFE	hemochromatosis	-3.981
AI845695	HIVP2	human immunodeficiency virus type I enhancer binding protein 2	-2.117
AI326012	HLA-DOB	major histocompatibility complex, class II, DO beta	-3.134
AI853649	HMBS	hydroxymethylbilane synthase	2.365
AI853499	HMGB3	high-mobility group box 3	4.433
AI838304	HMGN1	high-mobility group nucleosome binding domain 1	-2.792
AI848175	HMGN3	high mobility group nucleosomal binding domain 3	-2.363
AI430932	HMHA1	histocompatibility (minor) HA-1	-2.523
AI842171	HMOX1	heme oxygenase (decycling) 1	2.191
AI840973	HNMT	histamine N-methyltransferase	-2.043
AI465155	HNRPUL2	heterogeneous nuclear ribonucleoprotein U-like 2	2.126
AI840878	HOPX	HOP homeobox	-4.403
AI839103	HPGD	hydroxyprostaglandin dehydrogenase 15-(NAD)	-18.487
AI853214	HRH1	histamine receptor H1	-3.246
AI447937	HS2ST1	heparan sulfate 2-O-sulfotransferase 1	2.267
AI846654	HS6ST2	heparan sulfate 6-O-sulfotransferase 2	-5.633
AI847129	HSD11B1	hydroxysteroid (11-beta) dehydrogenase 1	-4.318
AI838302	HSD17B4	hydroxysteroid (17-beta) dehydrogenase 4	-2.747
AI841344	HSP90AA1	heat shock protein 90kDa alpha (cytosolic), class A member 1	-2.513
AI852541	HSP90B1	heat shock protein 90kDa beta (Grp94), member 1	-2.671
AI841289	HSPA1B	heat shock 70kDa protein 1B	2.461
AI847573	HTRA2	HtrA serine peptidase 2	2.001
AI850948	IBTK	inhibitor of Bruton agammaglobulinemia tyrosine kinase	2.038
AI843407	ICA1	islet cell autoantigen 1, 69kDa	-2.406
AI843699	ICA1	islet cell autoantigen 1, 69kDa	-2.173
AI428577	ICAM4	intercellular adhesion molecule 4 (Landsteiner-Wiener blood group)	2.561
AI836808	ID2	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	-3.508
AI839283	ID3	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	-4.488
AI848083	ID4	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	-3.825
AI323287	ID4	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	2.056
AI837750	IDH1	isocitrate dehydrogenase 1 (NADP+), soluble	-3.542
AI853903	IER5	immediate early response 5	2.047
AI854552	IFIT3	interferon-induced protein with tetratricopeptide repeats 3	-2.605

AI464509	IFITM1	interferon induced transmembrane protein 1 (9-27)	-2.56
AI842277	IGFBP3	insulin-like growth factor binding protein 3	-2.614
AI848717	IGFBP7	insulin-like growth factor binding protein 7	-4.668
AI838580	IIGP2	interferon inducible GTPase 2	2.259
AI451483	IKZF5	IKAROS family zinc finger 5 (Pegasus)	2.136
AI851902	IL16	interleukin 16 (lymphocyte chemoattractant factor)	-2.455
AI850443	ILVBL	ilvB (bacterial acetolactate synthase)-like	-4.657
AI413228	INSIG2	insulin induced gene 2	-2.147
AI847975	INTS1	integrator complex subunit 1	-2.433
AI450172	IQWD1	IQ motif and WD repeats 1	2.702
AI847031	ITGA6	integrin, alpha 6	-3.543
AI447669	ITGA8 (includes EG:8516)	integrin, alpha 8	-2.411
AI323739	ITGAE	integrin, alpha E (antigen CD103, human mucosal lymphocyte antigen 1; alpha polypeptide)	-2.025
AI528527	ITGB2	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)	-2.329
AI528630	ITK	IL2-inducible T-cell kinase	-2.259
AI839869	ITM2C	integral membrane protein 2C	-3.043
AI427528	ITSN1	intersectin 1 (SH3 domain protein)	2.333
AI850940	ITSN2	intersectin 2	-2.134
AI848982	JAG1	jagged 1 (Alagille syndrome)	-2.01
AI850297	JAM3	junctional adhesion molecule 3	-2.716
AI449362	JMJD1C	jumonji domain containing 1C	-12.728
AI846165	JOSD2	Josephin domain containing 2	2.043
AI448900	KCNJ8	potassium inwardly-rectifying channel, subfamily J, member 8	-2.523
AI851891	KCTD12	potassium channel tetramerisation domain containing 12	-3.278
AI429228	KIAA0776	KIAA0776	-2.002
AI853026	KIAA1109	KIAA1109	-2.843
AI851362	KIAA1211	KIAA1211 protein	2.595
AI849666	KIAA1370	KIAA1370	2.4
AI835009	KIAA1383	KIAA1383	-2.375
AI450547	KIF14	kinesin family member 14	2.435
AI852487	KIF1B	kinesin family member 1B	-2.132
AI327056	KIF22	kinesin family member 22	2.047
AI323435	KIF4A	kinesin family member 4A	3.217
AI451567	KLF2	Kruppel-like factor 2 (lung)	-3.082
AI448727	KLF6	Kruppel-like factor 6	-3.255
AI413227	KLF7	Kruppel-like factor 7 (ubiquitous)	-2.998

AI851500	KLHL24	kelch-like 24 (Drosophila)	-2.566
AI839140	KTELC1	KTEL (Lys-Tyr-Glu-Leu) containing 1	-2.631
AI850263	LAMP2	lysosomal-associated membrane protein 2	-2.403
AI449243	LANCL2	LanC lantibiotic synthetase component C-like 2 (bacterial)	3.373
AI846864	LAPTM4A	lysosomal-associated protein transmembrane 4 alpha	-2.362
AI426288	LCE1F (includes EG:67828)	late cornified envelope 1F	-2.939
AI573454	LCK	lymphocyte-specific protein tyrosine kinase	-4.942
AI853861	LDB2	LIM domain binding 2	-2.027
AI893459	LEFTY1	left-right determination factor 1	-4.137
AI465143	LGALS1	lectin, galactoside-binding, soluble, 1 (galectin 1)	-2.763
AI844613	LHFP	lipoma HMGIC fusion partner	-5.995
AI837076	LHFPL4	lipoma HMGIC fusion partner-like 4	2.584
AI851348	LIMCH1	LIM and calponin homology domains 1	-7.534
AI852632	LITAF	lipopolysaccharide-induced TNF factor	-2.681
AI853173	LOC100039220	similar to RNA polymerase 1-3	2.079
AI848093	LOC124512	hypothetical protein LOC124512	-2.481
AI840497	LOC285636	hypothetical protein LOC285636	-2.355
AI836224	LOC389541	similar to CG14977-PA	-2.455
AI851514	LOC51149	hypothetical LOC51149	-2.643
AI451723	LONRF3	LON peptidase N-terminal domain and ring finger 3	-3.984
AI845269	LPCAT2	lysophosphatidylcholine acyltransferase 2	-3.074
AI325918	LPCAT2	lysophosphatidylcholine acyltransferase 2	-2.106
AI852132	LPIN1	lipin 1	-5.58
AI326787	LPL	lipoprotein lipase	-4.91
AI848829	LRP12	low density lipoprotein-related protein 12	-2.069
AI428119	LRRFIP1	leucine rich repeat (in FLII) interacting protein 1	-2.652
AI385625	LRRN1	leucine rich repeat neuronal 1	-3.182
AI843624	LTA4H	leukotriene A4 hydrolase	-2.391
AI854869	LUZP1	leucine zipper protein 1	2.407
AI853670	LUZP1	leucine zipper protein 1	2.185
AI844280	LY6C1	lymphocyte antigen 6 complex, locus C1	-3.534
AI841111	LY6E (includes EG:4061)	lymphocyte antigen 6 complex, locus E	-2.171
AI843659	LY86	lymphocyte antigen 86	-3.542
AI834910	LYSMD4	LysM, putative peptidoglycan-binding, domain containing 4	-2.089
AI843799	MACF1	microtubule-actin crosslinking factor 1	-2.552
AI844115	MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)	-2.526

AI413961	MAFB	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)	-2.467
AI848445	MAGED1	melanoma antigen family D, 1	-2.07
AI854878	MAP4	microtubule-associated protein 4	-2.401
AI852857	MAP4K3	mitogen-activated protein kinase kinase kinase kinase 3	-2.209
AI845248	MASP1	mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)	-5.129
AI448995	MCART6	mitochondrial carrier triple repeat 6	2.53
AI837599	MCFD2	multiple coagulation factor deficiency 2	-2.666
AI325074	MCM4	minichromosome maintenance complex component 4	2.786
AI854485	MDC1	mediator of DNA damage checkpoint 1	2.014
AI839535	MDGA2	MAM domain containing glycosylphosphatidylinositol anchor 2	-2.653
AI415666	MDK	midkine (neurite growth-promoting factor 2)	3.231
AI448103	MDM2 (includes EG:4193)	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)	-2.001
AI841479	MECR	mitochondrial trans-2-enoyl-CoA reductase	-4.395
AI854259	MED13L	mediator complex subunit 13-like	-2.186
AI839384	MED14	mediator complex subunit 14	-10.302
AI853220	MED17	mediator complex subunit 17	2.27
AI849762	MEF2A	myocyte enhancer factor 2A	-2.19
AI528587	MERTK	c-mer proto-oncogene tyrosine kinase	-4.245
AI848908	METAP2	methionyl aminopeptidase 2	2.036
AI838645	MFAP5	microfibrillar associated protein 5	-2.191
AI326835	MFGE8	milk fat globule-EGF factor 8 protein	-4.247
AI846600	MGLL	monoglyceride lipase	2.117
AI854373	MGRN1	mahogunin, ring finger 1	-3.787
AI451243	MGST1	microsomal glutathione S-transferase 1	-7.154
AI843448	MGST3	microsomal glutathione S-transferase 3	2.721
AI854577	MIB1	mindbomb homolog 1 (Drosophila)	2.07
AI852140	MICAL-L1	MICAL-like 1	-2.027
AI847663	MKRN1	makorin, ring finger protein, 1	2.535
AI465514	MLL5	myeloid/lymphoid or mixed-lineage leukemia 5 (trithorax homolog, Drosophila)	-2.34
AI447812	MOCS2	molybdenum cofactor synthesis 2	-2.193
AI841901	MOCS2	molybdenum cofactor synthesis 2	-2.067
AI429226	MOV10L1	Mov10L1, Moloney leukemia virus 10-like 1, homolog (mouse)	-2.086
AI528706	MPEG1	macrophage expressed gene 1	-5.662
AI844833	MPHOSPH10	M-phase phosphoprotein 10 (U3 small nucleolar ribonucleoprotein)	-5.216
AI853199	MRPL40	mitochondrial ribosomal protein L40	-5.586
AI854257	MRPS24	mitochondrial ribosomal protein S24	2.542

AI662494	MS4A4B	membrane-spanning 4-domains, subfamily A, member 4B	-16.699
AI449185	MS4A4C	membrane-spanning 4-domains, subfamily A, member 4C	-8.65
AI835093	MS4A6A	membrane-spanning 4-domains, subfamily A, member 6A	-2.496
AI851996	MS4A6C	membrane-spanning 4-domains, subfamily A, member 6C	-2.753
AI447446	MS4A6D	membrane-spanning 4-domains, subfamily A, member 6D	-4.976
AI851462	MSRA	methionine sulfoxide reductase A	-2.599
AI849635	MTDH	metadherin	-2.322
AI836552	MTF2	metal response element binding transcription factor 2	-3.113
AI327236	MTMR7	myotubularin related protein 7	-5.083
AI853833	MTX3 (includes EG:382793)	metaxin 3	-2.003
AI842069	MXI1	MAX interactor 1	2.281
AI850319	MYCBP2	MYC binding protein 2	-2.048
AI838002	MYH11	myosin, heavy chain 11, smooth muscle	-2.481
AI839296	MYL9 (includes EG:10398)	myosin, light chain 9, regulatory	-3.12
AI842649	MYL9 (includes EG:98932)	myosin, light polypeptide 9, regulatory	-2.262
AI842846	MYO5A	myosin VA (heavy chain 12, myoxin)	-3.859
AI447706	MYO9A	myosin IXA	-2.667
AI854284	MYT1	myelin transcription factor 1	2.288
AI429738	NACAD	NAC alpha domain containing	7.226
AI848449	NAG	neuroblastoma-amplified protein	-2.605
AI844239	NAGK	N-acetylglucosamine kinase	-2.247
AI845387	NAPB	N-ethylmaleimide-sensitive factor attachment protein, beta	-2.102
AI427126	NCAPG2	non-SMC condensin II complex, subunit G2	2.367
AI843284	NCOR1	nuclear receptor co-repressor 1	-2.689
AI837293	NDFIP1	Nedd4 family interacting protein 1	-2.551
AI852317	NDRG1	N-myc downstream regulated gene 1	-3.199
AI851103	NDUFB4	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 4, 15kDa	-2.608
AI853434	NECAB3	N-terminal EF-hand calcium binding protein 3	2.962
AI661037	NEIL3	nei endonuclease VIII-like 3 (E. coli)	3.452
AI848918	NEU1	sialidase 1 (lysosomal sialidase)	-4.781
AI662131	NFAT5	nuclear factor of activated T-cells 5, tonicity-responsive	-2.52
AI845242	NFATC1	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	-2.212
AI845477	NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	3.679
AI845865	NID2	nidogen 2 (osteonidogen)	-2.019
AI845807	NIN	ninein (GSK3B interacting protein)	-2.795
AI844644	NINJ1	ninjurin 1	-3.218

AI844559	NMI	N-myc (and STAT) interactor	-3.015
AI847331	NPC1	Niemann-Pick disease, type C1	-2.066
AI854135	NRSN1	neurensin 1	3.052
AI850456	NSDHL	NAD(P) dependent steroid dehydrogenase-like	-2.165
AI838320	NSMCE1	non-SMC element 1 homolog (S. cerevisiae)	-2.209
AI848680	NT5DC2	5'-nucleotidase domain containing 2	-2.071
AI849795	NUCB2	nucleobindin 2	-3.723
AI854103	NUDT4	nudix (nucleoside diphosphate linked moiety X)-type motif 4	2.194
AI852641	NUPR1	nuclear protein 1	-2.037
AI848253	NXN	nucleoredoxin	-2.312
AI528785	ODF1	outer dense fiber of sperm tails 1	2.008
AI844033	OLIG2	oligodendrocyte lineage transcription factor 2	-2.16
AI852078	OPA3	optic atrophy 3 (autosomal recessive, with chorea and spastic paraplegia)	2.017
AI447692	ORC2L	origin recognition complex, subunit 2-like (yeast)	2.443
AI840215	OSGEP	O-sialoglycoprotein endopeptidase	-7.332
AI846866	OSTF1	osteoclast stimulating factor 1	-2.954
AI839228	P2RX1	purinergic receptor P2X, ligand-gated ion channel, 1	-2.258
AI448454	P2RY14	purinergic receptor P2Y, G-protein coupled, 14	-3.017
AI846283	PACSIN2	protein kinase C and casein kinase substrate in neurons 2	-2.009
AI666339	PADI4	peptidyl arginine deiminase, type IV	-2.056
AI323455	PAM	peptidylglycine alpha-amidating monooxygenase	2.13
AI847747	PANX1	pannexin 1	-2.184
AI451081	PARP3	poly (ADP-ribose) polymerase family, member 3	-2.724
AI848790	PBX1	pre-B-cell leukemia homeobox 1	-2.029
AI843903	PCDH17	protocadherin 17	-2.804
AI847306	PCDH17	protocadherin 17	-2.602
AI851189	PCGF2	polycomb group ring finger 2	2.149
AI849831	PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	2.356
AI452258	PDE4B	phosphodiesterase 4B, cAMP-specific (phosphodiesterase E4 dunce homolog, Drosophila)	-2.408
AI385687	PDGFA	platelet-derived growth factor alpha polypeptide	-2.011
AI840738	PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	-2.617
AI845602	PDGFRB	platelet-derived growth factor receptor, beta polypeptide	-2.084
AI848783	PDK2	pyruvate dehydrogenase kinase, isozyme 2	2.258
AI845446	PDLIM7	PDZ and LIM domain 7 (enigma)	-2.263
AI449816	PDZK1IP1	PDZK1 interacting protein 1	2.139
AI836252	PENK	proenkephalin	-3.39

AI853212	PEX26	peroxisome biogenesis factor 26	-4.492
AI846339	PFN1	profilin 1	-2.014
AI845608	PFN2	profilin 2	-2.902
AI852019	PGCP	plasma glutamate carboxypeptidase	-3.548
AI451286	PGGT1B	protein geranylgeranyltransferase type I, beta subunit	-2.078
AI844383	PGRMC1	progesterone receptor membrane component 1	-4.474
AI850079	PIB5PA	phosphatidylinositol (4,5) bisphosphate 5-phosphatase, A	-2.762
AI838939	PICALM	phosphatidylinositol binding clathrin assembly protein	2.536
AI847987	PIGA	phosphatidylinositol glycan anchor biosynthesis, class A (paroxysmal nocturnal hemoglobinuria)	2.202
AI450664	PIP4K2A	phosphatidylinositol-5-phosphate 4-kinase, type II, alpha	-2.116
AI844522	PIP5K1B	phosphatidylinositol-4-phosphate 5-kinase, type I, beta	3.067
AI835305	PIP5K1C	phosphatidylinositol-4-phosphate 5-kinase, type I, gamma	-2.091
AI836137	PKM2	pyruvate kinase, muscle	-3.243
AI845798	PLA2G12A	phospholipase A2, group XIIA	2.206
AI842940	PLCG2	phospholipase C, gamma 2 (phosphatidylinositol-specific)	-2.301
AI844893	PLEC1	plectin 1, intermediate filament binding protein 500kDa	-3.72
AI854890	PLOD1	procollagen-lysine 1, 2-oxoglutarate 5-dioxygenase 1	-2.201
AI448193	PLS3	plastin 3 (T isoform)	-3.393
AI852430	PMP22	peripheral myelin protein 22	-2.337
AI326372	PNLIP	pancreatic lipase	-19.674
AI850222	PNN	pinin, desmosome associated protein	-4.771
AI837771	PNO1	partner of NOB1 homolog (S. cerevisiae)	-2.866
AI851158	POLB	polymerase (DNA directed), beta	3.268
AI844222	POLE3	polymerase (DNA directed), epsilon 3 (p17 subunit)	2.12
AI844414	POLG	polymerase (DNA directed), gamma	2.04
AI850411	POLR3F	polymerase (RNA) III (DNA directed) polypeptide F, 39 kDa	-4.902
AI847054	PPAP2B	phosphatidic acid phosphatase type 2B	-5.906
AI854500	PPBP	pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)	-4.065
AI847551	PPM1G	protein phosphatase 1G (formerly 2C), magnesium-dependent, gamma isoform	2.16
AI847940	PPP1R14B	protein phosphatase 1, regulatory (inhibitor) subunit 14B	-2.041
AI528708	PPP1R15A (includes EG:23645)	protein phosphatase 1, regulatory (inhibitor) subunit 15A	2.752
AI850402	PPP1R16B	protein phosphatase 1, regulatory (inhibitor) subunit 16B	-2.404
AI844687	PPP3CB	protein phosphatase 3 (formerly 2B), catalytic subunit, beta isoform	-3.245
AI323727	PPP3CC	protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform	-3.496
AI449368	PPT1	palmitoyl-protein thioesterase 1 (ceroid-lipofuscinosis, neuronal 1, infantile)	-3.864
AI851427	PRKAR2B	protein kinase, cAMP-dependent, regulatory, type II, beta	2.099

