

Dissertation

**The effects of systemic inflammation on
brain lipid homeostasis**

submitted by

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STATUTORY DECLARATION

I hereby declare that this dissertation is my own original work and that I have fully acknowledged by name all of those individuals and organisations that have contributed to the research for this dissertation. Due acknowledgement has been made in the text to all other material used. Throughout this dissertation and in all related publications I followed the guidelines of “Good Scientific Practice “.

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ZUSAMMENFASSUNG

Entzündungen des zentralen Nervensystems sind ein Hauptmerkmal vieler chronisch-entzündlicher Erkrankungen des Gehirns, wie z. B. Alzheimer, Parkinson und Multiple Sklerose. In dieser Studie wurde ein Mausmodell verwendet, um die durch Entzündungsreaktionen ausgelösten Veränderungen im Gehirn zu charakterisieren. Um Änderungen der Genexpression zu untersuchen, wurde ein Microarray eingesetzt, der das ganze Mausgenom abdeckt. Signifikante Regulationen gab es vor allem in Transkripten, die in Lipid Metabolismus, Funktion der Blut-Hirn-Schranke (BBB) sowie Entzündung involviert sind. Eines dieser Transkripte, CH25H (Cholesterol 25-Hydroxylase) war auf mRNA- und Protein-Ebene stark induziert. CH25H katalysiert die Reaktion von Cholesterol zu 25-Hydroxycholesterol (25-HC), wodurch das Molekül hydrophiler wird. Zwei mögliche Konsequenzen der Induktion von CH25H im Gehirn, 1) Modulierung der BBB sowie 2) Einfluss auf die Cholesterol-Homeostase durch liver X receptor (LXR) wurden im Detail untersucht. Um Effekte von 25-HC auf die Permeabilität und Zellintegrität der BBB zu testen, wurde ein *in vitro* Modell der BBB verwendet. Die Ergebnisse zeigen, dass sowohl Permeabilität als auch Zellintegrität zeitabhängig beeinflusst werden. Ein Vergleich mit 24-HC, dem Hauptprodukt des Cholesterol Abbauweges im Gehirn zeigt, dass beide Oxysterole die Permeabilität und Integrität der BBB *in vitro* in gleichem Ausmaß beeinflussen. Um den Einfluss von CH25H auf die Homeostase von Cholesterol in Neuronen und Astrozyten zu überprüfen, wurden die Zellen mit 25-HC inkubiert. Die Regulation von Transkripten involviert in Biosynthese, die Prozessierung von sterol regulatory element binding protein (SREBP), sowie die Cholesterol Biosynthese wurden untersucht. Die Ergebnisse zeigen eine Induktion von Cholesterol-Abbauwegen sowie eine Reduktion der Cholesterol-Biosynthese auf transkriptioneller Ebene. Weiters konnte gezeigt werden, dass die Biosynthese von Cholesterol in Neuronen durch 25-HC konzentrationsabhängig reduziert wird. Zusammenfassend zeigt diese Studie, dass Neuroinflammation zu drastischen transkriptionellen Änderungen der BBB, des Lipid Metabolismus sowie inflammatorischer Wege führt. Weiters konnte gezeigt werden, dass CH25H als auch das Produkt 25-HC unter neuroinflammatorischen Bedingungen *in vivo* induziert wird und 25-HC ein potenter Regulator der Cholesterol-Homeostase *in vitro* ist.

ABSTRACT

Neuroinflammation is involved in the progression of several neurodegenerative diseases, like Alzheimer's disease, Parkinson's disease and Multiple sclerosis. I used a murine model of systemic inflammation and concomitant neuroinflammation to characterize transcriptional changes that underlie the physiological and pathophysiological effects of neuroinflammation. To investigate changes in gene expression upon peripherally induced neuroinflammation, a whole genome microarray was performed. I found pronounced changes in the regulation of genes involved in lipid metabolism, blood-brain barrier (BBB) function and inflammation. Furthermore, neuroinflammation mediated a strong upregulation of the transcript and protein levels of cholesterol 25-hydroxylase (CH25H). CH25H catalyzes the conversion from cholesterol to the more hydrophilic 25-hydroxycholesterol (25-HC). Two possible effects of increased 25-HC in the brain which I investigated here are modulation of BBB permeability and cholesterol homeostasis via liver X receptor (LXR) activation. I used an *in vitro* model of the BBB to evaluate the effect of 25-HC on cerebrovascular permeability and cell integrity. These experiments revealed that permeability and cell integrity decreased in a time-dependent manner. A comparison with the predominant end product of brain cholesterol elimination, namely 24-HC, demonstrated that both oxysterols affect permeability and cell integrity of porcine brain microvascular endothelial cells to a similar extent. In the second part of the study I sought to clarify effects of exogenously added 25-HC on transcriptional regulation of cholesterol biosynthetic genes, SREBP processing, and cholesterol biosynthesis in the murine CATH.a neuronal cell line as well as the astrocytoma cell line CCF-STTG1. I found that transcript levels of well known LXR target genes were regulated leading an upregulation of cholesterol dissiminating pathways and reduced transcripts of cholesterol biosynthetic pathways. This finding was supported by the fact, that the CATH.a neuronal cell line, treated with 25-HC showed a reduction of cholesterol biosynthesis in a concentration-dependent manner. Data of the present study indicate that neuroinflammation leads to pronounced changes in gene products of lipid metabolism, BBB integrity as well as inflammation. Furthermore, it shows that CH25H as well as its enzymatic endproduct 25-HC is upregulated under neuroinflammatory conditions *in vivo* and that 25-HC is a potent regulator of neuronal cholesterol homeostasis *in vitro*.

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1 INTRODUCTION

1.1 Short overview of the Central Nervous System

1.1.1 Development and anatomy of the nervous system

The human nervous system is divided into the central and the peripheral nervous system. These two systems control motor and cognitive functions and integrate sensory information in concert. The brain is an organ with high metabolic demands, which needs a tight water and ion homeostasis. It occupies approximately 2 % of total body weight, but receives approximately 20 % of cardiac output and caloric supply to the body. During early development the neural tube expands in the front of the embryo and forms the three primary brain divisions: the forebrain (prosencephalon), midbrain (mesencephalon) and hindbrain (rhombencephalon). These three vesicles further differentiate into five subdivisions: telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon. The mesencephalon, metencephalon and myelencephalon comprise the brain stem (Gilbert 2000) (Figure 1).

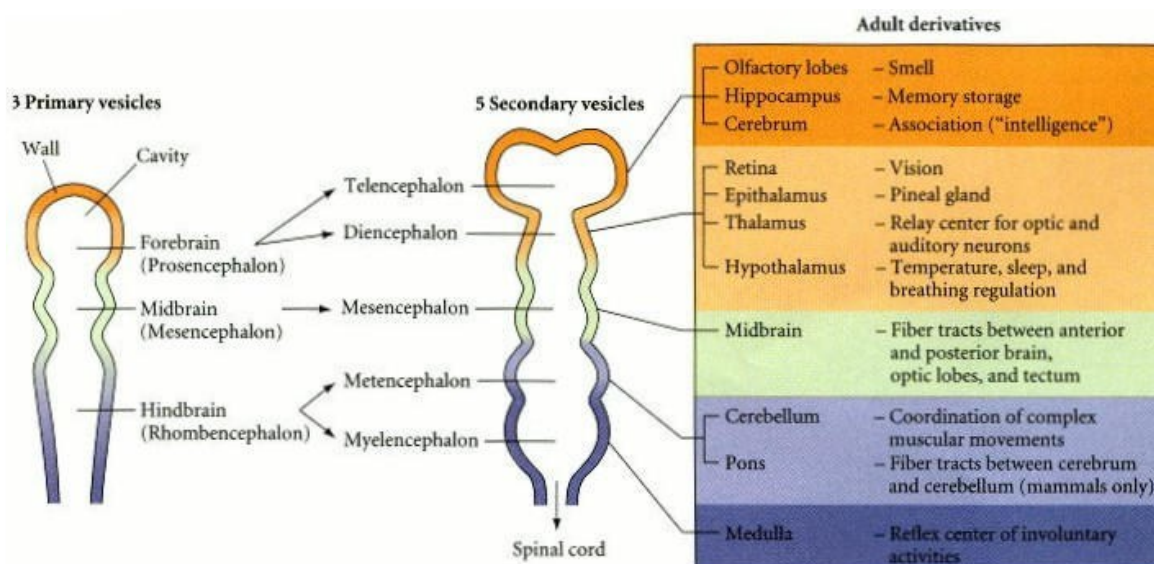


Figure 1: During the early brain development the neural tube forms the three primary brain divisions (forebrain, midbrain and hindbrain). These three vesicles further differentiate into five subdivisions (Gilbert 2000).

Three major structures are visible in most human brain images: cerebrum, cerebellum and caudal portion of the brainstem. The cerebrum is the biggest part and has a highly convoluted surface. The ridges of these convolutions are named gyri, and the valleys between them are named sulci (Figure 2). Scientists agree that the infolding of the cerebral hemispheres allows much more cortical surface area (about 1.6 m^2 on average), and as a consequence more place for neurons (Purves, Augustine et al. 2001). The brain is protected by bones and the three meninges: dura mater, arachnoid mater and pia mater.

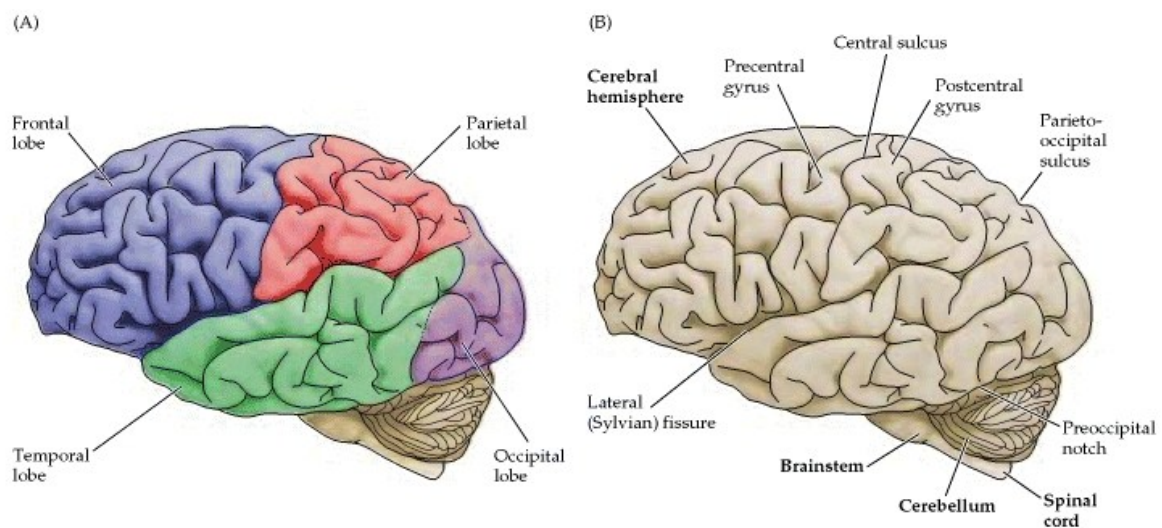


Figure 2: Lateral view of the human brain. (A) The brain can be divided into four lobes. (B) Location of the hemispheres and major sulci and gyri visible from this perspective (Purves, Augustine et al. 2001).

1.1.2 The cellular components of the central nervous system

Neurons – center of attention

Neurons have a complex morphology. Four distinct regions with different functions can be distinguished: cell body, dendrites, axon and axon terminal. The cell body contains the nucleus, and is the place of synthesis for neuronal proteins and membranes. Just in a few cases proteins are also synthesized in dendrites. Axons and axon terminals are dependent on the supply of material from the cell body, as they don't contain ribosomes and can therefore not synthesize proteins. Newly synthesized proteins and membranes are sent from the cell body to the axons and axon terminals via a process called anterograde transport. During this process material is sent along microtubules to the place they are required. The reverse

transport, in which damaged membranes and organelles move from the axon towards the cell body, is called retrograde transport. This form of transport is necessary as lysosomes, which degrade this material are only found in the cell body. Most neurons have a single fine axon and multiple broad dendrites emerging from the opposite pole, which are specialized to receive chemical signals from axon termini of other neurons (Raine 1999). In the awake state, approximately 16 % of total energy is used for neuronal functions, including neuronal firing and cycling of γ -amino butyric acid (GABA) and glutamate neurotransmitters. Energy is supplied through the blood via the microvasculature. Depending on the neural activity, the magnitude and spatial location of cerebral blood flow changes. This complex phenomenon is called neurovascular coupling (Drake and Iadecola 2007).

Microglia - the brains macrophages

Microglia are the primary immunocompetent and phagocytic cells in the central nervous system (CNS) (Vance and Hayashi 2010). They are derived from myeloid cells in the periphery and comprise about 10 % of all cells in the brain. The distribution of microglia in the brain varies, from 0.5 to 16.6 % with the largest number residing in the grey matter of the hippocampus, olfactory bulb, telencephalon, basal ganglia and substantia nigra. These cells, which show a phenotype similar to dendritic cells are constantly scavenging the CNS for brain injury and immunological stimuli and are readily activated in response to a stimulus. Activated microglia change their morphology from a resting ramified to an active amoeboid state, express certain surface molecules like CD (cluster of differentiation) 14, MHC (major histocompatibility complex) class II and chemokine receptors. They clear toxic cellular debris and act as antigen presenting cells. Microglia are also involved in regulating brain development by enforcing programmed elimination of neural cells, and enhancing neuronal survival through the release of trophic and anti-inflammatory factors. In the mature brain microglia facilitate repair by guiding stem cells to the site of inflammation or injury. However, microglia may become overactivated for reasons, which are not completely known yet (Figure 3). They might induce highly detrimental neurotoxic effects by the excessive production of cytotoxic factors like superoxide (O_2^-), nitric oxide (NO) and tumor necrosis factor α (TNF α) and thereby can be involved in the development of certain diseases, like Alzheimer's disease (AD),

Parkinson's disease (PD), Huntington's disease (HD) or Multiple sclerosis (MS) (Purves, Augustine et al. 2001; Block, Zecca et al. 2007).

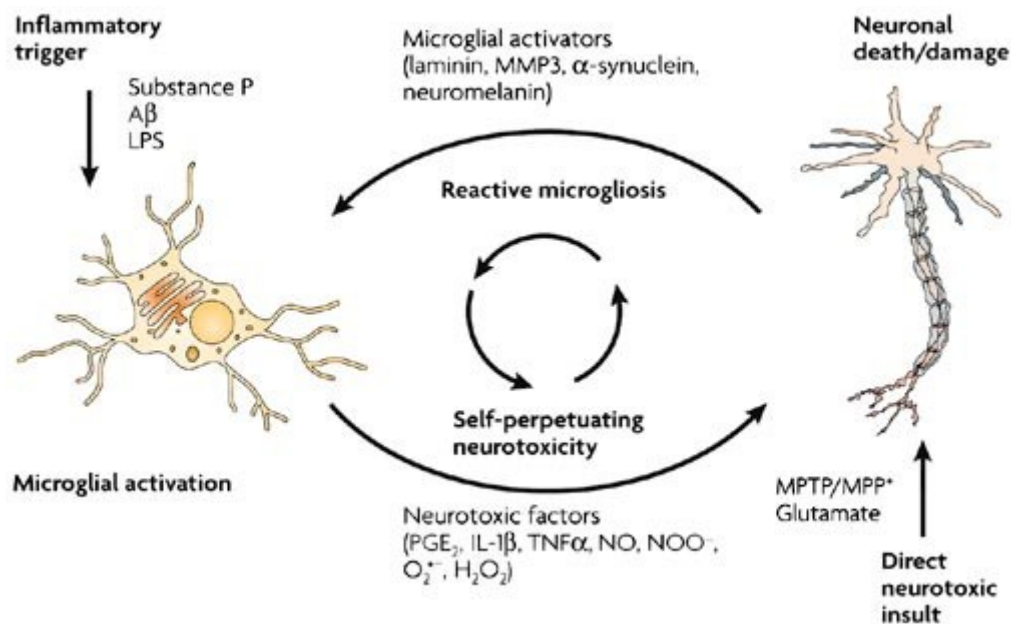


Figure 3: Overactivation of microglia leads to neurotoxicity. Triggers can be pro-inflammatory stimuli like bacterial lipopolysaccharide or neuronal damage. Microglia become activated and release neurotoxic proinflammatory factors, which lead to a self-perpetuating cycle of neurotoxicity. Reactive microgliosis might be an underlying reason for progressive neuronal damage in certain neurodegenerative diseases (Block, Zecca et al. 2007).

Astrocytes – neurovascular couplers

Astrocytes, which are the most abundant glial cells in the CNS (Vance and Hayashi 2010) were originally regarded to be passive elements of the CNS. It was thought that they are mainly supportive to neurons, a guiding structure during development and regulators of ion homeostasis. In fact, they are also bidirectional communication partners in the CNS by receiving signals from neighboring neuronal cells and releasing neuroactive substances. Furthermore, they transmit signals from neurons to the vasculature. Astrocytes have been shown to be involved in regulating the blood flow, proliferation of stem cells and determination of synaptic number. (Oberheim, Wang et al. 2006) Astrocytes, which have a star like appearance, express receptors for most neurotransmitters and neuromodulators (Purves, Augustine et al. 2001). They are able to detect deleterious effects to the CNS and react by a strong proliferation of “reactive astrocytes”. This proliferation can lead to the formation of “glial scars” (Purves, Augustine et al. 2001).

Oligodendrocytes – myelin producing cells

Oligodendrocytes are the myelin-producing cells in the CNS. One single oligodendrocyte is able to myelinate several adjacent axons. Myelin is formed by oligodendrocytes wrapping large amounts of spiral layers of their plasma membrane around the axon (Hartline and Colman 2007; Simons and Trotter 2007). Myelin is formed of a stack of uniformly thick membranes, in which electron-dense and –light layers are alternating (Figure 4). These layers are called major dense line and intraperiod line (Hartline 2008). This process insulates the axon and facilitates transmission of nerve impulses by salutatory conduction. Sodium channels are clustered into nodes of Ranvier, and this enables action potentials to jump from one node to the other (Waxman 2006). This process leads to a reduction of energy and space utilization requirement of the nerve cell. Myelin has an *in situ* water content of around 40 %. The dry mass is characterized by high lipid content (approx. 80 %) and a simple array of myelin-specific proteins (approx. 20 %). Compared to other biological membranes, the proportion of protein is quite low (Siegel *et al.*, 1999a).

Studies in mutant animals defective in these proteins showed, that except of myelin-associated glycoprotein (MAC) all other structural myelin-specific proteins are necessary for the proper function of the nervous system, as an elimination disrupts the myelination process. In case of MAC the animals could still form functional myelin, although subtle morphological abnormalities were present (Adlkofer *et al.*, 1995; Boison *et al.*, 1994; Campagnoni *et al.*, 1988; Giese *et al.*, 1992; Li *et al.*, 1994; Lunn *et al.*, 1995; Montag *et al.*, 1994).

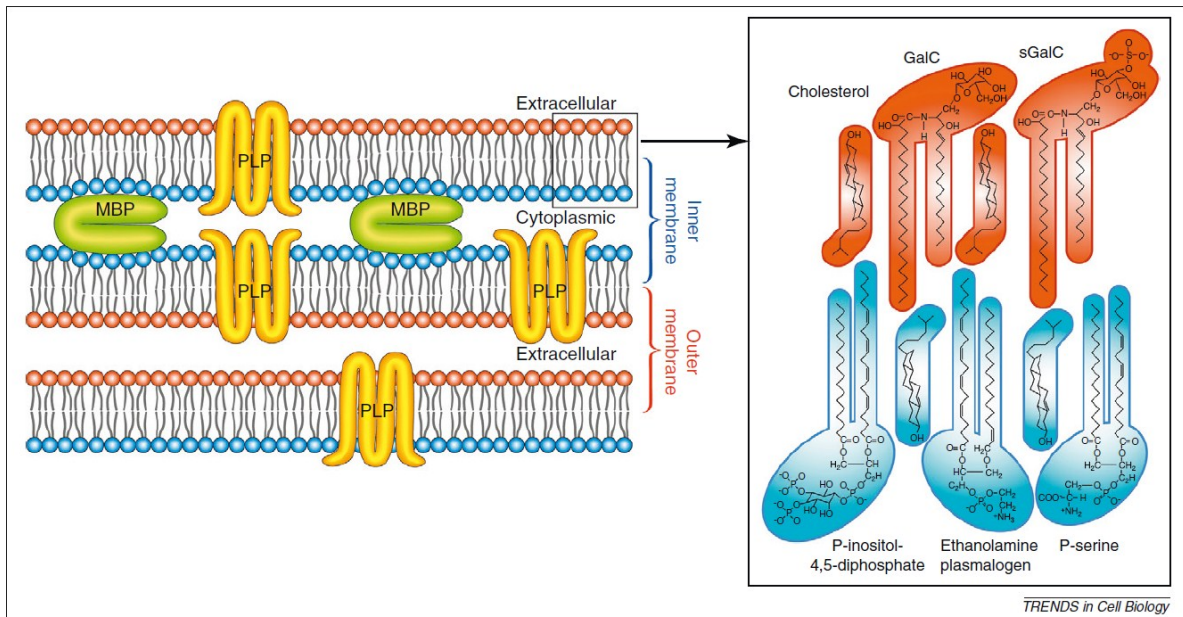


Figure 4: The structure of the myelin sheath and major lipid classes. Inner membrane and outer membrane constitute the intraperiodic and major dense line. This leads to the asymmetric lipid composition of the bilayer. On the right, the major lipid classes are shown (Aggarwal, Yurlova et al. 2011).

Myelin contains a highly characteristic combination of lipids, although there is no absolutely “myelin-specific” lipid. One of them is cerebroside (also called galactosylceramide), which is typical for myelin. The concentration of cerebroside is directly proportional to the amount of myelin. Furthermore, around 20 % of the galactolipid in myelin is present as sulfatide, in which the 3-hydroxy group on the galactose of cerebroside is sulfated. Galactocerebroside together with its sulfated derivative, sulfatide, constitute approx. 27 % of myelin lipids (Norton and Cammer 1984). Because of the specificity and quantitative significance it was considered that galactocerebroside is essentially required for the differentiation of oligodendrocytes as well as the structure and function of myelin. Surprisingly, Coetzee *et al.* found that knockout mice lacking UDP-galactose:ceramide galactosyltransferase, an obligate enzyme in the pathway of cerebroside biosynthesis did not show any big abnormalities regarding the myelin structure, although small differences in structure and changes in conduction velocity of axons could be demonstrated. Later in life these animals developed a progressive hindlimb paralysis and vacuolation of myelin in the spinal cord. These findings show that cerebroside and/or sulfatides play important roles for insulation of neurons and stability, rather than for myelin formation

(Coetzee, Fujita et al. 1996). Additional to cerebroside, the major lipid species in myelin are cholesterol, ethanolamine-containing plasmalogens, phospho- and sphingolipids. Additionally to the highly characteristic lipid composition of myelin the fatty acid composition of the individual lipids is distinctive. Table 1 shows that major amounts of myelin are found in the white matter, whereas the lipid composition of the gray matter is quite different from the lipid composition of myelin (Morell and Quarles 1999).

Table 1: Composition of myelin and brain. Protein and lipid is given in percent of dry weight. Others are given in percent of total lipid weight. Plasmalogens are primarily ethanolamine phosphatides (Morell and Quarles 1999).

Substance	Myelin			White matter		Gray matter	Whole brain
	Hs	Bt	Rn	Hs	Bt	Hs	Rn
Protein	30.0	24.7	29.5	39.0	39.5	55.3	56.9
Lipid	70.0	75.3	70.5	54.9	55.0	32.7	37.0
Cholesterol	27.7	28.1	27.3	27.5	23.6	22.0	23.0
Cerebroside	22.7	24.0	23.7	19.8	22.5	5.4	14.6
Sulfatide	3.8	3.6	7.1	5.4	5.0	1.7	4.8
Total galactolipid	27.5	29.3	31.5	26.4	28.6	7.3	21.3
Ethanolamine phosphatides	15.6	17.4	16.7	14.9	13.6	22.7	19.8
Lecithin	11.2	10.9	11.3	12.8	12.9	26.7	22.0
Sphingomyelin	7.9	7.1	3.2	7.7	6.7	6.9	3.8
Phosphatidylserine	4.8	6.5	7.0	7.9	11.4	8.7	7.2
Phosphatidylinositol	0.6	0.8	1.2	0.9	0.9	2.7	2.4
Plasmalogens	12.3	14.1	14.1	11.2	12.2	8.8	11.6
Total phospholipid	43.1	43.0	44.0	45.9	46.3	69.5	57.6

Hs: Homo sapiens, Bt: Bos Taurus, Rn: Rattus norvegicus.

In the peripheral nervous system (PNS), myelin is formed by Schwann cells, which myelinate only a single axon. The myelin in the CNS is targeted in certain serious diseases such as MS or metachromatic leukodystrophies (Purves *et al.*, 2001).

Pericytes

Pericytes belong to the smooth muscle cell lineage and are located adjacent to capillaries. They share a basement membrane with endothelial cells and encircle approx. 30-70 % of the capillaries with their cytoplasmic processes (Allt and Lawrenson 2001). Pericytes are in close relation to endothelial cells. With their processes they form focal contacts through specialized junctions. In the CNS, these cells provide mechanical stability to blood vessels, and regulate microvascular permeability, angiogenesis and remodeling. In addition, recent studies have reported, that pericytes can regulate brain capillary blood flow by contraction and relaxation. These facts led to the speculation that pericytes are involved in the development of neuropathology in certain diseases, like hypertension, diabetes, MS or AD (Zlokovic 2008).

Brain capillary endothelial cells

Brain capillary endothelial cells (BCEC) show a distinct morphology, which makes them different to endothelial cells of the peripheral circulatory system (Figure 5). They are thinner, possess less caveolae at the luminal surface, a 2-5-fold increase in number of mitochondria (Oldendorf, Cornford et al. 1977) and a 40 % thinner capillary wall (Coomber and Stewart 1985). One BCEC forms the whole circumference of a brain capillary. BCEC are interconnected by tight junctions, which prevent movement of polar molecules (proteins and ions) of various size and lead to a polarized cell surface protein expression (Dejana 2004). At the basolateral side of the cells they are underlined by a collagen-containing extracellular matrix, the basal lamina (Lattera, Keep et al. 1999).

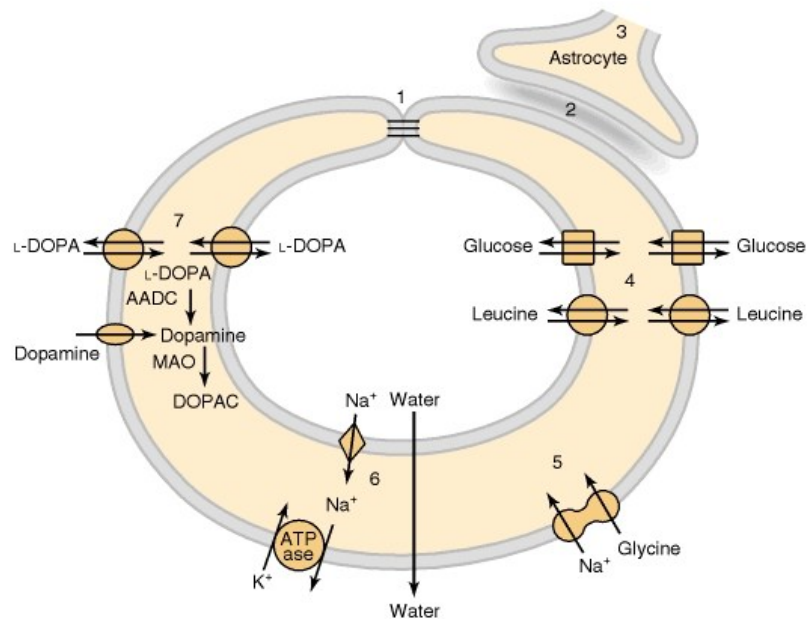


Figure 5: Schematic cross section through a brain capillary endothelial cell. (1) Tight junctions limit diffusion across the barrier. (2) The basement membrane supports the structure of the capillary and possibly, together with (3) astrocytic endfeet, influences endothelial cell function. (4) Transport for glucose, the main energy source of the brain and essential amino acids is facilitated by carriers. (5) Secondary transport systems cause efflux from brain to blood for small, nonessential amino acids. (6) Sodium ion transporters are located on the luminal side and Na, K-ATPase on the antiluminal side, which might be important for providing an osmotic driving force to facilitate secretion of interstitial fluid by brain capillaries. (7) Neurotransmitter precursors like L-DOPA are taken up by a large neutral amino acid carrier (Lattera, Keep et al. 1999).

1.1.3 Blood-Brain Barrier

In 1885 Paul Ehrlich performed a series of experiments to demonstrate the existence of a barrier between the blood and the brain. After infusing Evan's blue dye intravenously in rats he found all organs except of the brain and the spinal cord to be stained. However, he incorrectly postulated that the dye didn't adhere because of the brain's tissue (Cipolla 2009). A few years later, Lewandowsky (1900) was the first to draw the right conclusions, after he saw that pharmacological actions due to intravenously administered bile acids were missing in the CNS and correctly postulated the existence of a mechanical membrane which separates the circulating blood from the nervous system. His finding was proven by the lack of staining in the brain and spinal cord after intravenous administration of the dye trypan blue. With time the concept of the blood-brain barrier (BBB) as being impermeable changed. It could be shown that the brain endothelium maintained the chemical composition of the neuronal milieu by regulating an active transport of ions and carrier-mediated

transport of glucose and amino acids across the barrier, whereas it blocked/limited the transport of plasma components, leukocytes and red blood cells into the brain. The pathophysiological consequences of a breakdown i.e. disruption of the tight junctions, followed by a progressive synaptic and neuronal dysfunction can be deleterious. This scenario was reported for AD, PD, amyotrophic lateral sclerosis (ALS) and others (Zlokovic 2008).

The BBB, which separates the circulating blood from the nervous system, is composed of a tight monolayer of brain endothelial cells (BEC). These cells, together with pericytes, astrocytes and microglia prevent any free exchange of molecules between blood and brain (Figure 6). Exceptions are lipid-soluble molecules which are smaller than 400 kDa in size and have less than 9 hydrogen bonds. These molecules are able to pass the monolayer by lipid-mediated diffusion. This knowledge is the corner stone for brain drug development. The BECs are connected by protein complexes called tight junctions (including proteins like occludin, claudins, junctional adhesion molecule A, ZO-1 proteins, and actin) and adherence junctions (including proteins like VE-cadherin, Caveolin-1, and PECAM-1) (Zlokovic 2008). A few regions in the brain are not protected by a BBB. These are pituitary gland, median eminence, area postrema, preoptic recess, paraphysis, pineal gland and the endothelium of the choroid plexus (Laterra, Keep et al. 1999).

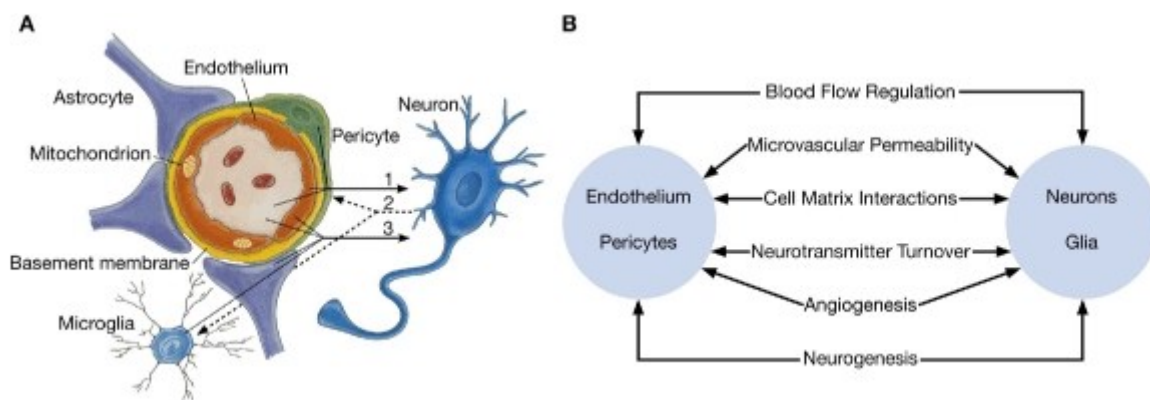


Figure 6: The neurovascular unit comprises endothelial cells, basal lamina, extracellular matrix, neurons, astrocytes and pericytes. These cells are in close contact to each other and regulate the development and synaptic activity in the CNS. The communication between these cells regulates the permeability properties of the BBB (Banerjee and Bhat 2007; Zlokovic 2008).

1.2 Cholesterol

1.2.1 Main functions of cholesterol

Unesterified cholesterol is essential for every cell. It is used as a structural component to control fluidity and permeability of the plasma membrane. Cholesterol molecules are located between phospholipid molecules of each monolayer to reduce the movement of their acyl chains. Unesterified cholesterol is usually located at the outer or inner leaflet of the plasma membrane. Only a small fraction of cholesterol is located at intracellular structures like the endoplasmic reticulum (ER), Golgi apparatus or the nucleus. Nevertheless this small intracellular fraction regulates cellular cholesterol homeostasis. Furthermore, cholesterol interacts with hedgehog proteins, which control embryonic development (Clayton 1998). Sonic hedgehog (SHH) influences patterning and development of the CNS (Porter, Young et al. 1996). Cholesterol is a precursor for steroid hormones (such as progesterone, testosterone, cortisol, and estradiol), bile acids (Berg, Tymoczko et al. 2002) and Vitamin D (Clayton 1998). Given these facts, it becomes clear that misregulations related to the cholesterol-pathway can lead to major malformations and diseases, e.g. Niemann-Pick type C, Smith-Lemi-Opitz syndrome or Tangier disease (Benarroch 2008).

1.2.2 Sources of cholesterol

Cholesterol can either be synthesized *de novo* or taken up by the cell through cholesterol-rich circulating lipoproteins. For the organism as a whole, sources can be biosynthesis (usually in the liver) and diet. It is known that already in the fetal brain most cholesterol is synthesized *in situ* (Clayton 1998). In the fully developed brain exchange of cholesterol across the BBB is inhibited, hence cholesterol must be formed *in situ* (Pfriege and Ungerer 2011).

1.2.3 Biosynthesis of cholesterol

The biosynthesis of cholesterol starts with acetyl-CoA (acetyl Coenzyme A). In the brain, acetyl CoA is mainly derived from glucose. In other tissues other molecules, like fatty acids contribute to cholesterol biosynthesis. Generally, the first steps of the

pathway take place in peroxisomes. Later steps take place in the cytoplasm or ER. For a simplified pathway see Figure 7.

In a first step of reactions, which takes place in the cytosol, acetyl-CoA is converted into 3-hydroxy-3-methylglutaryl CoA with help of the enzyme hydroxymethylglutaryl-CoA synthase. In the next step, which takes place in the ER, mevalonate is formed. This committed step is enforced by the enzyme HMGCR (3-hydroxy-3-methylglutaryl-Coenzyme-A-reductase), which is an integral ER membrane protein. It is the rate limiting step in the pathway of cholesterol biosynthesis.

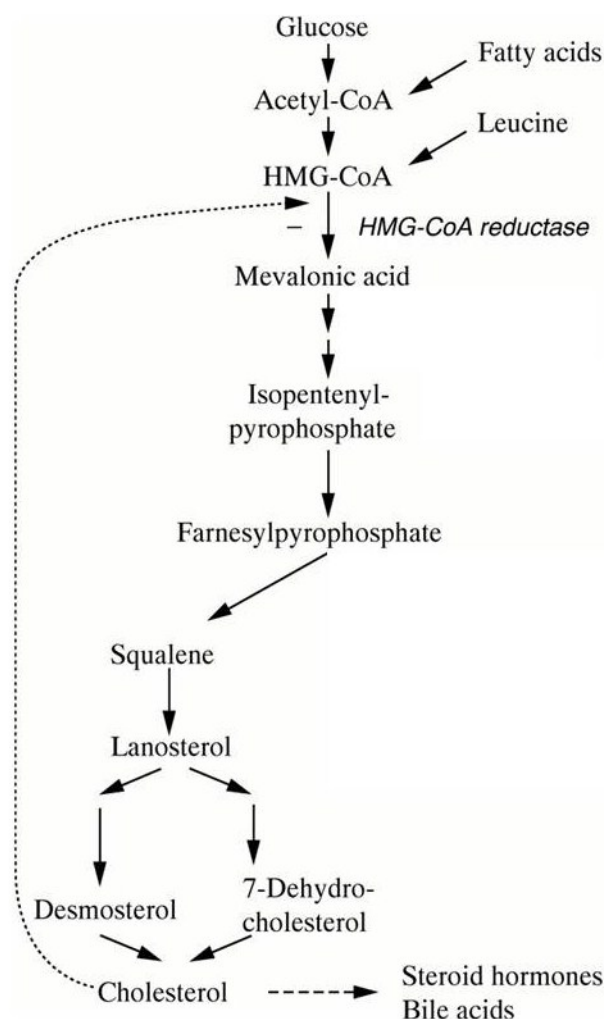


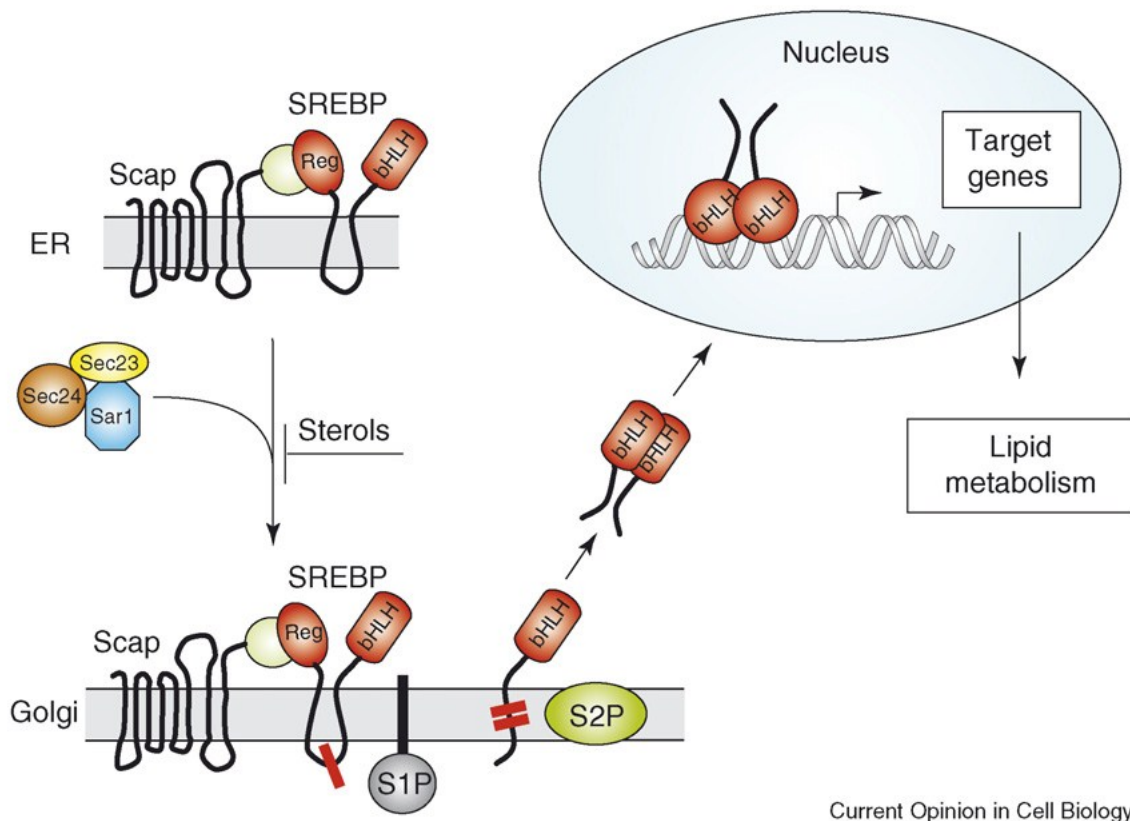
Figure 7: Simplified pathway of cholesterol biosynthesis (modified from (Clayton 1998)).

Mevalonate is then converted to squalene through steps forming isopentenyl pyrophosphate (C₅), dimethylallyl pyrophosphate (C₅), geranyl pyrophosphate (C₁₀)

and farnesyl pyrophosphate (C_{15}). These steps take place in the cytoplasm and peroxisome. The last step in the synthesis of squalene is catalyzed by the ER enzyme squalene synthase. In the final step of cholesterol biosynthesis lanosterol is formed from squalene condensation, which is then converted to cholesterol (C_{27}). These last steps take place at the ER membrane (Berg, Tymoczko et al. 2002).

1.2.4 Regulation of cholesterol biosynthesis

Cholesterol and lipid metabolism are controlled by sterol regulatory element binding protein family of transcription factors (SREBP) (Espenshade 2006). The family consists of three transcription factors, SREBP1a, SREBP1c and SREBP2. The SREBPs are synthesized as precursor proteins and are located in the ER membrane through two membrane-spanning domains. As long as the proteins are membrane-associated, they are transcriptionally inactive. A sterol sensor protein, called SREBP-cleavage-activating protein (SCAP) interacts with the C-terminus of the SREBP (Edwards, Tabor et al. 2000; Goldstein, DeBose-Boyd et al. 2006). In case of sterol depletion, SCAP interacts with vesicle proteins Sar1 and Sec23/24, binds to SREBP and escorts it in COPII-coated vesicles from the ER to the Golgi, where SREBP is processed by two proteases called site 1 and site 2 protease (S1P and S2P) and thereby activated (Figure 8). The transcriptionally active mature form of SREBP translocates to the nucleus and binds to promoters of SREBP target genes, thereby initiating their transcription. The target genes are involved in synthesis and metabolism of cholesterol. When cholesterol levels reach a critical threshold, cholesterol itself binds to Scap thereby initiating a conformational change in Scap. As a result, Scap cannot bind to COPII coat proteins Sar1 and Sec23/24 anymore, which is needed to incorporate the SREBPs into COPII-coated vesicles. A group of proteins, called Insigs (Insig-1 and Insig-2) are further enhancing the sterol-mediated inhibition of the Scap-COPII complex formation by interacting with Scap and HMGR. The Scap/SREBP complex is retained in the ER, and proteolytic activation of SREBPs is stopped. This leads to a decline of transcription of SREBP target genes, including the SREBPs themselves (Bengoechea-Alonso and Ericsson 2007).



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Figure 8: Activation of SREBPs. Scap senses a lack of cholesterol and escorts SREBP to the Golgi, where it is processed by proteases to reach its active form. Mature SREBPs then translocate to the nucleus to initiate transcription of cholesterol synthesis and metabolism (Bengoechea-Alonso and Ericsson 2007).

1.2.5 Cholesterol homeostasis in the central nervous system

In the CNS cholesterol has a big impact as it is a major component of myelin. The majority of sterol in the brain is unesterified cholesterol, followed by smaller amounts of desmosterol and very little cholesteryl ester. The concentration of unesterified cholesterol in the human brain is approximately 23 mg/g brain tissue, which is far the highest in all tissues of the human body. The human brain accounts for 2.1 % of the total body weight, but contains 23 % of the sterol pool (Dietschy and Turley 2004). Although the basal metabolic rate in the brain is very high, the turnover rate of cholesterol and other sterols is exceptionally low. In the mouse it reaches 0.4 % per day, whereas in the human it only reaches up to 0.03 % per day. In contrast, the whole-body turnover of cholesterol in the mouse reaches approx. 8 % per day (Dietschy and Turley 2001). There is solid evidence that the entire cholesterol which is required in the brain is synthesized *in situ* rather than imported from the blood, as lipoprotein transport across the BBB is inhibited (Edmond, Korsak et al. 1991;

Jurevics and Morell 1995; Pfrieger 2003). It is also known that the synthesis of cholesterol exceeds the actual requirements, indicating that homeostatic pathways that mediate efflux of excess cerebral cholesterol to the peripheral circulation must exist (Dietschy and Turley 2004).

It was shown, that in the developing brain, neurons cover their cholesterol requirements by biosynthesis (Vance, Pan et al. 1994; de Chaves, Rusinol et al. 1997). As soon as astrocytes are fully developed, neurons reduce their own synthesis and import cholesterol for axon repair or remodeling from astrocytes via cholesterol-laden lipoproteins (Figure 9) (Mahley 1988). An explanation for this change might be the high cost of this pathway for neurons. Cholesterol synthesis requires large amounts of energy. For one cholesterol-molecule, 20 enzymes, 18 acetyl-CoA, 18 ATP and 29 NADPH are needed (Vance, Pan et al. 1994; de Chaves, Rusinol et al. 1997). Due to the big surface area and long distances to the presynaptic terminals and dendritic spines it is probably more cost-effective for neurons to outsource this function to astrocytes (Kabara 1973). Furthermore, there is evidence that the expression level of cholesterol-specific enzymes and the cholesterol content (Turley, Burns et al. 1998) vary among the brain regions (Zhang, Appelkvist et al. 1996; Bae, Lee et al. 1999; Pfrieger 2003).

1.2.6 Trafficking of cholesterol

Cholesterol exchange between cells is mediated by lipoproteins. Since the BBB separates the cerebrospinal fluid (CSF) from the plasma, the lipoprotein composition in the CNS is distinct from that in the plasma. The level of apo A1 (apolipoprotein A1), the most abundant lipoprotein in the plasma, is lower in the brain. It is not synthesized directly in the brain, but rather in endothelial cells at the BBB and partly taken up from the plasma by unknown mechanisms (Pitas, Boyles et al. 1987; Mockel, Zinke et al. 1994; Panzenboeck, Balazs et al. 2002). The major lipoprotein in the brain is apo E, which is synthesized by astrocytes and assembled in HDL-like particles (Fagan, Holtzman et al. 1999). It is involved in recycling phospholipids and cholesterol locally (Beffert, Danik et al. 1998). Furthermore, apo J (DeMattos, Brendza et al. 2001) and apo D (Patel, Asotra et al. 1995) are also found in the CNS. Members of the ATP-binding cassette (ABC) transporter family mediate the efflux of lipid from cells to apolipoproteins and are key players in the regulation of formation of

apo E containing lipoproteins in the brain. It is known that ABCA1 and ABCG1 are expressed in the brain. ABCA1 seems to regulate the level of apo E in the CNS. Furthermore, it is necessary for the production of normally-lipidated apo E by astrocytes *in vivo* and *in vitro* (Hirsch-Reinshagen, Zhou et al. 2004; Vance and Hayashi 2010). Defects in the gene encoding ABCA1 were shown to be the cause of Tangier disease, characterized by a low level or even lack of HDL and high risk of coronary artery disease (Rust, Rosier et al. 1999). ABCG1 and the related ABCG4, which are highly expressed in astrocytes and neurons (Karten, Campenot et al. 2006; Tarr and Edwards 2008; Wang, Yvan-Charvet et al. 2008; Bojanic, Tarr et al. 2010), have been shown to have a function in promoting sterol efflux from astrocytes (Vance and Hayashi 2010).

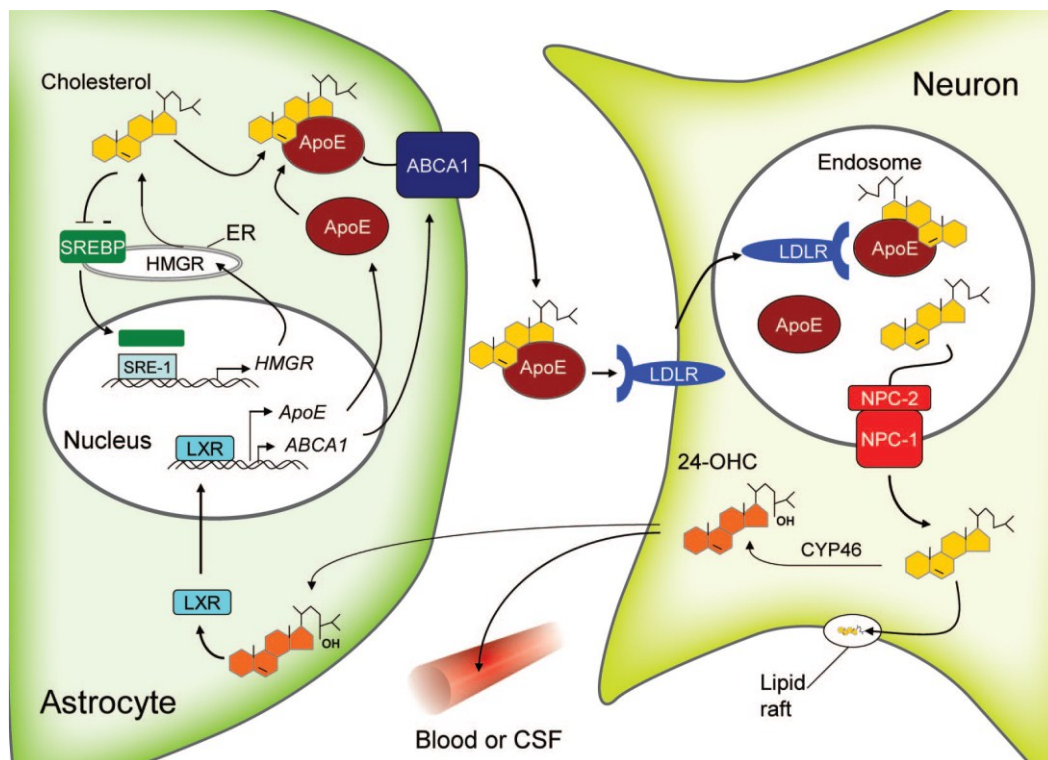


Figure 9: Neurons outsource cholesterol synthesis to nearby astrocytes. In the developed brain, astrocytes synthesize cholesterol via the enzyme HMGCR (and several others) and secrete it in form of cholesterol-loaden apo E through the transporter ABCA1 to nearby neurons. HMGCR is regulated by a feedback inhibition via SREBP. On the side of the neuron, the cholesterol-loaden lipoprotein is internalized via LDLR. NPC1 and NPC2 are required for intracellular shuttling of cholesterol in the neuron. Excess of cholesterol is converted to 24-hydroxycholesterol by the cytochrome P450 enzyme CYP46. Oxysterols, which are important ligands for LXRs can cross the BBB (Benarroch 2008).

1.3 Oxysterols – modulators of cholesterol metabolism and other processes

1.3.1 Main functions of oxysterols

Oxysterols are potent transcriptional regulators of lipid metabolism. The half-life of most oxysterols is short, as they are converted into bile-acids and eliminated from the body immediately (Bjorkhem 2006). Side-chain hydroxylation of cholesterol makes the sterol more hydrophilic and enhances the ability of the molecule to pass lipophilic membranes. It was shown that oxysterols are potent transcriptional regulators of sterol and fatty acid metabolism (Brown and Goldstein 1974; Kandutsch and Chen 1974). Oxysterols are found in trace amounts in biological membranes and lipoproteins, and are always accompanied by a great excess of cholesterol (10^3 - 10^6 fold). They play important roles in several biological pathways, e.g. apoptosis, necrosis, immunosuppression and inflammation as well as in states of diseases like atherosclerosis or the development of gallstones (Bjorkhem and Diczfalussy 2002).

1.3.2 Brain oxysterol formation and metabolism

Several oxysterols were discovered so far. The most abundant ones in the brain are in this order 24(S)-hydroxycholesterol (24-HC), 27-hydroxycholesterol (27-HC) and 25-hydroxycholesterol (25-HC). The first two are generated by cytochrome P450 enzymes, while the generation of the last one is catalyzed by a non-heme di-iron containing enzyme (Figure 10).

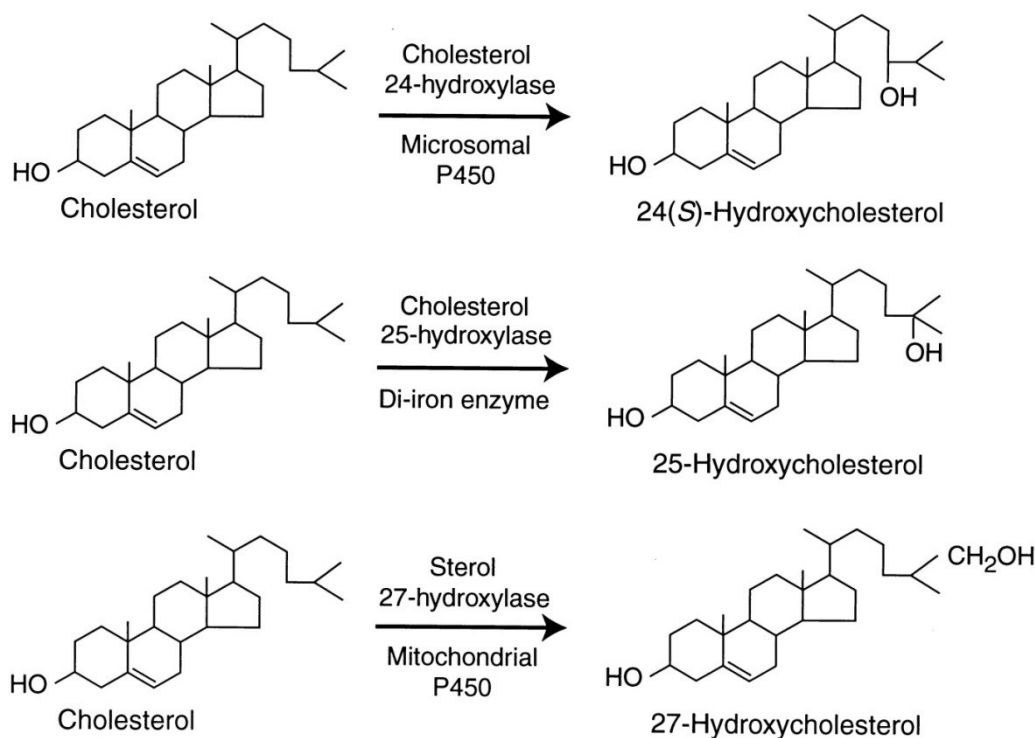


Figure 10: Cholesterol is converted into oxysterols by different enzymes. The conversion to 24-HC as well as 27-HC is catalyzed by two different cytochrome P450 enzymes, namely cholesterol-24 hydroxylase and sterol 27-hydroxylase, whereas the conversion to 25-HC is catalyzed by the di-iron enzyme cholesterol 25-hydroxylase (Russell 2000).

24(S)-hydroxycholesterol

24-HC is known to be synthesized in order to get rid of excess cholesterol in the brain. Through this mechanism the sterol gathers the ability to pass lipophilic phospholipid-containing membranes up to 3,000 times faster than cholesterol itself, thereby diffusing easily through the BBB (Bjorkhem 2006) into the circulation, where it is transported to the liver and converted into bile acids (Lutjohann, Breuer et al. 1996). The monooxygenase catalyzing this conversion, cholesterol 24-hydroxylase (CYP46A1), is expressed predominantly in the brain, in the ER of a specific subset of neurons only (Bjorkhem 2006). However, its expression is restricted to certain regions in the brain, as well as certain neurons in these regions (Russell 2000). The levels of 24-HC in the circulation seem to be remarkably stable (Bjorkhem and Diczfalusy 2002). In the brain, the levels of 24-HC were found to be 4-15 ng/mg wet weight (Lutjohann, Breuer et al. 1996).