AI846727	PRKCB1	protein kinase C, beta 1	-2.163
AI836106	PRKD3	protein kinase D3	-2.194
AI451496	PRKRIP1	PRKR interacting protein 1 (IL11 inducible)	-2.133
AI450264	PRND	prion protein 2 (dublet)	-2.413
AI836611	PRNP	prion protein (p27-30) (Creutzfeldt-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia)	3.179
AI845688	PRO1073	PRO1073 protein	3.112
AI853644	PRO1073	PRO1073 protein	2.695
AI840668	PRPF18	PRP18 pre-mRNA processing factor 18 homolog (S. cerevisiae)	2.151
AI845192	PRPH	peripherin	-3.428
AI851674	PRPSAP2	phosphoribosyl pyrophosphate synthetase-associated protein 2	-2.228
AI851714	PRRX1	paired related homeobox 1	-2.516
AI452270	PRSS23	protease, serine, 23	-3.047
AI838336	PSAP	prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy)	-2.561
AI846077	PSCD3	pleckstrin homology, Sec7 and coiled-coil domains 3	2.612
AI845984	PSCD4	pleckstrin homology, Sec7 and coiled-coil domains 4	-2.137
AI836233	PSMB10	proteasome (prosome, macropain) subunit, beta type, 10	-2.339
AI385718	PSMB8	proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional peptidase 7)	-3.625
AI323618	PSMB9	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)	-2.648
AI847977	PSMD10	proteasome (prosome, macropain) 26S subunit, non-ATPase, 10	-4.935
AI840922	PTDSS2	phosphatidylserine synthase 2	2.698
AI425937	PTOV1	prostate tumor overexpressed gene 1	3.603
AI845299	PTPN1	protein tyrosine phosphatase, non-receptor type 1	-2.768
AI323869	PTPRE	protein tyrosine phosphatase, receptor type, E	-2.817
AI850339	PTPRZ1	protein tyrosine phosphatase, receptor-type, Z polypeptide 1	-2.242
AI845915	PTRF	polymerase I and transcript release factor	-2.419
AI837793	PUF60	poly-U binding splicing factor 60KDa	-3.002
AI853843	PUSL1	pseudouridylate synthase-like 1	-3.048
AI853174	PVRL1	poliovirus receptor-related 1 (herpesvirus entry mediator C)	-2.773
AI853209	QKI (includes EG:9444)	quaking homolog, KH domain RNA binding (mouse)	-5.059
AI848325	RAB12	RAB12, member RAS oncogene family	-2.093
AI850349	RAB1A	RAB1A, member RAS oncogene family	-8.086
AI846385	RAB23	RAB23, member RAS oncogene family	-2.166
AI845375	RAB3A	RAB3A, member RAS oncogene family	-2.636
AI429661	RAB3IL1	RAB3A interacting protein (rabin3)-like 1	2.051
AI844664	RAB4B	RAB4B, member RAS oncogene family	-2.267
AI852389	RAB7L1	RAB7, member RAS oncogene family-like 1	-2.299

AI325991	RAB9A	RAB9A, member RAS oncogene family	-2.734
AI323670	RABAC1	Rab acceptor 1 (prenylated)	-2.126
AI843623	RAD21	RAD21 homolog (S. pombe)	3.131
AI845531	RAF1	v-raf-1 murine leukemia viral oncogene homolog 1	-2.083
AI837488	RALA	v-ral simian leukemia viral oncogene homolog A (ras related)	-2.347
AI839876	RALGPS2	Ral GEF with PH domain and SH3 binding motif 2	-5.942
AI852621	RAP1GDS1	RAP1, GTP-GDP dissociation stimulator 1	-2.62
AI843837	RAPGEF2	Rap guanine nucleotide exchange factor (GEF) 2	-2.71
AI844405	RAPGEF5	Rap guanine nucleotide exchange factor (GEF) 5	-2.487
AI846922	RASA1	RAS p21 protein activator (GTPase activating protein) 1	-2.573
AI843392	RASGEF1B	RasGEF domain family, member 1B	-2.631
AI836866	RASGRP1	RAS guanyl releasing protein 1 (calcium and DAG-regulated)	-2.06
AI851058	RASGRP2	RAS guanyl releasing protein 2 (calcium and DAG-regulated)	-2.513
AI846239	RASL10B	RAS-like, family 10, member B	2.147
AI850136	RASSF8	Ras association (RalGDS/AF-6) domain family 8	-2.658
AI323835	RB1	retinoblastoma 1 (including osteosarcoma)	2.585
AI841402	RB1CC1	RB1-inducible coiled-coil 1	-3.128
AI447702	RBM39	RNA binding motif protein 39	-2.067
AI839071	RBM5	RNA binding motif protein 5	-2.059
AI429645	RCAN1	regulator of calcineurin 1	-2.059
AI837029	RCN1	reticulocalbin 1, EF-hand calcium binding domain	-2.514
AI851795	REEP3	receptor accessory protein 3	-2.363
AI838188	REEP3	receptor accessory protein 3	-2.251
AI451037	REEP3	receptor accessory protein 3	-2.09
AI450210	RERE	arginine-glutamic acid dipeptide (RE) repeats	-2.355
AI838568	RG9MTD1	RNA (guanine-9-) methyltransferase domain containing 1	-2.291
AI449289	RGS12	regulator of G-protein signaling 12	-2.602
AI327093	RGS19	regulator of G-protein signaling 19	-2.039
AI847151	RGS5	regulator of G-protein signaling 5	-2.754
AI834967	RHOG	ras homolog gene family, member G (rho G)	-2.347
AI324882	RHOQ	ras homolog gene family, member Q	-3.066
AI850172	RIOK2	RIO kinase 2 (yeast)	-2.033
AI661042	RIPK5	receptor interacting protein kinase 5	-2.874
AI853140	RMST	rhabdomyosarcoma 2 associated transcript (non-coding RNA)	2.007
AI851631	RNASET2	ribonuclease T2	-2.247
AI849183	RNF123	ring finger protein 123	2.333

AI839936	RNF13	ring finger protein 13	-2.058
AI848923	RNF149	ring finger protein 149	-2.202
AI850285	RNF44	ring finger protein 44	-2.894
AI853111	RNMT	RNA (guanine-7-) methyltransferase	-2.603
AI854172	RNMTL1	RNA methyltransferase like 1	3.033
AI448833	RNPC3	RNA-binding region (RNP1, RRM) containing 3	-2.622
AI846531	RNPEP	arginyl aminopeptidase (aminopeptidase B)	-2.032
AI666717	RP4-691N24.1	ninein-like	2.016
AI845529	RPP40	ribonuclease P/MRP 40kDa subunit	-2.012
AI851166	RQCD1	RCD1 required for cell differentiation1 homolog (S. pombe)	-2.434
AI573426	RRAS	related RAS viral (r-ras) oncogene homolog	-2.322
AI836229	RSF1	remodeling and spacing factor 1	-2.965
AI850607	RSU1	Ras suppressor protein 1	-3.41
AI842679	RTP4	receptor (chemosensory) transporter protein 4	-3.848
AI852705	RUFY2	RUN and FYVE domain containing 2	-2.176
AI853842	RWDD1 (includes EG:51389)	RWD domain containing 1	-3.399
AI844800	S100A10	S100 calcium binding protein A10	-2.125
AI841112	S100A16	S100 calcium binding protein A16	-2.545
AI854782	SAMD14	sterile alpha motif domain containing 14	2.247
AI426455	SAP30	Sin3A-associated protein, 30kDa	6.089
AI836978	SAP30BP	SAP30 binding protein	-2.16
AI839993	SCARB2	scavenger receptor class B, member 2	-2.527
AI854680	SCN2B	sodium channel, voltage-gated, type II, beta	2.253
AI323707	SCP2	sterol carrier protein 2	-2
AI854112	SCPEP1	serine carboxypeptidase 1	-2.331
AI427019	SCRN3	secernin 3	2.672
AI854113	SDC2	syndecan 2	-3.175
AI854015	SDC3	syndecan 3	-3.026
AI835777	SDF4	stromal cell derived factor 4	8.057
AI450397	SEC24D	SEC24 related gene family, member D (S. cerevisiae)	-9.589
AI452230	SELI	selenoprotein I	2.161
AI838678	SELPLG	selectin P ligand	-2.556
AI385610	SEMA5A	sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5A	-4.99
AI848774	SEMA6D	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6D	-2.975
AI838693	SEPP1	selenoprotein P, plasma, 1	-11.527
AI840037	SEPX1	selenoprotein X, 1	-2.555

AI854679	SERINC1	serine incorporator 1	-2.283
AI843466	SERP1	stress-associated endoplasmic reticulum protein 1	-2.014
AI844072	SERPINE2	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2	-2.869
AI326777	SERPINH1	serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)	-3.076
AI848855	SERTAD2	SERTA domain containing 2	2.671
AI836681	SESN3	sestrin 3	-2.085
AI845668	SETD7	SET domain containing (lysine methyltransferase) 7	-2.317
AI666563	SF3B1	splicing factor 3b, subunit 1, 155kDa	-2.221
AI465457	SFRS11	splicing factor, arginine/serine-rich 11	2.535
AI450790	SFXN1	sideroflexin 1	-2.784
AI854349	SGK1	serum/glucocorticoid regulated kinase 1	-2.239
AI849233	SGMS1	sphingomyelin synthase 1	2.869
AI854585	SGSM3	small G protein signaling modulator 3	2.312
AI661046	SH2D1A (includes EG:4068)	SH2 domain protein 1A, Duncan's disease (lymphoproliferative syndrome)	-4.271
AI852760	SH3BGRL2	SH3 domain binding glutamic acid-rich protein like 2	-3.162
AI415349	SH3BGRL3	SH3 domain binding glutamic acid-rich protein like 3	-2.986
AI851647	SH3BGRL3	SH3 domain binding glutamic acid-rich protein like 3	-2.813
AI447735	SH3YL1	SH3 domain containing, Ysc84-like 1 (S. cerevisiae)	3.088
AI844042	SIRPA	signal-regulatory protein alpha	-2.099
AI451572	SLA	Src-like-adaptor	-2.162
AI854524	SLC15A3	solute carrier family 15, member 3	-2.007
AI854532	SLC16A1	solute carrier family 16, member 1 (monocarboxylic acid transporter 1)	3.431
AI836636	SLC16A2	solute carrier family 16, member 2 (monocarboxylic acid transporter 8)	2.087
AI849989	SLC25A12	solute carrier family 25 (mitochondrial carrier, Aralar), member 12	-5.886
AI852122	SLC25A19	solute carrier family 25 (mitochondrial thiamine pyrophosphate carrier), member 19	-2.16
AI465324	SLC25A21	solute carrier family 25 (mitochondrial oxodicarboxylate carrier), member 21	2.737
AI841357	SLC25A4	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4	-2.034
AI838647	SLC25A42	solute carrier family 25, member 42	-2.945
AI413193	SLC28A2	solute carrier family 28 (sodium-coupled nucleoside transporter), member 2	-3.553
AI851366	SLC29A2	solute carrier family 29 (nucleoside transporters), member 2	2.113
AI846980	SLC30A4	solute carrier family 30 (zinc transporter), member 4	-2.895
AI843695	SLC37A3	solute carrier family 37 (glycerol-3-phosphate transporter), member 3	-2.703
AI839742	SLC38A3	solute carrier family 38, member 3	-3.239
AI451193	SLC38A5	solute carrier family 38, member 5	2.078
AI465146	SLC39A5	solute carrier family 39 (metal ion transporter), member 5	-2.09
AI846650	SLC6A4	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	-3.541

AI844828	SLC6A9	solute carrier family 6 (neurotransmitter transporter, glycine), member 9	2.783
AI451155	SLC7A11	solute carrier family 7, (cationic amino acid transporter, y+ system) member 11	-4.127
AI844125	SLU7 (includes EG:10569)	SLU7 splicing factor homolog (S. cerevisiae)	-3.513
AI853768	SMARCA1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1	-2.094
AI447889	SMARCA2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2	-2.331
AI426152	SMARCE1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1	2.176
AI838562	SMC6	structural maintenance of chromosomes 6	2.973
AI849987	SMPDL3A	sphingomyelin phosphodiesterase, acid-like 3A	-2.937
AI426386	SNAP23	synaptosomal-associated protein, 23kDa	-5.385
AI840449	SNRPD1	small nuclear ribonucleoprotein D1 polypeptide 16kDa	-3.013
AI842754	SNX2	sorting nexin 2	-3.199
AI840437	SNX5	sorting nexin 5	-2.302
AI662507	SNX9	sorting nexin 9	-2.2
AI854469	SON	SON DNA binding protein	2.167
AI851251	SORL1	sortilin-related receptor, L(DLR class) A repeats-containing	-4.126
AI841913	SOSTDC1	sclerostin domain containing 1	-2.693
AI840197	SPARC	secreted protein, acidic, cysteine-rich (osteonectin)	-5.85
AI851745	SPARCL1	SPARC-like 1 (mast9, hevjin)	-2.197
AI452278	SPG7	spastic paraplegia 7 (pure and complicated autosomal recessive)	-2.108
AI451311	SPIC	Spi-C transcription factor (Spi-1/PU.1 related)	-11.247
AI415299	SPIRE1	spire homolog 1 (Drosophila)	2.074
AI847805	SPP1	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	-6.251
AI451697	SPTA1	spectrin, alpha, erythrocytic 1 (elliptocytosis 2)	3.732
AI840676	SPTBN1	spectrin, beta, non-erythrocytic 1	-6.449
AI853605	SPTLC2 (includes EG:9517)	serine palmitoyltransferase, long chain base subunit 2	-2.207
AI327055	SRGN	serglycin	-3.032
AI849950	SSFA2	sperm specific antigen 2	-4.495
AI851690	SSH2	slingshot homolog 2 (Drosophila)	-2.107
AI844081	SSPN	sarcospan (Kras oncogene-associated gene)	-2.44
AI429591	ST3GAL2	ST3 beta-galactoside alpha-2,3-sialyltransferase 2	2.057
AI385650	ST3GAL4	ST3 beta-galactoside alpha-2,3-sialyltransferase 4	2.4
AI852814	STAB2	stabilin 2	-3.503
AI449540	STAT1	signal transducer and activator of transcription 1, 91kDa	-4.271
AI837104	STAT3	signal transducer and activator of transcription 3 (acute-phase response factor)	-2.115
AI413829	STBD1	starch binding domain 1	-2.081

AI415710	STK17B	serine/threonine kinase 17b	-2.705
AI850672	STK39	serine threonine kinase 39 (STE20/SPS1 homolog, yeast)	-2.844
AI447339	STK4	serine/threonine kinase 4	-2.047
AI426660	STRN3	striatin, calmodulin binding protein 3	-2.383
AI846963	STX4	syntaxin 4	-2.22
AI845861	STX6	syntaxin 6	2.286
AI451887	STXBP5	syntaxin binding protein 5 (tomosyn)	-3.639
AI844608	SUB1	SUB1 homolog (S. cerevisiae)	-3.764
AI841809	SUMO3	SMT3 suppressor of mif two 3 homolog 3 (S. cerevisiae)	-2.206
AI428264	SUPT16H	suppressor of Ty 16 homolog (S. cerevisiae)	2.061
AI852103	SUV39H1	suppressor of variegation 3-9 homolog 1 (Drosophila)	2.054
AI413198	SYNPO	synaptopodin	-2.092
AI851970	SYT11	synaptotagmin XI	-4.224
AI838544	SYT5	synaptotagmin V	-2.172
AI850390	TAF9B	TAF9B RNA polymerase II, TATA box binding protein (TBP)-associated factor, 31kDa	-6.493
AI846743	TAGLN	transgelin	-2.936
AI852545	TAGLN2	transgelin 2	-2.042
AI451692	TAL1	T-cell acute lymphocytic leukemia 1	5.049
AI850346	TAX1BP1	Tax1 (human T-cell leukemia virus type I) binding protein 1	-3.14
AI428527	TBC1D10C	TBC1 domain family, member 10C	-2.928
AI834833	TBL1XR1	transducin (beta)-like 1X-linked receptor 1	-2.392
AI849958	TBL3	transducin (beta)-like 3	-2.028
AI836560	TCN2	transcobalamin II; macrocytic anemia	-2.826
AI325946	TES	testis derived transcript (3 LIM domains)	-2.289
AI845325	TEX9	testis expressed 9	-3.078
AI841326	TF	transferrin	-4.328
AI451300	TFDP2	transcription factor Dp-2 (E2F dimerization partner 2)	3.312
AI426448	TFRC	transferrin receptor (p90, CD71)	5.745
AI849214	TGFB3	transforming growth factor, beta 3	-2.55
AI661287	TGFBI	transforming growth factor, beta-induced, 68kDa	-3.375
AI843821	THEX1	three prime histone mRNA exonuclease 1	2.036
AI843835	THY1	Thy-1 cell surface antigen	-2.139
AI850670	TIMP2	TIMP metalloproteinase inhibitor 2	-4.964
AI843384	TK2	thymidine kinase 2, mitochondrial	-2.702
AI450864	TLR4	toll-like receptor 4	-2.405
AI465383	TM4SF1	transmembrane 4 L six family member 1	-4.136

AI428514	TM6SF1	transmembrane 6 superfamily member 1	-2.647
AI838784	TM9SF2	transmembrane 9 superfamily member 2	-2.13
AI852088	TM9SF3	transmembrane 9 superfamily member 3	-2.065
AI852222	TMCC2	transmembrane and coiled-coil domain family 2	2.176
AI415143	TMCC3	transmembrane and coiled-coil domain family 3	-2.025
AI841979	TMEM151A	transmembrane protein 151A	-2.359
AI851095	TMEM179	transmembrane protein 179	-2.447
AI850198	TMEM181	transmembrane protein 181	-4.391
AI841526	TMEM35	transmembrane protein 35	-2.781
AI854863	TMEM43	transmembrane protein 43	-2.054
AI848275	TMEM66	transmembrane protein 66	-2.297
AI849532	TMEM86A	transmembrane protein 86A	-4.276
AI324819	TMSB10	thymosin, beta 10	-2.439
AI851379	TNFAIP8L2	tumor necrosis factor, alpha-induced protein 8-like 2	-2.253
AI450229	TNIP2	TNFAIP3 interacting protein 2	-2.027
AI841866	TNRC6A	trinucleotide repeat containing 6A	-2.18
AI845157	TNS1	tensin 1	-2.453
AI840048	TOM1	target of myb1 (chicken)	-2.115
AI854363	TOR1A	torsin family 1, member A (torsin A)	-2.516
AI851125	TP53INP2	tumor protein p53 inducible nuclear protein 2	-2.393
AI851246	TPD52	tumor protein D52	-2.061
AI849835	TPPP	tubulin polymerization promoting protein	-2.041
AI450999	TRAF3IP3	TRAF3 interacting protein 3	-2.379
AI851288	TRAF6	TNF receptor-associated factor 6	2.256
AI447612	TRAFFD1	TRAF-type zinc finger domain containing 1	-3.735
AI847775	TRAK2	trafficking protein, kinesin binding 2	2.057
AI323471	TRIM25	tripartite motif-containing 25	-2.47
AI451148	TRIM26	tripartite motif-containing 26	-3.243
AI851571	TRIM35	tripartite motif-containing 35	-2.377
AI845098	TRNT1	tRNA nucleotidyl transferase, CCA-adding, 1	2.133
AI326808	TSC22D3	TSC22 domain family, member 3	-3.767
AI848441	TSPAN14	tetraspanin 14	-2.277
AI844703	TSPAN17	tetraspanin 17	-2.261
AI325509	TSPAN4	tetraspanin 4	-2.434
AI847962	TSPAN7	tetraspanin 7	-2.021
AI851701	TSPAN9	tetraspanin 9	2.209