27-hydroxycholesterol

27-HC was shown to be quantitatively the most important oxysterol in the circulation of mice (Li-Hawkins, Lund et al. 2000) and adult humans (Dzeletovic, Breuer et al. 1995). However, its level in the brain is low, less than 10 % of those of 24-HC. There is evidence that a significant net uptake of 27-HC by the brain takes place. The enzyme responsible for the conversion of cholesterol to 27-HC, sterol 27-hydroxylase (CYP27A1), is located in the mitochondria of several, if not all cell types. Around 25 % of the bile-acid pool originates in oxysterols which are produced by CYP27A1 (Russell 2003). A lack of CYP27A1 in humans leads to cerebrotendinous xanthomatosis (CTX), a disease characterized by cholestanol-containing xanthomas in the brain and tendons. Mice lacking CYP27A1 do not develop xanthomas and accumulate less cholestanol than humans with CTX (Bavner, Shafaati et al. 2010).

25-hydroxycholesterol

25-HC is the most potent regulator of gene transcription involving lipid metabolism, gene expression and immune activation (Lund, Kerr et al. 1998; Bauman, Bitmansour et al. 2009). Cholesterol 25-hydroxylase (CH25H), the enzyme catalyzing the synthesis of 25-HC is expressed in most tissues at a low level. It is a glycoprotein and contains iron as a cofactor, which is present as Fe-O-Fe or Fe-OH-Fe. The iron is located within clusters of conserved histidine or glutamate residues (Fox, Shanklin et al. 1994; Shanklin, Whittle et al. 1994; Broun, Shanklin et al. 1998; Lund, Kerr et al. 1998). The gene CH25H, which is located on chromosome 10q contains no introns and is conserved throughout vertebrates (Papassotiropoulos, Lambert et al. 2005). Human and murine sequences show an identity of 78 % and even C-4 sterol methyl oxidase from *S. cerevisiae* shows 27 % identity to CH25H. Lund *et al.* showed that CH25H, transfected into COS cells is located at the ER or Golgi compartment. Furthermore, the enzyme is predicted to span the membrane multiple times. The enzyme CH25H is capable to hydroxylate cholesterol as well as 24-HC, leading to the products 25-HC and 24, 25-HC, respectively (Lund, Kerr et al. 1998). In humans, the level of 25-HC in plasma is equal to 3-5 ng/ml, but it is not clear, what the relative contribution of CH25H is, as the enzyme CYP27A also contributes to the formation of 25-HC in the plasma (Dzeletovic, Breuer et al. 1995). Some contradictory data on the implication of CH25H in AD were published by two groups (Papassotiropoulos, Lambert et al. 2005; Shibata, Kawarai et al. 2006). Furthermore it was reported that

metabolites of Vitamin D₃, namely 25-hydroxy vitamin D₃ and 1,25-dihydroxy vitamin D₃ upregulate CH25H in human primary prostate stromal cells (Wang and Tuohimaa 2006).

1.3.3 The role of 25-hydroxycholesterol in the regulation of lipid metabolism

25-HC is a potent regulator of cholesterol biosynthesis. 25-HC binds to the membrane-bound oxysterol binding protein insulin induced gene 2 (INSIG2), which specifically binds cholesterol derivatives with hydroxyl groups on the side chain. Subsequently, INSIG2 (and probably the same is true for INSIG1) undergoes a conformational change and binds to SREBF chaperone (SCAP), which leads to a retention of SREBP in the ER thereby inhibiting SREBP activation. Furthermore, INSIG2 also binds to HMGCR, resulting in ubiquitination and degradation of HMGCR (Radhakrishnan, Ikeda et al. 2007).

Oxysterols are natural ligands for liver X receptor α (LXR α) and liver X receptor β (LXR β) (Ulven, Dalen et al. 2005; Wong, Quinn et al. 2008). It was shown, that 25-HC has an intermediate capability ($EC_{50}=3.5 \mu M$) to activate LXR α , an orphan member of the nuclear receptor superfamily (Janowski, Willy et al. 1996). LXRs are involved in the regulation of fatty acid and cholesterol metabolism (Willy, Umesono et al. 1995; Tontonoz and Mangelsdorf 2003; Li and Glass 2004). LXR α is mainly expressed in adipose tissue, liver and enterocytes, whereas LXR β is expressed ubiquitously (Lehmann, Kliewer et al. 1997). Both isotypes regulate the concentration of free cholesterol in peripheral tissues by regulating the transcription of the cholesterol efflux pumps ABCA1 and ABCG1 (Tontonoz and Mangelsdorf 2003; Levin, Bischoff et al. 2005). They have the potential to act as ligand-dependent transcription factors, together with their heterodimeric partner retinoic-X receptor (RXR) or even alone. The findings regarding the regulation of lipid metabolism were also supported by a study with triple knockout mice deficient in CYP46, CYP27 and CH25H, which showed an impaired LXR response upon cholesterol feeding (Chen, Chen et al. 2007). LXR was shown to be a key transcriptional regulator of apo E gene in a tissue specific manner (Tam and Ramharack 1992; Brahimi, Bertrand et al. 2001; Laffitte, Repa et al. 2001). It was shown that 25-HC is able to stimulate the

secretion of apo E. This effect could be compensated by adding TNF α , which if added alone, had the opposite effect (Gueguen, Bertrand et al. 2001).

1.3.4 The role of 25-hydroxycholesterol in innate immunity

Recent work has put 25-HC and its enzyme CH25H into limelight for its role in modulation of immune response and inflammation (Bauman, Bitmansour et al. 2009; Diczfalusy, Olofsson et al. 2009; Park and Scott 2010). It was reported that the expression of CH25H in macrophages is induced rapidly upon treatment with lipopolysaccharide (LPS), an integral cell wall component of gram-negative bacteria. Furthermore, an injection of a low dose of LPS into healthy volunteers led to an increase in plasma 25-HC concentration. It was shown that the upregulation was dependent on toll-like receptor 4 (TLR4) signaling but independent of Myeloid differentiation protein 88 (MyD88) signaling (Diczfalusy, Olofsson et al. 2009). Studies by Baumann *et al.* showed that LPS treatment induced CH25H expression levels in heart, brain, lung, muscle, kidney and thymus in mice. The group suggests that the activation of the innate immune response through the activation of TLRs (especially TLR4 and to a lesser extent TLR3) stimulates the synthesis of 25-HC. Subsequently, 25-HC suppresses the class switch recombination (CSR) in B cells from IgM to IgA by a repression of the induction of activation-induced cytidine deaminase (AID) in response to transforming growth factor β 1 (TGF β 1) and interleukin 5 (IL5). Furthermore, it suppresses the cell division of B cells, which is required for CSR. Park *et al.* reported that the expression of CH25H is dependent on a pathway involving TRIF, and the IFN α /JAK/STAT1 pathway as well as the production of type I interferon (IFN) (Park and Scott 2010). On the other hand, it was reported that 25-HC suppresses TLR-mediated innate immune response through the activation of LXR (Castrillo, Joseph et al. 2003; Joseph, Castrillo et al. 2003). A possible role of 25-HC in airway diseases was tested by Koarai *et al.* It was shown that 25-HC increases the release of IL-8 and IL-6, both via a NF- κ B signaling pathway. Furthermore, it enhanced the release of IL-8 and IFN β in airway epithelial cells after stimulation of TLR3 (Koarai, Yanagisawa et al. 2012). Adding up these data, 25-HC seems to be an immunoregulatory lipid which negatively regulates the adaptive immune response.

1.4 Innate immune response

The immune response to microbial pathogens depends on the innate as well as the adaptive immune system (Hoffmann, Kafatos et al. 1999). The first line of defence is made up mostly by white blood cells, including neutrophil granulocytes and macrophages. These cells efficiently bind and kill pathogens and coordinate additional host responses by releasing a wide range of inflammatory mediators and cytokines. Furthermore, macrophages, which kill and degrade pathogens within the maturing phagosome, present components of the pathogen on their surface to T cells, thereby activating the adaptive immune response (Aderem and Underhill 1999; Aderem and Ulevitch 2000).

1.4.1 Neutrophils

Neutrophils account for 60 % of all white blood cells in humans (Bainton, Ulliyot et al. 1971). Their morphology is unique, as they have a polymorphic nucleus and numerous granules in the cytoplasm (Borregaard, Sehested et al. 1995). Three major types of granules can be distinguished in neutrophils. The primary “azurophilic” granules develop first and contain myeloperoxidase (MPO), neutrophil elastase, cathepsin G and bactericidal permeability increasing protein (BPI), secondary granules specifically contain lactoferrin and tertiary granules contain gelatinase and surface receptors (Bainton, Ulliyot et al. 1971; Brinkmann, Reichard et al. 2004). Neutrophils are driven by chemotactic gradients to the site of infection. They bind to E- or P-selectins on endothelial cells and roll with the blood stream along the endothelial surface until they manage to establish a tight adhesion to endothelial cells. They become flat and traverse through endothelial gaps to migrate to the site of infection. This whole process is accompanied by expression of chemokines, complement factors, integrins and others (Delves and Roitt 2000). At the site of infection, neutrophils engulf pathogens into a membrane-bound compartment, called phagosome. The phagosome fuses with granules and in this phagolysosome microorganisms are killed very effectively by a mixture of antimicrobial peptides, enzymes and reactive oxygen species (ROS). Afterwards, most neutrophils undergo apoptosis in a process requiring CD11b/CD18, ROS and caspases and are removed by macrophages (Haslett 1997; Serhan and Savill 2005; Brinkmann and Zychlinsky 2007). However, neutrophils are also able to kill bacteria and other microorganisms

extracellularly by degranulating and releasing antimicrobial factors into the extracellular medium (Henson 1992).

1.4.2 Two ways to fight/kill pathogens

Neutrophils are able to disarm microorganisms oxygen-dependently or independently. The oxygen-independent killing pathway involves the release of highly cationic proteins and peptides from neutrophil granules, thereby initiating the binding of the anionic microorganisms to the cell surface of neutrophils. However, the exact mechanisms of how these cells proceed to kill pathogens are not known yet (Lehrer and Ganz 1999). The oxygen-dependent way to kill microorganisms involves the NADPH oxidase enzyme complex, which is located at the phagosomal membrane. The hydrogen peroxide (H_2O_2), which is produced by this enzyme complex is used by the MPO to generate highly reactive hypochlorite (HOCl), which oxidizes proteins, nucleic acids and other molecules (Hampton, Kettle et al. 1998; Brinkmann and Zychlinsky 2007).

1.4.3 NETosis

Neutrophil extracellular traps (NETs) were discovered by Brinkmann *et al.* in 2004 (Figure 11). It was shown that approximately one-third of activated neutrophils generate extracellular fibers. These extracellular fibers contain chromatin bound to proteins from azurophilic granules, such as MPO. NETs surround the cell from which they originate from and immobilize (and possibly kill) bacteria extracellularly (Brinkmann, Reichard et al. 2004). However, the release of NETs is accompanied by neutrophil cell death, as in the moment the components of NETs are released the neutrophil cell membrane ruptures (Fuchs, Abed et al. 2007). Therefore, the stimulation of neutrophils to form NETs requires the activation of several receptors, such as TLRs, as well as cytokine and Fc receptors in concert and is usually the last line of defence after phagocytosis of bacteria (Clark, Ma et al. 2007). Upon stimulation of NET formation, protein kinase C (PKC) is activated, and through a signal transduction cascade the NADPH oxidase complex is formed and activated (Fuchs, Abed et al. 2007). There is evidence that ROS formed by the complex is required for the formation of NETs and it was shown that H_2O_2 is a potent inducer of NET formation (Droge 2002). However, the formation of NETs might also have

negative consequences. In case of patients with autoimmune diseases such as systemic lupus erythematoses (SLE) NETs probably induce a pathological reaction against self, as the immune system develops antibodies against NET components (Fairhurst, Wandstrat et al. 2006).

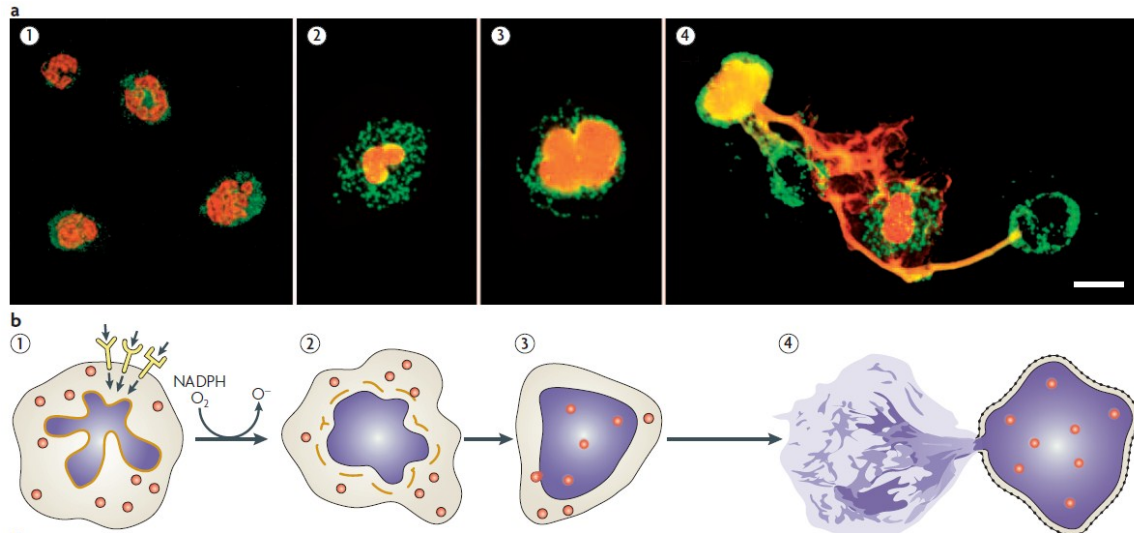


Figure 11: The steps in NETosis. 1: Several receptors on the surface of neutrophils are stimulated simultaneously by pathogens, thereby activating the neutrophil NET formation. 2: In the first 60 minutes, no major changes in the morphology of the nucleus or the granules occur. 3: Thereafter, the nucleus starts losing the lobules, chromatin segregates into euchromatin and heterochromatin. 4: 180 min after activation, NET components are freely mixed in the cell and shortly after that NETs are released and neutrophils die. a) Fluorescent micrographs and b) schematic overview (Brinkmann and Zychlinsky 2007).

1.5 Myeloperoxidase

1.5.1 Sources of myeloperoxidase

MPO is mainly expressed by polymorphonuclear neutrophils (PMN), where it is stored in primary/azurophilic granules and released in the phagosome as well as the extracellular space. It makes up approximately 5 % of total protein in these cells. Besides that, MPO is also expressed in monocytes, tissue macrophages, Kupffer cells, microglia and neurons although to a much lesser extent (Bos, Wever et al. 1978; Daugherty, Dunn et al. 1994; Kettle, van Dalen et al. 1997; Klebanoff 1999). During maturation to macrophages, monocytes gradually lose their ability to synthesize MPO. However, macrophages can take up MPO by endocytosis of whole neutrophils or even MPO alone.

1.5.2 Structure of MPO

MPO is a strongly cationic glycosylated protein with a molecular weight of 140 kDa. It consists of a heme-containing homo-dimer linked by a disulfide bridge (Nauseef and Malech 1986). Each homo-dimer consists of a heavy (55-64 kDa) and light (10-15 kDa) chain (Akin and Kinkade 1986; Taylor, Guzman et al. 1990). The secondary structure is mostly α -helical, with 5 helices per monomer (Zeng and Fenna 1992). The heavy chain contains a protoporphyrin IX group with an iron ion in the center. The two heme groups, which are linked with the apoprotein by one sulfonium ion linkage and two ester linkages are functionally identical (Andrews, Parnes et al. 1984). The sulfonium ion linkage, which is unique to MPO in contrast to other mammalian peroxidases, seems to be responsible for its chlorination properties (Fenna, Zeng et al. 1995; Taylor, Strobel et al. 1995).

1.5.3 Enzymatic reactions catalyzed by MPO

MPO is able to form several oxidants, as well as oxidize different substrates. The complete MPO-system contains the enzyme MPO, H_2O_2 and oxidizable cofactors (Figure 12). The native enzyme, in which the heme iron is in the ferric state (Fe^{3+}) can bind either superoxide anion radicals (O_2^-) or hydrogen peroxide (H_2O_2) and be oxidized by these substrates (Kettle and Winterbourn 1994). Oxidation by H_2O_2 leads to transformation of MPO into a short-lived redox intermediate called compound I. In compound I the iron is in its ferryl state (Fe^{4+}) (Kettle, van Dalen et al. 1997; Everse 1998). Compound I, which is the primary catalytic complex of MPO is able to oxidize several substrates like halides (e.g. Cl^- , Br^- , I^-) or pseudohalides (e.g. SCN^- thiocyanate) to the corresponding hypo(pseudo)halous acid (Lau and Baldus 2006).

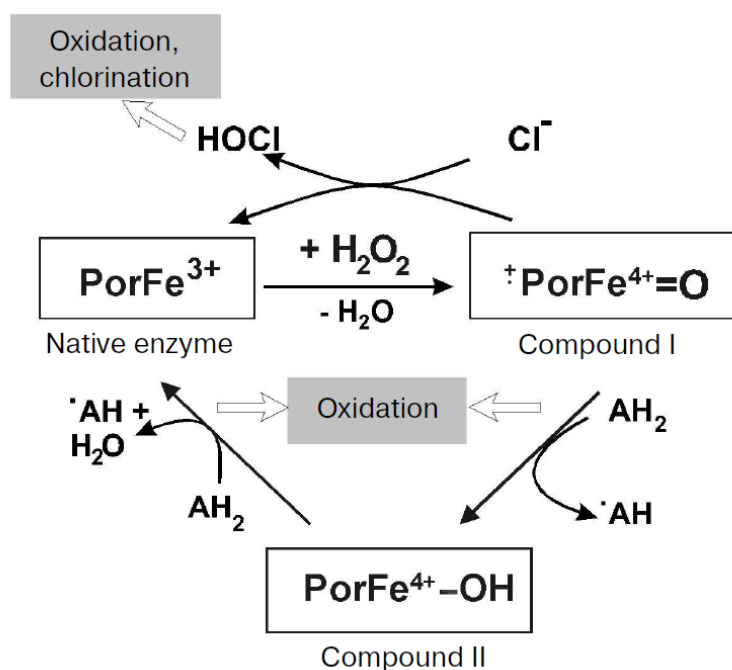


Figure 12: MPO complex formations. MPO in its native state is bound to iron in its ferric state (Fe³⁺). In the presence of its substrate H₂O₂ the iron is oxidized and MPO is transformed into a short-lived intermediate called compound I, which contains the iron in its ferryl state (Fe⁴⁺). Compound I oxidizes several substrates like Cl⁻, thereby regenerating the native Fe³⁺-MPO. The reaction of compound I with excessive H₂O₂ results in the formation of compound II. This compound is inactive regarding the oxidation of chloride, but can be reduced to the active, native enzyme (Arnhold 2004).

1.5.4 MPO reaction products

In physiological fluids like plasma Cl⁻ is present in a 1000-fold molar excess (100-140 mM) compared to other (pseudo-)halogens like Br⁻ (20-100 μM), SCN⁻ (20-120 μM) and I⁻ (<1 μM). Hence it is assumed that the powerful chlorinating oxidant HOCl is the main product of the MPO-H₂O₂ mediated reaction (Fliss 1988). Nevertheless, the exact nature of product formation is not clear, as the affinity of Cl⁻ to MPO is much lower compared to other anions (1:60:730 for Cl⁻, Br⁻, and SCN⁻). HOCl is a major reactive oxidant critical for the antibacterial and antiviral properties of neutrophils. Approximately 45-80 % of the H₂O₂ generated by activated neutrophils forms 20-400 μM HOCl per hour. Almost all remaining H₂O₂ is converted to HSCN, only a small amount (< 5%) to HOBr and HOI (Hawkins C. L. 2009, Malle et al. 2006a, Yap et al. 2007). Most species of bacteria are killed readily by the MPO/H₂O₂/chloride system (Klebanoff, Waltersdorff et al. 1984; Winterbourn 1985; van Dalen, Whitehouse et al. 1997; Eiserich, Hristova et al. 1998; Winterbourn, Vissers et al.

2000; Malle, Marsche et al. 2006; Yap, Whiteman et al. 2007; Hawkins 2009). An overview of the reactions with HOCl is shown in Figure 13.

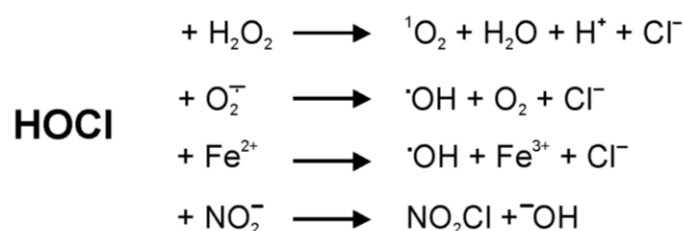


Figure 13: HOCl-derived reactive oxygen and nitrogen species (Arnhold 2004).

1.5.5 Physiological and pathophysiological properties of MPO

At physiologic pH the product of MPO, HOCl (pK_a : 7.53) exists as a mixture of hypochlorite ion and undissociated acid. In case of a lower pH, as it exists in the phagosome of neutrophils, HOCl predominates and can react with excess chloride to form molecular chlorine (Cl_2) (Hazen, Hsu et al. 1996; Hazen, Hsu et al. 1996; Henderson, Byun et al. 1999). These highly reactive and short-lived products can attack microorganisms from several sites (Winterbourn 2002). Attacks can happen at any oxidizable group on proteins (e.g. iron-sulfur proteins, membrane transport proteins, sulfhydryl and thioether groups of proteins), nucleic acids (e.g. origin of replication site for DNA synthesis) and lipids (e.g. cholesterol, plasmalogens) (Albrich and Hurst 1982; McKenna and Davies 1988; Rosen, Michel et al. 1998; Ullen, Fauler et al. 2010). Furthermore, nitrogen-chlorine derivatives (e.g. monochloramines and dichloramines) can form upon the reaction of HOCl with nitrogen-containing compounds (Figure 14) (Hazen, d'Avignon et al. 1998; Hazen, Hsu et al. 1998; Klebanoff 2005). Nevertheless, HOCl also has the potential to damage host tissue. It can convert free and protein-bound tyrosine residues to 3-chlorotyrosine, which is a specific marker for MPO activity (Domigan, Charlton et al. 1995; Hazen, Hsu et al. 1996). 3-chlorotyrosine was found in LDL isolated from human atherosclerotic tissue, suggesting an involvement of MPO in the oxidative damage of the human artery wall (Hazen and Heinecke 1997). Furthermore, oxidation of macrophage-released NO produces nitrite, which might be converted to nitrogen dioxide radical by MPO (Eiserich, Hristova et al. 1998; Gaut, Byun et al. 2002). ONOO^- as well as $\text{NO}_2\cdot$ generate 3-nitrotyrosine from tyrosine residues, which is a potent nitrating

intermediate. Elevated levels of 3-nitrotyrosine have been detected in LDL isolated from human atherosclerotic tissue (Shao, Oda et al. 2010).

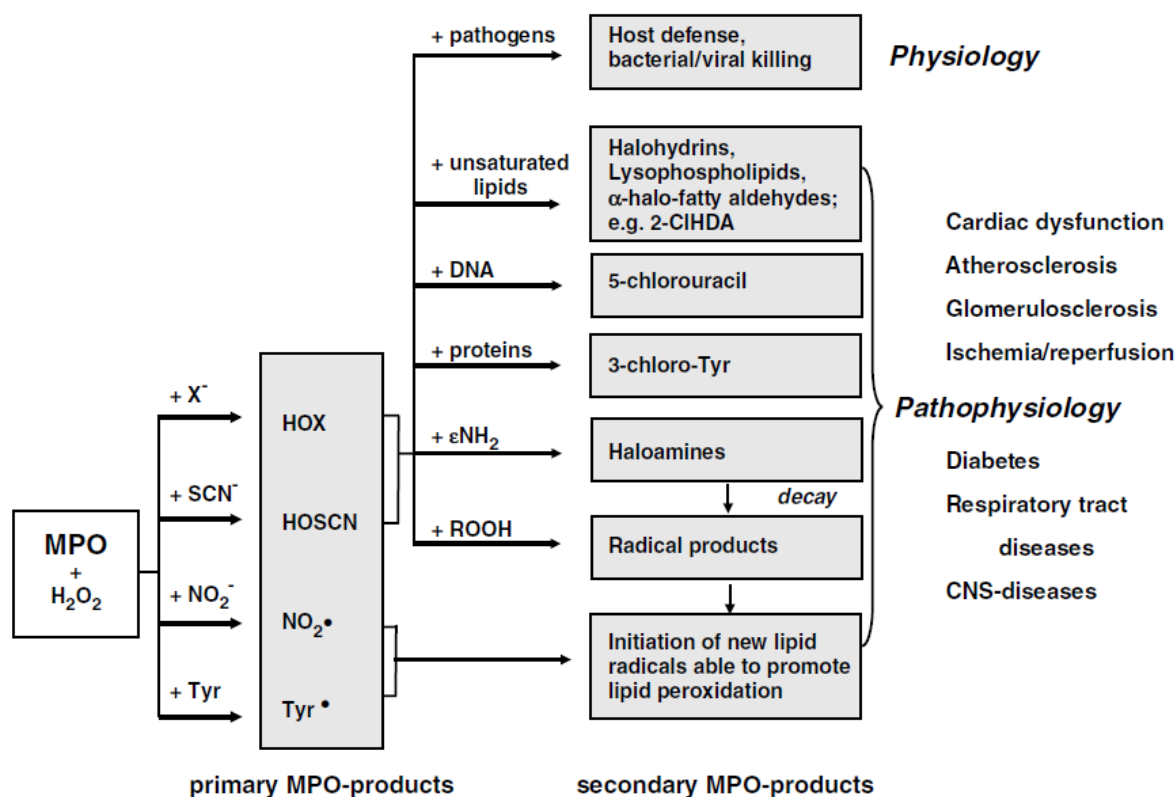


Figure 14: The MPO-H₂O₂-system and its physiological and pathophysiological consequences to the organism (Malle, Furtmuller et al. 2007).

1.5.6 Implications of MPO in neuroinflammatory and neurodegenerative diseases

Overactivation of MPO is assumed to be involved in the pathology of different diseases, such as MS, AD, PD and HD.

Multiple Sclerosis

MS is an inflammatory autoimmune disease of the CNS, which is associated with demyelinated axons leading to multifocal lesions. It is initiated by cross-reactive T-cells, which recognize myelin-associated antigens, like myelin basic protein (MBP) (Steinman, Waisman et al. 1995). It was shown that MPO mRNA and protein is located in microglia/macrophages in and around the lesions in MS in contrast to

normal brain tissue, where MPO was not detected. Cortical demyelination was associated with increased activity of MPO (Gray, Thomas et al. 2008). Furthermore, a -463G/A polymorphism in the MPO gene, which is associated with increased MPO production, was found to be increased in females with early-onset MS (Nagra, Becher et al. 1997). A well known mouse model of MS is the experimental autoimmune encephalitis model (EAE). EAE is an inflammatory disease which targets the central nervous system. It is induced by immunizing susceptible mice with components of the myelin sheath, e.g. myelin oligodendrocytic glycoprotein. Autoreactive T cells are generated, which infiltrate the CNS and lead to an inflammatory circle involving invading macrophages and resident microglia. The consequences involve regional demyelination, associated with nerve dysfunction and accompanied by a paralysis from the tail to the hind limbs, in severe cases also including the fore limbs. Brennan *et al.* showed that mice lacking MPO have a higher incidence and severity of EAE than wild-type littermates. They propose a protective role of MPO in EAE (Brennan, Gaur et al. 2001). Contradictory results were published by Forghani *et al.* recently. They found an increase of myeloid cells and MPO protein together with MPO-specific lesions in the brains of EAE mice, which were partly inhibited when injecting the irreversible MPO inhibitor 4-aminobenzoic acid hydrazide (ABAH) (Forghani, Wojtkiewicz et al. 2012).

Alzheimer's disease

AD is characterized by a cellular accumulation of amyloid β ($A\beta$)-peptide in the core of senile plaques in the brain as well as the intracellular accumulation of tau protein, so called neurofibrillary tangles and neuropil threads (Duyckaerts, Potier et al. 2008). Mounting evidence suggests oxidative damage to be an early event in AD (Lovell and Markesbery 2007). It was shown that lipid peroxidation in autopsy tissue of AD patients is increased, along with oxidized proteins and nucleic acids (Keller, Schmitt et al. 2005; Markesbery and Lovell 2007; Bader Lange, Cenini et al. 2008). In line with this finding, MPO colocalized with $A\beta$ and apo E within senile plaques in the cerebral cortex of AD patients. It was shown that apo E is highly susceptible to oxidation by the MPO-HOCl-system. Furthermore, MPO expression was induced *in vitro* in cultured rodent microglial cells by $A\beta$ (Jolivalt, Leininger-Muller et al. 1996; Jolivalt, Leininger-Muller et al. 2000). In a study of Maki *et al.*, a transgenic mouse model for AD, expressing the human MPO-463G allele and the human APP751 gene

was used (Maki, Tyurin et al. 2009). Mice expressing human APP751 are known to develop amyloid plaques and the MPO -463G allele is linked to higher MPO gene expression (Sturchler-Pierrat, Abramowski et al. 1997; Kumar, Piedrafita et al. 2004). In these mice, MPO was strongly expressed in astrocytes and deposited in A β plaques. The expression of MPO correlated with an increased level of 4-hydroxynonenal, a product of lipid peroxidation (Maki, Tyurin et al. 2009).

Parkinson's disease

MPO also seems to be involved in the pathogenesis of PD. PD, a neurodegenerative disorder affecting approx. 1 % of the population above the age of 60, is clinically characterized by disabling motor abnormalities (e.g. rigidity, resting tremor, bradykinesia and postural instability) (Dauer and Przedborski 2003). Its major neuropathological features are the loss of nigrostriatal dopaminergic neurons from the substantia nigra pars compacta (SNpc) area of the brain, which can be modeled by the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). Furthermore, intracytoplasmic inclusion bodies called “Lewy bodies”, which are mostly composed of fibrillar α -synuclein, can be found (Spillantini, Schmidt et al. 1997). Except for a few inherited cases related to known gene defects the outbreak of PD seems to be sporadic (Vila and Przedborski 2004). However, inflammation increases the risk of developing PD (Chen, Zhang et al. 2003). It was shown that inflammation and subsequent ROS formation are crucial for the development of the lesions. MPO protein and mRNA was reported to be upregulated in the ventral midbrain of human PD and MPTP mice. Furthermore, it was shown that MPO deficient mice are more resistant to MPTP-induced cytotoxicity than wild-type mice. The MPO-specific biomarkers 3-chlorotyrosine and HOCl-modified proteins, as well as reactive nitrogen species were increased in the brains of MPTP-injected mice (Pennathur, Jackson-Lewis et al. 1999; Choi, Pennathur et al. 2005).

1.6 Lipopolysaccharide as a model for neuroinflammation and neurodegeneration

The endotoxin lipopolysaccharide (LPS) is the major component of the outer leaflet of gram-negative bacteria. When administered, it immediately leads to an activation of

the innate immune system. It is responsible for alterations in the central and peripheral tissues, which are associated with gram-negative infections. Banks *et al.* and Singh *et al.* showed, that when administered systemically, only very little LPS directly enters the brain (Singh and Jiang 2004; Banks and Robinson 2010). In fact, LPS binds to its receptors CD14 and TLR4 and triggers a series of signaling events, subsequently leading to the development of an inflammatory response in the brain (Banks and Robinson 2010). Qin *et al.* showed that systemic LPS administration leads to a chronic neuroinflammation and progressive neurodegeneration. They reported that brain TNF α increases upon systemic LPS injection and remains elevated for 10 months, whereas TNF α in the serum drops back to basal level after 9 hours (Figure 15). The treatment activates microglia and increases the expression of pro-inflammatory factors, like TNF α , IL-1 β , MCP-1 and NF- κ B p65 in the brain. Furthermore, the number of tyrosine hydroxylase-immunoreactive neurons in the SNpc decreases by 23 % at 7 months, and 47 % at 10 months post treatment (Qin, Wu et al. 2007). Similar results were reported by Gao *et al.*, who postulated that LPS activation of microglia causes a progressive and cumulative loss of dopaminergic neurons over time (Gao, Jiang et al. 2002). Therefore, introduction of systemic LPS can be used as a model to study neuroinflammation and neurodegeneration.

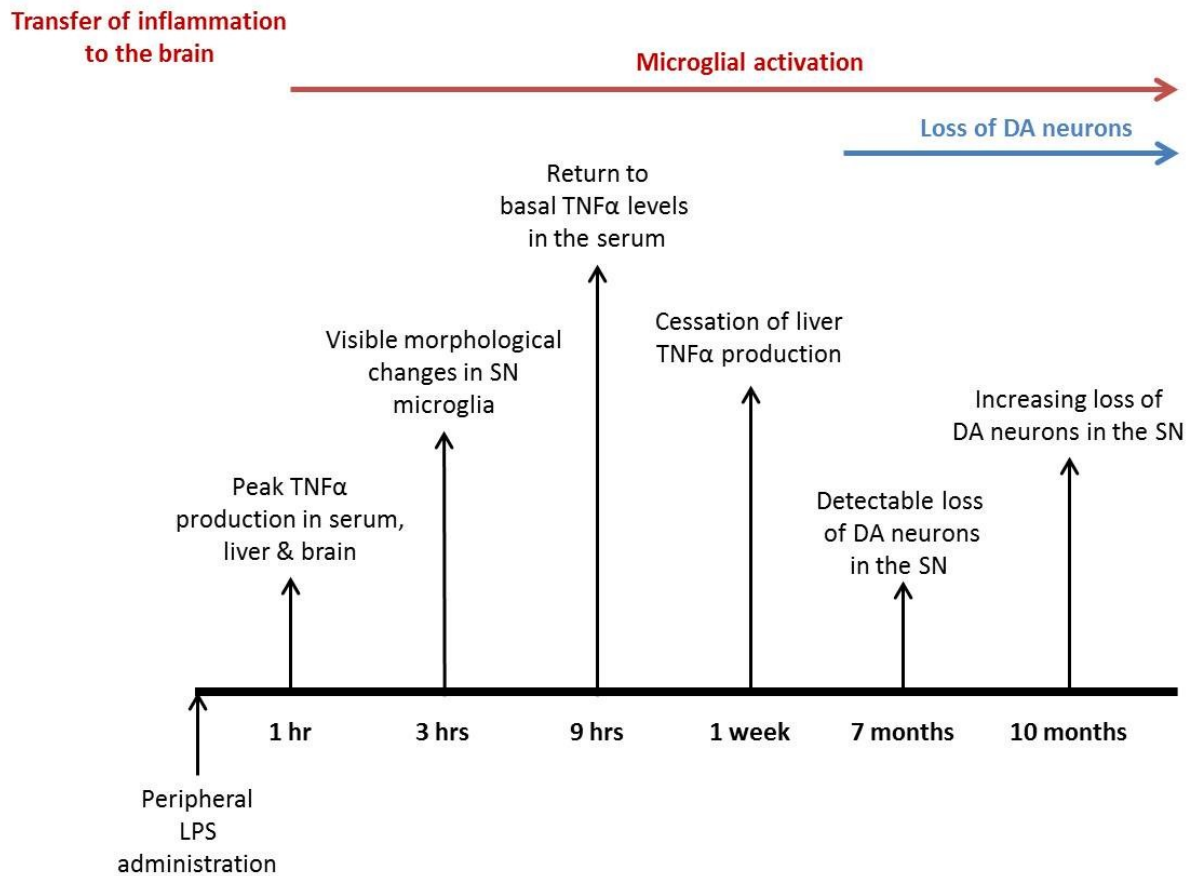


Figure 15: Systemic LPS leads to a chronic microglial activation and progressive loss of dopaminergic neurons (Qin, Wu et al. 2007).

2 AIMS

2.1 Aims – part 1

Myeloperoxidase is implicated in the progression of neurodegenerative diseases like Alzheimer's disease, Parkinson's disease and Multiple sclerosis. An inflammatory signal and subsequent disruption of the blood brain barrier lead to infiltration of plasma proteins and extravasation of immune cells e.g. macrophages, monocytes and neutrophil granulocytes from the peripheral circulation. During the inflammatory process, cytokines and reactive oxygen species are produced. In cooperation with the NADPH oxidase complex MPO generates the strong oxidant hypochlorous acid (HOCl) in the presence of physiological concentrations of chloride ions. HOCl is capable of modifying proteins, nucleic acids, and lipids. Especially unsaturated lipids, present in high amounts in the central nervous system, are susceptible to modification by HOCl. As unsaturated lipids fulfill a variety of important functions in the brain, HOCl-mediated modification might have deleterious consequences on CNS function.

Therefore, the first set of aims of this thesis was:

1. to obtain *in vivo* evidence for MPO expression in the brain upon neuroinflammation
2. to characterize alterations in gene expression during neuroinflammation in the CNS

2.2 Aims – part 2

The results of microarray analyses, which were established during part 1 of the thesis, showed a strong elevation of cholesterol-25 hydroxylase (CH25H) mRNA in brains of LPS treated mice. Therefore, we decided to further investigate the underlying mechanisms governing this upregulation *in vivo* and *in vitro* and to clarify the impact of 25-HC on cholesterol homeostasis in neurons and astrocytes.

In the central nervous system cholesterol has a big impact as it is the major architectural component of compact myelin. Cholesterol is not able to cross the intact blood-brain barrier and therefore is synthesized in the brain. Excessive cholesterol is hydroxylated, released into the peripheral circulation, and degraded in the liver. Side-chain hydroxylation of cholesterol makes the sterol more hydrophilic and enhances the ability of the molecule to pass the blood brain barrier. It is also known, that hydroxysterols are potent activators of SREBP (sterol regulatory element binding protein) and LXR (liver X receptor), thereby inhibiting cholesterol biosynthesis and increasing cholesterol efflux.

Therefore, the second set of aims of this thesis was:

1. To investigate whether CH25H mRNA upregulation parallels an increase of CH25H protein as well as its enzymatic end product 25-hydroxycholesterol
2. to measure the effect of 25-hydroxycholesterol on barrier function and cell integrity
3. to investigate the effects of 25-hydroxycholesterol on cholesterol metabolism *in vivo* as well as *in vitro* in a neuronal and astrocytic cell line

3 MATERIALS AND METHODS

3.1 Materials

Table 2: List of materials and companies they were obtained from

Material	Company
Earl's medium M199	Gibco, Vienna, Austria
Dispase	Gibco, Vienna, Austria
Penicillin/Streptomycin	Gibco, Vienna, Austria
Gentamycin	Gibco, Vienna, Austria
Trypsin	Gibco, Vienna, Austria
Glutamine	Gibco, Vienna, Austria
Dispase (<i>Bacillus polymyxa</i>)	Gibco, Vienna, Austria
Ox serum	PAA Laboratories, Linz, Austria
Cell culture plastic ware	Greiner, Kremsmuenster, Austria; Costar, Vienna, Austria; VWR, Austria
Opti-Fluor Scintillation cocktail	Perkin Elmer, Vienna, Austria
MTT test kit	Sigma Aldrich, Vienna, Austria
Protease Inhibitors	Sigma Aldrich, Vienna, Austria
Lipopolysaccharide from <i>E. coli</i> (0111:B4)	Sigma Aldrich, Vienna, Austria
Heparin sodium salt	Sigma Aldrich, Vienna, Austria
Bovine serum albumin	Sigma Aldrich, Vienna, Austria
DMEM Ham's 12	Sigma Aldrich, Vienna, Austria
Hydrocortisone	Sigma Aldrich, Vienna, Austria
DMSO	Sigma Aldrich, Vienna, Austria
Sodium hypochlorite	Sigma Aldrich, Vienna, Austria
Hydrogen peroxide	Sigma Aldrich, Vienna, Austria
3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide	Sigma Aldrich, Vienna, Austria
Phenylmethylsulfonyl fluoride	Sigma Aldrich, Vienna, Austria
4', 6'-diamindino-2-phenylindole dihydrochloride	Sigma Aldrich, Vienna, Austria
ECL Plus TM Western Blotting Detection Reagent	GE Healthcare, Vienna, Austria
dNTPs	GE Healthcare, Vienna, Austria
RNA guard	GE Healthcare, Vienna, Austria
Random hexamer primers	GE Healthcare, Vienna, Austria
ECL Western Blotting Substrate	Pierce Biotech., Vienna, Austria
ECL SuperSignal Western Blotting Substrate	Pierce Biotech., Vienna, Austria
Polyvinylidene difluoride transfer membrane (PVDF)	Pall Corporation, Vienna, Austria

Material	Company
Mowiol	Calbiochem-Novabiochem, La Jolla, CA, USA
RNeasy Kit	Qiagen, Vienna, Austria
Primer assays	Qiagen, Vienna, Austria
Turbo RNasefree DNase	Ambion, Mannheim, Germany
Dithiothreitol	Invitrogen, Vienna, Austria
First strand buffer	Invitrogen, Vienna, Austria
SuperScript II reverse transcriptase	Invitrogen, Vienna, Austria
Primers (sequence self-designed)	Invitrogen, Vienna, Austria
Recombinant human and mouse TNFa	Invitrogen, Vienna, Austria
Recombinant human IL-1b	Invitrogen, Vienna, Austria
PCR buffer	Gencraft, Vienna, Austria
Biotherm Taq DNA polymerase	Gencraft, Vienna, Austria
25-hydroxycholesterol-26,26,26,27,27,27-d6	Dr. Ehrenstorfer GmbH, Augsburg, Germany
C57BL/6 mice	Inst. f. Versuchstierkunde, Himberg, Austria
Injection needles	Henke Sass Wolf, Tuttlingen, Germany
Surgery supplies	Henke Sass Wolf, Tuttlingen, Germany
ECIS electrode arrays (8W10E+)	Ibidi, Germany
Trimethylchlorosilane	Pierce, Rockford, IL, USA
N-methyl-N-trifluoroacetamide	Pierce, Rockford, IL, USA
Acetonitrile	Pierce, Rockford, IL, USA
Myeloperoxidase	Planta Naturstoffe, Vienna, Austria
Bradford protein assay	Bio-Rad, Vienna, Austria
CURIX Ultra UV-G X-ray films	Agfa, Mortsel, Belgium
Ultra V Blocking solution	Lab Vision Corp., Fremont, CA, USA
AEC Substrate System	Lab Vision Corp., Fremont, CA, USA
Antibody diluent for immunohistochemistry	Dako, Carpinteria, CA, USA
PeqGOLD TriFast	Peqlab, Erlangen, Germany
SlimFast®	SlimFast, Germany, Messel
Agarose	Gibco
MOPS (morpholinopropanesulfonic acid)	Roth
Formaldehyd	Sigma
DEPC (diethylpyrocarbonate)	Sigma
[¹⁴ C]-acetate	ARC, St. Louis, MO, USA

Table 3: Cells used in this work

Cell type	Origin	Description
CATH.a	mouse	Brain neuron
CCF-STTG1	human	Astrocytoma
pBMVEC	porcine	Primary brain microvascular endothelial cells

Table 4: Buffers

Buffer	Components	pH
Cell culture & mouse experiments		
PBS	140 mM NaCl, 2.7 mM KCl, 8.1 mM Na ₂ HPO ₄ , 1.5 mM KH ₂ PO ₄	7.4
Lysis Buffer (RIPA Buffer)	50 mM Tris-HCl, 1 % (v/v) NP-40, 150 mM NaCl, 1 mM Na ₃ VO ₄ , 1 mM NaF, 1 mM EDTA, aprotinin, leupeptin, pepstatin: 1 µg/ml	7.4
SDS-PAGE		
Sample Buffer	150 mM Tris-HCl, 4 % (w/v) SDS, 20 % (v/v) glycerol, 5 % (v/v) 2-mercaptoethanol, 0.04 % (w/v) bromphenol-blue	6.8
Gel Buffers	1.5 M Tris-HCl	8.8
	500 mM Tris-HCl	6.8
Running Buffer	250 mM Tris-HCl, 2 M glycine, 35 mM SDS	
Immunoblotting		
Blotting Buffer	150 mM Tris-HCl, 400 mM glycine, 10-20 % (v/v) MeOH	
TBS-T	25 mM Tris-HCl, 150 mM NaCl, 0.05 % (v/v) Tween 20	7.5
Stripping Buffer	60 mM Tris-HCl, 2 % (w/v) SDS, 100 mM 2-mercaptoethanol	6.8
Gel electrophoresis (DNA & RNA)		
TAE buffer	40 mM Tris, 20 mM acetic acid, 1 mM EDTA	8.5
MOPS 10x (250 ml)	10.5 g MOPS, 1.7 g NaAc, 5 ml EDTA, ~1.5 ml 10 M NaOH, DEPC water	7.0
RNA loading buffer	1.44 ml formamid, 0.28 ml 10x MOPS, 0.52 ml 37 % formaldehyde, 0.4 ml DEPC-water, 0.2 ml glycerin (80 %), bromphenolblue, 10 µl EtBr (10 mg/ml)	

3.2 Methods

3.2.1 Animal treatment

Neuroinflammation was induced by an intraperitoneal (i.p.) injection of a single dose of LPS (Ullen, Fauler et al. 2010). Concentrations are given as mg/kg body weight. Control mice were injected with PBS. At the indicated time points, mice were killed by cervical dislocation and organs were removed and processed for further analysis (Ullen, Fauler et al. 2010).

Animal care and ethics

Animal experiments using male C57BL/6 mice were performed in accordance with animal care ethics approval and guidelines, as per Animal Care Certificate BMWF-66.010/0104-II/10b/2009 of the Austrian Federal Ministry of Science and Research (Vienna, Austria). Animals were maintained under a 12 h light/dark cycle. Water and normal chow diet were available ad libitum.

3.2.2 Whole genome mouse microarray

Experimental setup & RNA isolation

Six male C57BL/6 mice, aged 12 weeks were injected i.p. with 16.6 mg/kg LPS. At each timepoint (3 hours and 48 hours) 3 mice were killed. As a control three mice were injected with PBS only and killed after 48 hours. The brains were snap-frozen for further analysis. RNA was isolated using PeqGOLD TriFast, and purified using RNeasy Kit. RNA quality was tested using a Bioanalyzer.

Microarray hybridisation

Hybridisation was performed at the Microarray Facility at the “Zentrum für Medizinische Forschung” (ZMF), Medical University of Graz. An AB1700 mouse genome survey array (Applied Biosystems) was used. This microarray consists of 32,996 probes, which represent 32,381 curated genes that target 44,498 transcripts.

Data analysis/Transformation (by ZMF)

Data extracted from the scanned arrays were processed using GeneSpring GX (Agilent Technologies). Values below 10 were set to 10. Each measurement was

divided by the 50th percentile of all measurements in that sample. Each gene was divided by the median of its measurements in all samples. If the median of the normalized values was below 0.01 then each measurement for that gene was divided by 0.01 if the numerator was above 0.01, otherwise the measurement was thrown out.

Data filtering (by ZMF)

15,535 genes had an S/N>3. 11,672 genes were identified as non-changing. QCd list: contains changing genes; S/N>3 (3,863 genes).

Statistical analysis (by ZMF)

For statistical analysis a one-way parametric test was used, without assuming equal variances. Accepted false discovery rate was 0.05. 3 hour and 48 hour data were compared with control data. A Benjamini-Hochberg multiple testing correction was employed.

Data Mining Using GeneGo®

Data lists were uploaded into a trial version of GeneGo® MetaCore by gene bank number. GeneGo defines an enrichment procedure as mapping gene IDs from the dataset onto gene IDs in entities of built-in functional ontologies (represented by canonical pathway maps, cellular process networks, disease biomarker networks, drug target networks, toxicity networks, and metabolic networks). The terms in each analysis are statistically ranked, based on their relevance within the dataset. First, GeneGo “Analyze Single Experiment” feature was generated. This workflow resulted in the top ten “Statistically Significant Pathway Maps” as well as “Process Networks”. This analysis gives a *p*-value distribution of GeneGo folders for each dataset. These procedures are limited to canonical pathways represented in the GeneGo database. Relevant pathways and protein complexes of interest were identified manually (junctional complexes as well as lipid metabolism).

3.2.3 Cell culture

Catecholaminergic CATH.a neurons (an established *in vitro* model to study neurodegenerative pathways (Son, Lee et al. 2012)) were obtained from ATCC and cultured in poly-L-lysine-coated 75 cm² flasks containing RPMI 1640 medium supplemented with 10 % (v/v) horse serum, 5 % (v/v) FCS, 0.4 % (v/v) HEPES buffer,

0.4 % (w/v) sodium pyruvate, 0.4 % (w/v) L-glutamine, penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37 °C (5 % CO₂). CCF-STTG1 astrocytoma cells were cultured in 75 cm² flasks containing RPMI 1640 high glucose medium supplemented with 10 % (v/v) FCS and 1 % (v/v) P/S (penicillin/streptomycin) at 37 °C (5 % CO₂). The split ratio of cells was 1:5 and passages below 30 were used for experiments.

3.2.4 SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting

To determine the levels of CH25H, SREBP2 and β-actin as loading control, SDS-PAGE followed by Western blotting was performed on mouse tissue lysates of brain and liver as well as whole cell lysates of CATH.a neuronal cells.

Preparation of cells

Cells were washed twice with PBS and lysed on ice in 30 µl RIPA buffer for 10 min. Lysates were scraped, transferred into tubes and treated with ultrasound for 2 x 3 min. Cell debris was removed by spinning at 13,000 g (4 °C) for 10 min. The protein concentration of the supernatant was measured with Bradford protein assay reagent as described in the manual. An aliquot was mixed with 6x sample buffer, incubated at 95 °C for 5 min and subjected to SDS-PAGE.

Preparation of tissue

Tissue was collected and snap-frozen immediately. Tissue was homogenized with pestil and mortar under liquid nitrogen. Approx. 50 mg of tissue-homogenate was transferred to a tube containing 1 ml of RIPA buffer. Homogenate was vortexed and kept on ice for 20 min, treated with ultrasound for 2 x 3 min and cell debris was removed by spinning at 13,000 g (4 °C) for 10 min. Protein concentration was assayed with Bradford reagent in the supernatant, which was kept in a separate tube. An aliquot was mixed with 6x sample buffer, incubated at 95 °C for 5 min and subjected to SDS-PAGE.

SDS-PAGE

Gels were made in advance and kept at 4 °C. Samples (amount loaded is indicated in the respective figure) were loaded on the gel, and the gel was run for approx. 1.5 h at 120 V.

Immunoblotting

Gel was transferred to PVDF (SREBP2 blots) or nitrocellulose (CH25H blots) membranes for approx. 1 h in blotting buffer. Blots were stained with Ponceau S solution to check for transfer quality. Blots were washed for 3 x 5 min with TBS-T, and blocked for 2 hours in Blocking Solution (5 % milk in TBS-T (pH 7.4) or 3 % SlimFast® in TBS-T). Blots were washed for 8 x 15 min and incubated with the respective 1st antibody (see table 5) diluted in blocking solution overnight at 4 °C. Blots were washed for 8 x 15 min and incubated with 2nd antibody (see table 6) for 2 h at room temperature (RT) diluted in blocking solution. Blots were washed for 8 x 15 minutes and subjected to detection.

Detection

Immunoreactive bands were visualized using ECL, ECL Plus™ or ECL SuperSignal Western Blotting Substrate (depending on the expected intensity) and CURIX Ultra UV-G X-ray films.

In case membranes were subjected to reprobing with another set of antibodies, membranes were stripped after detection using stripping buffer for 30 min.

Table 5: Primary antibodies

Target	Type	Source	Company	Product code
β-Actin	monoclonal	mouse	Sigma	A 5316
CH25H	polyclonal	goat	Santa Cruz Biotech.	Sc-107479
CH25H	polyclonal	goat	Santa Cruz Biotech.	Sc-107480
MBP	monoclonal	mouse	Abcam	Ab24567
MPO	polyclonal	rabbit	Dako	A0398
MPO	polyclonal	rabbit	Santa Cruz Biotech.	Sc-16128
SREBP-2	polyclonal	rabbit	Santa Cruz Biotech.	Sc-5603

* Both CH25H and both MPO antibodies showed equal results.

Table 6: Secondary antibodies

Target	Conjugation	Source	Company	Product No.
mouse IgG	HRP	goat	Santa Cruz Biotech.	Sc-2302
rabbit IgG	HRP	goat	Pierce Biotech.	0031460
goat IgG	HRP	rabbit	Santa Cruz Biotech.	Sc-2922

3.2.5 Quantitative Real Time PCR (qPCR)

Isolation of total RNA

Total RNA from tissue was isolated from snap-frozen tissue using peqGOLD TriFast followed by a cleanup using RNeasy Kit according to the protocol. Total RNA from cells was isolated using RNeasy Kit only.

To check for integrity of the RNA a quality control was performed randomly by analyzing the RNA on an RNA-gel (1 %: 1.1 g agarose, 11 ml 10xMOPS, 88.8 ml DEPC water, boil in microwave, cool down to 54 °C, add 18.7 ml formaldehyde). The bands for the 28S rRNA and 18S rRNA were clearly visible. No smear was detected.

Reverse transcription of RNA

The RNA concentration and purity was checked by using the NanoDrop (PeqLab). For each set of experiments an equal amount of RNA was reverse transcribed (max. 5 µg of RNA) using 200 U of SuperScript II reverse transcriptase (Invitrogen) and random hexamer primers (Amersham Biosciences) according to the protocol from Invitrogen.

qPCR

Samples were diluted with RNase-free water and primer efficiency was tested via qPCR prior to further analyses. qPCR was performed with a 7900HT fast real-time PCR system (Applied Biosystems), the QuantiFast SYBR Green PCR kit (Qiagen), and primers (see Table 7 and Table 8). Five ng of cDNA was used as template for each qPCR reaction (for very low expressed RNA, up to 10 ng of cDNA was used). Samples were run in triplicates for each experiment, with TBP1 or GAPDH as an internal control (indicated in the graphs).

Analysis of qPCR

For analysis of the expression profiles the public domain program “Relative Expression Software Tool—REST 384 version 2” was used (<http://www.gene-quantification.com/download.html>).