AI848701	TTC28	tetratricopeptide repeat domain 28	-2.095
AI834826	TTC3	tetratricopeptide repeat domain 3	-2.113
AI465319	TXNDC1	thioredoxin domain containing 1	2.177
AI840235	UBAC1	UBA domain containing 1	2.961
AI850365	UBE2M	ubiquitin-conjugating enzyme E2M (UBC12 homolog, yeast)	-3.717
AI848676	UBL3	ubiquitin-like 3	-2.208
AI841927	UBXD2	UBX domain containing 2	-3.394
AI853172	UGCG	UDP-glucose ceramide glucosyltransferase	-2.975
AI465374	USMG5	upregulated during skeletal muscle growth 5 homolog (mouse)	2.157
AI848382	USP1	ubiquitin specific peptidase 1	2.019
AI851327	USP30	ubiquitin specific peptidase 30	-2.256
AI465456	USP45	ubiquitin specific peptidase 45	2.233
AI842867	USP54	ubiquitin specific peptidase 54	-2.144
AI385712	VCL	vinculin	-2.126
AI845820	VIM	vimentin	-6.262
AI528709	VPREB3	pre-B lymphocyte gene 3	-2.945
AI850060	VPS8	vacuolar protein sorting 8 homolog (S. cerevisiae)	-4.542
AI449667	VSIG2	V-set and immunoglobulin domain containing 2	2.224
AI849245	WARS	tryptophanyl-tRNA synthetase	-2.598
AI852574	WDFY2	WD repeat and FYVE domain containing 2	-2.727
AI850110	WDR1	WD repeat domain 1	-2.118
AI854008	WDR26	WD repeat domain 26	2.66
AI428454	WDR48	WD repeat domain 48	4.92
AI847307	WIPF1	WAS/WASL interacting protein family, member 1	-2.632
AI844065	WIPF3	WAS/WASL interacting protein family, member 3	-4.074
AI839342	XAB1	XPA binding protein 1, GTPase	-4.662
AI844927	YKT6	YKT6 v-SNARE homolog (S. cerevisiae)	-3.506
AI854645	ZBTB4	zinc finger and BTB domain containing 4	-4.117
AI851226	ZBTB8OS	zinc finger and BTB domain containing 8 opposite strand	-2.229
AI427093	ZC3H11A	zinc finger CCCH-type containing 11A	-2.276
AI846301	ZC3H11A	zinc finger CCCH-type containing 11A	-2.041
AI448873	ZC3H8	zinc finger CCCH-type containing 8	-2.464
AI841298	ZCCHC18	zinc finger, CCHC domain containing 18	-2.318
AI843361	ZDHHC14	zinc finger, DHHC-type containing 14	2.025
AI850103	ZDHHC20	zinc finger, DHHC-type containing 20	-2.262
AI835128	ZDHHC20	zinc finger, DHHC-type containing 20	-2.237

AI851177	ZFH3	zinc finger homeobox 3	-2.287
AI528715	ZFP36L1	zinc finger protein 36, C3H type-like 1	-2.552
AI451054	ZFP53	zinc finger protein 53	-2.043
AI839189	ZFYVE21	zinc finger, FYVE domain containing 21	-2.437
AI849851	ZMYND11	zinc finger, MYND domain containing 11	-2.103
AI848462	ZNF148	zinc finger protein 148	-2.793
AI449016	ZNF292	zinc finger protein 292	-2.072
AI464386	ZNF330	zinc finger protein 330	-2.014
AI834982	ZNF364	zinc finger protein 364	-2.555
AI415055	ZNF498	zinc finger protein 498	2.375
AI852587	ZNF521	zinc finger protein 521	-2.801
AI464551	ZNF618	zinc finger protein 618	-3.16

7.4 Appendix 4

Table 11: Complete List of Differentially Regulated Genes in the Splenic Cell Population of Clinical Scrapie VM Mice

ID	Genes	Description	Fold Change
A_52_P109521	AATF	apoptosis antagonizing transcription factor	-2.772
A_51_P144696	ABCB10	ATP-binding cassette, sub-family B (MDR/TAP), member 10	-24.07
A_51_P245368	ABCB1B	ATP-binding cassette, sub-family B (MDR/TAP), member 1B	3.209
A_52_P223194	ABCB4	ATP-binding cassette, sub-family B (MDR/TAP), member 4	-4.547
A_52_P336142	ABCG2	ATP-binding cassette, sub-family G (WHITE), member 2	-10.798
A_51_P406020	ABHD4	abhydrolase domain containing 4	2.316
A_51_P407165	ABHD5	abhydrolase domain containing 5	-3.535
A_52_P367675	ACIN1	apoptotic chromatin condensation inducer 1	-3.709
A_52_P589735	ACP1	acid phosphatase 1, soluble	-5.55
A_51_P518823	ACSL6	acyl-CoA synthetase long-chain family member 6	-4.598
A_52_P420504	ACTA2	actin, alpha 2, smooth muscle, aorta	2.371
A_51_P145735	ACYP1	acylphosphatase 1, erythrocyte (common) type	-2.258
A1661341	ADAM10	ADAM metallopeptidase domain 10	-2.541
A_51_P227222	ADAMTS2	ADAM metallopeptidase with thrombospondin type 1 motif, 2	5.06
A_51_P243134	ADCY6	adenylate cyclase 6	-4.579
A_52_P586141	ADCY7	adenylate cyclase 7	5.448
A_51_P514270	ADD2	adducin 2 (beta)	-9.119
A_52_P629895	ADH1C (includes EG:126)	alcohol dehydrogenase 1C (class I), gamma polypeptide	5.46
A_51_P411527	AFMID	arylformamidase	-3.86
A_52_P649568	AIPL1	aryl hydrocarbon receptor interacting protein-like 1	2.121
A_51_P496779	AKAP9	A kinase (PRKA) anchor protein (yotiao) 9	2.278
A_52_P257058	AKR1B10	aldo-keto reductase family 1, member B10 (aldose reductase)	6.001
A_51_P237553	AKT1	v-akt murine thymoma viral oncogene homolog 1	2.615
A_51_P512072	ALAD	aminolevulinate, delta-, dehydratase	-8.011
A_51_P216905	ALDOA	aldolase A, fructose-bisphosphate	2.215
A_52_P145349	ALS2CR2	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 2	-3.044
A_52_P484807	AMD1	adenosylmethionine decarboxylase 1	-5.623

A_51_P383638	AMY2A	amylase, alpha 2A; pancreatic	5.612
A_51_P269375	ANK1	ankyrin 1, erythrocytic	-10.049
A_51_P467971	ANK3	ankyrin 3, node of Ranvier (ankyrin G)	2.294
A_51_P134801	ANKRD12	ankyrin repeat domain 12	4.335
A_52_P512955	ANLN	anillin, actin binding protein	-7.197
A_51_P499755	ANP32A	acidic (leucine-rich) nuclear phosphoprotein 32 family, member A	-3.448
A_52_P755439	ANP32B	acidic (leucine-rich) nuclear phosphoprotein 32 family, member B	-8.891
A_51_P113144	AP1S2	adaptor-related protein complex 1, sigma 2 subunit	2.998
A_51_P419935	AP2S1	adaptor-related protein complex 2, sigma 1 subunit	-2.686
A_51_P391904	APBB1P	amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein	2.336
A_51_P149562	APBB2	amyloid beta (A4) precursor protein-binding, family B, member 2 (Fe65-like)	2.892
A_51_P407977	APEH	N-acylaminoacyl-peptide hydrolase	-2.232
A_51_P237593	APLP2	amyloid beta (A4) precursor-like protein 2	-3.287
A_51_P125205	AQP1	aquaporin 1 (Colton blood group)	-6.631
A_52_P136092	AQP9	aquaporin 9	-5.357
A_52_P83557	ARFGEF2	ADP-ribosylation factor guanine nucleotide-exchange factor 2 (brefeldin A-inhibited)	-2.055
A_52_P29785	ARFIP2	ADP-ribosylation factor interacting protein 2 (arfaptin 2)	2.215
A_51_P409496	ARHGAP11A	Rho GTPase activating protein 11A	-5.23
A_52_P323525	ARHGEF11	Rho guanine nucleotide exchange factor (GEF) 11	3.936
A_52_P390227	ARHGEF12	Rho guanine nucleotide exchange factor (GEF) 12	-2.948
A_52_P119301	ARHGEF17	Rho guanine nucleotide exchange factor (GEF) 17	2.533
A_52_P577205	ARHGEF3	Rho guanine nucleotide exchange factor (GEF) 3	3.193
A_51_P373226	ARL2	ADP-ribosylation factor-like 2	-2.449
A_52_P155849	ARL4A	ADP-ribosylation factor-like 4A	-6.3
A_52_P104885	ARL5B	ADP-ribosylation factor-like 5B	2.969
A_51_P301964	ARL6IP1	ADP-ribosylation factor-like 6 interacting protein 1	-3.912
A_51_P163834	ARL6IP2	ADP-ribosylation factor-like 6 interacting protein 2	-2.452
A_52_P592429	ARL6IP6	ADP-ribosylation-like factor 6 interacting protein 6	-4.63
A_51_P436727	ARRB1	arrestin, beta 1	-4.317
A_51_P111757	ASB1	ankyrin repeat and SOCS box-containing 1	-3.044
A_52_P625171	ASPH	aspartate beta-hydroxylase	-8.347
A_51_P464769	ASPM	asp (abnormal spindle) homolog, microcephaly associated (Drosophila)	-18.486
A_51_P343129	ATAD2	ATPase family, AAA domain containing 2	-12.495
A_51_P512859	ATBF1 (includes EG:463)	AT-binding transcription factor 1	9.261
A_52_P32756	ATF1	activating transcription factor 1	-3.413

A_51_P269216	ATF5	activating transcription factor 5	2.884
A_52_P131490	ATG4B	ATG4 autophagy related 4 homolog B (S. cerevisiae)	-3.839
A_51_P507942	ATP13A2	ATPase type 13A2	2.061
A_51_P151484	ATP1B1	ATPase, Na ⁺ /K ⁺ transporting, beta 1 polypeptide	6.509
A_51_P207636	ATP5B	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, beta polypeptide	-2.815
A_52_P106929	ATP5F1	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit B1	-3.821
A_52_P75415	ATP5L	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit G	-3.271
A_52_P321318	ATP6	ATP synthase F0 subunit 6	19.528
A_52_P163766	ATP6V0A2	ATPase, H ⁺ transporting, lysosomal V0 subunit a2	2.652
A_52_P412167	ATP6V0A4	ATPase, H ⁺ transporting, lysosomal V0 subunit a4	-4.908
A_51_P166434	ATP6V1E1	ATPase, H ⁺ transporting, lysosomal 31kDa, V1 subunit E1	-3.032
A_52_P403420	ATP6V1H	ATPase, H ⁺ transporting, lysosomal 50/57kDa, V1 subunit H	-2.868
A_51_P518488	ATPIF1	ATPase inhibitory factor 1	-3.062
A_51_P405089	AYTL2	acyltransferase like 2	-10.105
A_52_P24631	AZIN1	antizyme inhibitor 1	-3.625
A_51_P364432	B3GAT3	beta-1,3-glucuronyltransferase 3 (glucuronosyltransferase I)	-3.223
A_51_P438952	BAG4	BCL2-associated athanogene 4	-2.342
A_52_P162744	BAZ1B	bromodomain adjacent to zinc finger domain, 1B	-3.512
A_51_P467990	BCAS2	breast carcinoma amplified sequence 2	-3.877
A_52_P534870	BCL2	B-cell CLL/lymphoma 2	4.823
A_52_P510877	BCL2L1	BCL2-like 1	-3.196
A_51_P356493	BICC1	bicaudal C homolog 1 (Drosophila)	3.023
A_51_P230103	BIRC5	baculoviral IAP repeat-containing 5 (survivin)	-10.232
A_51_P314418	BLM	Bloom syndrome	-5.396
A_52_P674578	BLMH	bleomycin hydrolase	-2.128
A_51_P279841	BLNK	B-cell linker	4.313
A_51_P295206	BRIP1	BRCA1 interacting protein C-terminal helicase 1	-2.703
AA015403	BTF3	basic transcription factor 3	-3.205
A_51_P490509	BUB1B	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)	-4.52
A_52_P148212	C14ORF106	chromosome 14 open reading frame 106	-4.961
A_52_P595663	C15ORF23	chromosome 15 open reading frame 23	-11.525
A_52_P339543	C1D	nuclear DNA-binding protein	-4.592
A_51_P357094	C1ORF96	chromosome 1 open reading frame 96	-3.118
A_51_P497985	C2	complement component 2	5.041
A_52_P190339	C21ORF45	chromosome 21 open reading frame 45	-3.496
A_52_P127239	C6ORF167	chromosome 6 open reading frame 167	-5.814

A_52_P265584	C8ORF4	chromosome 8 open reading frame 4	2.571
A_51_P455647	CA2	carbonic anhydrase II	-4.317
A_51_P280384	CACNA1H	calcium channel, voltage-dependent, T type, alpha 1H subunit	5.465
A_51_P324529	CALCOCO2	calcium binding and coiled-coil domain 2	-2.536
A_51_P185971	CALM2	calmodulin 2 (phosphorylase kinase, delta)	-2.956
A_52_P1157979	CALM3	calmodulin 3 (phosphorylase kinase, delta)	-3.396
A_52_P188261	CAMK2D	calcium/calmodulin-dependent protein kinase (CaM kinase) II delta	3.728
A_52_P260818	CARD10	caspase recruitment domain family, member 10	3.199
A_51_P163444	CARHSP1	calcium regulated heat stable protein 1, 24kDa	-2.805
A_52_P444804	CARS	cysteinyI-tRNA synthetase	-3.614
A_52_P1147844	CASC5	cancer susceptibility candidate 5	-11.874
A_52_P117576	CASP3	caspase 3, apoptosis-related cysteine peptidase	-2.28
A_52_P279658	CAST	calpastatin	-4.047
A_51_P117162	CBX3	chromobox homolog 3 (HP1 gamma homolog, Drosophila)	-4.737
A_51_P133582	CBX4	chromobox homolog 4 (Pc class homolog, Drosophila)	3.178
A_52_P676573	CBX5	chromobox homolog 5 (HP1 alpha homolog, Drosophila)	-3.884
A_52_P263774	CBX7	chromobox homolog 7	3.47
A_52_P644114	CCDC18	coiled-coil domain containing 18	-5.416
A_51_P248786	CCDC80	coiled-coil domain containing 80	4.832
A_51_P227564	CCDC99	coiled-coil domain containing 99	-5.424
A_51_P322640	CCL24	chemokine (C-C motif) ligand 24	-2.651
A_51_P481920	CCNA2	cyclin A2	-10.27
A_52_P202770	CCNB1	cyclin B1	-4.224
A_51_P433228	CCND2	cyclin D2	2.492
A_51_P238448	CCND3	cyclin D3	-4.272
A_52_P796682	CCNE1	cyclin E1	-7.254
A_52_P58558	CCNE2 (includes EG:9134)	cyclin E2	-13.629
A_52_P283835	CCNK	cyclin K	5.026
A_52_P668146	CCPG1	cell cycle progression 1	3.146
A_52_P236176	CCT4	chaperonin containing TCP1, subunit 4 (delta)	-2.36
A_51_P144783	CCT5	chaperonin containing TCP1, subunit 5 (epsilon)	-2.018
A_52_P244193	CD24	CD24 molecule	-2.996
A_52_P162967	CD28	CD28 molecule	3.203
A_51_P375146	CD36	CD36 molecule (thrombospondin receptor)	-4.699
A_52_P232693	CD59	CD59 molecule, complement regulatory protein	-7.273
A_51_P342652	CD79B	CD79b molecule, immunoglobulin-associated beta	2.703

A_52_P106294	CDC14B	CDC14 cell division cycle 14 homolog B (<i>S. cerevisiae</i>)	4.347
A_51_P450033	CDC2	cell division cycle 2, G1 to S and G2 to M	-5.03
A_51_P361022	CDC20	cell division cycle 20 homolog (<i>S. cerevisiae</i>)	-3.135
A_52_P615877	CDC25B	cell division cycle 25 homolog B (<i>S. pombe</i>)	-6.041
A_51_P279575	CDC25C	cell division cycle 25 homolog C (<i>S. pombe</i>)	-6.449
A_52_P50959	CDC26	cell division cycle 26 homolog (<i>S. cerevisiae</i>)	-2.044
A_51_P401451	CDC45L	CDC45 cell division cycle 45-like (<i>S. cerevisiae</i>)	-5.147
A_52_P219473	CDC6	cell division cycle 6 homolog (<i>S. cerevisiae</i>)	-8.811
A_51_P206616	CDC7	cell division cycle 7 homolog (<i>S. cerevisiae</i>)	-3.044
A_51_P444696	CDCA2	cell division cycle associated 2	-7.638
A_51_P127195	CDCA3	cell division cycle associated 3	-5.692
A_52_P61988	CDCA5	cell division cycle associated 5	-6.608
A_52_P392544	CDCA8	cell division cycle associated 8	-9.914
A_52_P248604	CDH5	cadherin 5, type 2, VE-cadherin (vascular epithelium)	2.153
A_52_P673863	CDKN1B	cyclin-dependent kinase inhibitor 1B (p27, Kip1)	-2.708
A_52_P128818	CDKN2AIP	CDKN2A interacting protein	-2.985
A_51_P122356	CDKN2D	cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4)	-4.742
A_52_P30989	CDKN3	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	-10.094
A_51_P356353	CDS2	CDP-diacylglycerol synthase (phosphatidate cytidyltransferase) 2	2.116
A_51_P171728	CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)	11.164
A_51_P273979	CENPA	centromere protein A	-6.619
A_51_P164021	CENPE	centromere protein E, 312kDa	-9.739
A_52_P227880	CENPF	centromere protein F, 350/400ka (mitosin)	-13.812
A_51_P492830	CENPH	centromere protein H	-7.515
A_52_P4666	CENPK	centromere protein K	-10.336
A_51_P191087	CEP250	centrosomal protein 250kDa	2.014
A_52_P309381	CFH	complement factor H	3.377
A_51_P134812	CHAC1	ChaC, cation transport regulator homolog 1 (<i>E. coli</i>)	5.12
A_52_P547965	CHD1	chromodomain helicase DNA binding protein 1	-3.499
A_51_P326502	CHEK1	CHK1 checkpoint homolog (<i>S. pombe</i>)	-4.027
A_51_P137111	CHEK2	CHK2 checkpoint homolog (<i>S. pombe</i>)	-8.739
A_51_P191669	CHGB	chromogranin B (secretogranin 1)	3.703
A_52_P674808	CHRD1	chordin-like 1	10.224
A_52_P640686	CIT	citron (rho-interacting, serine/threonine kinase 21)	-4.389
A_51_P105837	CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	3.161