Table 7: Self-designed primers

Gene name	Accession No.	Organism	Sequence (5'-3')	length (bp)
ABCA1	NM_005502	Human	For: AAGGCTACCAGTTACATTTGAC Rev: TTCTCAGGATTGGCTTCAGG	171
ABCA1	NM_013454.3	Mouse	For: CTCTTCATGACTCTAGCCTGGA Rev: ACACAGACAGGAAGACGAACAC	250
APOE	NM_000041.2	Human	For: TCTGAGCAGGTGCAGGAGGA Rev: TGGTAACTGCCAGGCGCTT	359
APOE	NM_009696.3	Mouse	For: CTGACAGGATGCCTAGCCG Rev: CGCAGGTAATCCCAGAAGC	107
β -actin	NM_001101.3	Human	For: AAAGACCTGTACGCCAACACA Rev: AGTACTTGCGCTCAGGAGGA	254
β -actin	NM_007393.3	Mouse	For: GTCAGAAGGACTCCTATGTG Rev: CATGAGGTAGTCTGTCAGGT	428
FASN	NM_004104.4	Human	For: TGTGGACATGGTCACGGAC Rev: GGCATCAAACCTAGACAGGTC	100
FASN	NM_007988.3	Mouse	For: GAAGCCGAACACCTCTGTGCAGT Rev: CTCCTTGCTGCCATCTGTATTG	199
FDFT1	NM_004462.3	Human	For: CCTCAAGAGGTTTGGAGCAG Rev: TCTTCACTGCCCCTTTGAAC	253
FDFT1	NM_010191.2	Mouse	For: ATGGAGTTCGTCAAGTGTCTAGG Rev: CGTGCCGTATGTCCCCATC	202
HMGCR	NM_000859.2	Human	For: GGAACCTCGGCCTAATGAAG Rev: CGGCGAATAGATACACCACG	168
HMGCR	NM_008255.2	Mouse	For: CTATTGCACCGACAAGAAGCCT Rev: GCCATCACAGTGCCACATACAA	486
HMGCS1	NM_002130.6	Human	For: CTCTTGGGATGGACGGTATGC Rev: GCTCCAACCTCCACCTGTAGG	90
HMGCS1	NM_145942.4	Mouse	For: TTGGCAGGGAAGAGGGTTGGAGTG Rev: GGCCGAGGTTGCAGGGAGTCTTG	432
MPO	NM_010824.2	Mouse	For: GCCAGCAGCCATGAAGAAGT Rev: CCGGATCTCATCCACCACAA	304
SREBP2	NM_004599.2	Human	For: TAGGCAGTCTGGTGGACAATGAGGT Rev: CACAGGAAGGTGAGGACACACAGAA	247
SREBP2	NM_033218.1	Mouse	For: GTGCGCTCTCGTTTTACTGAAGT Rev: GTATAGAAGACGGCCTTCACCAA	86

Table 8: Primer assays obtained from Qiagen

Gene name	Accession No.	Organism	Product No.	length (bp)
CH25H	NM_003956	Human	QT00202370	133
CH25H	NM_009890	Mouse	QT00253001	97
COX2	NM_000963	Human	QT00040586	68
COX2	NM_011198	Mouse	QT00165347	95
GAPDH (3' end)	NM_002046	Human	QT00079247	95

GAPDH (5' end)	NM_002046	Human	QT01192646	119
MPO	NM_000250	Human	QT00067613	164
NOS2	NM_153292	Human	QT00068740	92
NOS2	NM_010927	Mouse	QT00100275	118
TBP1	NM_013684	Mouse	QT00198443	114
TNF α	NM_000594	Human	QT01079561	104
TNF α	NM_013693	Mouse	QT00104006	112

3.2.6 Isolation and cultivation of primary porcine brain microvascular endothelial cells

Isolation of pBMVEC

pBMVECs (porcine brain microvascular endothelial cells) were isolated by mechanical disintegration, sequential enzymatic digestion and centrifugation steps according to Tewes and colleagues (Tewes and Galla 2001) and characterized as described previously (Goti, Balazs et al. 2002). Briefly, porcine brains were obtained from freshly slaughtered pigs at the local slaughterhouse. Meninges and secretory areas were removed and brains were minced and suspended in “preparation medium”. The suspension was incubated with solid dispase (0.1 %, w/v) for 60-120 minutes at 37 °C under gentle stirring. Dextran solution (16 %, w/v) was added to a final concentration of 10 % (w/v) and the suspension was centrifuged at 6,800 g for 10 min at 4 °C. The pellet was resuspended in “Medium A” and filtered through a 180 μ m nylon mesh to get rid of larger vessels. Capillaries were digested with 0.03 % (w/v) collagenase/dispase for 5 min at 37 °C under gentle stirring. Cell aggregates were collected by centrifuging at 140 g for 10 min at RT. The cell aggregate pellet of 1 brain was resuspended in 5 ml “Medium A” and centrifuged on a discontinuous Percoll gradient (20 ml 1.03 g/ml bottom-layered with 15 ml of 1.07 g/ml) at 1,300 g for 10 min at RT in a swinging bucket rotor without brake. The cell clusters of the pBMVEC which were gathered at the interface of the two phases were washed in “Medium A” and centrifuged at 140 g for 10 min. Subsequently, cell clusters from one brain were seeded in “Medium A”, onto six to eight 75 cm² collagen-coated culture flasks. Therefore, the culture flasks were preincubated with 60 μ g/ml collagen in PBS for 5 min. Solution was aspirated afterwards. After 1 day in culture cells were washed with PBS rigorously and cultivated in “Medium B”.

Cultivation of pBMVEC

Cells reached confluency around 3 days post seeding. Cells were split by trypsinisation and seeded onto collagen-coated multiwell cell culture plates (coated with 60 µg/ml collagen in PBS) at a density of ~20,000 cells/cm² or 8W10E+ ECIS arrays (coated with 200 µg/ml collagen in 150 mM NaCl) at a density of 40,000 cells/cm².

Table 9: Media for preparation and cultivation of pBMVEC

<i>Preparation medium</i>	M199, 1 % P/S (v/v), 1 % gentamycine (v/v), 0.35 % glutamine (v/v)
<i>Medium A</i>	10 % ox-serum, 1 % P/S, 1 % gentamycine and 0.35 % glutamine (v/v)
<i>Medium B</i>	medium A without gentamycine
<i>Incubation medium</i>	DMEM/Ham's F12 medium 1:1 (v/v), 1 % P/S, 0.35 % glutamine, 500 nM hydrocortisone

Electrical Cell-Substrate Impedance Sensing – a model for in vitro BBB function

ECIS® is an impedance-based method to study the effects of compounds on the barrier-integrity of pBMVECs. For this work, ECIS Z system (Applied Biophysics, Troy, NY) was used. To determine the effects of 25-HC and 24-HC on barrier integrity, cells were grown on 250 µm collagen-coated gold electrodes of 8W10E+ arrays (Applied Biophysics) and cultured to confluency while barrier function at 4 kHz and membrane capacitance at 64 kHz were measured once a minute. After reaching confluency, the cells were exposed to 10 µM of 24-HC and 25-HC, respectively. Hydroxycholesterols were added from ethanolic stock solutions with a concentration of 50 mM.

3.2.7 Quantification of 25-hydroxycholesterol in LPS mouse brains

Gas chromatography-mass spectroscopy (GC-MS) analysis

Brains of vehicle (PBS) and LPS-treated animals (8.3 mg/kg) were removed, frozen in liquid nitrogen and homogenized using a BioPulverizer (BioSpec Products, Bartlesville, USA). Lipids were extracted as described (Lutjohann, Breuer et al. 1996). Before extraction, 51.6 µg of 24-hydroxycholesterol D₇ was added as internal standard. Lipids were hydrolyzed in ethanolic KOH, after neutralization with acetic acid and addition of NaCl (200 µmol per extraction) lipids were re-extracted with

chloroform/methanol (2:1; v/v). The chloroform phase was collected and samples were dried under a gentle stream of nitrogen. The dried lipids were derivatized with MSTFA/pyridine (2:1; v/v) containing 1% (v/v) TMCS at 37 °C for 30 min. A Fisons 8000 gas chromatograph coupled with a Fisons MD 800 quadrupole mass spectrometer was used for detection. A DB-5MS capillary column was used. Helium was used as carrier gas and the temperature was increased from 160 °C to a final temperature of 270 °C. The connection between GC and MS instrument was kept at 300 °C. The ion source temperature was 200 °C. Mass spectra were recorded with electron ionization energy of 70 eV and an emission current of 100 μ A. The diagnostic ions used for (two-fold) silylated 25-HC were at m/z = 131.1, 456.4 and 546.4. Silylated 25-HC D₆ was detected at m/z = 137.1, 462.4 and 552.4. For quantification of endogenous 25-HC, m/z 131 was used and normalized to 24-HC D₇ standard ion m/z 334.

3.2.8 Immunofluorescence with brains of LPS treated mice

Serial sagittal cryosections (5 μ M) were collected on glass slides, air dried for 2 h at RT, fixed in acetone for 5 min at RT and stored at -40 °C until required. Prior to immunostaining sections were thawed for 30 min at RT followed by fixation in acetone for 10 min at -20 °C. After this step, slides were air-dried for 20 min. Sections were blocked with Lab Vision Blocking Solution for 5 min at RT. First antibody was added as a dilution in antibody diluent from DAKO (4 μ g/ml dilution) for 30 min at RT. After incubation slides were washed several times with PBS and secondary antibody, diluted with antibody diluent (1:400 dilution) was added for 30 min at RT. After incubation period was over slides were washed several times with PBS. Sections were stained for nuclei using DAPI solution (1:250 dilution) in PBS for 10 min at RT. Slides were mounted with Mowiol and analyzed on a confocal laser scanning microscope (Leica SP2, Leica Lasertechnik GmbH, Heidelberg, Germany).

3.2.9 Cholesterol biosynthesis assay

CATH.a cells were seeded in 6-well dishes coated with poly-L-lysine and grown in a humidified incubator under standard conditions (5 % CO₂ at 37 °C) in medium. On day 1 medium with serum and 25-HC (solubilized in ethanol, stock solution: 50 mM) was added in concentrations ranging from 100 ng/ml (250 nM) to 2 μ g/ml (5 μ M). The

ethanol concentration in the medium was kept at or below 0.5 %. Control cells were treated with ethanol only. On day 2 medium was replaced by fresh medium containing 25-HC (same concentrations than above) and [^{14}C]-acetate (2 $\mu\text{Ci}/\text{well}$) and incubated for 6 h. Cells were washed carefully and incubated with medium with serum. After treatment, medium was collected for measurement and total lipids were extracted from cells with hexane isopropanol (3:2 v/v) and analyzed by thin-layer chromatography as previously described. A lipid extract of human serum was used as a standard. The TLC plate was developed in hexane:ether:glacial acetic acid 70:30:1 (v/v/v) for approx. 45 minutes. Bands were visualized with iodine vapor. Bands co-migrating with the unlabeled free cholesterol and cholesterol ester fractions were cut out and subjected to liquid scintillation counting. Extracted cells were hydrolyzed with NaOH (0.3 M) and the protein content was determined using the Lowry procedure.

3.2.10 Statistical analysis

One-way or two-way ANOVA and Bonferroni post-test or students t-test were performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA) software as indicated in the graphs, reported as mean $\pm$ SEM or SD for the specified number of animals/replicates. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. For analysis of gene expression profiles the public domain program Relative Expression Software Tool - REST 384 version 2 (<http://www.gene-quantification.com/download.html>) was used (Pfaffl, Horgan et al. 2002).

4 RESULTS

4.1 Systemic lipopolysaccharide induces neuroinflammation

Previous studies have shown that peripheral LPS administration induces a robust neuroinflammatory response (Qin, Wu et al. 2007). To establish this mouse model in our group mice received 5 mg/kg LPS intraperitoneally. mRNA was isolated from the brains of treated animals at different time points and qPCR was used to detect transcriptional regulation of inflammatory genes. As expected, neuroinflammation was clearly induced in this mouse model. We detected significant transcriptional upregulation of the inflammatory markers MPO, TNF α , iNOS and COX2 (Figure 16).

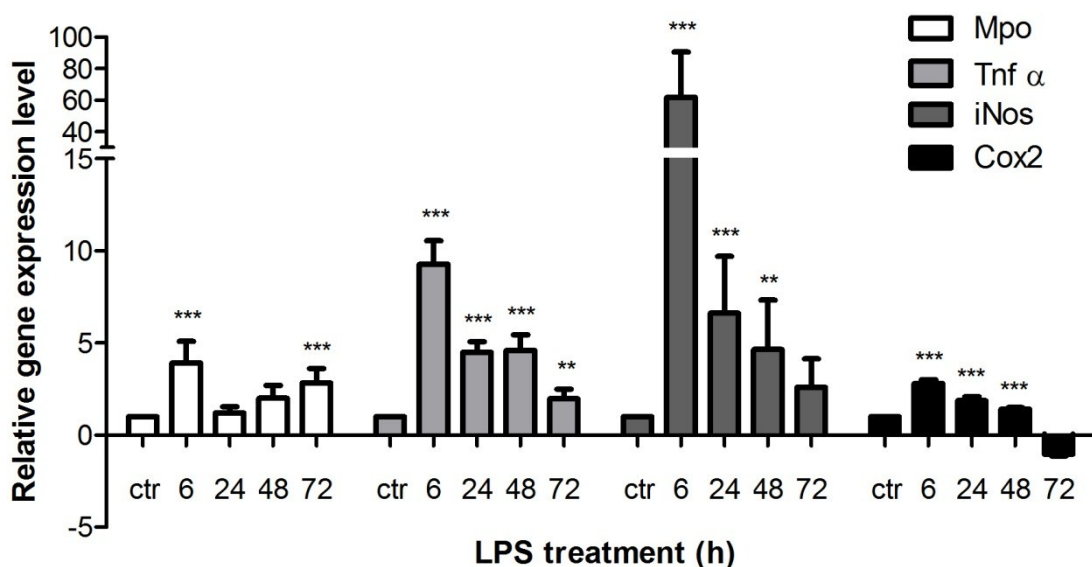


Figure 16: Systemic LPS induces neuroinflammation in mice. Male C57BL/6 mice aged 8 weeks were i.p. injected with a single dose of LPS (5 mg/kg). At the indicated time points mice were killed by cervical dislocation, brains were removed and mRNA was isolated and reverse transcribed. cDNA levels of the inflammatory markers MPO, TNF α , iNOS and COX2 were normalized to the reference gene TBP1 and expressed relative to gene expression in control mice (injected with PBS). Values represent means $\pm$ SEM of 4 mice per group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. These data are published (Ullen, Fauler et al. 2010).

In a second set of experiments a higher dose of LPS (16.6 mg/kg) was used and transcriptional changes of inflammatory markers were measured in blood and

brain of LPS-injected mice (Figure 17). The results show that MPO and iNOS mRNA expressions are higher and changes occur faster in the blood compared to the brain. TNF α mRNA was upregulated to a lesser extent in the blood than in the brain. COX2 transcription, however, was inversely regulated upon LPS administration in the blood compared to the brain.

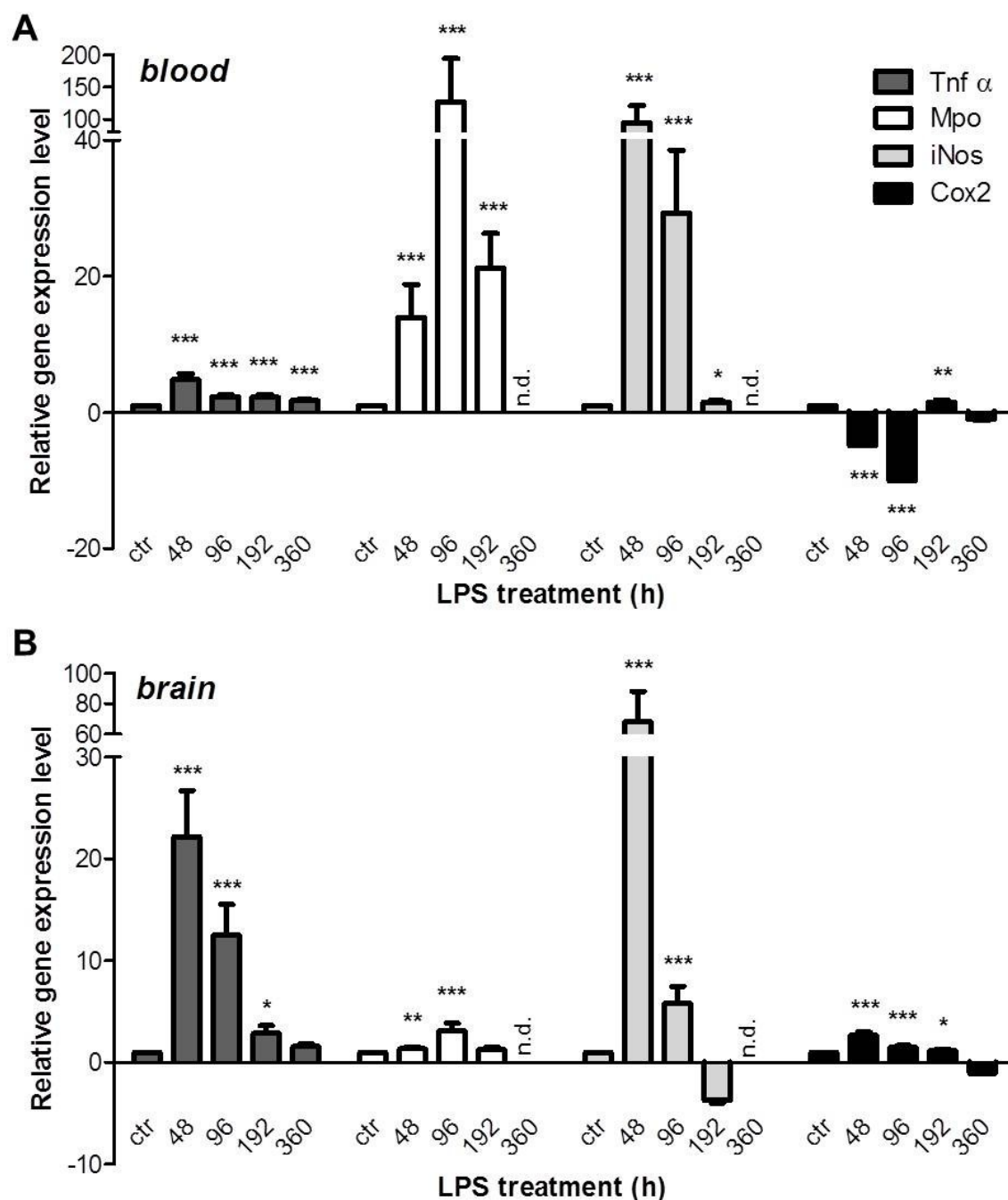


Figure 17: Comparison of inflammatory markers in blood (A) and brain (B) post LPS treatment. Male C57BL/6 mice aged 11 weeks were i.p. injected with a single dose of LPS (16.6 mg/kg). At the indicated time points, mice were anesthetized and blood and

brains were taken. mRNA was isolated and reverse transcribed. Gene expression levels of the inflammatory marker genes MPO, COX2, TNF α and iNOS were normalized to GAPDH as a reference gene and expressed relative to gene expression in control mice (injected with PBS). Values represent quadruplicate estimations of 2 mice per group, shown as means $\pm$ SEM. * P <0.05, ** P <0.01, *** P <0.001, n.d.: not detected.

4.2 Neuroinflammation is accompanied by invasion of MPO-positive cells into the brain

Our next aim was to detect the presence of MPO positive cells in the brains of LPS treated mice. Therefore, we injected 8.3 mg/kg LPS i.p. and chose a time-point where we expected MPO protein expression in the brain (96 h). Brain cryosections (5 μ m) were stained for MPO and DAPI (DNA binding dye) using immunofluorescence (Figure 18). We were able to detect MPO in certain cells of brain capillaries, especially at the meninges, but failed to detect MPO in neuronal cells. No staining was detected in control mice injected with PBS only.

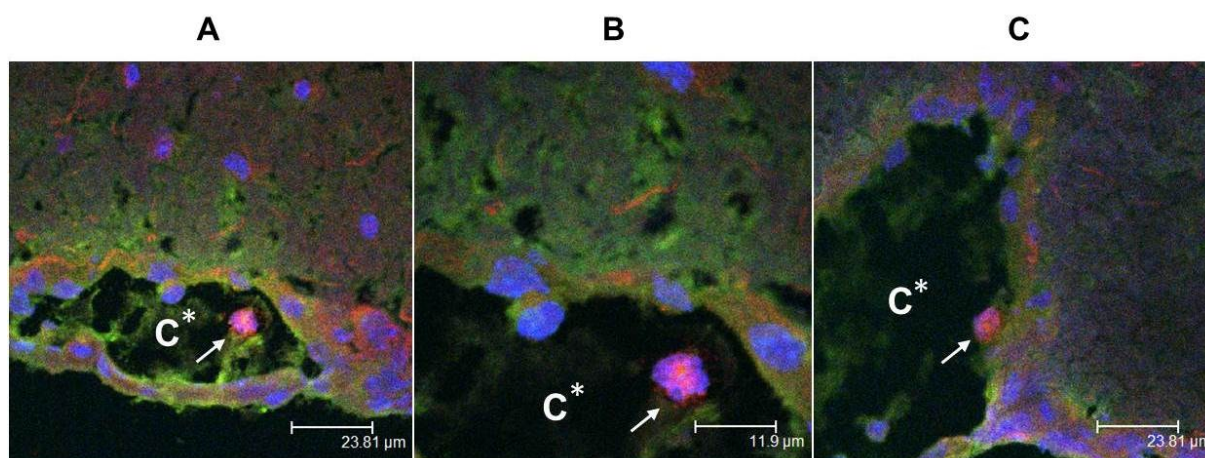


Figure 18: Immunofluorescence analysis of LPS mouse brains shows MPO-positive cells (arrow) in brain capillaries. Mice were sacrificed 96 h following LPS (8.3 mg/kg) or PBS injection i.p. Brain cryosections (5 μ m) were stained for MPO (red), DAPI (blue) and MBP1 (green). All images are from the same mouse, B is a magnification of A. C*: capillary.

4.3 LPS induced neuroinflammation leads to pronounced body weight loss

In response to a single LPS injection i.p. mice developed severe peripheral inflammation and fully blown neuroinflammation. At 96 h post treatment mice had lost 28 % of their body weight while brain weight showed a slight tendency to increase.

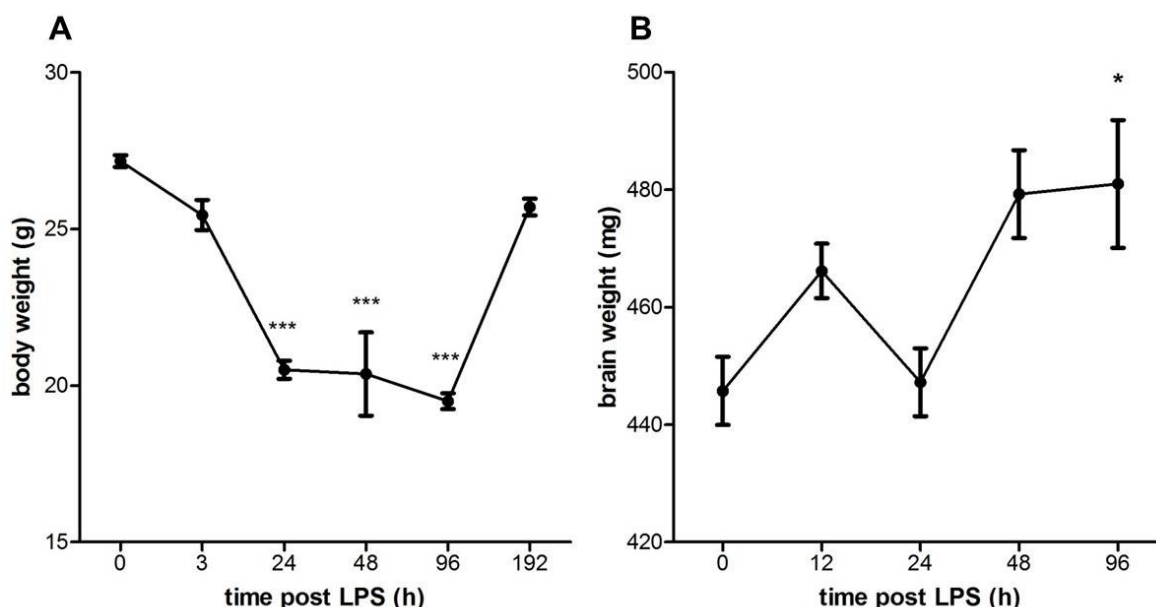


Figure 19: Brain- and body weights are affected in LPS treated mice. Male C57BL/6 mice aged 12 weeks received a single LPS dose i.p. At the indicated time points mice were weighted (A) and killed by cervical dislocation. Brains, which were taken for other studies were weighted immediately after death (B). Results are shown as means of 3 mice/group $\pm$ SEM. Significances were calculated with 1-way ANOVA followed by a Bonferroni post-test. * $P \leq 0.05$, ** $P \leq 0.005$, *** $P \leq 0.001$.

4.4 Effects of neuroinflammation on cerebral gene expression - a microarray study

To investigate global changes in gene expression upon LPS treatment, a whole genome microarray was performed. Twelve week old male C57BL/6 mice were i.p. injected with 16.6 mg/kg LPS to induce a severe inflammation, and brains were taken at the indicated time points. Results from microarray studies provide an overall picture of the changes in gene expression in the mouse brain upon the induction of a global inflammatory response. The results revealed that 1218

transcripts were differentially regulated at 3 h, and 504 transcripts were differentially regulated at 48 h post LPS treatment ($P \leq 0.05$). Fold changes (FC) ranged from 517 (chemokine ligand 10) to 0.04 (mitofusin 2) in 3 h post treatment and from 132 (serum amyloid A3) to 0.02 (synaptosomal-associated protein, 25 kDa) at 48 h post treatment. Out of these genes, 327 genes were induced and 891 genes were repressed at 3 hours post LPS treatment and 167 were induced and 337 genes were repressed at 48 hours post LPS treatment (Figure 20). Interestingly, the majority of these changes were unique to either the 3 h or 48 h time point, reflecting the regulation of distinct sets of genes with time. Nevertheless, 39 genes were found to be commonly induced, and 171 genes were commonly repressed at both time points. The entire list of regulated genes is given in Appendix 1 and 2.

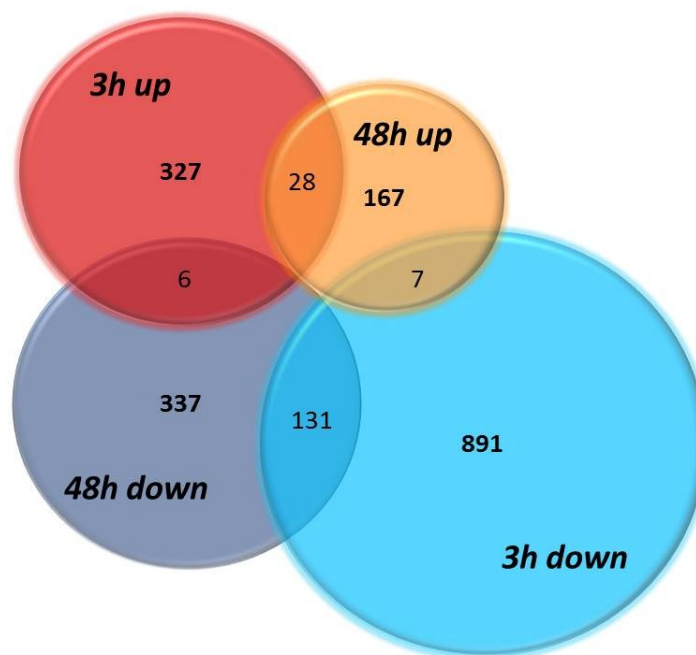


Figure 20: Temporal regulation of neuroinflammation. Comparison between 3 h and 48 h time point. Unique genes that overlap between the sets are shown in the intersections. Overall 1,218 genes were regulated at 3 h and 504 transcripts were regulated at 48 h.

For the following analyses, a free trial of the online platform MetaCore (MetaCore <http://www.genego.com/metacore.php>) from GeneGo was used.

4.4.1 Most affected pathway maps and process networks through LPS treatment

First, the affected genes were categorized on the basis of their function under gene ontology (GO) terms of biological processes. A pathway map integrating the most affected pathways involved in a specified biological process was generated.

The top 10 highly affected pathways with the respective p values as a measure for the degree of impact are shown in Figure 21. These mostly comprise of pathways involved in development, neurogenesis and cancer signaling. The top 4 pathways affected at both time points were vascular endothelial growth factor (VEGF) signaling, WNT signaling, Retinoid signaling and associated with spindles and kinetochores protein 1 (ASK1) signaling.

GeneGo Pathway Maps

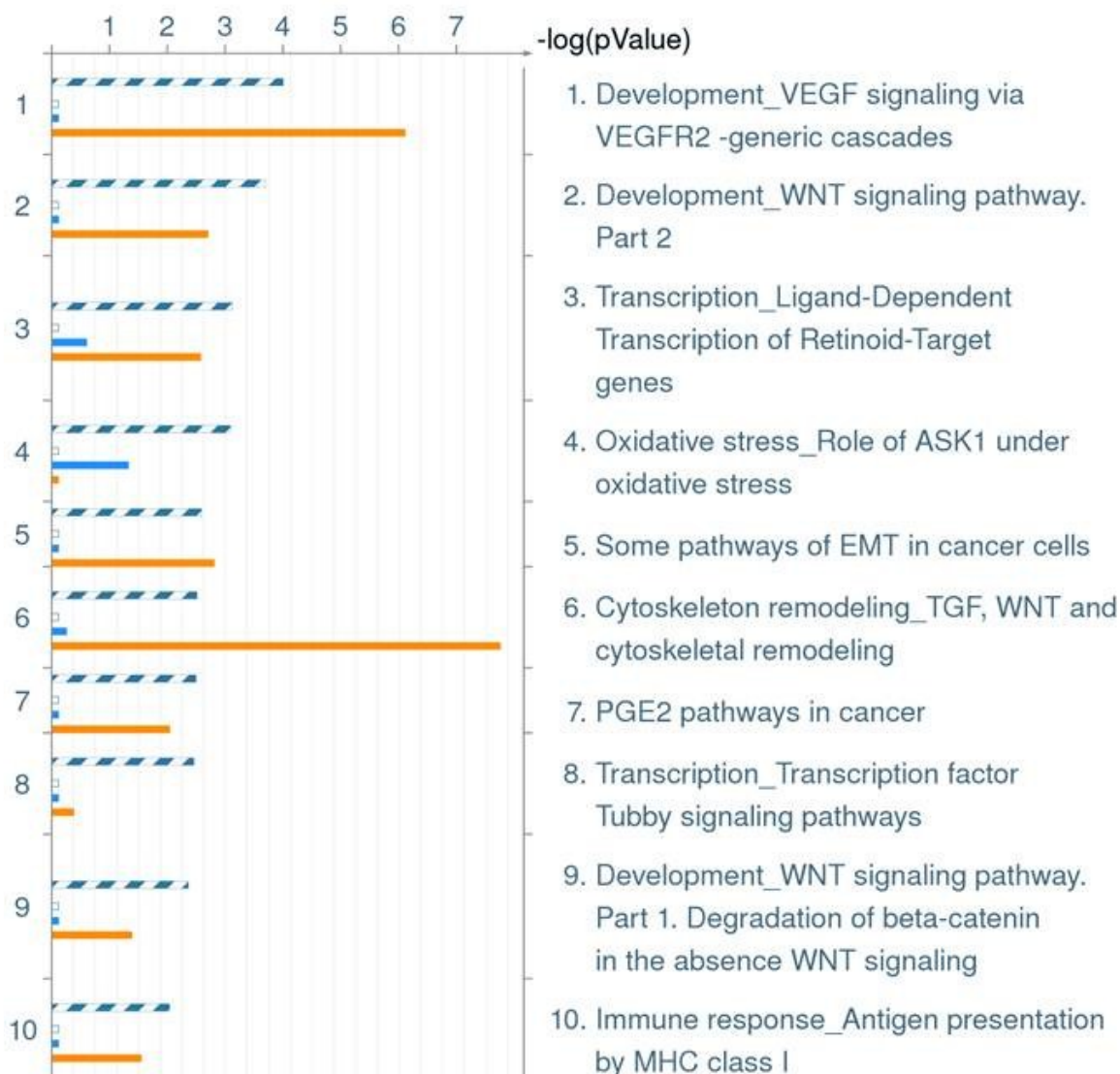


Figure 21: Categorization in terms of pathways of the differentially expressed genes based on Gene Ontology (GO). Histograms representing top GeneGo pathway maps. Bars represent the inverse log of the p -value. Orange: 3 h, blue: 48 h, hashed: affected at both time points.

Next, a categorization in terms of most affected process networks was established. The top 10 highly affected process networks with the respective p values as a measure for the degree of impact are shown in Figure 22. The top 4 affected process networks were neurogenesis, synaptogenesis, protein folding and cadherins.

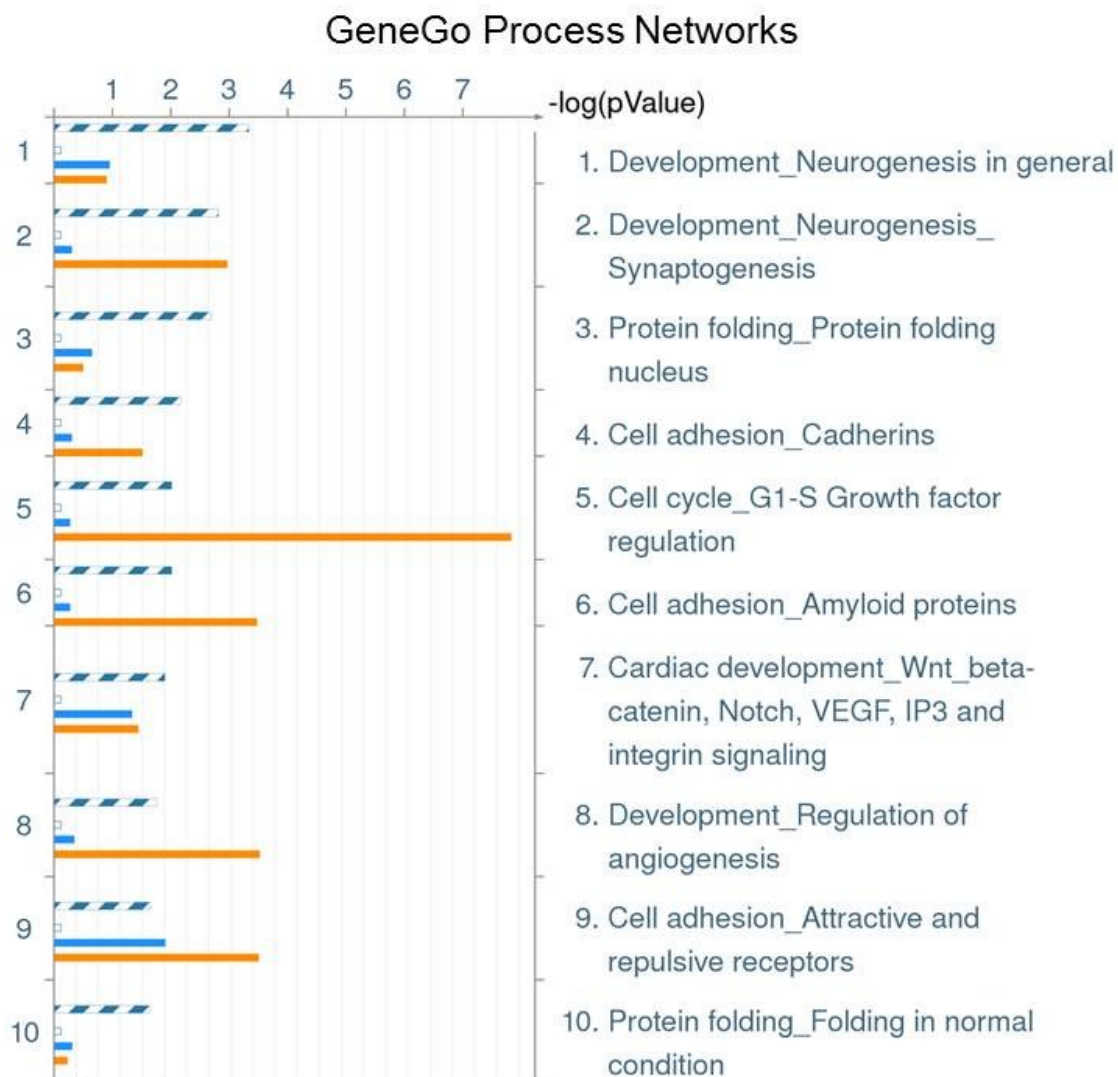


Figure 22: Categorization of process networks of the differentially expressed genes based on Gene Ontology (GO). Bars represent the inverse log of the p -value. Orange: 3 h, blue: 48 h, hashed: affected at both time points.

4.4.2 Most affected pathways through LPS treatment

Next, the most affected pathways in the brains of mice receiving peripheral LPS were detected. We found that the high mobility group box 1/toll-like receptor signaling pathway (HMGB1/TLR) was predominantly affected in mice at 3 h post treatment (Figure 23). The microarray data showed that toll-like receptor 2 (TLR2; 3 h: 17) was significantly upregulated. Furthermore, the upregulated genes in this pathway included interferon regulatory factor 7 (IRF7; 3 h: 4.5), nuclear factor of κ light polypeptide gene enhancer in B cells inhibitor β (I- κ B; 3 h: 6) and v-rel reticuloendotheliosis viral oncogene homolog A (RelA; 3 h: 2.0). The proinflammatory chemokines macrophage inflammatory protein 1-beta

(MIP-1 β ; 3 h: 98), interleukin 6 (IL-6; 3 h: 25.6), interleukin 1 β (IL-1 β ; 3 h: 21.6) and tumor necrosis factor α (TNF α ; 3 h: 9.3) were up-regulated. The down-regulated genes included TGF- β activated kinase 1/MAP3K7 binding protein 2 (TAB; 3 h: 0.3), ubiquitin-conjugating enzyme E2 variant 1 (UEV1A; 3 h: 0.4), inhibitor of kappaB kinase γ (IKK γ ; 3 h: 0.3), mitogen-activated protein kinase p38 alpha (p38 alpha; 3 h: 0.27) and interferon β (IFN β ; 3 h: 0.4).

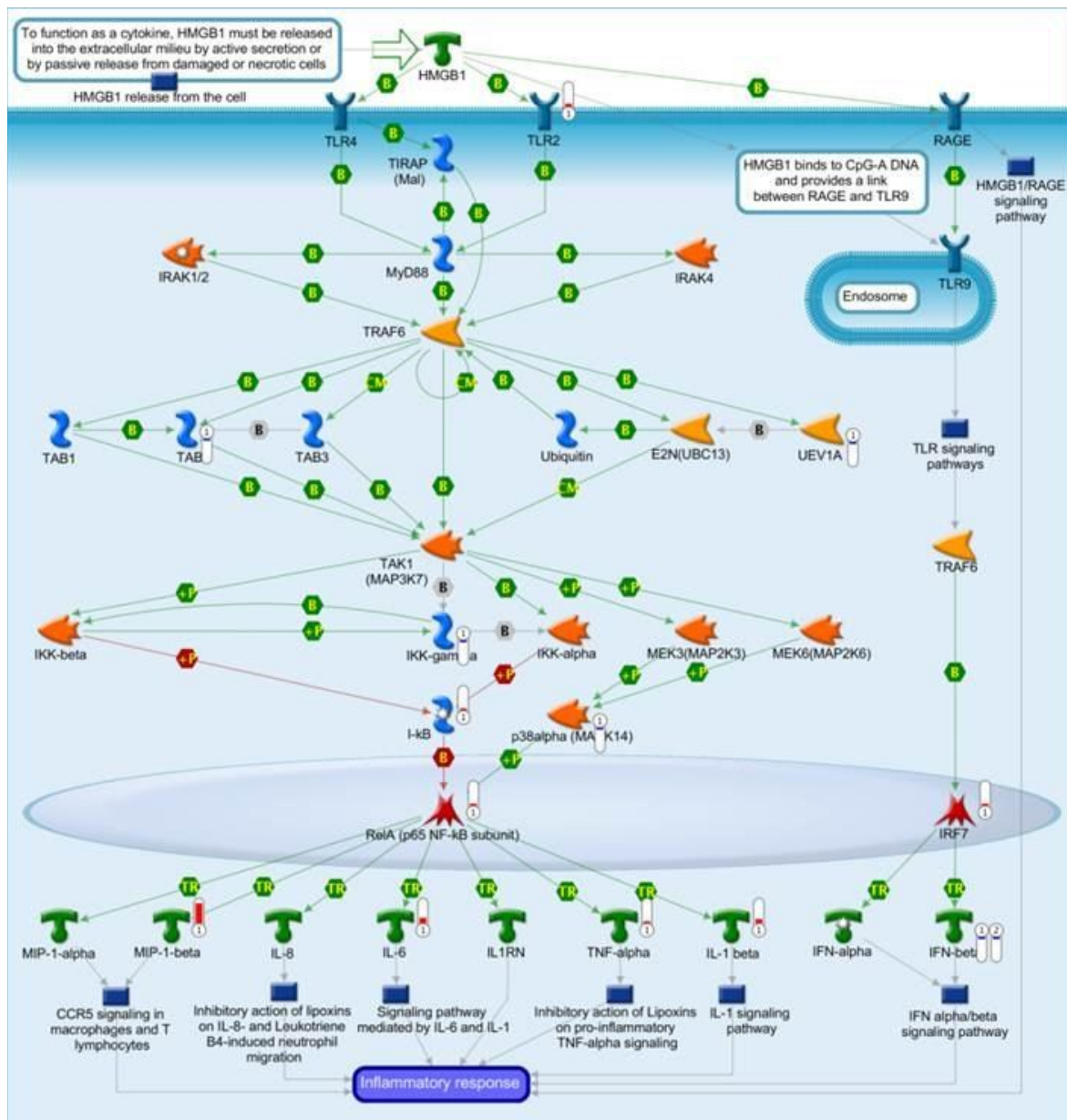


Figure 23: TLR signaling pathway is highly affected by LPS treatment. Thermometers indicate up- (red) and downregulation (blue) of genes involved in this pathway. Numbers written below the thermometer indicate 1: 3 h, and 2: 48 h regulations. Detailed legends are shown in Appendix III.

The interleukin 1 (IL-1) pathway was affected in LPS treated mice (Figure 24). Both, interleukin 1 α (IL 1 α : 22-fold) and interleukin 1 β (IL 1 β : 22-fold) were up-regulated 3 hours post treatment. Furthermore, a whole cascade of transcription factors affected by IL-1 was positively regulated through LPS treatment. These include nuclear factor of kappa light polypeptide gene enhancer in B cells 1, p105 (NF- κ B p50/p65: 3-fold), interferon regulatory factor 7 (IRF: 7-fold), and activator protein 1 (AP-1: 4-fold).

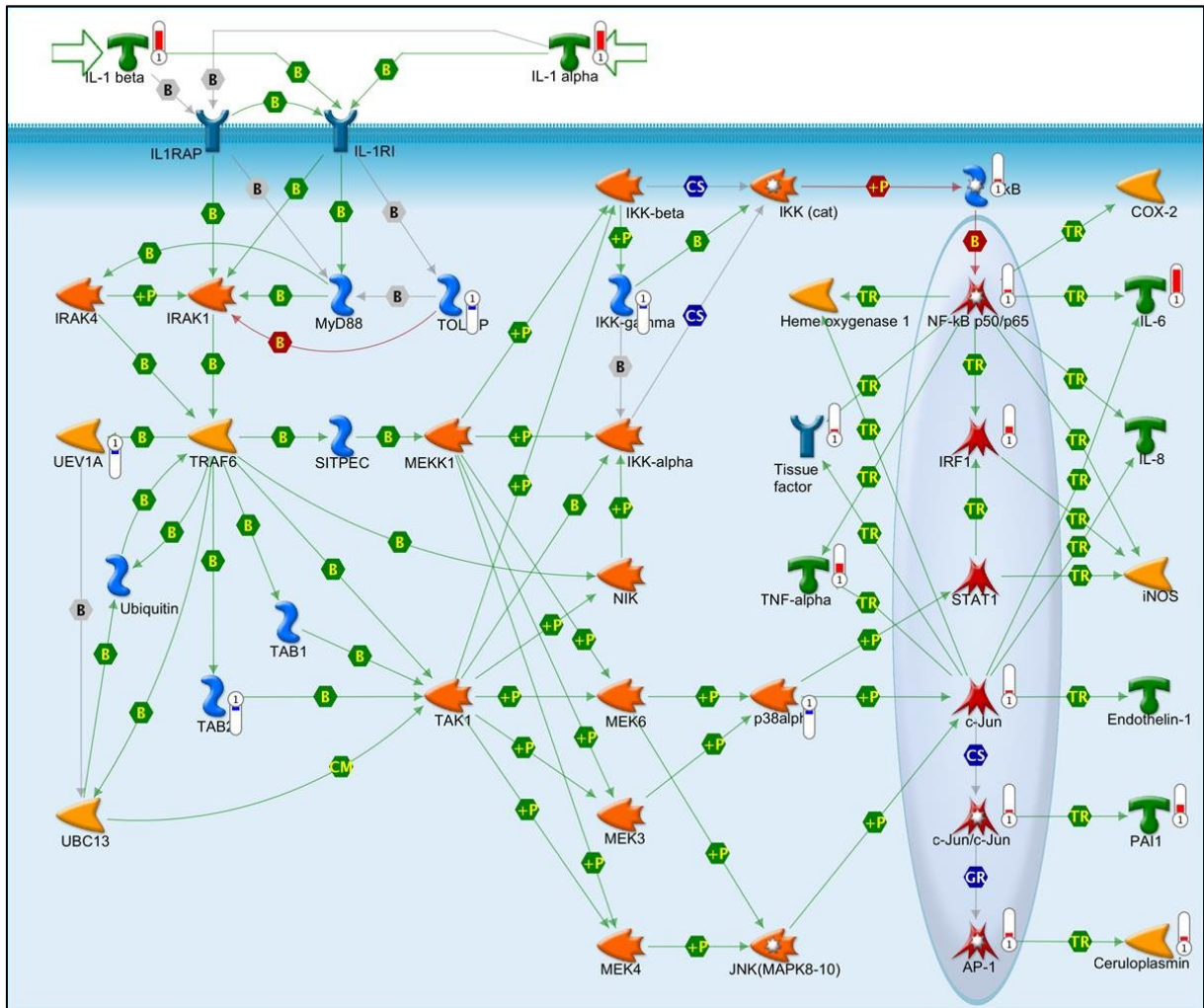


Figure 24: IL-1 signaling pathway map showing affected genes in LPS treated mice. Thermometers indicate up- (red) and downregulation (blue) of genes involved in this pathway. Numbers written below the thermometer indicate 1: 3 h, and 2: 48 h regulations. Detailed legends are shown in Appendix III.

Next, we found the oxidative stress pathway to be affected in LPS treated mice (Figure 25). The microarray data revealed a regulation of thioredoxin (TRX) and related genes. We saw a downregulation of thioredoxin reductase 1 (Txnrd1: 0.3),

thioredoxin domain containing 4 (Txndc4: 0.6) as well as thioredoxin like 4b (Txnl4b: 0.3) and an elevation of thioredoxin interacting protein (Txnip: 2.5) at 3 h post LPS treatment. At 48 hours post treatment Txn1 (0.3), Txndc4 (0.7) as well as Txndc9 (0.5) were downregulated.

Furthermore, glycogen synthase kinase 3, a kinase which is particularly abundant in the brain was downregulated in the microarray (Gsk3b: 0.5 at 3 h and 0.6 at 48 h post treatment). Next, the molecular chaperone heatshock protein 90 (HSP90) and homeodomain interacting protein kinase 1 (HIPK1) were downregulated in LPS treated mice at both time points.

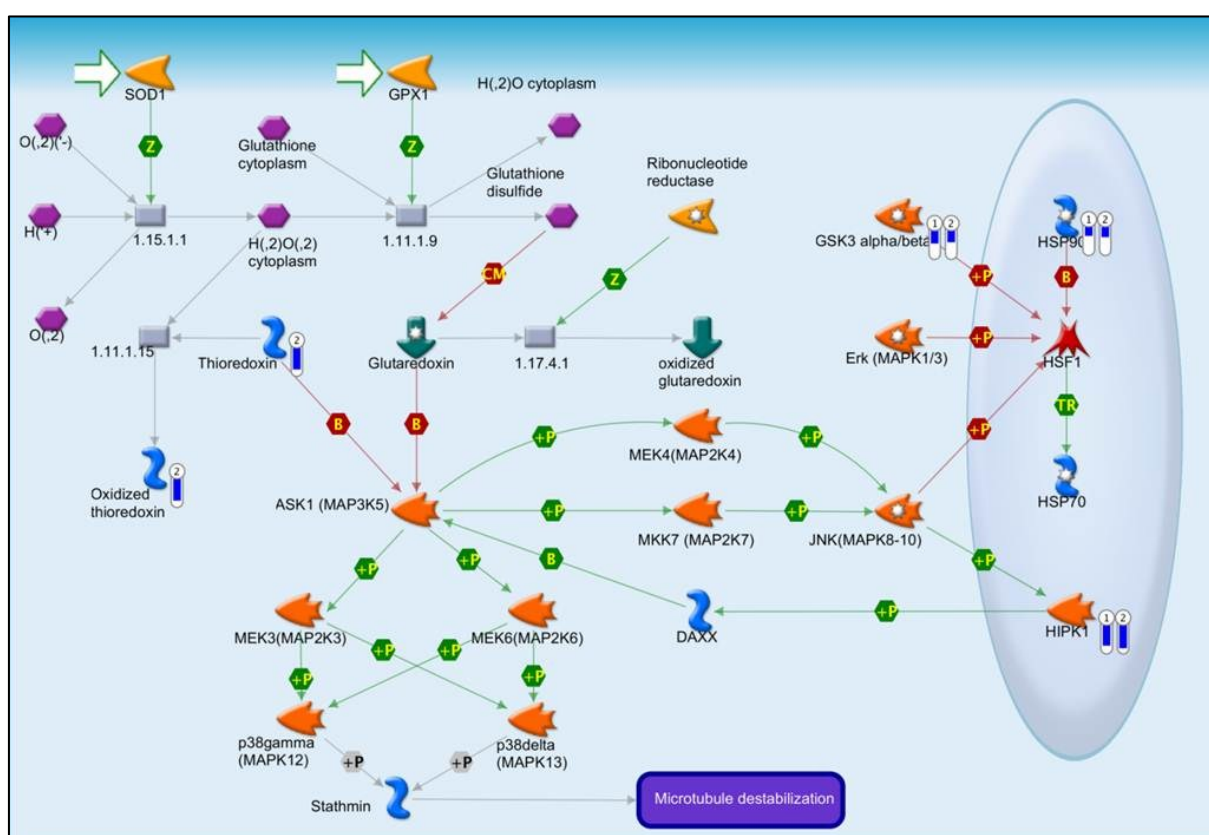


Figure 25: Oxidative stress is induced in LPS treated mice. Thermometers indicate up- (red) and downregulation (blue) of genes involved in this pathway. Numbers written below the thermometer indicate 1: 3 h, and 2: 48 h regulations. Detailed legends are shown in Appendix III.

4.4.1 Affected pathways and functional complexes – tight junctions and lipid metabolism

During this part the affected genes were categorized on the basis of their function using the KEGG pathway maps (Kyoto Encyclopedia of Genes and Genomes; <http://www.genome.jp/kegg/pathway.html>).

The microarray study revealed a downregulation of several genes involved in the formation of junctional complexes due to LPS treatment. These included the junctional adhesion molecules occludin (OCLN; 3 h: 0.5) and claudin 5 (CLDN5; 3 h: 0.2), as well as par-3 partitioning defective 3 homolog (PARD3; 3 h: 0.2). Furthermore, cortactin (CTTN; 3 h: 0.2), was downregulated. The downregulated genes involved in the formation of junctional complexes are represented in Figure 26. All gene regulations shown were seen at 3 h post LPS treatment except of symplekin (SYMPK), which was upregulated at both time points.

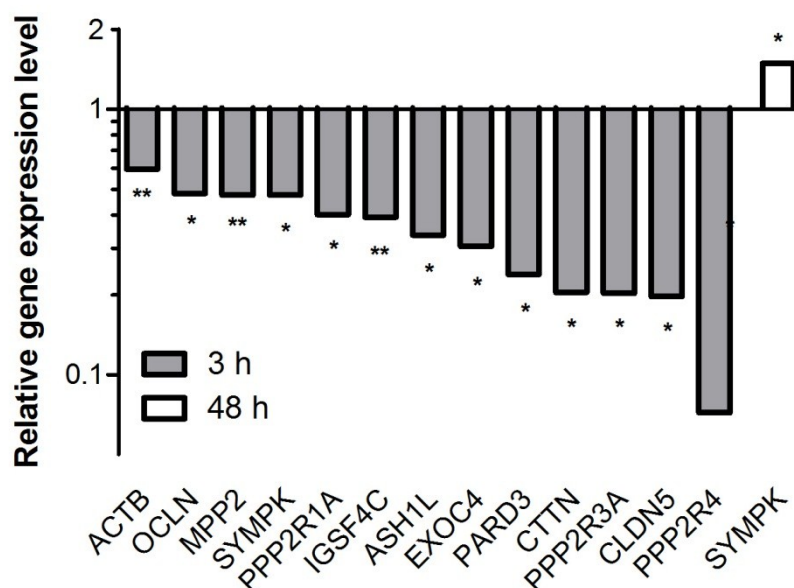


Figure 26: Regulators and constituents of junctional complexes are affected in LPS treated mice. Microarray data revealed a downregulation of several tight-junction related genes. Respective relative gene expression values are represented in the graph. * $P \leq 0.05$, ** $P \leq 0.005$, *** $P \leq 0.001$. ACTB: beta actin; OCLN: occludin; MPP2: Palmitoylated membrane protein 2; SYMPK: symplekin; PPP2R1A: protein phosphatase 2 regulatory subunit A α ; IGSF4C: cell adhesion molecule 4; ASH1L: absent small and homeotic disks protein 1 homolog; EXOC4: exocyst complex component 4; PARD3: partitioning defective 3 homolog; CTTN: cortactin; PPP2R3A: protein phosphatase 2, regulatory subunit B alpha; CLDN5: claudin 5; PPP2R4: protein phosphatase 2A activator, regulatory subunit 4

Next, we focused on genes involved in lipid metabolism and found several regulated transcripts upon LPS treatment (Figure 27). Most of the affected genes were found at 3 h post LPS treatment. According to the KEGG pathway they were categorized into three major pathways based on their function:

1. Sphingolipid metabolism: Sphingosine kinase 1 (SPHK1; 3 h: 2.6) is involved in the synthesis of sphingosine-1-phosphate (S1P). Other enzymes regulated during neuroinflammatory conditions were δ (4)-desaturase sphingolipid 2 (DEGS2; 3 h: 0.2), an enzyme catalyzing the formation of ceramide from dihydroceramide, galactose-3-O-sulfotransferase 1 (GAL3ST1; 48 h: 1.7), which is involved in the synthesis of sulfatide and digalactosyl-ceramidesulfate and galactose-3-O-sulfotransferase 1 (B4GALT6; 48 h: 0.7), which is responsible for the conversion of glucosylceramide to lactosylceramide.
2. Glycerophospholipid metabolism: The expression of phosphatidylethanolamine N-methyl-transferase (PEMT; 3 h: 0.6) a key enzyme involved in the biosynthesis of phosphatidylcholine was decreased in LPS treated mice. Furthermore, 1-acylglycerol-3-phosphate O-acyltransferase 6 (AGPAT6; 3 h: 0.5) was downregulated. This enzyme catalyzes the conversion of lysophosphatidic acid to phosphatidic acid by transferring an acyl-residue to phosphatidylcholine to generate 1-acyl-sn-glycero-3-phosphocholine. Next, phospholipase A2 (PLA2G6; 3 h: 0.5), which catalyzes the release of fatty acids from phospholipids was downregulated. Other enzymes in the glycerophospholipid pathway regulated during LPS treatment in mouse brains were diacylglycerol kinase zeta (DGKZ; 3 h: 0.5), which generates glycerol-3-phosphate from diacylglycerol, phosphatidylserine decarboxylase (PISD; 3 h: 0.2), which converts phosphatidylserine to phosphatidylethanolamine and mitochondrial glycerol-3-phosphate acyltransferase (GPAM; 48 h: 0.1), which converts glycerol-3-phosphate to acyl-glycerol-3-phosphate.