A_51_P481592	CKAP2 (includes EG:26586)	cytoskeleton associated protein 2	-7.76
A_51_P227004	CKS1B	CDC28 protein kinase regulatory subunit 1B	-2.324
A_51_P462249	CKS2 (includes EG:1164)	CDC28 protein kinase regulatory subunit 2	-3.326
A_52_P462049	CLCN3	chloride channel 3	-12.518
A_51_P388325	CLGN	calmegin	-3.092
A_51_P127681	CLIC4	chloride intracellular channel 4	-2.571
A_51_P420547	CLIC5	chloride intracellular channel 5	2.716
A_52_P559498	CLK3	CDC-like kinase 3	-3.237
A_51_P320888	CLN5	ceroid-lipofuscinosis, neuronal 5	2.333
A_52_P430523	CLN8	ceroid-lipofuscinosis, neuronal 8 (epilepsy, progressive with mental retardation)	-7.847
A_51_P370423	CLSTN2	calsyntenin 2	-2.435
A_51_P311525	CLTC	clathrin, heavy chain (Hc)	-3.26
A_51_P367720	CLU	clusterin	3.121
A_52_P88818	CLYBL	citrate lyase beta like	-2.439
A_51_P343309	CMAS	cytidine monophosphate N-acetylneuraminic acid synthetase	-3.556
A_51_P101763	CNTN1	contactin 1	3.486
A_51_P191700	COL18A1	collagen, type XVIII, alpha 1	3.028
AI844874	COL1A1	collagen, type I, alpha 1	2.992
A_51_P491350	COL4A2	collagen, type IV, alpha 2	4.594
A_51_P414637	COL5A1	collagen, type V, alpha 1	-4.445
A_52_P479262	COL6A3	collagen, type VI, alpha 3	5.142
A_52_P527625	COLEC12	collectin sub-family member 12	4.431
A_52_P23616	COMMD1	copper metabolism (Murr1) domain containing 1	-4.217
A_51_P109335	COPA	coatamer protein complex, subunit alpha	3.07
A_51_P296361	COX17	COX17 cytochrome c oxidase assembly homolog (S. cerevisiae)	-2.334
A_52_P323415	CPEB4	cytoplasmic polyadenylation element binding protein 4	-7.489
A_51_P257258	CPOX	coproporphyrinogen oxidase	-3.974
A_52_P940077	CSAD	cysteine sulfinic acid decarboxylase	2.678
A_51_P311977	CSDA	cold shock domain protein A	-3.438
A_51_P146753	CSF2RB	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)	4.49
A_52_P161674	CSTF2	cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa	-3.311
AI852172	CSTF3	cleavage stimulation factor, 3' pre-RNA, subunit 3, 77kDa	-2.001
A_52_P35960	CTSD	cathepsin D	-2.764
A_51_P431558	CUGBP1	CUG triplet repeat, RNA binding protein 1	-3.153
A_52_P478051	CUGBP2	CUG triplet repeat, RNA binding protein 2	2.34
A_52_P36616	CUL4A	cullin 4A	-5.111

A_51_P172502	CXCL12	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	6.695
A_52_P536947	CYFIP2	cytoplasmic FMR1 interacting protein 2	2.296
A_51_P506328	CYP2J2	cytochrome P450, family 2, subfamily J, polypeptide 2	2.653
A_51_P394574	DAAM1	dishevelled associated activator of morphogenesis 1	-4.3
A_51_P148675	DAG1	dystroglycan 1 (dystrophin-associated glycoprotein 1)	2.479
A_51_P126198	DAPK2 (includes EG:23604)	death-associated protein kinase 2	-4.244
A_51_P311611	DARC	Duffy blood group, chemokine receptor	-3.56
A_52_P416584	DARS	aspartyl-tRNA synthetase	2.058
A_52_P397418	DBF4	DBF4 homolog (S. cerevisiae)	-6.535
A_52_P362603	DCC1	defective in sister chromatid cohesion homolog 1 (S. cerevisiae)	-7.892
A_51_P500718	DCK	deoxycytidine kinase	-12.547
A_52_P26991	DCLK1	doublecortin-like kinase 1	2.987
A_52_P327627	DCLRE1A	DNA cross-link repair 1A (PSO2 homolog, S. cerevisiae)	-2.445
A_51_P334104	DCN	decorin	27.619
A_52_P201627	DCTN4	dynactin 4 (p62)	-2.05
A_52_P536731	DDEF2	development and differentiation enhancing factor 2	-3.94
A_52_P477709	DEK	DEK oncogene (DNA binding)	-4.007
A_52_P207194	DGKG	diacylglycerol kinase, gamma 90kDa	2.592
A_52_P329197	DHFR	dihydrofolate reductase	-4.268
A_51_P403260	DIAPH3	diaphanous homolog 3 (Drosophila)	-7.075
A_52_P377416	DISP1	dispatched homolog 1 (Drosophila)	4.046
A_51_P469633	DKC1	dyskeratosis congenita 1, dyskerin	-3.432
A_52_P221554	DKFZP762E1312	hypothetical protein DKFZp762E1312	-4.316
A_52_P411003	DLG7	discs, large homolog 7 (Drosophila)	-4.325
A_52_P529073	DMTF1	cyclin D binding myb-like transcription factor 1	2.822
A_51_P392593	DMXL1	Dmx-like 1	2.866
A_52_P475653	DNAJB4	DnaJ (Hsp40) homolog, subfamily B, member 4	3.312
A_52_P630493	DNAJB6	DnaJ (Hsp40) homolog, subfamily B, member 6	-3.427
A_51_P159896	DNAJC1	DnaJ (Hsp40) homolog, subfamily C, member 1	2.714
A_52_P665436	DNAJC10	DnaJ (Hsp40) homolog, subfamily C, member 10	-2.3
A_52_P438359	DNAJC19	DnaJ (Hsp40) homolog, subfamily C, member 19	3.422
A_52_P329875	DNAJC3	DnaJ (Hsp40) homolog, subfamily C, member 3	2.93
A_51_P195573	DNMT1	DNA (cytosine-5-)-methyltransferase 1	-3.934
A_51_P373163	DOCK1	dedicator of cytokinesis 1	2.979
A_51_P393332	DOCK4	dedicator of cytokinesis 4	-3.019
A_52_P157158	DSC2	desmocollin 2	-3.085

A_52_P14686	DTL	denticleless homolog (Drosophila)	-4.537
A_51_P405766	DTYMK	deoxythymidylate kinase (thymidylate kinase)	-4.484
A_51_P418469	DUT (includes EG:1854)	dUTP pyrophosphatase	-3.417
A_52_P102783	DYNC2H1	dynein, cytoplasmic 2, heavy chain 1	2.258
A_52_P27249	DYNLT1	dynein, light chain, Tctex-type 1	4.039
A_51_P120636	E2F1	E2F transcription factor 1	-9.926
A_51_P157524	E2F4	E2F transcription factor 4, p107/p130-binding	-2.976
A_52_P374997	E2F7	E2F transcription factor 7	-8.225
A_51_P419959	EAF1	ELL associated factor 1	-2.581
A_51_P130015	ECT2	epithelial cell transforming sequence 2 oncogene	-9.953
A_52_P354520	EDEM1	ER degradation enhancer, mannosidase alpha-like 1	2.562
A_51_P475573	EDG2	endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2	3.093
A_51_P222475	EHBP1L1	EH domain binding protein 1-like 1	-2.531
A_51_P205564	EID1	EP300 interacting inhibitor of differentiation 1	-2.312
A_52_P379876	EIF2AK1	eukaryotic translation initiation factor 2-alpha kinase 1	-4.195
A_51_P424079	EIF2B4	eukaryotic translation initiation factor 2B, subunit 4 delta, 67kDa	-2.127
A_52_P314042	EIF2S3	eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa	-2.469
A_51_P341025	EIF3S12	eukaryotic translation initiation factor 3, subunit 12	-3.526
A_51_P131895	EIF4E	eukaryotic translation initiation factor 4E	-2.167
A_52_P278497	EIF4EBP3	eukaryotic translation initiation factor 4E binding protein 3	2.153
A_52_P637573	EIF4G3	eukaryotic translation initiation factor 4 gamma, 3	3.653
A_52_P50009	EIF5B	eukaryotic translation initiation factor 5B	-2.246
A_52_P568217	ENAH	enabled homolog (Drosophila)	4.095
A_51_P231499	ENPP1	ectonucleotide pyrophosphatase/phosphodiesterase 1	3.507
A_51_P506402	EPB41	erythrocyte membrane protein band 4.1 (elliptocytosis 1, RH-linked)	2.544
A_52_P73126	EPB42	erythrocyte membrane protein band 4.2	-10.245
A_52_P360595	EPS15	epidermal growth factor receptor pathway substrate 15	-2.062
A_51_P223665	ERH	enhancer of rudimentary homolog (Drosophila)	-2.049
A_52_P519882	ETF1	eukaryotic translation termination factor 1	-2.751
A_52_P628455	EWSR1	Ewing sarcoma breakpoint region 1	-2.969
A_52_P496260	EXOSC8	exosome component 8	-3.03
A_51_P416243	EXOSC9	exosome component 9	-2.386
A_51_P248067	EZH2	enhancer of zeste homolog 2 (Drosophila)	-5.289
A_51_P336833	FABP4	fatty acid binding protein 4, adipocyte	2.367
A_51_P358112	FADS1	fatty acid desaturase 1	2.025
A_52_P85292	FAM53B	family with sequence similarity 53, member B	-8.539

A_52_P556462	FANCD2	Fanconi anemia, complementation group D2	-6.398
A_52_P588483	FBLN1	fibulin 1	3.692
A_52_P451600	FBXL3	F-box and leucine-rich repeat protein 3	-3.596
A_51_P393958	FBXO5	F-box protein 5	-4.767
A_52_P1128347	FBXO7	F-box protein 7	3.931
A_52_P643001	FBXW2	F-box and WD repeat domain containing 2	-2.807
A_51_P405476	FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	2.186
A_52_P408757	FCGR2A	Fc fragment of IgG, low affinity IIa, receptor (CD32)	3.226
A_51_P130095	FCGR2B	Fc fragment of IgG, low affinity IIb, receptor (CD32)	2.426
A_51_P352924	FEM1B	fem-1 homolog b (C. elegans)	2.097
A_52_P502684	FEN1	flap structure-specific endonuclease 1	-7.775
A_51_P378589	FER1L3	fer-1-like 3, myoferlin (C. elegans)	2.316
A_51_P383489	FIGNL1	fidgetin-like 1	-12.779
A_51_P130727	FKBP11	FK506 binding protein 11, 19 kDa	2.575
A_52_P585104	FKBP4	FK506 binding protein 4, 59kDa	-3.33
A_51_P446856	FLNB	filamin B, beta (actin binding protein 278)	2.963
A_51_P398723	FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	2.362
A_51_P100856	FN1	fibronectin 1	2.439
A_51_P398525	FN3K	fructosamine 3 kinase	-16.405
A_52_P235241	FNTB	farnesyltransferase, CAAX box, beta	-2.441
A_52_P262219	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	2.611
A_51_P138044	FOXO1A	forkhead box O1A	2.497
AI849135	FOXO3A (includes EG:2309)	forkhead box O3A	-4.805
A_52_P604022	FOXP1	forkhead box P1	3.183
A_51_P119633	FRAT1	frequently rearranged in advanced T-cell lymphomas	-2.188
A_52_P478532	FUSIP1	FUS interacting protein (serine/arginine-rich) 1	-6.211
A_51_P279389	FUT8	fucosyltransferase 8 (alpha (1,6) fucosyltransferase)	3.884
A_52_P486322	FZR1	fizzy/cell division cycle 20 related 1 (Drosophila)	-2.446
A_51_P282538	GAD1	glutamate decarboxylase 1 (brain, 67kDa)	-2.121
A_51_P296608	GADD45A	growth arrest and DNA-damage-inducible, alpha	-6.677
A_52_P85020	GADD45GIP1	growth arrest and DNA-damage-inducible, gamma interacting protein 1	-2.051
A_51_P343569	GALNT10	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 10 (GalNAc-T10)	-2.861
A_52_P589321	GAPDH (includes EG:14433)	glyceraldehyde-3-phosphate dehydrogenase	2.853
A_51_P157083	GAS1	growth arrest-specific 1	5.167

A_52_P233441	GATA2	GATA binding protein 2	2.097
A_51_P297579	GCH1	GTP cyclohydrolase 1 (dopa-responsive dystonia)	-11.807
A_51_P365019	GCLC	glutamate-cysteine ligase, catalytic subunit	-4.027
A_51_P119670	GCNT1	glucosaminyl (N-acetyl) transferase 1, core 2 (beta-1,6-N-acetylglucosaminyltransferase)	-2.074
A_52_P52303	GFAP	glial fibrillary acidic protein	-44.07
A_51_P349988	GGTA1	glycoprotein, alpha-galactosyltransferase 1	2.103
A_52_P680935	GHR	growth hormone receptor	10.054
A_51_P337083	GINS1	GINS complex subunit 1 (Psf1 homolog)	-8.114
A_51_P479865	GLB1L	galactosidase, beta 1-like	2.454
A_52_P670745	GLO1	glyoxalase I	-2.277
A_51_P365489	GLS	glutaminase	3.225
A_51_P208580	GLTSCR2	glioma tumor suppressor candidate region gene 2	-2.181
A_52_P101279	GLUL	glutamate-ammonia ligase (glutamine synthetase)	-2.231
A_52_P570738	GMEB2	glucocorticoid modulatory element binding protein 2	-2.568
A_52_P492176	GMFB	glia maturation factor, beta	-4.095
A_51_P166155	GMNN	geminin, DNA replication inhibitor	-3.902
A_52_P47255	GMPS	guanine monophosphate synthetase	-2.657
A_51_P311659	GNAQ	guanine nucleotide binding protein (G protein), q polypeptide	3.559
A_51_P217047	GNG10	guanine nucleotide binding protein (G protein), gamma 10	-2.246
A_51_P237166	GNG5	guanine nucleotide binding protein (G protein), gamma 5	3.948
A_51_P102809	GNL1	guanine nucleotide binding protein-like 1	-4.383
A_51_P491017	GNPTG	N-acetylglucosamine-1-phosphate transferase, gamma subunit	9.999
A_52_P140497	GOLGA3	golgi autoantigen, golgin subfamily a, 3	2.722
A_52_P467096	GOLGA4	golgi autoantigen, golgin subfamily a, 4	2.33
A_51_P173197	GORASP2	golgi reassembly stacking protein 2, 55kDa	2.95
A_52_P536383	GPBP1	GC-rich promoter binding protein 1	4.506
A_52_P216613	GPR18	G protein-coupled receptor 18	2.29
A_52_P6045	GPSM2	G-protein signalling modulator 2 (AGS3-like, C. elegans)	-13.871
A_51_P221008	GRAMD1B	GRAM domain containing 1B	3.236
A_52_P619738	GRAMD3	GRAM domain containing 3	2.829
A_51_P186374	GRAP2	GRB2-related adaptor protein 2	-6.792
A_52_P40363	GRB10	growth factor receptor-bound protein 10	2.05
A_52_P272364	GRIA3	glutamate receptor, ionotropic, AMPA 3	2.277
A_51_P152873	GRIPAP1	GRIP1 associated protein 1	-2.439
A_52_P306792	GRWD1	glutamate-rich WD repeat containing 1	-2.85
A_51_P503625	GSTA3	glutathione S-transferase A3	5.662

A_51_P260169	GSTM3 (includes EG:2947)	glutathione S-transferase M3 (brain)	-3.591
A_51_P479311	GSTM5	glutathione S-transferase M5	5.939
A_51_P320377	GTF2A2	general transcription factor IIA, 2, 12kDa	-3.34
A_51_P381522	GTF2IRD1	GTF2I repeat domain containing 1	2.578
A_52_P493965	GUCY1A3	guanylate cyclase 1, soluble, alpha 3	6.102
A_52_P77080	GYPA	glycophorin A (MNS blood group)	-11.611
A_51_P191199	GYPC	glycophorin C (Gerbich blood group)	-6.101
A_51_P245275	H2AFX	H2A histone family, member X	-3.288
A_52_P76734	H3F3A	H3 histone, family 3A	-2.702
A_51_P125368	HARS	histidyl-tRNA synthetase	-3.815
A_52_P278538	HBA2	hemoglobin, alpha 2	-3.014
A_51_P374468	HBD	hemoglobin, delta	-3.873
A_51_P179672	HBG1	hemoglobin, gamma A	-2.064
A_52_P207261	HBP1	HMG-box transcription factor 1	4.534
A_51_P116007	HDAC2	histone deacetylase 2	-3.965
A_52_P339791	HDGF	hepatoma-derived growth factor (high-mobility group protein 1-like)	-3.532
A_52_P545066	HDGFRP3	hepatoma-derived growth factor, related protein 3	3.668
A_51_P416585	HDLBP	high density lipoprotein binding protein (vigilin)	2.089
A_51_P376347	HEBP1	heme binding protein 1	-2.328
A_51_P129160	HECTD1	HECT domain containing 1	2.504
A_52_P268720	HELZ	helicase with zinc finger	2.261
A_52_P311276	HEMGN	hemogen	-32.651
A_51_P173957	HERC1	hect (homologous to the E6-AP (UBE3A) carboxyl terminus) domain and RCC1 (CHC1)-like domain (RLD) 1	-2.854
A_52_P679860	HERC5	hect domain and RLD 5	-3.914
A_51_P304076	HIF1AN	hypoxia-inducible factor 1, alpha subunit inhibitor	-3.304
AK020117	HIST1H1B	histone cluster 1, H1b	-2.983
A_51_P501260	HIST1H1D	histone cluster 1, H1d	-6.961
A_52_P651235	HIST1H2AB	histone cluster 1, H2ab	-6.061
A_52_P498210	HIST1H2AE	histone cluster 1, H2ae	-6.201
A_51_P426975	HIST2H4	histone cluster 2, H4	-2.632
A_52_P479599	HK1	hexokinase 1	-3.39
A_52_P341161	HM13	histocompatibility (minor) 13	6.967
A_51_P155361	HMBS	hydroxymethylbilane synthase	-8.601
A_52_P323044	HMGB1	high-mobility group box 1	-4.235
A_51_P499940	HMGB2	high-mobility group box 2	-8.617

A_51_P138548	HMGB3	high-mobility group box 3	-4.716
A_52_P137371	HMGCR	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	-3.432
A_52_P149864	HMGN2	high-mobility group nucleosomal binding domain 2	-2.069
A_52_P9656	HMMR	hyaluronan-mediated motility receptor (RHAMM)	-2.616
A_51_P419981	HNRPA2B1	heterogeneous nuclear ribonucleoprotein A2/B1	-2.415
A_52_P339966	HNRPA3	heterogeneous nuclear ribonucleoprotein A3	-2.433
A_52_P596080	HNRPC	heterogeneous nuclear ribonucleoprotein C (C1/C2)	-4.507
A_52_P535623	HOOK1	hook homolog 1 (Drosophila)	2.654
A_52_P15300	HOXB2	homeobox B2	2.879
A_51_P232384	HRBL	HIV-1 Rev binding protein-like	-3.495
A_52_P599317	HS6ST2	heparan sulfate 6-O-sulfotransferase 2	2.305
A_51_P117952	HSPA1A	heat shock 70kDa protein 1A	7.299
A_52_P1172972	HSPD1	heat shock 60kDa protein 1 (chaperonin)	-4.13
A_52_P144173	HYAL1	hyaluronoglucosaminidase 1	-2.371
A_51_P453142	IBTK	inhibitor of Bruton agammaglobulinemia tyrosine kinase	-5.799
AF296283	ICAM4	intercellular adhesion molecule 4 (Landsteiner-Wiener blood group)	-2.832
AF225918	ICK	intestinal cell (MAK-like) kinase	2.002
A_52_P533809	ID1	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	2.331
A_52_P441634	IDI1	isopentenyl-diphosphate delta isomerase 1	-2.807
A_52_P435154	IFI16	interferon, gamma-inducible protein 16	4.251
A_52_P134762	IFI203	interferon activated gene 203	5.989
A_52_P542388	IFIT2	interferon-induced protein with tetratricopeptide repeats 2	-3.46
A_52_P322006	IFRD2	interferon-related developmental regulator 2	-4.801
A_52_P634111	IGF1R	insulin-like growth factor 1 receptor	11.675
A_52_P851352	IGFBP3	insulin-like growth factor binding protein 3	5.367
A_51_P472292	IGFBP7	insulin-like growth factor binding protein 7	11.345
A_51_P155336	IGH	immunoglobulin heavy chain complex	4.92
A_51_P311235	IGH-1A	immunoglobulin heavy chain 1a (serum IgG2a)	4.063
A_51_P391836	IGHA1	immunoglobulin heavy constant alpha 1	6.152
A_51_P461067	IGHG1	immunoglobulin heavy constant gamma 1 (G1m marker)	5.817
A_52_P311042	IGHM	immunoglobulin heavy constant mu	12.605
A_51_P327632	IGSF3	immunoglobulin superfamily, member 3	-3.268
A_51_P112355	IGTP	interferon gamma induced GTPase	-3.236
A_51_P344507	IL10RB	interleukin 10 receptor, beta	3.431
A_52_P558502	IL16	interleukin 16 (lymphocyte chemoattractant factor)	3.853
A_51_P348280	IL17RA	interleukin 17 receptor A	-2.462

A_52_P12243	IL17RD	interleukin 17 receptor D	3.806
A_52_P467814	IL1RAP	interleukin 1 receptor accessory protein	4.371
A_51_P339793	IL1RL1	interleukin 1 receptor-like 1	-5.677
A_52_P177054	IL22	interleukin 22	3.566
A_51_P118945	IL2RA	interleukin 2 receptor, alpha	2.888
A_51_P153624	IMPG2	interphotoreceptor matrix proteoglycan 2	-3.053
A_51_P389597	INS	insulin	2.57
A_52_P644273	IRAK3	interleukin-1 receptor-associated kinase 3	2.29
A_52_P354823	IRF8	interferon regulatory factor 8	2.898
A_52_P463936	ISG15	ISG15 ubiquitin-like modifier	-3.238
A_51_P309920	ITGA8 (includes EG:8516)	integrin, alpha 8	4.68
A_51_P352076	ITGAE	integrin, alpha E (antigen CD103, human mucosal lymphocyte antigen 1; alpha polypeptide)	2.751
A_52_P371949	ITGB4BP	integrin beta 4 binding protein	2.094
A_51_P500051	ITPR2	inositol 1,4,5-triphosphate receptor, type 2	3.147
A_51_P104897	ITPR3	inositol 1,4,5-triphosphate receptor, type 3	2.881
A_52_P342880	ITSN1	intersectin 1 (SH3 domain protein)	-6.334
A_52_P67794	ITSN2	intersectin 2	3.254
A_52_P501805	IWS1	IWS1 homolog (S. cerevisiae)	-3.229
A_51_P355360	JAK3	Janus kinase 3 (a protein tyrosine kinase, leukocyte)	2.232
A_51_P113865	JAKMIP1	janus kinase and microtubule interacting protein 1	2.942
A_51_P418375	JAM2	junctional adhesion molecule 2	4.444
A_52_P42459	JMY	junction-mediating and regulatory protein	-2.836
A_52_P225898	KCNJ8	potassium inwardly-rectifying channel, subfamily J, member 8	3.928
A_51_P437847	KCTD1	potassium channel tetramerisation domain containing 1	4.149
A_51_P299062	KEL	Kell blood group, metallo-endorpeptidase	-7.147
A_51_P268010	KHDRBS1	KH domain containing, RNA binding, signal transduction associated 1	-2.268
A_52_P165001	KIAA0310	KIAA0310	3.389
A_51_P124467	KIAA0999	KIAA0999 protein	2.293
A_52_P151465	KIAA0999	KIAA0999 protein	2.172
A_52_P636608	KIAA1276	KIAA1276 protein	4.9
A_51_P167551	KIAA1370	KIAA1370	5.195
A_52_P584374	KIAA1524	KIAA1524	-7.707
A_52_P177802	KIF11	kinesin family member 11	-14.076
A_52_P670275	KIF14	kinesin family member 14	-6.105
A_51_P133138	KIF20A	kinesin family member 20A	-2.158
A_51_P493467	KIF22	kinesin family member 22	-4.161

A_51_P153734	KIF23	kinesin family member 23	-19.985
A_51_P324287	KIF23	kinesin family member 23	-4.458
A_51_P254805	KIF4A	kinesin family member 4A	-7.014
A_51_P137433	KIFC1	kinesin family member C1	-10.732
A_51_P168630	KLF1	Kruppel-like factor 1 (erythroid)	-6.634
A_51_P405280	KLF7	Kruppel-like factor 7 (ubiquitous)	4.058
A_51_P341571	KLHDC2	kelch domain containing 2	-3.662
A_51_P245414	KLK1 (includes EG:16612)	kallikrein 1	3.577
A_51_P395309	KLK1B5	kallikrein 1-related peptidase b5	11.413
A_51_P474643	KPNA2	karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	-3.833
A_51_P240768	KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	-2.668
A_51_P191865	LAMA2	laminin, alpha 2 (merosin, congenital muscular dystrophy)	5.287
A_52_P572284	LAPTM4A	lysosomal-associated protein transmembrane 4 alpha	2.976
A_51_P401668	LAPTM5	lysosomal associated multispinning membrane protein 5	2.867
A_51_P133349	LATS2	LATS, large tumor suppressor, homolog 2 (Drosophila)	2.759
A_51_P201137	LBR	lamin B receptor	-7.08
A_51_P384756	LDB1	LIM domain binding 1	-2.604
A_51_P497100	LGALS4	lectin, galactoside-binding, soluble, 4 (galectin 4)	3.035
A_52_P579352	LIFR	leukemia inhibitory factor receptor alpha	3.819
A_51_P285446	LIG1	ligase I, DNA, ATP-dependent	-3.751
A_52_P506407	LILRB3	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	4.891
A_52_P488640	LIMA1	LIM domain and actin binding 1	4.135
A_52_P572218	LIN9	lin-9 homolog (C. elegans)	-4.858
A_52_P330984	LIN9	lin-9 homolog (C. elegans)	-3.502
A_52_P652513	LMAN1	lectin, mannose-binding, 1	3.481
A_51_P313561	LMNA	lamin A/C	-7.843
A_52_P330314	LMO4	LIM domain only 4	3.166
A_52_P186937	LOC129607	hypothetical protein LOC129607	-5.322
A_51_P378602	LOC340571	seven in absentia homolog 1 (Drosophila) pseudogene	-3.66
A_51_P306067	LPP	LIM domain containing preferred translocation partner in lipoma	2.498
A_51_P121236	LRDD	leucine-rich repeats and death domain containing	-2.482
A_51_P392303	LRRN3	leucine rich repeat neuronal 3	4.557
A_51_P265495	LY6A	lymphocyte antigen 6 complex, locus A	3.277
A_52_P201655	LYK5	protein kinase LYK5	2.99
A_51_P150722	LYST	lysosomal trafficking regulator	3.253
A_52_P238027	LYZ	lysozyme (renal amyloidosis)	2.359

A_51_P230873	MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	-4.575
A_51_P379230	MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)	4.845
A_52_P240754	MAFG	v-maf musculoaponeurotic fibrosarcoma oncogene homolog G (avian)	-2.401
A_52_P171019	MAN1A1	mannosidase, alpha, class 1A, member 1	3.946
A_51_P336161	MAN2B1	mannosidase, alpha, class 2B, member 1	3.315
A_51_P170807	MAP3K6	mitogen-activated protein kinase kinase kinase 6	3.008
A_52_P320044	MAP4K3	mitogen-activated protein kinase kinase kinase kinase 3	2.321
A_51_P162612	MAP4K5	mitogen-activated protein kinase kinase kinase kinase 5	-4.925
A_52_P252258	MAPK12	mitogen-activated protein kinase 12	2.619
A_51_P359018	MAPK7	mitogen-activated protein kinase 7	2.34
A_51_P140576	MAPT	microtubule-associated protein tau	2.73
A_52_P210011	MARCH2	membrane-associated ring finger (C3HC4) 2	-8.036
A_51_P405668	MARCH5	membrane-associated ring finger (C3HC4) 5	-2.042
A_51_P200586	MARCH8	membrane-associated ring finger (C3HC4) 8	-6.992
A_51_P189269	MBNL2	muscleblind-like 2 (Drosophila)	2.716
A_51_P433281	MBOAT2	membrane bound O-acyltransferase domain containing 2	-7.184
A_52_P530405	MBTPS2	membrane-bound transcription factor peptidase, site 2	2.271
A_51_P268439	MCAM	melanoma cell adhesion molecule	-2.902
A_52_P364776	MCM3	minichromosome maintenance complex component 3	-7.19
A_51_P356762	MCM4	minichromosome maintenance complex component 4	-2.889
A_51_P190111	MCM5	minichromosome maintenance complex component 5	-2.628
A_52_P197666	MED12	mediator of RNA polymerase II transcription, subunit 12 homolog (S. cerevisiae)	2.346
A_52_P453577	MED19	mediator of RNA polymerase II transcription, subunit 19 homolog (S. cerevisiae)	-2.7
A_52_P175157	MEF2A	myocyte enhancer factor 2A	2.659
A_51_P135802	MEF2C	myocyte enhancer factor 2C	3.251
A_52_P670612	MEIS1	Meis homeobox 1	4.273
A_52_P277641	MEIS2	Meis homeobox 2	7.966
A_52_P674414	MKLN1	muskelin 1, intracellular mediator containing kelch motifs	-2.178
A_52_P246082	MKNK1	MAP kinase interacting serine/threonine kinase 1	5.416
A_51_P368660	MPHOSPH10	M-phase phosphoprotein 10 (U3 small nucleolar ribonucleoprotein)	-2.16
A_52_P212429	MR1	major histocompatibility complex, class I-related	3.392
A_51_P160913	MR1	major histocompatibility complex, class I-related	2.371
A_52_P256929	MRAS	muscle RAS oncogene homolog	2.438
A_51_P338542	MRPS23	mitochondrial ribosomal protein S23	-2.667
A_52_P121491	MSRB3	methionine sulfoxide reductase B3	3.034

A_52_P282279	MTHFD1	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1, methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthetase	-3.359
A_52_P413947	MTHFR	5,10-methylenetetrahydrofolate reductase (NADPH)	2.096
A_51_P408310	MTR	5-methyltetrahydrofolate-homocysteine methyltransferase	-3.467
A_52_P538447	MUTED	mutated homolog (mouse)	-3.583
A_51_P257619	MUTYH	mutY homolog (E. coli)	2.314
A_52_P190647	MXD3	MAX dimerization protein 3	-7.529
A_52_P83704	MYB	v-myb myeloblastosis viral oncogene homolog (avian)	2.132
A_51_P281844	MYEF2	myelin expression factor 2	-2.416
A_52_P680681	MYH10	myosin, heavy chain 10, non-muscle	-4.763
A_52_P635898	MYO5A	myosin VA (heavy chain 12, myoxin)	3.283
A_52_P564166	MYOM1	myomesin 1, 185kDa	4.957
A_51_P140350	NADSYN1	NAD synthetase 1	2.297
A_52_P231232	NANOS1	nanos homolog 1 (Drosophila)	-4.16
A_52_P339054	NAP1L4	nucleosome assembly protein 1-like 4	-4.34
A_51_P335981	NARF	nuclear prelamin A recognition factor	-4.77
A_51_P467889	NAT9	N-acetyltransferase 9	-2.502
A_52_P236671	NBEA	neurobeachin	4.18
A_51_P380309	NCAM1	neural cell adhesion molecule 1	2.985
A_52_P139399	NCAPD2	non-SMC condensin I complex, subunit D2	-5.945
A_51_P424810	NCAPG2	non-SMC condensin II complex, subunit G2	-3.672
A_52_P510852	NCBP1 (includes EG:4686)	nuclear cap binding protein subunit 1, 80kDa	-2.963
AK077841	NCBP2	nuclear cap binding protein subunit 2, 20kDa	4.567
A_51_P134007	NCL	nucleolin	-3.165
A_51_P502888	NCOA1	nuclear receptor coactivator 1	2.398
A_51_P151983	NCOA4	nuclear receptor coactivator 4	-3.328
A_52_P311903	NCOA7	nuclear receptor coactivator 7	-2.88
A_52_P561934	NCOR1	nuclear receptor co-repressor 1	3.525
A_51_P286814	NCOR2	nuclear receptor co-repressor 2	3.01
A_51_P191649	NDC80	NDC80 homolog, kinetochore complex component (S. cerevisiae)	-8.774
A_52_P13436	NDUFA11 (includes EG:69875)	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 11	-3.335
A_52_P327927	NDUFB2	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2, 8kDa	2.471
A_52_P298112	NEBL	nebulin	3.986
A_52_P27103	NECAP2	NECAP endocytosis associated 2	-2.135
A_52_P544878	NEDD4L	neural precursor cell expressed, developmentally down-regulated 4-like	2.879
A_52_P70479	NEK1	NIMA (never in mitosis gene a)-related kinase 1	4.842

AF007247	NEK2	NIMA (never in mitosis gene a)-related kinase 2	-2.612
A_52_P58524	NEU1	sialidase 1 (lysosomal sialidase)	2.326
A_52_P608444	NFAT5	nuclear factor of activated T-cells 5, tonicity-responsive	3.936
A_51_P515965	NFE2	nuclear factor (erythroid-derived 2), 45kDa	-5.418
A_52_P641684	NFIA	nuclear factor I/A	-2.781
A_51_P304412	NFIB	nuclear factor I/B	4.847
A_51_P477418	NFIC	nuclear factor I/C (CCAAT-binding transcription factor)	2.817
A_52_P239707	NFYB	nuclear transcription factor Y, beta	-2.355
A_51_P355416	NISCH	nischarin	2.599
A_52_P395228	NNT	nicotinamide nucleotide transhydrogenase	2.941
A_51_P508510	NOTCH1	Notch homolog 1, translocation-associated (Drosophila)	2.292
A_51_P488888	NP	nucleoside phosphorylase	-6.349
A_51_P382347	NPHP3	nephronophthisis 3 (adolescent)	2.719
A_52_P598895	NPNT	nephronectin	2.002
A_51_P426195	NPPB	natriuretic peptide precursor B	3.828
A_51_P147684	NR2F2	nuclear receptor subfamily 2, group F, member 2	5.288
A_52_P566348	NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog	-2.535
A_52_P8430	NRF1	nuclear respiratory factor 1	-5.947
A_51_P229547	NRXN1	neurexin 1	6.613
A_52_P498608	NSBP1	nucleosomal binding protein 1	-5.215
A_52_P297803	NSDHL	NAD(P) dependent steroid dehydrogenase-like	4.218
A_51_P397176	NSL1	NSL1, MIND kinetochore complex component, homolog (S. cerevisiae)	-2.854
A_51_P255360	NT5C3	5'-nucleotidase, cytosolic III	-3.494
A_52_P1126526	NUCKS1	nuclear casein kinase and cyclin-dependent kinase substrate 1	-3.036
A_52_P305246	NUDT4	nudix (nucleoside diphosphate linked moiety X)-type motif 4	-8.566
A_52_P398432	NUDT5	nudix (nucleoside diphosphate linked moiety X)-type motif 5	-2.644
A_51_P288447	NUP133	nucleoporin 133kDa	-2.273
A_51_P420037	NUP210	nucleoporin 210kDa	-2.218
A_51_P411296	NUP50	nucleoporin 50kDa	-3.671
A_51_P520794	NUPL1	nucleoporin like 1	-2.925
A_52_P184398	NXT1	NTF2-like export factor 1	-2.681
A_51_P472867	OAS3	2'-5'-oligoadenylate synthetase 3, 100kDa	-3.183
A_52_P461292	OAZ1	ornithine decarboxylase antizyme 1	-2.744
A_52_P436238	ODC1	ornithine decarboxylase 1	-6.415
A_52_P618197	ODF2	outer dense fiber of sperm tails 2	-2.457
A_52_P272217	OGFR	opioid growth factor receptor	-3.101

A_52_P656545	OGT	O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase)	2.116
A_51_P356931	ORC1L	origin recognition complex, subunit 1-like (yeast)	-7.349
A_51_P296416	ORC6L	origin recognition complex, subunit 6 like (yeast)	-2.391
A_51_P221460	ORMDL3	ORM1-like 3 (<i>S. cerevisiae</i>)	-2.957
A_52_P370878	OSBPL8	oxysterol binding protein-like 8	-2.778
A_52_P229981	OSTALPHA	organic solute transporter alpha	-7.331
A_51_P326709	OTUD7B	OTU domain containing 7B	-3.959
A_51_P189959	OXSRI	oxidative-stress responsive 1	-2.345
A_51_P234728	P2RY5	purinergic receptor P2Y, G-protein coupled, 5	3.363
A_52_P601148	P2RY5	purinergic receptor P2Y, G-protein coupled, 5	3.089
A_52_P393396	PABPC4	poly(A) binding protein, cytoplasmic 4 (inducible form)	-7.784
A_51_P452153	PACAP	hypothetical protein MGC29506	2.798
A_51_P461844	PAFAH1B2	platelet-activating factor acetylhydrolase, isoform Ib, beta subunit 30kDa	-2.848
A_51_P373814	PANK1	pantothenate kinase 1	-2.266
A_51_P198645	PARVB	parvin, beta	-3.636
A_51_P230098	PBK	PDZ binding kinase	-6.026
A_51_P396351	PCNA	proliferating cell nuclear antigen	-4.967
A_51_P176487	PCNX	pecanex homolog (<i>Drosophila</i>)	2.115
A_51_P294705	PCYT1A	phosphate cytidylyltransferase 1, choline, alpha	-4.457
A_51_P178435	PDCD5	programmed cell death 5	-2.85
A_52_P526565	PDE1A	phosphodiesterase 1A, calmodulin-dependent	3.766
A_51_P120990	PDE4D	phosphodiesterase 4D, cAMP-specific (phosphodiesterase E3 dunce homolog, <i>Drosophila</i>)	-8.193
A_51_P157677	PDLIM3	PDZ and LIM domain 3	4.352
A_52_P359502	PDXK	pyridoxal (pyridoxine, vitamin B6) kinase	2.141
A_51_P491329	PDZK1IP1	PDZK1 interacting protein 1	-7.182
A_51_P102987	PENK	proenkephalin	6.51
A_52_P666257	PEX11B	peroxisomal biogenesis factor 11B	3.993
A_52_P456059	PFDN1	prefoldin subunit 1	-2.582
A_51_P184849	PFKFB2	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2	-2.215
A_51_P117995	PFKM	phosphofructokinase, muscle	-3.026
A_52_P510119	PGM2L1	phosphoglucomutase 2-like 1	-3.958
A_52_P48814	PHF20	PHD finger protein 20	2.483
A_51_P471779	PHF5A	PHD finger protein 5A	-2.85
A_52_P153700	PHLDB2	pleckstrin homology-like domain, family B, member 2	3.95
A_52_P353567	PI4K2B	phosphatidylinositol 4-kinase type 2 beta	-6.412