3. Plasmalogen metabolism: The expression of platelet-activating factor acetylhydrolase 1b, regulatory subunit 1 (PAFAH1B1; 3 h: 0.6), which is responsible for the generation of 1-alkyl-sn-glycero-3-phosphocholine was decreased in our study.

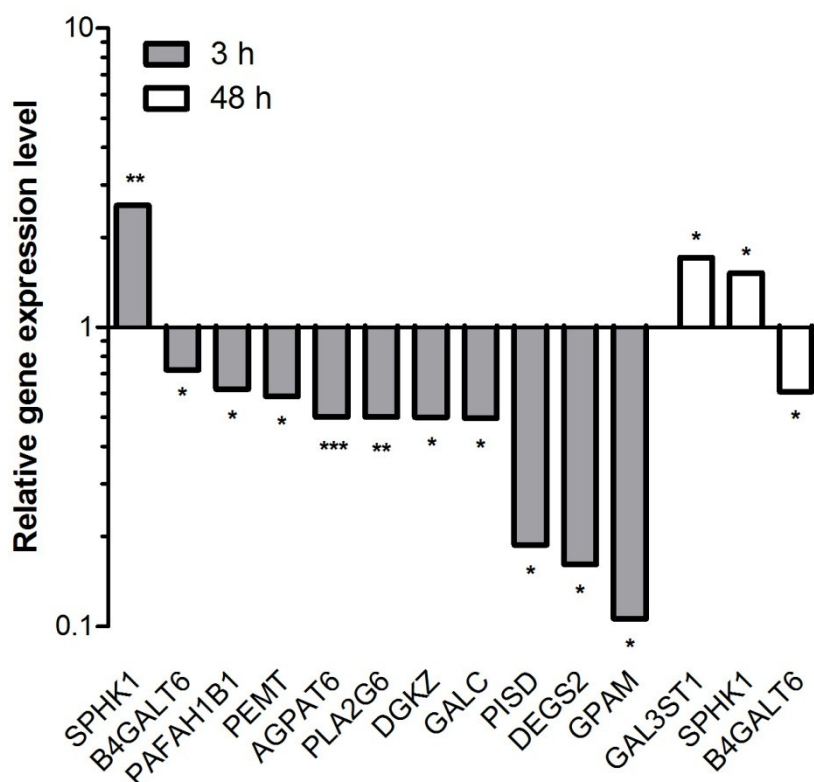


Figure 27: Lipid metabolism is affected in LPS treated mice. Microarray data revealed a regulation of several genes involved in sphingolipid, glycerophospholipid and plasmalogen metabolism. Respective relative gene expression values are represented in the graph. * $P \leq 0.05$, ** $P \leq 0.005$, *** $P \leq 0.001$. SPHK1: sphingosine kinase 1; B4GALT6: UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 6; PAFAH1B1: platelet-activating factor acetylhydrolase 1b, regulatory subunit 1; PEMT: phosphatidylethanolamine N-methyltransferase; AGPAT6: 1-acylglycerol-3-phosphate O-acyltransferase 6; PLA2G6: phospholipase A2, group VI; DGKZ: diacylglycerol kinase, zeta; GALC: galactosylceramidase; PISD: phosphatidylserine decarboxylase; DEGS2: sphingolipid delta 4 desaturase/C-4 hydroxylase; GPAM: glycerol-3-phosphate acyltransferase, mitochondrial; GAL3ST1: galactose-3-O-sulfotransferase 1

4.5 Cholesterol 25-hydroxylase expression is induced in brains and livers of LPS injected mice

The microarray data revealed a significant induction of CH25H under neuroinflammatory conditions in the brain. To confirm microarray data, qPCR was

performed. Furthermore, the mRNA expression of CH25H was measured in the livers of the same mice, too. These data show a 41-fold induction at 3 h and a 7-fold induction at 48 h post treatment in the brain (Figure 28). The induction in the liver was 34-fold at 3 h and 10-fold at 48 h post LPS treatment.

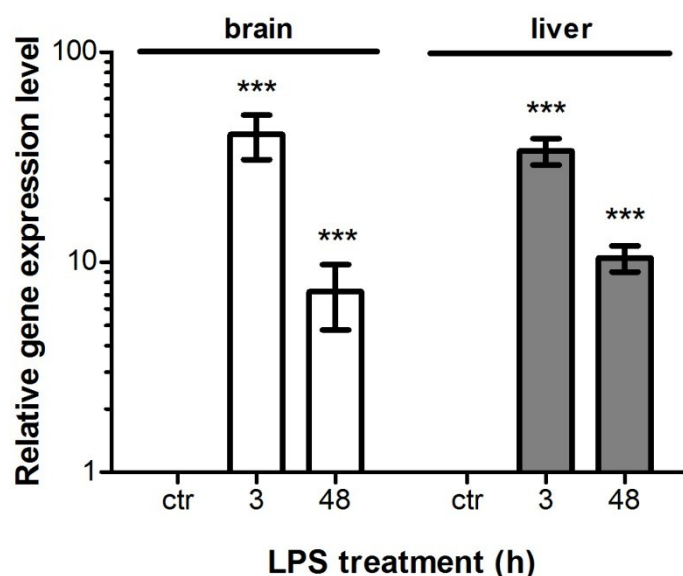


Figure 28: Effect of systemic LPS on CH25H mRNA expression in brains and livers of mice. Systemic LPS (16.6 mg/kg) induces upregulation of CH25H mRNA in brains and livers of 12 week old male C57BL/6 mice. mRNA levels were normalized to GAPDH and expressed relative to gene expression in control mice receiving PBS. Results shown represent means $\pm$ SEM of three animals per time point. * $P \leq 0.05$, ** $P \leq 0.005$, *** $P \leq 0.001$. Gene expression ratios were calculated by REST as described under “Materials and Methods”.

4.6 Elevation of cholesterol 25-hydroxylase parallels with inflammatory response and is time- and concentration dependent

To investigate the time- and concentration dependence of CH25H expression upon LPS treatment in mouse brains, a qPCR experiment was performed using two different LPS doses (1.6 mg/kg and 8.3 mg/kg) and 3 different time points (3 h, 6 h, 24 h). The data showed an induction of CH25H mRNA expression up to 7-fold at 1.6 mg/kg LPS and 10-fold at 8.3 mg/kg LPS (Figure 29). Furthermore, TNF α gene expression peaked at 33-fold at 1.6 mg/kg LPS and 64-fold at 8.3 mg/kg LPS.

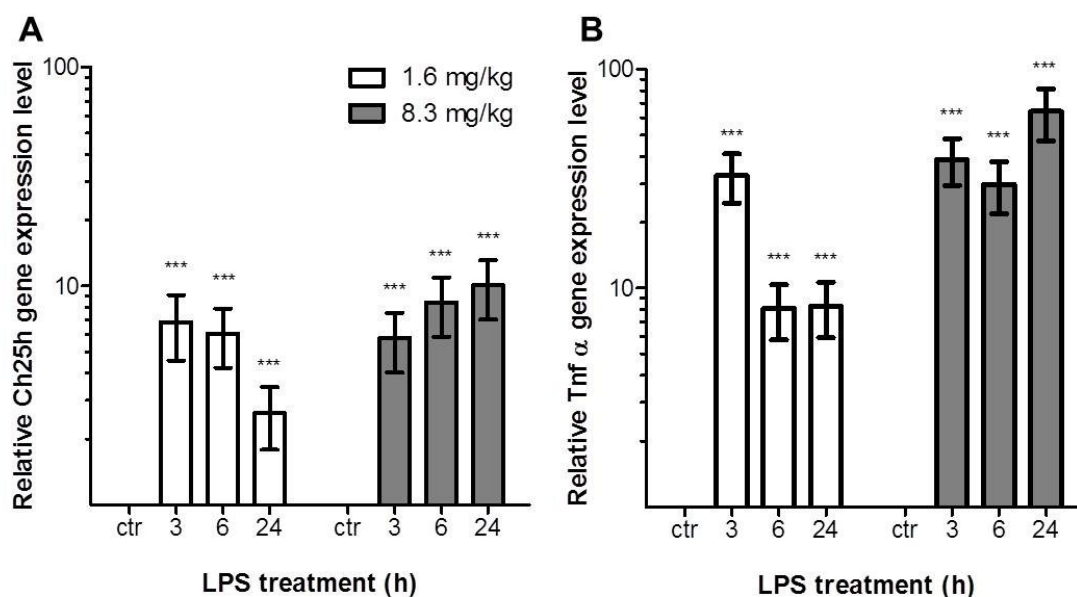


Figure 29: CH25H (A) and TNF α (B) mRNA increase time- and concentration-dependently in mouse brains upon treatment with LPS. C57BL/6 mice (18-20 week old) were used. mRNA levels were normalized to GAPDH and expressed relative to gene expression in control mice receiving PBS. Results shown represent means $\pm$ SEM of three animals per time point measured in triplicates. * $P \leq 0.05$, ** $P \leq 0.005$, *** $P \leq 0.001$. Gene expression ratios were calculated by REST as described under “Materials and Methods”.

4.7 Cholesterol 25-hydroxylase protein expression increases in brains of LPS treated mice

To verify whether the changes in the mRNA expression of CH25H are accompanied by changes in protein levels, Western Blot analysis of brains and livers of LPS treated mice was performed. LPS (16.8 mg/kg) was injected and mice were sacrificed at the indicated time points, protein homogenates were prepared and blotted for CH25H and β -actin (Figure 30). The results showed an increased protein expression (3-fold compared to control) 192 h post treatment in mouse brains, whereas in the livers of these mice protein levels were found to be decreased (2-fold at 24 h and 48 h post LPS treatment).

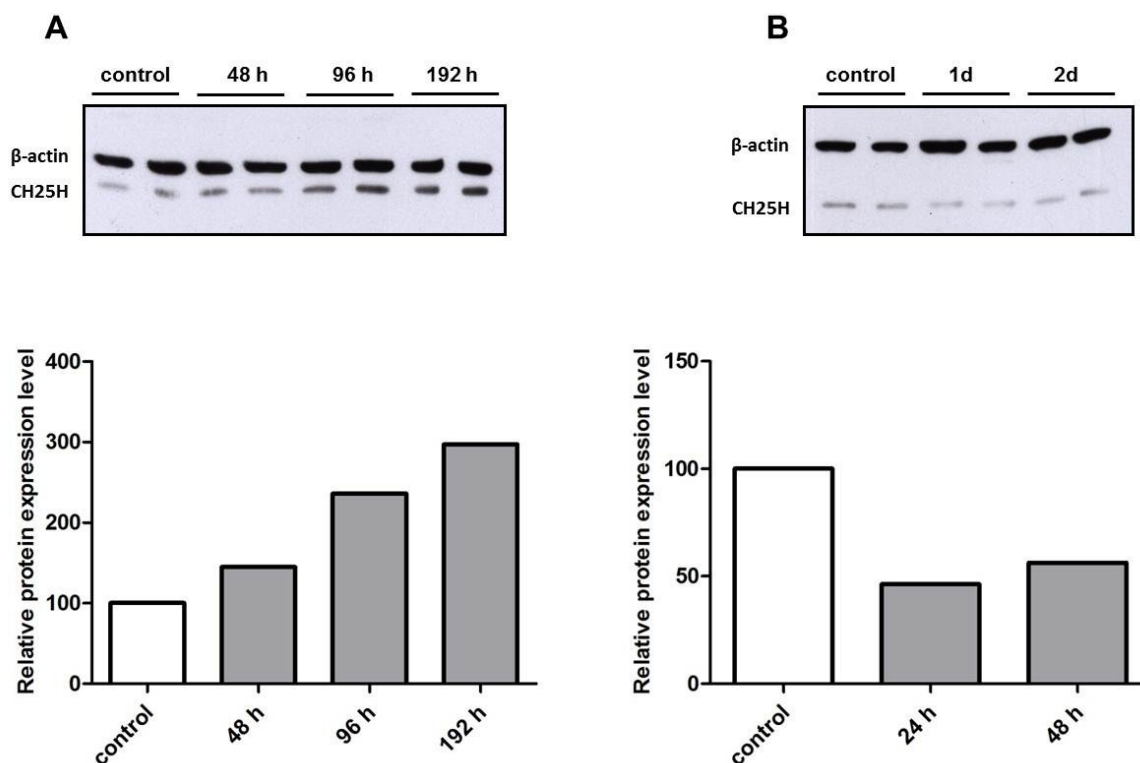


Figure 30: CH25H protein expression rises in brains (A) and livers (B) of LPS treated C57BL/6 male mice (11 week old). 16.6 mg/kg LPS was used. 20 µg whole protein extract was loaded on the gel. Western blots show protein levels of CH25H and β-actin. Protein levels were normalized to β-actin. Bar charts show results expressed relative to protein expression in control mice receiving PBS. Results shown in the graphs represent means of two animals per time point.

4.8 25-hydroxycholesterol increases in brains of LPS treated mice

Next the effect of increased levels of CH25H mRNA as well as protein expression on the levels of 25-HC in brains of LPS treated mice was investigated (Figure 31). For this, mice were injected with LPS, brains were surgically removed and lipids were isolated and analyzed by GC-MS. 25-hydroxycholesterol was detected at the diagnostic ions of m/z 131, 141, 456, 531 and 546. At 48 h post LPS treatment 25-HC increased by approx. 7 ng/mg from 4 to 11 ng/mg brain tissue. GC-MS measurements were performed by Gerald Eibinger in cooperation with Günter Fauler.

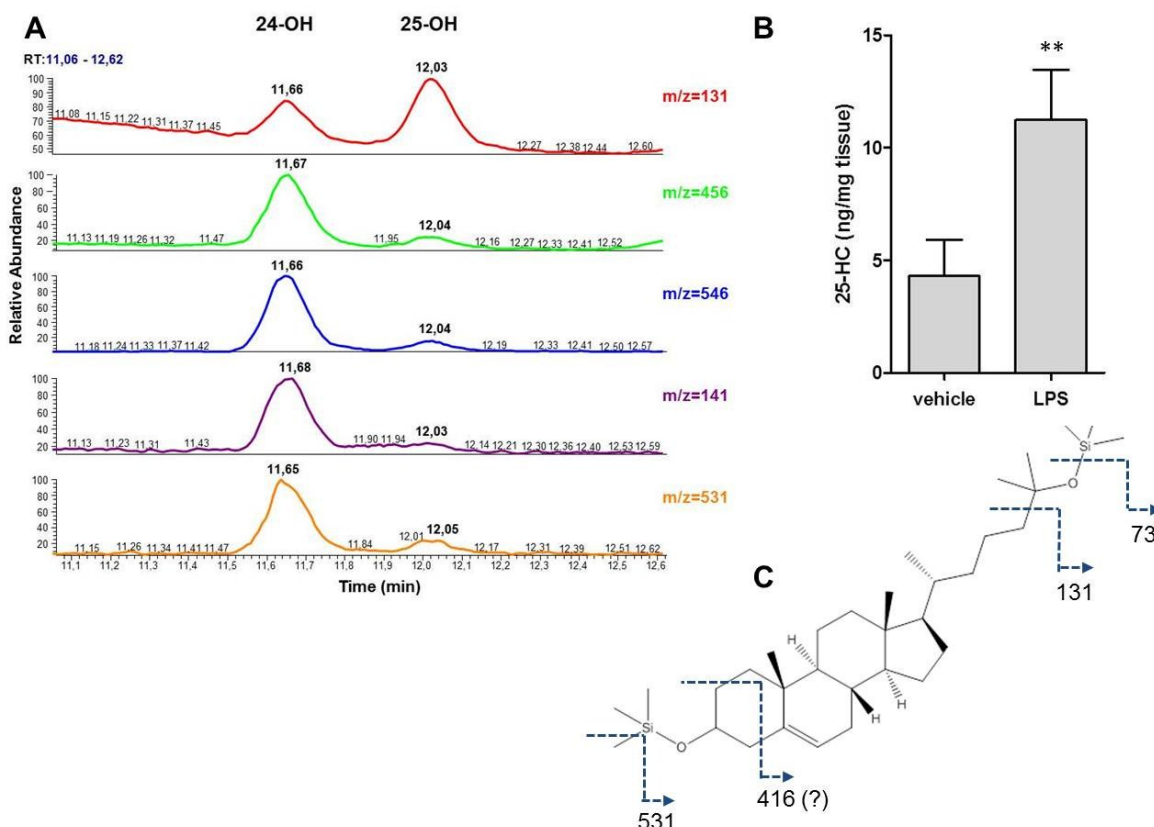


Figure 31: Quantification of 25-hydroxycholesterol in brains of LPS treated mice. GC-MS analysis of 25-HC D₆ and 24-HC D₆ standards (A) at m/z ratios 131, 456, 546, 141 and 531). Quantitative analyses of 25-HC (B) in mouse brain lipid extracts of LPS treated mice (20 week old, 24 h, 8.3 mg/kg LPS) and vehicle control (PBS). (C) Proposed fragmentation pattern with m/z ratios for 25-hydroxycholesterol trimethylsilyl ether fragments (by Andreas Üllen). Results shown are means of 3 mice/group $\pm$ SEM. Statistics were calculated with students t-test. * $P \leq 0.05$, ** $P \leq 0.005$, *** $P \leq 0.001$.

4.9 CH25H expression is comparable in different brain regions

Furthermore I have performed preliminary western blot analyses to get an indication about the expression level of CH25H in different brain regions (Figure 32). Therefore, brain regions were separated immediately after removing the brain and Western Blot was performed with protein samples. The brain regions separated under the microscope were olfactory bulb, cerebrum, cerebellum, midbrain and brainstem. No differences in protein expression in these regions were detected.

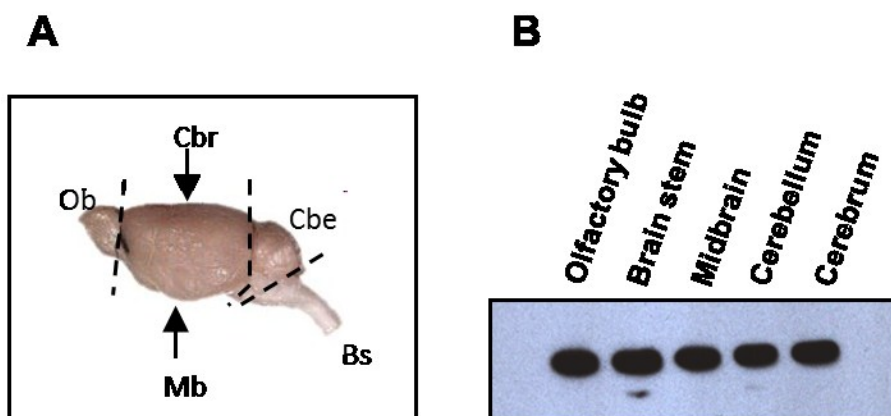


Figure 32: Protein expression of CH25H is comparable in different brain regions. A: overview of a mouse brain. B: Western blot for CH25H. Ob: olfactory bulb, Cbr: cerebrum, Cbe: cerebellum, Mb: midbrain, Bs: brain stem. 20 μ g of whole protein extract were loaded on the gel. Equal loading was tested using Ponceau S staining (not shown).

4.10 Transcript levels of cholesterol metabolic genes are affected in a mouse model of neuroinflammation

Neuroinflammation not only led to an increase in inflammatory markers in the brains of mice, but also influenced expression of genes involved in cholesterol metabolism. Here we show that in 20 week old male mice treated with 8.3 mg/kg LPS i.p. ABCA1 and ApoE mRNA increased time dependently. ABCA1 had its peak expression at 6 h post treatment, whereas for ApoE it was at 3 h post treatment (Figure 33). No significant differences were seen with HMGCS and HMGCR mRNA levels.

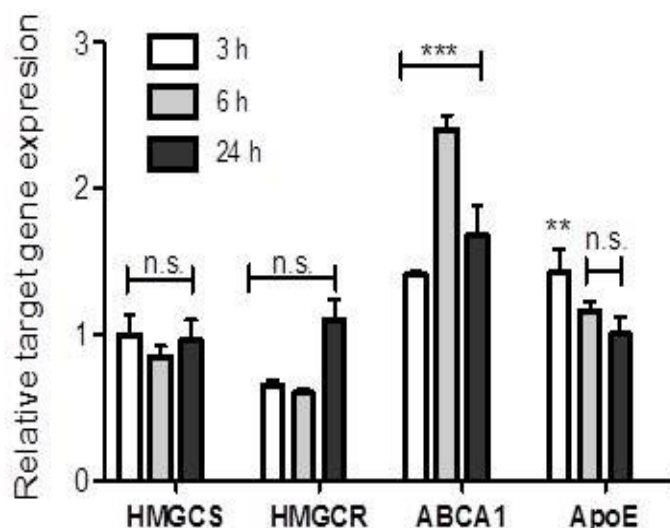


Figure 33: Cholesterol metabolic gene expression in brain is affected under neuroinflammation. Twenty week old male C57BL/6 mice were i.p. injected with LPS (8.3 mg/kg). At the indicated time points (3h, 6h or 24h) mice were killed with cervical dislocation, brains were taken and mRNA was isolated and reverse transcribed. cDNA levels of HMGCS, HMGCR, ABCA1 and ApoE were normalized to GAPDH as a reference gene and expressed relative to gene expressions in vehicle control (PBS). Values represent means of triplicates $\pm$ SEM. Statistics were calculated with REST program. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

4.11 Influence of 25-hydroxycholesterol on pBMVEC viability

This experiment aimed to evaluate cytotoxic properties of 25-HC in comparison to cholesterol in pBMVEC. Therefore, pBMVEC were incubated with the indicated concentrations of 25-HC and cholesterol for up to 24 h. At the indicated time points cell viability was measured using the MTT test. The results show that exposure of pBMVEC to 25-HC does not affect cell viability at shorter time periods (1.5 to 10 h), but a reduction of cell viability to 50-70 % of vehicle control at concentrations ranging from 100 to 250 μ M at 24 h of treatment were seen. Cell viability of cholesterol-treated cells decreased in a concentration-dependent manner (Figure 34). This experiment was performed by Christoph Nussold.

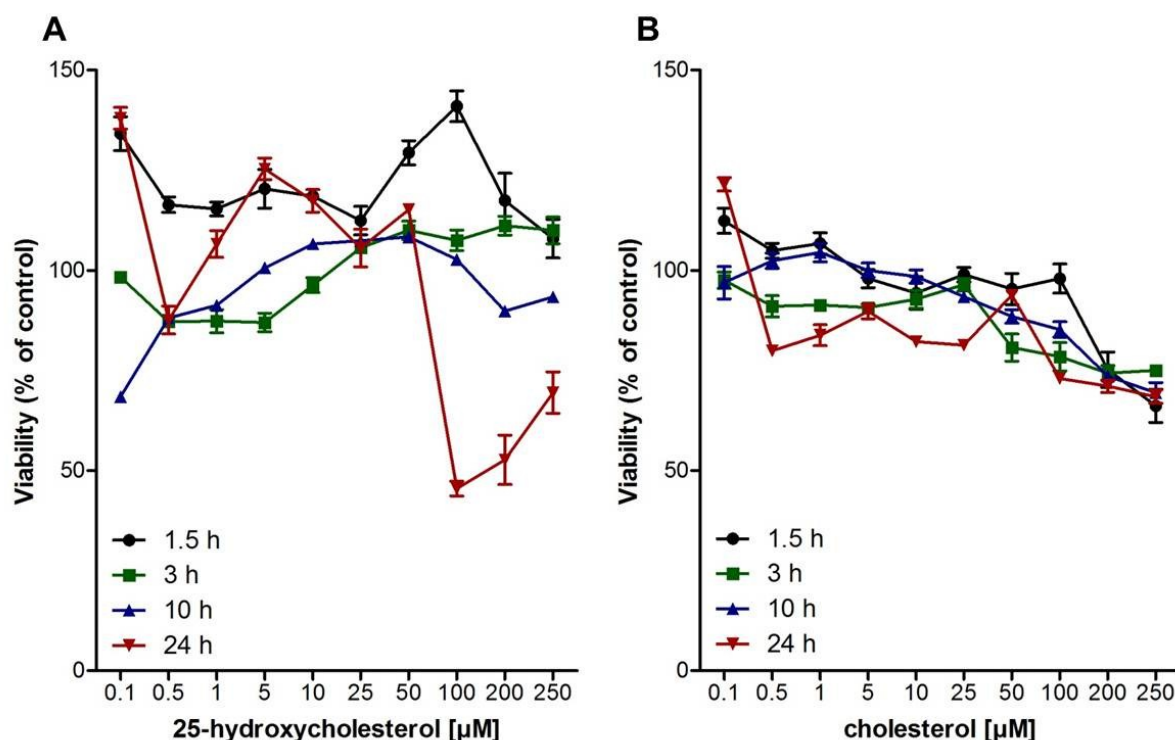


Figure 34: pBMVEC viability in the presence of 25-hydroxycholesterol. Cells were incubated in the absence (vehicle control, EtOH) or presence of 25-HC (A) or cholesterol (B) at indicated concentrations for the indicated time periods. Cell viability was analyzed using the MTT test. Results shown are % of controls and represent means $\pm$ SEM of triplicates. Experiment was done by Christoph Nussbold.

4.12 25-hydroxycholesterol slightly reduces barrier function in an blood-brain barrier model *in vitro*

Next, the effects of 25-HC on pBMVEC barrier function were investigated and compared to 24-HC. Real time monitoring of electrical impedance was performed at 4 kHz (a measure for barrier function) and 64 kHz (a measure for membrane integrity) with the ECIS system. The results show an equal reduction in barrier function upon treatment with 25-HC or 24-HC compared to vehicle control (EtOH). Furthermore, cell integrity is significantly disturbed at both treatments, as it can be seen at 64 kHz impedance measurement (Figure 35).