A_51_P183400	PICALM	phosphatidylinositol binding clathrin assembly protein	-2.057
A_51_P458839	PIGA	phosphatidylinositol glycan anchor biosynthesis, class A (paroxysmal nocturnal hemoglobinuria)	-4.35
A_52_P487483	PIGQ	phosphatidylinositol glycan anchor biosynthesis, class Q	-5.849
A_52_P344152	PIK3IP1	phosphoinositide-3-kinase interacting protein 1	3.741
A_51_P511236	PIK3R1	phosphoinositide-3-kinase, regulatory subunit 1 (p85 alpha)	-2.307
A_52_P454994	PIK4CA	phosphatidylinositol 4-kinase, catalytic, alpha polypeptide	2.512
A_52_P271910	PIP5K1C	phosphatidylinositol-4-phosphate 5-kinase, type I, gamma	4.119
A_51_P386612	PIR	pirin (iron-binding nuclear protein)	-7.8
A_52_P491933	PKIB	protein kinase (cAMP-dependent, catalytic) inhibitor beta	2.454
A_52_P290629	PKN2	protein kinase N2	-3.484
A_51_P207550	PLA2G12A	phospholipase A2, group XIIA	-3.082
A_52_P268662	PLA2R1	phospholipase A2 receptor 1, 180kDa	6.092
A_52_P409833	PLAT	plasminogen activator, tissue	5.211
A_52_P1100772	PLEC1	plectin 1, intermediate filament binding protein 500kDa	2.1
A_52_P27225	PLEC1	plectin 1, intermediate filament binding protein 500kDa	-2.919
A_51_P500869	PLEKHA5	pleckstrin homology domain containing, family A member 5	3.133
A_51_P135832	PLEKHC1	pleckstrin homology domain containing, family C (with FERM domain) member 1	3.04
A_52_P668285	PLK4	polo-like kinase 4 (Drosophila)	-6.91
A_51_P243532	PLP2	proteolipid protein 2 (colonic epithelium-enriched)	2.187
A_51_P251022	PLSCR2	phospholipid scramblase 2	3.578
A_51_P492087	POLE3	polymerase (DNA directed), epsilon 3 (p17 subunit)	-3.13
A_52_P411716	POLH	polymerase (DNA directed), eta	-4.324
A_52_P276222	POLR2F	polymerase (RNA) II (DNA directed) polypeptide F	-2.818
A_52_P405090	POLR3G	polymerase (RNA) III (DNA directed) polypeptide G (32kD)	-2.782
A_51_P256665	PON3	paraoxonase 3	2.581
A_51_P433026	PPAPDC2	phosphatidic acid phosphatase type 2 domain containing 2	2.812
A_51_P230439	PPFIBP2	PTPRF interacting protein, binding protein 2 (liprin beta 2)	3.258
A_52_P402175	PPM1B	protein phosphatase 1B (formerly 2C), magnesium-dependent, beta isoform	-3.247
A_52_P471967	PPM1L	protein phosphatase 1 (formerly 2C)-like	-4.812
A_51_P302503	PPP1R15A (includes EG:23645)	protein phosphatase 1, regulatory (inhibitor) subunit 15A	-5.476
A_51_P486132	PPP1R8	protein phosphatase 1, regulatory (inhibitor) subunit 8	-2.202
A_51_P270960	PPP2R3A (includes EG:5523)	protein phosphatase 2 (formerly 2A), regulatory subunit B", alpha	9.165
A_51_P245405	PPP3CC	protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform	5.556
A_52_P473866	PPT1	palmitoyl-protein thioesterase 1 (ceroid-lipofuscinosis, neuronal 1, infantile)	2.66

A_52_P96939	PQLC1	PQ loop repeat containing 1	-4.832
A_51_P332652	PQLC3	PQ loop repeat containing 3	5.342
A_52_P262118	PRDM15	PR domain containing 15	2.006
A_52_P550034	PRDX2	peroxiredoxin 2	-6.966
A_51_P406604	PRIC285 (includes EG:85441)	peroxisomal proliferator-activated receptor A interacting complex 285	3.67
A_51_P221062	PRKAR2B	protein kinase, cAMP-dependent, regulatory, type II, beta	-2.671
A_52_P375455	PRKCE	protein kinase C, epsilon	3.82
A_52_P284889	PRKCZ	protein kinase C, zeta	4.77
A_51_P413965	PRKD3	protein kinase D3	3.354
A_51_P168662	PRPF8	PRP8 pre-mRNA processing factor 8 homolog (S. cerevisiae)	2.51
A_52_P33097	PRR11	proline rich 11	-2.838
A_51_P209771	PRRX1	paired related homeobox 1	6.732
A_52_P448060	PRSS3 (includes EG:5646)	protease, serine, 3 (mesotrypsin)	9.929
A_51_P488928	PSCD3	pleckstrin homology, Sec7 and coiled-coil domains 3	-2.831
A_52_P398989	PSCDBP	pleckstrin homology, Sec7 and coiled-coil domains, binding protein	2.883
A_52_P4325451	PSMA1	proteasome (prosome, macropain) subunit, alpha type, 1	-2.126
A_51_P106227	PSMA4	proteasome (prosome, macropain) subunit, alpha type, 4	-2.808
A_52_P238044	PSMC3IP	PSMC3 interacting protein	-2.699
A_52_P469512	PSMD9	proteasome (prosome, macropain) 26S subunit, non-ATPase, 9	-5.057
A_51_P185713	PTDSS2	phosphatidylserine synthase 2	-4.01
A_51_P254855	PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	-2.083
A_52_P249402	PTMA	prothymosin, alpha (gene sequence 28)	-2.239
A_52_P677117	PTPRC	protein tyrosine phosphatase, receptor type, C	2.252
A_51_P181772	PTPRE	protein tyrosine phosphatase, receptor type, E	2.427
A_52_P148883	PTPRF	protein tyrosine phosphatase, receptor type, F	3.834
A_51_P443976	PTPRR	protein tyrosine phosphatase, receptor type, R	3.355
A_52_P628590	PVR	poliovirus receptor	2.641
A_51_P125009	PVRL3	poliovirus receptor-related 3	4.652
A_51_P503896	PYCR1	pyrroline-5-carboxylate reductase 1	2.715
A_52_P389310	RAB11A	RAB11A, member RAS oncogene family	-2.837
A_52_P410609	RAB31	RAB31, member RAS oncogene family	-2.192
A_52_P362161	RAB3B	RAB3B, member RAS oncogene family	4.394
A_51_P276418	RAB6B	RAB6B, member RAS oncogene family	7.658
A_51_P314763	RABGGTB	Rab geranylgeranyltransferase, beta subunit	-4.171
A_52_P488220	RAC1	ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)	-2.046

A_52_P298465	RACGAP1	Rac GTPase activating protein 1	-13.461
A_51_P480855	RAD18	RAD18 homolog (S. cerevisiae)	-3.635
A_52_P39237	RAD21	RAD21 homolog (S. pombe)	-3.794
A_52_P313728	RAD54B	RAD54 homolog B (S. cerevisiae)	-4.104
A_52_P259521	RAG2	recombination activating gene 2	4.496
A_51_P190106	RALGPS2	Ral GEF with PH domain and SH3 binding motif 2	2.69
A_51_P100227	RANBP5	RAN binding protein 5	-3.709
A_51_P487108	RANGAP1	Ran GTPase activating protein 1	-5.638
A_51_P116906	RAPGEF3	Rap guanine nucleotide exchange factor (GEF) 3	4.075
A_51_P516860	RAPGEF5	Rap guanine nucleotide exchange factor (GEF) 5	6.063
A_52_P466147	RARRES2	retinoic acid receptor responder (tazarotene induced) 2	4.318
A_52_P392485	RASA2	RAS p21 protein activator 2	3.602
A_52_P419873	RB1	retinoblastoma 1 (including osteosarcoma)	-4.662
A_52_P424767	RBBP4	retinoblastoma binding protein 4	-4.748
A_52_P557940	RBM4B	RNA binding motif protein 4B	3.478
A_52_P355014	RBM8A	RNA binding motif protein 8A	-3.902
A_52_P144263	RBMS1	RNA binding motif, single stranded interacting protein 1	4.648
A_52_P141404	RCOR3	REST corepressor 3	-3.31
A_52_P571684	RDX	radixin	-3.636
A_51_P365369	RELN	reelin	-4.177
A_51_P378450	RFC4	replication factor C (activator 1) 4, 37kDa	-3.449
A_52_P399175	RFFL	ring finger and FYVE-like domain containing 1	-3.59
A_51_P241769	RHD	Rh blood group, D antigen	-25.807
A_51_P452953	RHOD	ras homolog gene family, member D	3.455
A_51_P489289	RHOH	ras homolog gene family, member H	2.856
A_52_P592466	RHOJ	ras homolog gene family, member J	4.973
A_51_P305693	RIF1	RAP1 interacting factor homolog (yeast)	-2.375
A_52_P249798	RNASE1 (includes EG:6035)	ribonuclease, RNase A family, 1 (pancreatic)	9.107
A_51_P237383	RNASE4	ribonuclease, RNase A family, 4	6.194
A_51_P424128	RNASEH2A	ribonuclease H2, subunit A	-2.795
A_51_P251639	RNF123	ring finger protein 123	-4.053
A_52_P384079	RNF139	ring finger protein 139	-8.086
A_52_P674694	RNF141	ring finger protein 141	-2.57
A_52_P338833	RNF19	ring finger protein 19	-2.903
A_52_P580483	RNF8	ring finger protein 8	-3.745
A_52_P312563	RNMT	RNA (guanine-7-) methyltransferase	-4.098

A_51_P481494	ROBO3	roundabout, axon guidance receptor, homolog 3 (Drosophila)	3.235
A_52_P152428	ROCK1	Rho-associated, coiled-coil containing protein kinase 1	-2.429
A_52_P652442	RORA	RAR-related orphan receptor A	2.834
A_51_P333159	RPA1	replication protein A1, 70kDa	-3.252
A_51_P234303	RPA2	replication protein A2, 32kDa	-4.638
A_51_P438057	RPA3	replication protein A3, 14kDa	-4.459
K02928	RPL30	ribosomal protein L30	-3.361
A_51_P509107	RPL35A	ribosomal protein L35a	-2.714
X73331	RPL37A	ribosomal protein L37a	-4.12
A_51_P496480	RPL7	ribosomal protein L7	-3.853
A_52_P415440	RPS12	ribosomal protein S12	-2.496
A_52_P49886	RPS3A	ribosomal protein S3A	-3.524
A_51_P439092	RPSA	ribosomal protein SA	-2.726
A_51_P164686	RRBP1	ribosome binding protein 1 homolog 180kDa (dog)	3.139
A_51_P502082	RRM1	ribonucleotide reductase M1 polypeptide	-4.735
A_51_P110689	RRM2	ribonucleotide reductase M2 polypeptide	-14.016
A_52_P670026	RSAD2	radical S-adenosyl methionine domain containing 2	-4.253
A_51_P230942	RUNX2	runt-related transcription factor 2	2.645
A_52_P433	SAPS3	SAPS domain family, member 3	2.426
A_52_P504771	SART1	squamous cell carcinoma antigen recognized by T cells	-2.307
A_52_P62530	SCLT1	sodium channel and clathrin linker 1	-2.727
A_51_P184331	SCN3B	sodium channel, voltage-gated, type III, beta	2.75
A_52_P512817	SCOTIN	scotin	2.951
A_52_P201245	SDSL	serine dehydratase-like	-3.296
A_52_P488427	SEC14L2	SEC14-like 2 (S. cerevisiae)	-3.684
A_51_P174743	SEC24A	SEC24 related gene family, member A (S. cerevisiae)	3.365
A_52_P489729	SEC61A1	Sec61 alpha 1 subunit (S. cerevisiae)	3.479
L25086	SEC61G	Sec61 gamma subunit	-2.497
A_52_P629487	SELENBP1	selenium binding protein 1	-2.328
A_51_P415755	SEMA6D	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6D	3.997
A_52_P5765	SENP2	SUMO1/sentrin/SMT3 specific peptidase 2	-2.769
A_52_P470128	SENP6	SUMO1/sentrin specific peptidase 6	2.136
A_52_P3591	SEPT6	septin 6	3.369
A_51_P326191	SERPINA3G	serine (or cysteine) peptidase inhibitor, clade A, member 3G	3.651
A_51_P181297	SERPINB1	serpin peptidase inhibitor, clade B (ovalbumin), member 1	2.499
A_52_P639522	SERPINB2	serpin peptidase inhibitor, clade B (ovalbumin), member 2	2.243

A_51_P376238	SERPING1	serpin peptidase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary)	7.893
A_52_P50107	SET	SET translocation (myeloid leukemia-associated)	-3.413
A_51_P201971	SETD8	SET domain containing (lysine methyltransferase) 8	-3.06
A_51_P261150	SF3B4	splicing factor 3b, subunit 4, 49kDa	3.724
A_51_P485899	SFRS1	splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)	-2.688
AK011657	SFRS3	splicing factor, arginine/serine-rich 3	-2.723
A_52_P501697	SGCB	sarcoglycan, beta (43kDa dystrophin-associated glycoprotein)	2.993
A_52_P27864	SGCE	sarcoglycan, epsilon	8.521
A_51_P487999	SGOL1	shugoshin-like 1 (S. pombe)	-5.57
A_52_P411296	SH3KBP1	SH3-domain kinase binding protein 1	2.424
A_51_P204402	SHCBP1	SHC SH2-domain binding protein 1	-2.435
A_51_P434659	SIPA1L1	signal-induced proliferation-associated 1 like 1	3.403
A_51_P141152	SIRT1	sirtuin (silent mating type information regulation 2 homolog) 1 (S. cerevisiae)	-2.625
A_51_P110068	SIRT2	sirtuin (silent mating type information regulation 2 homolog) 2 (S. cerevisiae)	3.103
A_51_P138972	SIVA1	SIVA1, apoptosis-inducing factor	-2.222
A_51_P204454	SKP2	S-phase kinase-associated protein 2 (p45)	-5.185
A_52_P358090	SLA/LP	soluble liver antigen/liver pancreas antigen	-3.094
A_52_P261262	SLAMF7	SLAM family member 7	2.38
A_51_P127615	SLBP	stem-loop (histone) binding protein	-5.101
A_52_P196476	SLC11A2	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2	-6.14
A_51_P397363	SLC16A1	solute carrier family 16, member 1 (monocarboxylic acid transporter 1)	-16.276
A_51_P448618	SLC16A10	solute carrier family 16, member 10 (aromatic amino acid transporter)	-7.831
A_51_P408631	SLC20A1	solute carrier family 20 (phosphate transporter), member 1	-2.998
A_51_P343429	SLC25A37	solute carrier family 25, member 37	-3.3
A_51_P117477	SLC27A1	solute carrier family 27 (fatty acid transporter), member 1	3.158
A_51_P261092	SLC2A9	solute carrier family 2 (facilitated glucose transporter), member 9	3.122
A_51_P300572	SLC30A1	solute carrier family 30 (zinc transporter), member 1	-3.573
A_52_P376106	SLC30A9	solute carrier family 30 (zinc transporter), member 9	-5.488
A_52_P485939	SLC39A8	solute carrier family 39 (zinc transporter), member 8	-2.661
A_51_P362483	SLC43A1	solute carrier family 43, member 1	-6.598
A_52_P529049	SLK	STE20-like kinase (yeast)	-2.65
A_52_P207203	SMARCA5	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5	-4.438
A_51_P265660	SMC2	structural maintenance of chromosomes 2	-6.851
A_52_P138002	SMC3	structural maintenance of chromosomes 3	-5.925
A_51_P143212	SMC4	structural maintenance of chromosomes 4	-3.551

A_52_P430194	SMOX	spermine oxidase	-6.792
A_52_P461552	SMU1	smu-1 suppressor of mec-8 and unc-52 homolog (C. elegans)	-2.493
A_51_P485383	SNAP23	synaptosomal-associated protein, 23kDa	-2.448
A_52_P636084	SNCA	synuclein, alpha (non A4 component of amyloid precursor)	-6.104
A_52_P550147	SNED1	sushi, nidogen and EGF-like domains 1	9.008
A_52_P501767	SNRNP70	small nuclear ribonucleoprotein 70kDa polypeptide (RNP antigen)	2.145
A_52_P214661	SNRPD1	small nuclear ribonucleoprotein D1 polypeptide 16kDa	-4.347
A_51_P363634	SNRPE	small nuclear ribonucleoprotein polypeptide E	-3.195
A_51_P255613	SNRPF	small nuclear ribonucleoprotein polypeptide F	-2.066
A_52_P3283	SNX15	sorting nexin 15	-8.395
A_51_P367381	SOD1	superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	-2.065
A_51_P226962	SOLH	small optic lobes homolog (Drosophila)	3.114
A_52_P652999	SORBS1	sorbin and SH3 domain containing 1	-7.671
A_52_P282171	SOX6	SRY (sex determining region Y)-box 6	-24.699
A_52_P214630	SOX9	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	3.581
A_51_P513530	SPAG5	sperm associated antigen 5	-8.036
A_51_P431087	SPARC	secreted protein, acidic, cysteine-rich (osteonectin)	3.084
A_51_P210510	SPARCL1	SPARC-like 1 (mast9, hevin)	5.844
A_51_P514700	SPC25	SPC25, NDC80 kinetochore complex component, homolog (S. cerevisiae)	-15.271
A_52_P168271	SPG20	spastic paraplegia 20 (Troyer syndrome)	-3.018
A_52_P526396	SPG3A	spastic paraplegia 3A (autosomal dominant)	-5.092
A_52_P400436	SPHK1	sphingosine kinase 1	-8.337
A_52_P242321	SPON1	spondin 1, extracellular matrix protein	10.2
A_51_P358765	SPP1	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	2.903
A_51_P452371	SPTB	spectrin, beta, erythrocytic (includes spherocytosis, clinical type I)	-5.92
AI448706	SPTBN1	spectrin, beta, non-erythrocytic 1	2.289
A_51_P300709	SRM	spermidine synthase	2.448
A_52_P593200	SRXN1	sulfiredoxin 1 homolog (S. cerevisiae)	-2.972
A_51_P119055	SSB	Sjogren syndrome antigen B (autoantigen La)	-2.79
A_51_P401659	SSPN	sarcospan (Kras oncogene-associated gene)	2.092
A_51_P174463	SSR4	signal sequence receptor, delta (translocon-associated protein delta)	2.454
A_52_P546090	SSR4	signal sequence receptor, delta (translocon-associated protein delta)	2.406
A_51_P291339	SSRP1	structure specific recognition protein 1	-2.647
A_52_P83913	SSX2IP	synovial sarcoma, X breakpoint 2 interacting protein	-7.234
A_52_P577819	ST6GALNAC2	ST6 (alpha-N-acetyl-neuraminy-2,3-beta-galactosyl-1,3)-N-acetylglactosaminide alpha-2,6-sialyltransferase 2	7.596

A_52_P314353	ST6GALNAC6	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 6	2.127
A_52_P676744	ST6GALNAC6	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 6	-2.803
A_51_P431047	ST8SIA3	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 3	-2.925
A_52_P640204	STAM	signal transducing adaptor molecule (SH3 domain and ITAM motif) 1	-2.232
A_51_P191520	STARD10	START domain containing 10	-6.569
A_51_P154973	STARD3	START domain containing 3	2.585
A_52_P561205	STARD4	START domain containing 4, sterol regulated	-4.996
A_52_P625624	STAU2	staufen, RNA binding protein, homolog 2 (Drosophila)	2.782
A_52_P376804	STCH	stress 70 protein chaperone, microsome-associated, 60kDa	2.06
A_52_P256844	STK19	serine/threonine kinase 19	-2.893
A_51_P332900	STK4	serine/threonine kinase 4	-2.188
A_52_P319161	STMN1	stathmin 1/oncoprotein 18	-5.708
A_52_P301503	STX2	syntaxin 2	-6.348
A_52_P876257	STX8	syntaxin 8	4.88
AI844608	SUB1	SUB1 homolog (S. cerevisiae)	2.556
J03750	SUB1	SUB1 homolog (S. cerevisiae)	-3.443
A_51_P133229	SULF2	sulfatase 2	3.246
A_51_P396708	SURB7	SRB7 suppressor of RNA polymerase B homolog (yeast)	-2.191
A_51_P510441	SUV39H1	suppressor of variegation 3-9 homolog 1 (Drosophila)	-3.109
A_52_P16931	SUV39H2	suppressor of variegation 3-9 homolog 2 (Drosophila)	-4.231
A_52_P302203	SUZ12	suppressor of zeste 12 homolog (Drosophila)	-5.48
A_51_P214796	SUZ12	suppressor of zeste 12 homolog (Drosophila)	-2.654
A_52_P647102	SVEP1	sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1	3.508
A_52_P647260	SYNPO2	synaptopodin 2	-2.538
A_52_P103929	SYT1	synaptotagmin I	2.481
A_51_P260730	SYT11	synaptotagmin XI	2.176
A_51_P340583	TAF1	TAF1 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 250kDa	-3.351
A_51_P263227	TAF5	TAF5 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 100kDa	-3.365
A_52_P520940	TAF7	TAF7 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 55kDa	-3.645
AI850390	TAF9B	TAF9B RNA polymerase II, TATA box binding protein (TBP)-associated factor, 31kDa	3.848
AI848172	TAF9B	TAF9B RNA polymerase II, TATA box binding protein (TBP)-associated factor, 31kDa	2.711
A_51_P255853	TAL1	T-cell acute lymphocytic leukemia 1	-12.116
A_52_P64924	TBPL1	TBP-like 1	-4.081

A_52_P141662	TCF3	transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)	-3.856
A_52_P424847	TCF4	transcription factor 4	6.483
A_52_P387724	TCF7L1	transcription factor 7-like 1 (T-cell specific, HMG-box)	7.152
A_51_P164030	TCP1	t-complex 1	-3.171
A_52_P348287	TCTE3	t-complex-associated-testis-expressed 3	-3.997
A_52_P658073	TEF	thyrotrophic embryonic factor	3.313
A_52_P351785	TFAM	transcription factor A, mitochondrial	-3.074
A_52_P103550	TFDP1	transcription factor Dp-1	-3.262
A_51_P136014	TFDP2	transcription factor Dp-2 (E2F dimerization partner 2)	-3.266
A_52_P228236	TFRC	transferrin receptor (p90, CD71)	-15.971
A_51_P366344	TGFB111	transforming growth factor beta 1 induced transcript 1	2.997
A_52_P243025	THOC1	THO complex 1	-3.734
A_51_P154684	THOC4	THO complex 4	-2.647
A_51_P437559	TIMELESS	timeless homolog (Drosophila)	-2.843
A_51_P336721	TIPIN	TIMELESS interacting protein	-3.668
A_51_P278686	TIRAP	toll-interleukin 1 receptor (TIR) domain containing adaptor protein	5.09
A_51_P446865	TLN1	talin 1	2.347
A_51_P452629	TLR2	toll-like receptor 2	2.024
A_51_P300806	TLR4	toll-like receptor 4	2.86
A_51_P371993	TMED10	transmembrane emp24-like trafficking protein 10 (yeast)	3.665
A_52_P467072	TMEM33	transmembrane protein 33	-7.929
A_51_P297068	TMOD1	tropomodulin 1	-6.14
A_52_P201311	TMPO	thymopoietin	-3.869
A_51_P364485	TNFAIP2	tumor necrosis factor, alpha-induced protein 2	-2.748
A_51_P230583	TNFRSF14	tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator)	-3.652
A_52_P192426	TNFRSF1A	tumor necrosis factor receptor superfamily, member 1A	3.044
A_52_P167411	TNK1	tyrosine kinase, non-receptor, 1	-3.796
A_51_P102257	TNS1	tensin 1	2.061
A_52_P156502	TOP2A	topoisomerase (DNA) II alpha 170kDa	-14.14
A_51_P325173	TPM1	tropomyosin 1 (alpha)	-2.276
A_51_P417025	TPST1	tyrosylprotein sulfotransferase 1	2.91
A_51_P369200	TPX2	TPX2, microtubule-associated, homolog (Xenopus laevis)	-5.715
A_51_P513163	TRA2A	transformer-2 alpha	-3.683
A_51_P484329	TRA@	T cell receptor alpha locus	3.696
A_52_P202029	TRAK2	trafficking protein, kinesin binding 2	-9.304
A_51_P417891	TRIM10	tripartite motif-containing 10	-6.025

A_52_P212650	TRIM16	tripartite motif-containing 16	2.594
A_52_P255327	TRIM44	tripartite motif-containing 44	3.003
A_52_P151198	TRIP11	thyroid hormone receptor interactor 11	2.957
A_52_P571715	TROAP	trophinin associated protein (tastin)	-3.452
A_52_P94454	TRPC2	transient receptor potential cation channel, subfamily C, member 2	-3.228
A_52_P267779	TSC1	tuberous sclerosis 1	-2.491
A_52_P306744	TSPAN8	tetraspanin 8	-12.406
A_52_P112017	TSPO	translocator protein (18kDa)	-2.787
A_51_P303095	TTC28	tetratricopeptide repeat domain 28	2.983
A_51_P514300	TUBA4A	tubulin, alpha 4a	-4.522
A_51_P103659	TUBB2A	tubulin, beta 2A	-2.576
A_52_P598278	TUBB2C	tubulin, beta 2C	-6.882
A_51_P413545	TXNDC11	thioredoxin domain containing 11	3.934
A_51_P460181	TXNRD2	thioredoxin reductase 2	-5.604
A_52_P292651	TYMS	thymidylate synthetase	-2.826
A_51_P502838	UBE2H	ubiquitin-conjugating enzyme E2H (UBC8 homolog, yeast)	-5.366
A_51_P257107	UBE2J1	ubiquitin-conjugating enzyme E2, J1 (UBC6 homolog, yeast)	3.86
A_51_P416919	UBE2J2	ubiquitin-conjugating enzyme E2, J2 (UBC6 homolog, yeast)	-2.929
A_51_P188782	UBE2S	ubiquitin-conjugating enzyme E2S	-3.789
A_52_P452364	UBE2V2	ubiquitin-conjugating enzyme E2 variant 2	-2.506
A_52_P281879	UBTF	upstream binding transcription factor, RNA polymerase I	-2.607
A_51_P481342	UGCG	UDP-glucose ceramide glucosyltransferase	-3.12
A_51_P491742	UHRF1	ubiquitin-like, containing PHD and RING finger domains, 1	-8.791
A_51_P177242	UNC13B	unc-13 homolog B (C. elegans)	4.71
A_52_P541875	UQCRH	ubiquinol-cytochrome c reductase hinge protein	-2.57
A_51_P282092	UROD	uroporphyrinogen decarboxylase	-3.781
A_51_P257832	UROS	uroporphyrinogen III synthase (congenital erythropoietic porphyria)	-15.36
A_52_P276248	USP14	ubiquitin specific peptidase 14 (tRNA-guanine transglycosylase)	-4.4
A_52_P124812	USP15	ubiquitin specific peptidase 15	-3.546
A_52_P42834	USP25	ubiquitin specific peptidase 25	-3.322
A_52_P512806	USP37	ubiquitin specific peptidase 37	-2.865
A_52_P569608	USP47	ubiquitin specific peptidase 47	-3.097
A_52_P212412	USP54	ubiquitin specific peptidase 54	2.607
A_52_P569218	UTRN	utrophin	2.689
A_51_P227826	VAMP8	vesicle-associated membrane protein 8 (endobrevin)	-3.482
A_52_P350687	VANGL1	vang-like 1 (van gogh, Drosophila)	-6.003

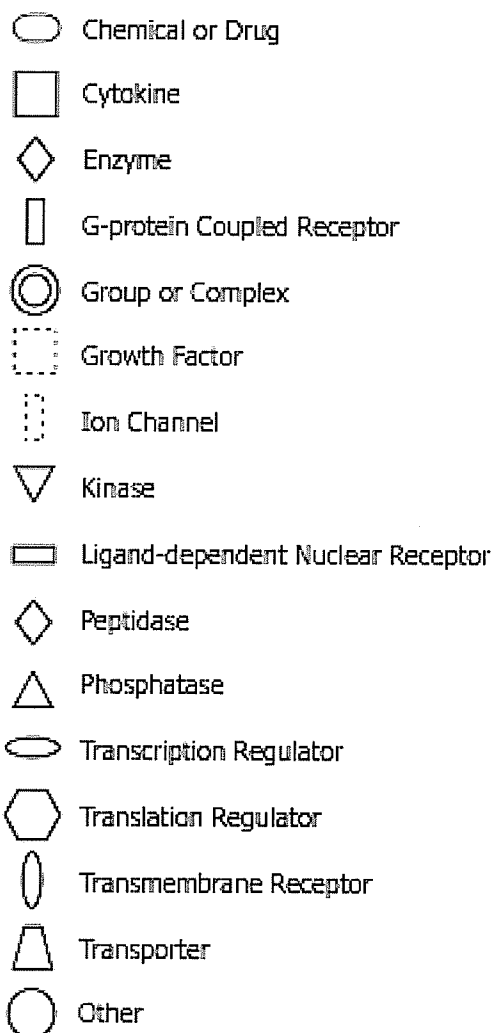
A_52_P363087	VBP1	von Hippel-Lindau binding protein 1	-3.393
A_52_P251703	VCAN	versican	3.45
A_52_P1044655	VCAN	versican	-2.031
A_51_P482552	VEGFA	vascular endothelial growth factor A	2.405
A_51_P381409	VIL1	villin 1	3.781
A_52_P493394	VPS24	vacuolar protein sorting 24 homolog (S. cerevisiae)	-3.295
A_51_P102339	VRK1	vaccinia related kinase 1	-3.133
A_52_P290391	VTI1A	vesicle transport through interaction with t-SNAREs homolog 1A (yeast)	2.78
A_52_P361664	VTI1A	vesicle transport through interaction with t-SNAREs homolog 1A (yeast)	-3.097
A_51_P103397	VWF	von Willebrand factor	2.136
A_51_P206886	VWF	von Willebrand factor	-2.844
A_52_P655890	WDHD1	WD repeat and HMG-box DNA binding protein 1	-4.236
A_52_P185568	WDR76	WD repeat domain 76	-2.721
A_52_P443330	WDR77	WD repeat domain 77	-2.643
A_51_P316951	WIPF3	WAS/WASL interacting protein family, member 3	2.238
A_51_P171616	WNT10A	wingless-type MMTV integration site family, member 10A	3.65
A_52_P498492	WRN	Werner syndrome	-2.597
A_52_P673458	WT1	Wilms tumor 1	4.69
A_52_P81214	WWP2	WW domain containing E3 ubiquitin protein ligase 2	-2.373
A_51_P502608	WWTR1	WW domain containing transcription regulator 1	4.112
A_52_P185079	XBP1	X-box binding protein 1	5.056
A_52_P490159	XCR1	chemokine (C motif) receptor 1	3.546
A_51_P312121	XDH	xanthine dehydrogenase	8.364
A_52_P89259	XPO1	exportin 1 (CRM1 homolog, yeast)	-4.01
AJ297360	XPO7	exportin 7	-3.238
A_51_P235594	XPO7	exportin 7	-6.715
A_52_P32466	YIF1A	Yip1 interacting factor homolog A (S. cerevisiae)	2.127
A_52_P175304	YME1L1	YME1-like 1 (S. cerevisiae)	3.204
A_52_P575874	YME1L1	YME1-like 1 (S. cerevisiae)	-2.378
A_52_P907995	YWHAE	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	2.063
A_52_P171715	YWHAE	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	-2.006
A_51_P141949	ZAK	sterile alpha motif and leucine zipper containing kinase AZK	-3.816
A_51_P169635	ZBTB1	zinc finger and BTB domain containing 1	-4.127
A_52_P256279	ZBTB11	zinc finger and BTB domain containing 11	-3.823
A_51_P108131	ZBTB4	zinc finger and BTB domain containing 4	2.659

A_52_P231610	ZC3HAV1	zinc finger CCCH-type, antiviral 1	-5.842
A_52_P429580	ZDHHC14	zinc finger, DHHC-type containing 14	2.155
A_51_P174081	ZDHHC14	zinc finger, DHHC-type containing 14	-2.032
A_52_P651248	ZDHHC2	zinc finger, DHHC-type containing 2	-3.483
A_52_P124173	ZDHHC8	zinc finger, DHHC-type containing 8	2.353
A_51_P220049	ZFP119	zinc finger protein 119	4.959
A_52_P558536	ZFP30	zinc finger protein 30 homolog (mouse)	7.561
A_51_P152203	ZFP386	zinc finger protein 386 (Kruppel-like)	3.666
A_51_P314077	ZFP53	zinc finger protein 53	3.86
A_52_P256105	ZFYVE9	zinc finger, FYVE domain containing 9	3.526
A_51_P390387	ZMYND19	zinc finger, MYND-type containing 19	-2.857
A_51_P223737	ZNF91	zinc finger protein 91	3.081
A_51_P473252	ZYX	zyxin	2.895

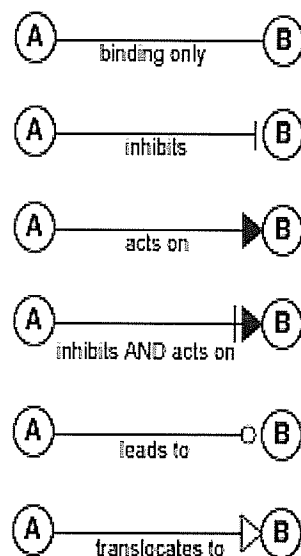
7.5 Appendix 5

Legend for IPA

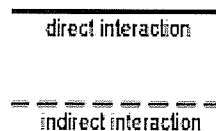
IPA Node Types



IPA Edge Types



Note: "Acts on" and "Inhibits" edge may also include a binding event.



(<https://analysis.ingenuity.com/pa/info/help/help.htm>)

8 References

Available at: http://www.ambion.com/techlib/misc/aama_dye_calc.html, 2007

Available at:

http://bakerinstitute.vet.cornell.edu/research/cytometry/flow_handbook.html, 2007.

Available at: <http://www.cjd.ed.ac.uk/>, 2008.

Available at: <http://david.abcc.ncifcrf.gov/>, 2007.

Available at: <http://www.ensembl.org/>, 2007

Available at: www.ingenuity.com, 2007.

Available at: www.proteinlounge.com, 2007.

Agilent G2567AA Feature Extraction Software (v.9.1) User Guide, 2006, Santa Clara, CA, Agilent technologies, Inc.

Aguzzi A, Glatzel M. Prion infections, blood and transfusions. *Nat Clin Pract Neurol* 2006; Jun;2(6):321-9.

Aguzzi A, Heikenwalder M. Pathogenesis of prion diseases: current status and future outlook. *Nat Rev Microbiol* 2006; Oct;4(10):765-75.

Alvarez FJ, Bisbe J, Bisbe V, Davalos A. Magnetic resonance imaging findings in pre-clinical Creutzfeldt-Jakob disease. *Int J Neurosci* 2005; Aug;115(8):1219-25.

Aucouturier P, Carnaud C. The immune system and prion diseases: a relationship of complicity and blindness. *J Leukoc Biol* 2002; Dec;72(6):1075-83.

Aucouturier P, Geissmann F, Damotte D, Saborio GP, Meeker HC, Kascsak R, *et al.*

Infected splenic dendritic cells are sufficient for prion transmission to the CNS in mouse scrapie. *J Clin Invest* 2001; Sep;108(5):703-8.

Aucouturier P, Kascsak RJ, Frangione B, Wisniewski T. Biochemical and conformational variability of human prion strains in sporadic Creutzfeldt-Jakob disease. *Neurosci Lett* 1999; Oct 15;274(1):33-6.

Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, *et al.* Immunobiology of dendritic cells. *Annu Rev Immunol* 2000;18:767-811.

Beekes M, McBride PA. Early accumulation of pathological PrP in the enteric nervous system and gut-associated lymphoid tissue of hamsters orally infected with scrapie. *Neurosci Lett* 2000; Jan 14;278(3):181-4.

Bencsik AA, Debeer SO, Baron TG. An alternative pretreatment procedure in animal transmissible spongiform encephalopathies diagnosis using PrPsc immunohistochemistry. *J Histochem Cytochem* 2005; Oct;53(10):1199-202.

Ben-Zaken O, Tzaban S, Tal Y, Horonchik L, Esko JD, Vlodavsky I, *et al.* Cellular heparan sulfate participates in the metabolism of prions. *J Biol Chem* 2003; Oct 10;278(41):40041-9.

Beranger F, Mange A, Solassol J, Lehmann S. Cell culture models of transmissible spongiform encephalopathies. *Biochem Biophys Res Commun* 2001; Nov 30;289(2):311-6.

Beringue V, Demoy M, Lasmezas CI, Gouritin B, Weingarten C, Deslys JP, Andreux JP, Couvreur P, Dormont D. Role of spleen macrophages in the clearance of scrapie agent early in pathogenesis. *J Pathol* 2000; 190: 495-502.

Blennow K, Johansson A, Zetterberg H. Diagnostic value of 14-3-3beta immunoblot and T-tau/P-tau ratio in clinically suspected Creutzfeldt-Jakob disease. *Int J Mol Med* 2005; Dec;16(6):1147-9.

Boellaard JW, Brown P, Tateishi J. Gerstmann-Straussler-Scheinker disease- the dilemma of molecular and clinical correlations. *Clin Neuropathol* 1999; Nov-Dec;18(6):271-85.

Bolton DC, McKinley MP, Prusiner SB. Identification of a protein that purifies with the scrapie prion. *Science* 1982; Dec 24;218(4579):1309-11.

Booth S, Bowman C, Baumgartner R, Sorensen G, Robertson C, Coulthart M, Phillipson C, Somorjai RL. Identification of central nervous system genes involved in the host response to the scrapie agent during preclinical and clinical infection. *J Gen Virol*. 2004 Nov;85(Pt 11):3459-71.

Brown KL, Stewart K, Ritchie D, Fraser H, Morrison WI, Bruce ME. Follicular dendritic cells in scrapie pathogenesis. *Arch Virol Suppl* 2000;(16)(16):13-21.

Brown KL, Stewart K, Ritchie DL, Mabbott NA, Williams A, Fraser H, *et al.* Scrapie replication in lymphoid tissues depends on prion protein-expressing follicular dendritic cells. *Nat Med* 1999; Nov;5(11):1308-12.

Brown P, Preece MA, Will *et al.* RG. "Friendly fire" in medicine: hormones, homografts, and Creutzfeldt-Jakob disease. *Lancet* 1992; Jul 4;340(8810):24-7.

Bruce ME, Brown KL, Mabbott NA, Farquhar CF, Jeffrey M. Follicular dendritic cells in TSE pathogenesis. *Immunol Today* 2000; Sep;21(9):442-6.

Bruce ME, McConnell I, Fraser H, Dickinson AG. The disease characteristics of different strains of scrapie in *Sinc* congenic mouse lines: implications for the nature of the agent and host control of pathogenesis. *J Gen Virol.* 1991; 72: 595-603.

Burthem J, Urban B, Pain A, Roberts DJ. The normal cellular prion protein is strongly expressed by myeloid dendritic cells. *Blood* 2001; Dec 15;98(13):3733-8.

Castilla J, Saa P, Hetz C, Soto C. *In vitro* generation of infectious scrapie prions. *Cell* 2005; Apr 22;121(2):195-206.

Caughey B, Raymond GJ, Bessen RA. Strain-dependent differences in beta-sheet conformations of abnormal prion protein. *J Biol Chem* 1998; Nov 27;273(48):32230-5.

- Caughey WS, Raymond LD, Horiuchi M, Caughey B. *et al.* Inhibition of protease-resistant prion protein formation by porphyrins and phthalocyanines. *Proc Natl Acad Sci USA* 1998; Oct 13;95(21):12117-22.
- Chakraborty C, Nandi S, Jana S. Prion disease: a deadly disease for protein misfolding. *Curr Pharm Biotechnol* 2005; Apr;6(2):167-77.
- Chaplin DD, Fu Y. Cytokine regulation of secondary lymphoid organ development. *Curr Opin Immunol* 1998; Jun;10(3):289-97.
- Cortelli P, Gambetti P, Montagna P, Lugaresi E. Fatal familial insomnia: clinical features and molecular genetics. *J Sleep Res* 1999; Jun;8 Suppl 1:23-9.
- Culmsee C, Landshamer S. Molecular insights into mechanisms of the cell death program: role in the progression of neurodegenerative disorders. *Curr Alzheimer Res* 2006; Sep;3(4):269-83.
- Cunningham C, Wilcockson DC, Boche D, Perry VH. Comparison of inflammatory and acute-phase responses in the brain and peripheral organs of the ME7 model of prion disease. *J Virol.* 2005 Apr;79(8):5174-84.
- Davies JE, Tang X, Bournat JC, Davies SJ. Decorin promotes plasminogen/plasmin expression within acute spinal cord injuries and by adult microglia *in vitro*. *J Neurotrauma* 2006; Mar-Apr;23(3-4):397-408.

Defaweux V, Dorban G, Demonceau C, Piret J, Jolois O, Thellin O, *et al.* Interfaces between dendritic cells, other immune cells, and nerve fibres in mouse Peyer's patches: potential sites for neuroinvasion in prion diseases. *Microsc Res Tech* 2005; Jan 1;66(1):1-9.

Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol.* 2003;4(5):P3.

Desnoyers L, Arnott D, Pennica D. WISP-1 binds to decorin and biglycan. *J Biol Chem* 2001; Dec 14;276(50):47599-607.

Doh-ura K, Tateishi J, Sasaki H, Kitamoto T, Sakaki Y. Pro----leu change at position 102 of prion protein is the most common but not the sole mutation related to Gerstmann-Straussler syndrome. *Biochem Biophys Res Commun* 1989; Sep 15;163(2):974-9.

Fischer MB, Roeckl C, Parizek P, Schwarz HP, Aguzzi A. Binding of disease-associated prion protein to plasminogen. *Nature* 2000; Nov 23;408(6811):479-83.

Fransson LA, Belting M, Jonsson M, Mani K, Moses J, Oldberg A. Biosynthesis of decorin and glypican. *Matrix Biol* 2000; Aug;19(4):367-76.

Furukawa H, Doh-ura K, Okuwaki R, Shirabe S, Yamamoto K, Udono H, *et al.* A pitfall in diagnosis of human prion diseases using detection of protease-resistant prion protein in

- urine. Contamination with bacterial outer membrane proteins. *J Biol Chem* 2004; May 28;279(22):23661-7.
- Gajdusek DC. Unconventional viruses and the origin and disappearance of kuru. *Science* 1977; Sep 2;197(4307):943-60.
- Glatzel M, Aguzzi A. The shifting biology of prions. *Brain Res Brain Res Rev* 2001; Oct;36(2-3):241-8.
- Goldgaber D, Goldfarb LG, Brown *et al.* P, Asher DM, Brown WT, Lin S, *et al.* Mutations in familial Creutzfeldt-Jakob disease and Gerstmann-Straussler-Scheinker's syndrome. *Exp Neurol* 1989; Nov;106(2):204-6.
- Hegde RS, Rane NS. Prion protein trafficking and the development of neurodegeneration. *Trends Neurosci* 2003; Jul;26(7):337-9.
- Heggebo R, Press CM, Gunnes G, Gonzalez L, Jeffrey M. Distribution and accumulation of PrP in gut-associated and peripheral lymphoid tissue of scrapie-affected Suffolk sheep. *J Gen Virol* 2002; Feb;83(Pt 2):479-89.
- Henry C, Knight R. Clinical features of variant Creutzfeldt-Jakob disease. *Rev Med Virol* 2002; May-Jun;12(3):143-50.
- Hijazi N, Shaked Y, Rosenmann H, Ben-Hur T, Gabizon R. Copper binding to PrPC may inhibit prion disease propagation. *Brain Res* 2003; Dec 12;993(1-2):192-200.

Hill AF, Collinge J. Prion strains and species barriers. *Contrib Microbiol* 2004;11:33-49.

Horonchik L, Tzaban S, Ben-Zaken O, Yedidia Y, Rouvinski A, Papy-Garcia D, *et al.*

Heparan sulfate is a cellular receptor for purified infectious prions. *J Biol Chem* 2005;

Apr 29;280(17):17062-7.

Hsiao K, Baker HF, Crow TJ, Poulter M, Owen F, TerWilliger JD, *et al.* Linkage of a prion protein missense variant to Gerstmann-Straussler syndrome. *Nature* 1989; Mar 23;338(6213):342-5.

Huang FP, Farquhar CF, Mabbott NA, Bruce ME, MacPherson GG. Migrating intestinal dendritic cells transport PrP(Sc) from the gut. *J Gen Virol* 2002; Jan;83(Pt 1):267-71.

Huang FP, Platt N, Wykes M, Major JR, Powell TJ, Jenkins CD, *et al.* A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. *J Exp Med* 2000; Feb 7;191(3):435-44.

Huber C, Thielen C, Seeger H, Schwarz P, Montrasio F, Wilson MR, *et al.* Lymphotoxin- β receptor-dependent genes in lymph node and follicular dendritic cell transcriptomes. *J Immunol* 2005; 174: 5526-5536.

Ingrosso L, Vetrugno V, Cardone F, Pocchiari M. Molecular diagnostics of transmissible spongiform encephalopathies. *Trends Mol Med* 2002; Jun;8(6):273-80.

Iozzo RV. The biology of the small leucine-rich proteoglycans. Functional network of interactive proteins. *J Biol Chem* 1999; Jul 2;274(27):18843-6.

Jeffrey M, Halliday WG, Bell J, Johnston AR, MacLeod NK, Ingham C, *et al.* Synapse loss associated with abnormal PrP precedes neuronal degeneration in the scrapie-infected murine hippocampus. *Neuropathol Appl Neurobiol* 2000; Feb;26(1):41-54.

Johnson RT. Prion diseases. *Lancet Neurol* 2005; Oct;4(10):635-42.

Kalamajski S, Aspberg A, Oldberg A. The decorin sequence SYIRIADTNIT binds collagen type I. *J Biol Chem* 2007; Jun 1;282(22):16062-7.

Kilic E, Kilic U, Wang Y, Bassetti CL, Marti HH, Hermann DM. The phosphatidylinositol-3 kinase/Akt pathway mediates VEGF's neuroprotective activity and induces blood brain barrier permeability after focal cerebral ischemia. *FASEB J* 2006; Jun;20(8):1185-7.

Klein MA, Kaeser PS, Schwarz P, Weyd H, Xenarios I, Zinkernagel RM, *et al.* Complement facilitates early prion pathogenesis. *Nat Med* 2001; Apr;7(4):488-92.

Klein MA, Frigg R, Flechsig E, Raeber AJ, Kalinke U, Bluethmann H, *et al.* A crucial role for B cells in neuroinvasive scrapie. *Nature* 1997; Dec 18-25;390(6661):687-90.

Klohn PC, Stoltze L, Flechsig E, Enari M, Weissman C. A quantitative, highly sensitive cell-based infectivity assay for mouse scrapie prions. *Proc Natl Acad Sci U S A* 2003; Sep 30;100(20):11666-71.

Koninger J, Giese NA, di Mola FF, Berberat P, Giese T, Esposito I, *et al.* Overexpressed decorin in pancreatic cancer: potential tumor growth inhibition and attenuation of chemotherapeutic action. *Clin Cancer Res* 2004; Jul 15;10(14):4776-83.

Kosco-Vilbois MH, Zentgraf H, Gerdes J, Bonnefoy JY. To 'B' or not to 'B' a germinal center?. *Immunol Today* 1997; May;18(5):225-30.

Kubler E, Oesch B, Raeber AJ. Diagnosis of prion diseases. *Br Med Bull* 2003;66:267-79.

Lam P. The Pathological Protein 2003; New York, NY, Copernicus Books.

Laplanche JL, Delasnerie-Laupretre N, Brandel JP, Chatelain J, Beaudry P, Alperovitch A, *et al.* Molecular genetics of prion diseases in France. French Research Group on Epidemiology of Human Spongiform Encephalopathies. *Neurology* 1994; Dec;44(12):2347-51.

Lasmezas CI, Deslys JP, Demaimay R, Adjou KT, Lamoury F, Dormont D, *et al.* BSE transmission to macaques. *Nature* 1996; Jun 27;381(6585):743-4.

Lee DW, Andersen JK, Kaur D. Iron dysregulation and neurodegeneration: the molecular connection. *Mol Interv* 2006; Apr;6(2):89-97.

Limviphuvadh V, Tanaka S, Goto S, Ueda K, Kanehisa M. The commonality of protein interaction networks determined in neurodegenerative disorders (NDDs). *Bioinformatics* 2007; Aug 15;23(16):2129-38.

Logan A, Baird A, Berry M. Decorin attenuates gliotic scar formation in the rat cerebral hemisphere. *Exp Neurol* 1999; Oct;159(2):504-10.

Lotscher M, Recher M, Hunziker L, Klein MA. Immunologically induced, complement-dependent up-regulation of the prion protein in the mouse spleen: follicular dendritic cells versus capsule and trabeculae. *J Immunol* 2003; Jun 15;170(12):6040-7.

Mabbott NA, MacPherson GG. Prions and their lethal journey to the brain. *Nat Rev Microbiol* 2006; Mar;4(3):201-11.

Mabbott NA, Young J, McConnell I, Bruce ME. Follicular dendritic cell dedifferentiation by treatment with an inhibitor of the lymphotoxin pathway dramatically reduces scrapie susceptibility. *J Virol* 2003; Jun;77(12):6845-54.

Mabbott NA, Bruce ME. The immunobiology of TSE diseases. *J Gen Virol* 2001; Oct;82(Pt 10):2307-18.

Mabbott NA, Mackay F, Minns F, Bruce ME. Temporary inactivation of follicular dendritic cells delays neuroinvasion of scrapie. *Nat Med* 2000; Jul;6(7):719-20.

Mabbott NA, Williams A, Farquhar CF, Pasparakis M, Kollias G, Bruce ME. Tumor necrosis factor alpha-deficient, but not interleukin-6-deficient, mice resist peripheral infection with scrapie. *J Virol* 2000; Apr;74(7):3338-44.

McBride PA, Eikelenboom P, Kraal G, Fraser H, Bruce ME. PrP protein is associated with follicular dendritic cells of spleens and lymph nodes in uninfected and scrapie-infected mice. *J Pathol* 1992; Dec;168(4):413-8.

McBride PA, Wilson MI, Eikelenboom P, Tunstall A, Bruce ME. Heparan sulfate proteoglycan is associated with amyloid plaques and neuroanatomically targeted PrP pathology throughout the incubation period of scrapie-infected mice. *Exp Neurol*. 1998 Feb;149(2):447-54.

McGovern G, Brown KL, Bruce ME, Jeffrey M. Murine scrapie infection causes an abnormal germinal centre reaction in the spleen. *J Comp Pathol* 2004; Feb-Apr;130(2-3):181-94.

Miele G, Manson J, Clinton M. A novel erythroid-specific marker of transmissible spongiform encephalopathies. *Nat Med* 2001; Mar;7(3):361-4.

Molina-Holgado F, Hider RC, Gaeta A, Williams R, Francis P. Metals ions and neurodegeneration. *Biometals* 2007; Jun;20(3-4):639-54.

Monari L, Chen SG, Brown P, Parchi P, Petersen RB, Mikol J, *et al.* Fatal familial insomnia and familial Creutzfeldt-Jakob disease: different prion proteins determined by a DNA polymorphism. *Proc Natl Acad Sci U S A* 1994; Mar 29;91(7):2839-42.

Nebert DW, Roe AL, Dieter MZ, Solis WA, Yang Y, Dalton TP. Role of the aromatic hydrocarbon receptor and [Ah] gene battery in the oxidative stress response, cell cycle control, and apoptosis. *Biochem Pharmacol* 2000; Jan 1;59(1):65-85.

Nielsen CH, Fischer EM, Leslie RG. The role of complement in the acquired immune response. *Immunology* 2000; May;100(1):4-12.

Prinz M, Heikenwalder M, Junt T, Schwarz P, Glatzel M, Heppner FL, *et al.* Positioning of follicular dendritic cells within the spleen controls prion neuroinvasion. *Nature* 2003; Oct 30;425(6961):957-62.

Prinz M, Montrasio F, Klein MA, Schwarz P, Priller J, Odermatt B, *et al.* Lymph nodal prion replication and neuroinvasion in mice devoid of follicular dendritic cells. *Proc Natl Acad Sci U S A* 2002; Jan 22;99(2):919-24.

Prusiner SB. Shattuck lecture--neurodegenerative diseases and prions. *N Engl J Med* 2001; May 17;344(20):1516-26.

Prusiner SB. Prions. *Proc Natl Acad Sci U S A* 1998; Nov 10;95(23):13363-83.

Prusiner SB. Novel proteinaceous infectious particles cause scrapie. *Science* 1982; Apr 9;216(4542):136-44.

Raeber AJ, Klein MA, Frigg R, Flechsig E, Aguzzi A, Weissman *et al.* n C. PrP-dependent association of prions with splenic but not circulating lymphocytes of scrapie-infected mice. *EMBO J* 1999; May 17;18(10):2702-6.

Riesner D. Biochemistry and structure of PrP(C) and PrP(Sc). *Br Med Bull* 2003;66:21-33.

Saborio GP, Permanne B, Soto C. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature* 2001; Jun 14;411(6839):810-3.

Schroter A, Zerr I, Henkel K, Tschampa HJ, Finkenstaedt M, Poser S. Magnetic resonance imaging in the clinical diagnosis of Creutzfeldt-Jakob disease. *Arch Neurol* 2000; Dec;57(12):1751-7.

Smith JP, Burton GF, Tew JG, Szakal AK. Tingible body macrophages in regulation of germinal center reactions. *Dev Immunol* 1998;6(3-4):285-94.

Smith JP, Lister AM, Tew JG, Szakal AK. Kinetics of the tingible body macrophage response in mouse germinal center development and its depression with age. *Anat Rec* 1991; Apr;229(4):511-20.

Snow AD, Mar H, Nochlin D, Kresse H, Wight TN. Peripheral distribution of dermatan sulfate proteoglycans (decorin) in amyloid-containing plaques and their presence in neurofibrillary tangles of Alzheimer's disease. *J Histochem Cytochem* 1992; Jan;40(1):105-13.

Sorensen G, Medina S, Parchaliuk D, Phillipson C, Robertson C, Booth SA. Comprehensive transcriptional profiling of prion infection in mouse models reveals networks of responsive genes. *BMC Genomics*. 2008 Mar 3;9(1):114.

Soto C. Diagnosing prion diseases: needs, challenges and hopes. *Nat Rev Microbiol* 2004; Oct;2(10):809-19.

Taylor DR, Watt NT, Perera WS, Hooper NM. Assigning functions to distinct regions of the N-terminus of the prion protein that are involved in its copper-stimulated, clathrin-dependent endocytosis. *J Cell Sci* 2005; Nov 1;118(Pt 21):5141-53.

Telling GC, Parchi P, DeArmond SJ, Cortelli P, Montagna P, Gabizon R, *et al.* Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. *Science* 1996; Dec 20;274(5295):2079-82.

Thackray AM, Klein MA, Bujdoso R. Subclinical prion disease induced by oral inoculation. *J Virol* 2003; Jul;77(14):7991-8.

Unterberger U, Voigtlander T, Budka H. Pathogenesis of prion diseases. *Acta Neuropathol (Berl)* 2005; Jan;109(1):32-48.

Van den Berg TK, Yoshida K, Dijkstra CD. Mechanism of immune complex trapping by follicular dendritic cells. *Curr Top Microbiol Immunol* 1995;201:49-67.

Vidal C, Meric P, Provost F, Herzog C, Lasmezas C, Gillet B, *et al.* Preclinical metabolic changes in mouse prion diseases detected by ¹H-nuclear magnetic resonance spectroscopy. *Neuroreport* 2006; Jan 23;17(1):89-93.

Weiss S, Proske D, Neumann M, Groschup MH, Kretzschmar HA, Famulok M, *et al.* RNA aptamers specifically interact with the prion protein PrP. *J Virol* 1997; Nov;71(11):8790-7.

Weissman C. The state of the prion. *Nat Rev Microbiol* 2004; Nov;2(11):861-71.

Weissman C, Flechsig E. PrP knock-out and PrP transgenic mice in prion research. *Br Med Bull* 2003;66:43-60.

White AR, Enever P, Tayebi M, Mushens R, Linehan J, Brandner S, *et al.* Monoclonal antibodies inhibit prion replication and delay the development of prion disease. *Nature* 2003; Mar 6;422(6927):80-3.

Wickner RB, Edskes HK, Roberts BT, Pierce M, Baxa U. Prions of yeast as epigenetic phenomena: high protein "copy number" inducing protein "silencing". *Adv Genet* 2002;46:485-525.

Wien TN, Sørby R, Omtvedt LA, Landsverk T, Husby G. Kinetics of glycosaminoglycan deposition in splenic AA amyloidosis induced in mink. *Scand J Immunol*; 2004 Dec;60(6):600-8. Erratum in: *Scand J Immunol*. 2005 Feb;61(2):215.

Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, *et al*. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 1996; Apr 6;347(9006):921-5.

Xaus J, Comalada M, Cardo M, Valledor AF, Celada A. Decorin inhibits macrophage colony-stimulating factor proliferation of macrophages and enhances cell survival through induction of p27(Kip1) and p21(Waf1). *Blood* 2001; Oct 1;98(7):2124-33.

Zerr I, Poser S. Clinical diagnosis and differential diagnosis of CJD and vCJD. With special emphasis on laboratory tests. *APMIS* 2002; Jan;110(1):88-98.