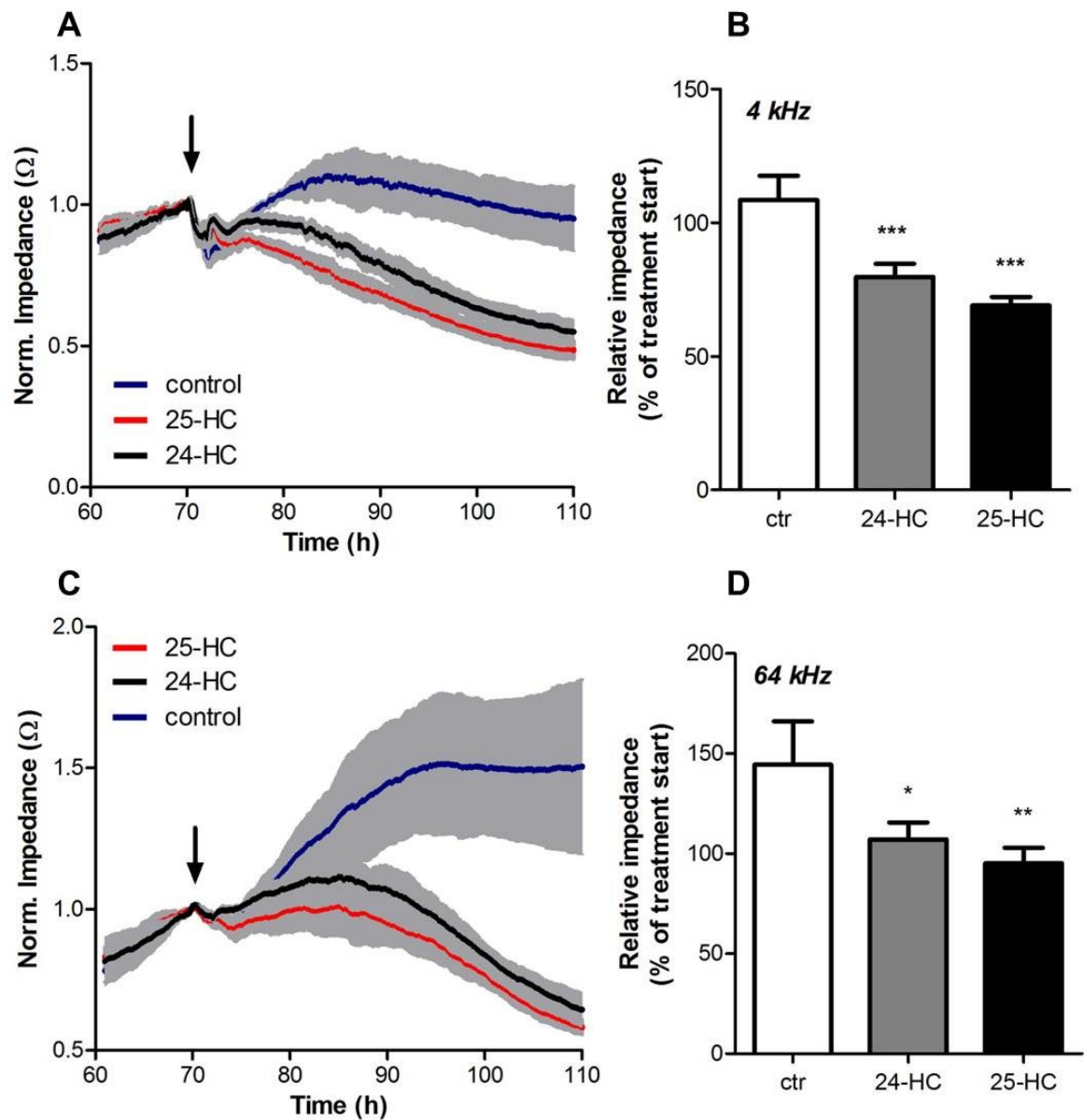


Figure 35: Impact of 25-hydroxycholesterol (25-HC) and 24-hydroxycholesterol (24-HC) on pBMVEC barrier function. pBMVECs were plated on gold microelectrodes and cultured to confluence. Barrier function of endothelial monolayers was continuously monitored by impedance sensing at 4 kHz (A) and 64 kHz (C). The 90 hour time points are shown as bar charts (B and D). After stabilization, cells were challenged (arrow) with 10 μ M 25-HC or 24-HC. Impedances were normalized to treatment start (addition of hydroxycholesterol). Results shown represent means $\pm$ SD of four independent experiments per group. Statistics were calculated with 1-way ANOVA followed by a Bonferroni post-test. * $P \leq 0.05$, ** $P \leq 0.005$, *** $P \leq 0.001$.

4.13 Cholesterol biosynthesis is reduced in mouse brain neurons upon 25-hydroxycholesterol treatment

To investigate the impact of 25-HC on the biosynthesis of cholesterol in neurons, an *in vitro* assay using CATH.a mouse neurons was performed. For this, cells were incubated with different concentrations of 25-HC over a time period of 6 h. Lipids were extracted as shown in Materials and Methods and cholesterol as well as cholesterol esters were measured in cells and media. The results (Figure 36) show a concentration-dependent reduction of cholesterol biosynthesis starting at 2.5 μ M 25-HC treatment. At 5 μ M 25-HC treatment cholesterol biosynthesis was reduced to approx. 45 % of vehicle control. Comparable results were obtained for the media, where the reduction in cholesterol reached 30 % of vehicle control.

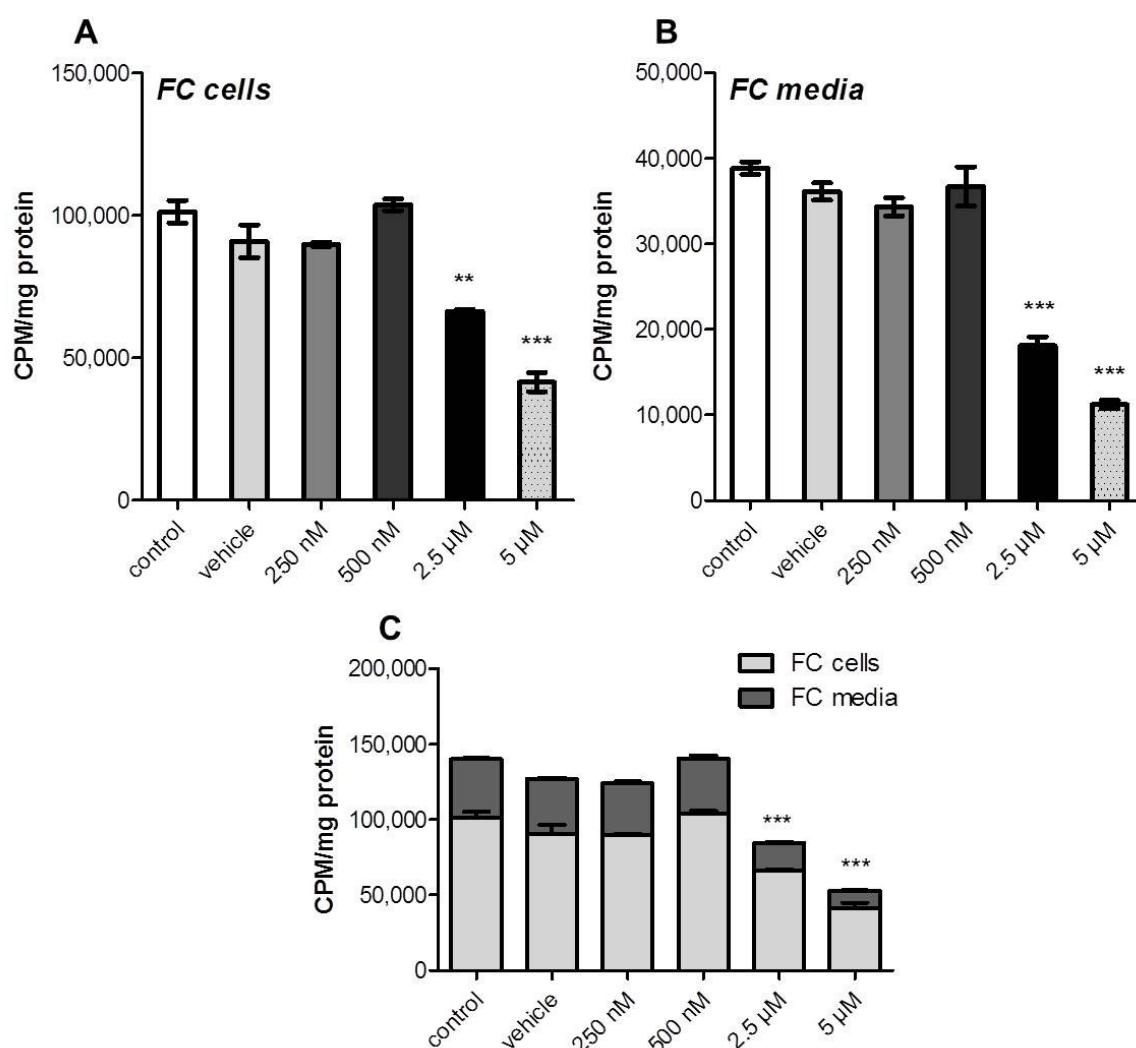


Figure 36: Cholesterol biosynthesis is reduced upon 25-HC treatment. CATH.a mouse neurons were treated with 25-HC for 6 h. Cells and media were taken and free cholesterol was separated by TLC and radioactivity was measured with a scintillation

counter. A: Free cholesterol (FC) cells. B: FC media. C: Total FC. Data were normalized to protein concentration and compared to vehicle control. Results shown represent means $\pm$ SEM of three independent experiments per group. Significances were calculated with 1-way ANOVA followed by a Bonferroni post-test. * $P \leq 0.05$, ** $P \leq 0.005$, *** $P \leq 0.001$.

Furthermore, also the radioactivity present in the cholesterylester (CE) fraction was quantitated (Figure 37). As expected cholesterol esterification in neurons is rather low. Upon treatment with 25-HC no concentration-dependent change in esterification of cholesterol occurred.

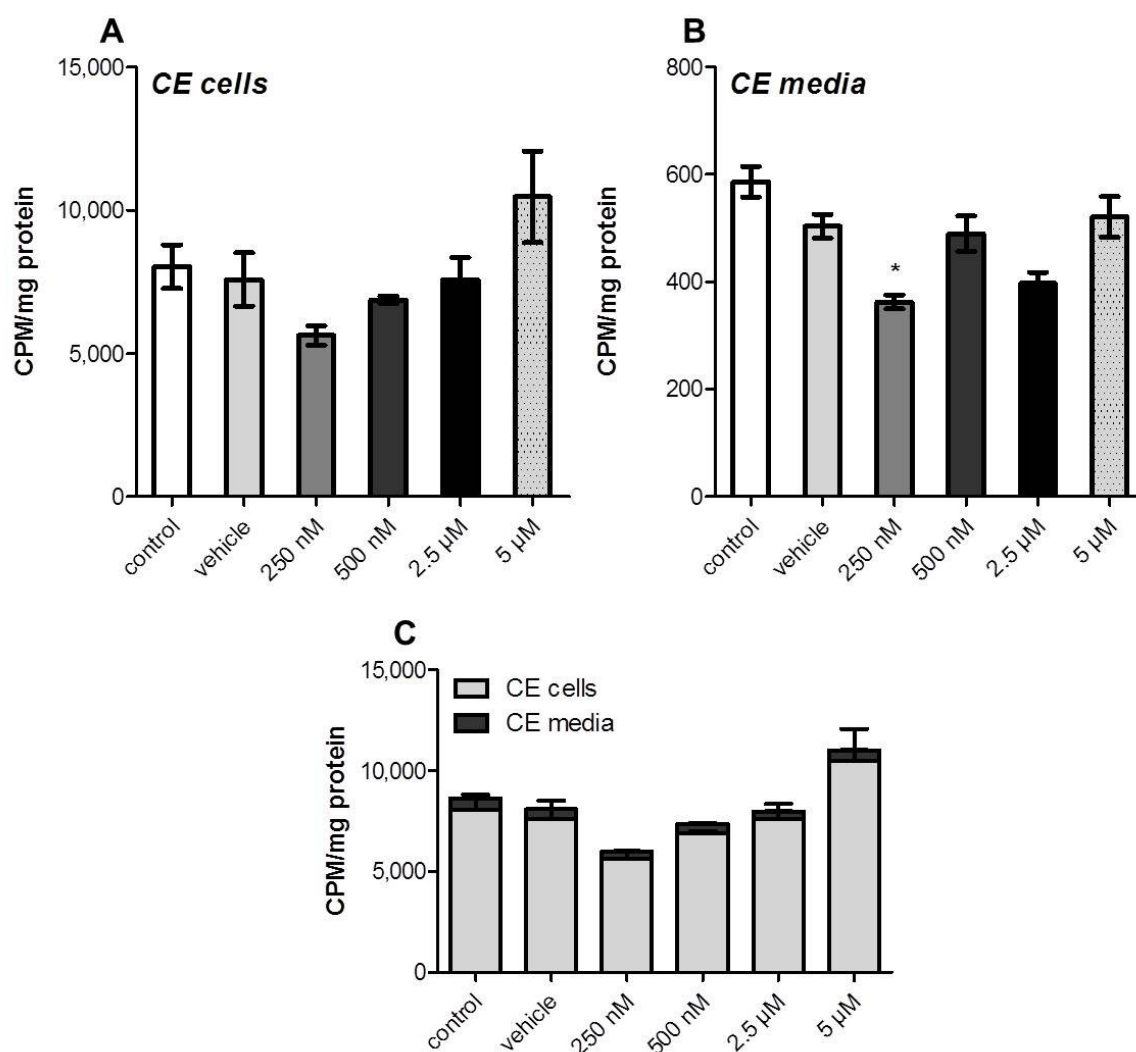


Figure 37: Cholesterol esterification does not change upon 25-HC treatment. CATH.a mouse neurons were treated with 25-HC for 6 h. Cells and media were taken and cholesterylester (CE) was separated by TLC and measured with a scintillator. A: CE cells. B: CE media. C: Total CE. Data were normalized to protein concentration and compared to vehicle control. Results shown represent means $\pm$ SEM of three independent experiments per group. Significances were calculated with 1-way ANOVA followed by a Bonferroni post-test. * $P \leq 0.05$, ** $P \leq 0.005$, *** $P \leq 0.001$.

4.14 25-hydroxycholesterol modulates expression of LXR target genes involved in cholesterol efflux and biosynthesis *in vitro*

I showed that the biosynthesis of cholesterol is significantly impaired upon treatment with 25-HC. The next approach aimed in studying the effect of 25-HC on expression of genes associated with lipid transport, synthesis or metabolism in neurons and astrocytoma cells *in vitro*. Therefore, the change in mRNA expression of lipid-associated genes in CATH.a mouse neurons and CCF-STTG1 human astrocytoma cells, which were challenged with 25-HC was analyzed by qPCR.

These analyses demonstrate that the expression of the cholesterol efflux transporter ATP-binding cassette sub-family A member 1 (ABCA1) is increased upon 25-HC treatment *in vitro* (Figure 38). In CATH.a mouse neurons the expression increases concentration-dependently with significant changes at 1 μ M 25-HC and higher. These changes are already present at 3 h of treatment and continue up to 12 h of treatment. The regulation ranges from 1.5 to 2.5-fold. Similar results were seen when treating CCF-STTG1 human astrocytoma cells with an upregulation of 5-fold at 12 h post treatment.

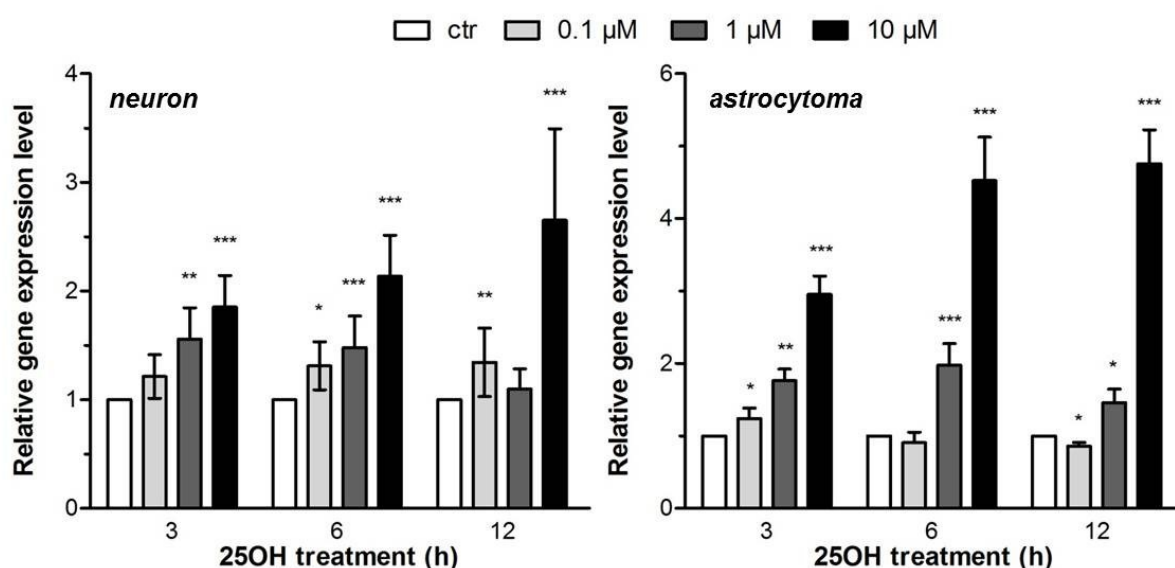


Figure 38: ABCA1 gene expression is induced upon 25-HC treatment in neurons and astrocytoma cells *in vitro*. At the indicated time points mRNA was isolated and reverse transcribed. cDNA levels of ABCA1 were normalized to β -actin as a reference gene and expressed relative to gene expressions in vehicle control. Values represent means of triplicates $\pm$ SEM. Statistics were calculated with REST program. * P <0.05, ** P <0.01, *** P <0.001.

Next the expression of the brain-specific lipoprotein apolipoprotein E (apo E) was investigated. The results show a slight change in expression in CATH.a neurons at the highest concentration (10 μ M) 12 h post treatment. Besides that no changes in mRNA expression were seen in CATH.a neurons. In the CCF-STTG1 expression changed more rapidly with an increase to about 2.5-fold at 3 h post treatment. At 6 h post treatment no change in expression was detected (Figure 39).

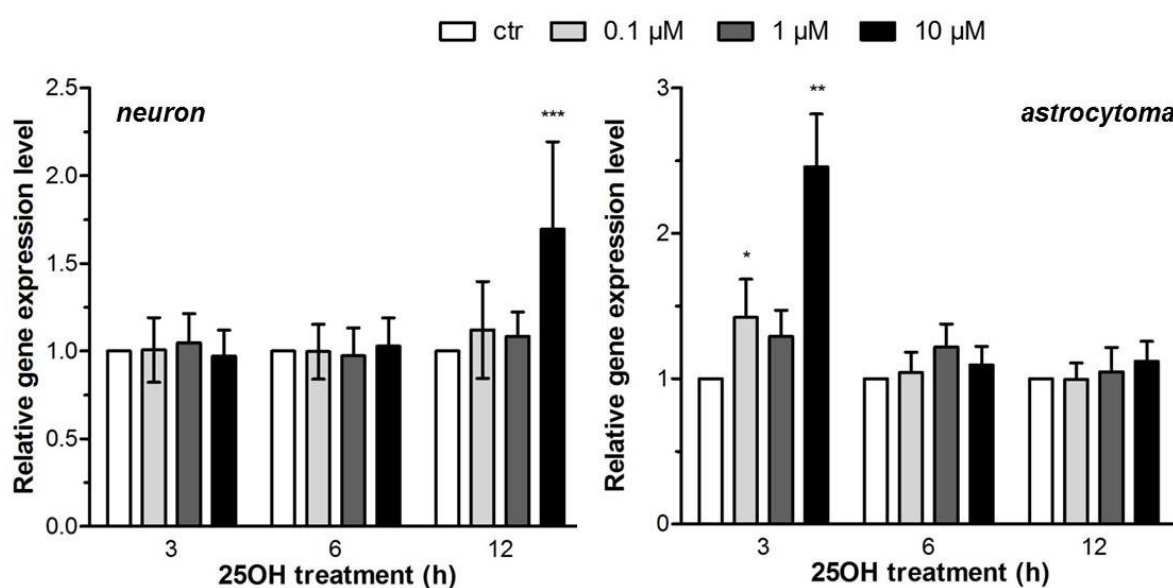


Figure 39: Apo E gene expression is induced upon 25-HC treatment in neurons and astrocytoma cells *in vitro*. At the indicated time points mRNA was isolated and reverse transcribed. cDNA levels of apo E were normalized to β -actin as a reference gene and expressed relative to gene expression in vehicle controls. Values represent means of triplicates $\pm$ SEM. Statistics were calculated with REST program. *P<0.05, **P<0.01, ***P<0.001.

Next we were interested in investigating the expression of fatty-acid synthase (FASN) upon 25-HC treatment. Fatty acid synthesis is an energy-consuming process that requires the multifunctional enzyme. Its main function is to catalyze the synthesis of palmitate or other long-chain saturated fatty acids by utilizing acetyl-CoA and malonyl-CoA as substrates, in the presence of the cofactor NADPH (Semenkovich 1997). FASN is expressed throughout all human tissues (Semenkovich, Coleman et al. 1995). Inactivation of FASN in brain or liver impairs expression of genes regulated by peroxisome proliferator-activated receptor α (PPAR α) that can be restored by PPAR α agonist treatment (Chakravarthy, Pan et al. 2005; Chakravarthy, Zhu et al. 2007) (Schneider, Yang et al. 2010). Our data show that the expression of FASN in 25-HC treated neurons was slightly reduced at 6 h of treatment, whereas in astrocytoma cells FASN expression slightly increased at 3 and 6 h post treatment (Figure 40).

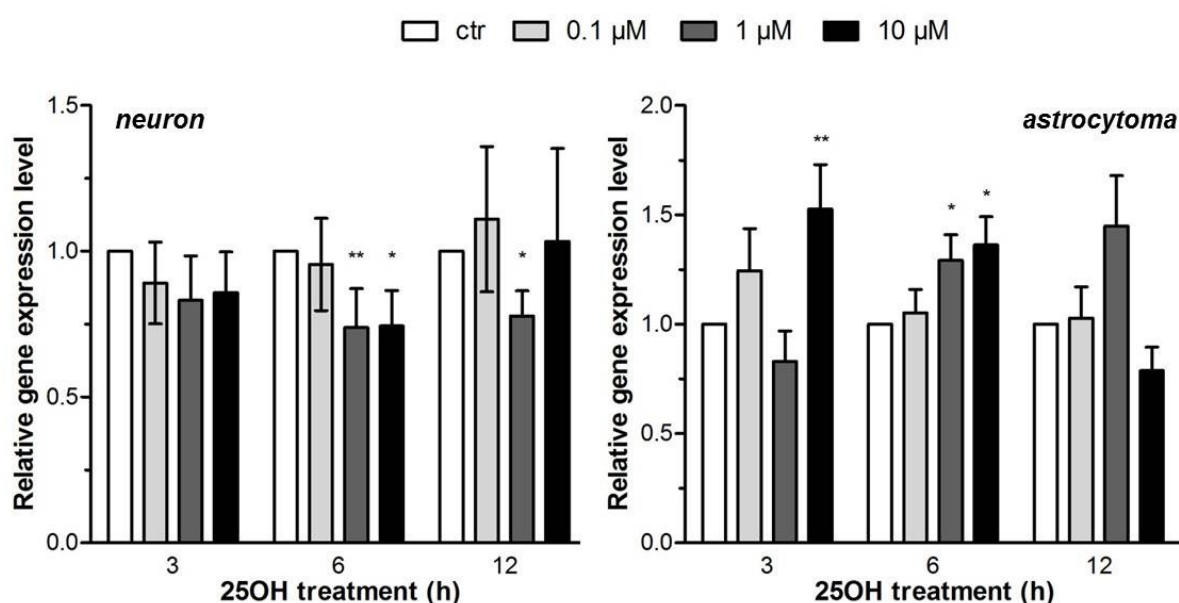


Figure 40: FASN gene expression in neurons and astrocytoma cells *in vitro*. At the indicated time points mRNA was isolated and reverse transcribed. cDNA levels of FASN were normalized to β -actin as a reference gene and expressed relative to gene expression in vehicle controls. Values represent means of triplicates $\pm$ SEM. Statistics were calculated with REST program. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Next we investigated the transcriptional changes in genes involved in cholesterol biosynthesis. Farnesyl-diphosphate farnesyltransferase (FDFT1), also called squalene synthase is responsible for the two-step reaction to form squalene from farnesyl diphosphate. It is known that squalene synthase is regulated by the cellular cholesterol status. At times of cholesterol deprivation, FDFT1 is upregulated whereas at times of cholesterol excess mRNA and protein levels are downregulated (Tansey and Shechter 2000). 25-HC led to a pronounced decrease in FDFT1 transcription (Figure 41). Neurons treated with 25-HC showed a strong decrease in mRNA expression at each time point. Expression decreased concentration-dependently to about half of the control level. In astrocytoma cells expression increased at short incubation periods, whereas it decreases at longer periods of incubation (6 h and 12 h).

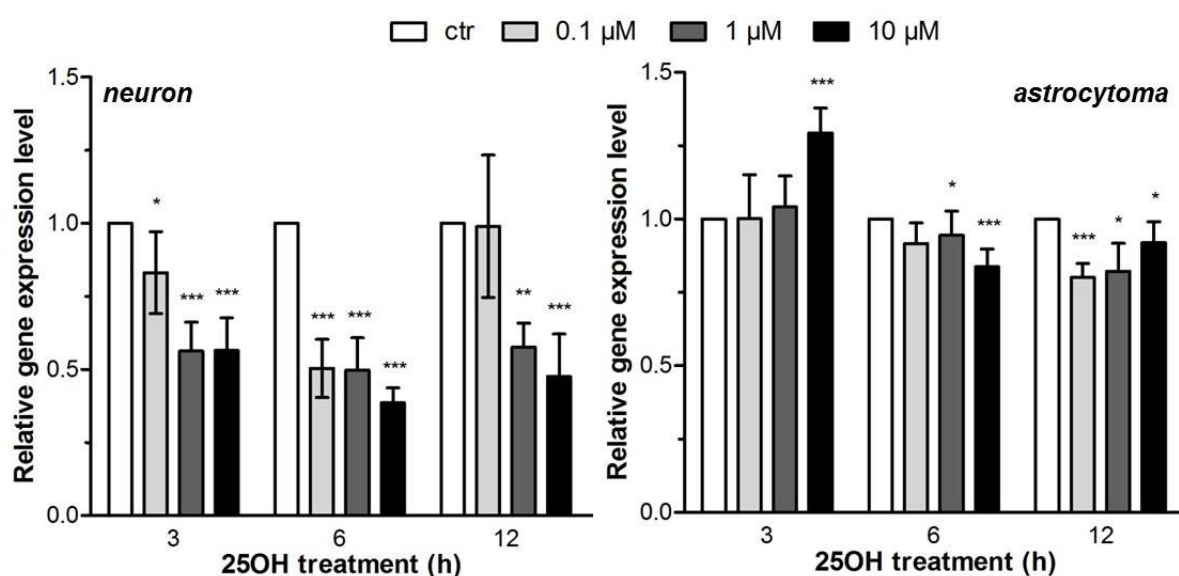


Figure 41: FDFT1 gene expression in neurons and astrocytoma cells *in vitro*. At the indicated time points mRNA was isolated and reverse transcribed. cDNA levels of FDFT1 were normalized to β -actin as a reference gene and expressed relative to gene expression in vehicle controls. Values represent means of triplicates $\pm$ SEM. Statistics were calculated with REST program. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Next, the levels of HMG-CoA-Synthase (HMGCS) were examined in 25-HC treated neurons and astrocytoma cells. HMGCS is an enzyme in the cholesterol biosynthesis pathway, where it catalyzes the reaction in which acetyl-CoA and acetoacetyl-CoA form 3-hydroxy-3-methylglutaryl-CoA. Treatment of cells with 25-HC led to a robust downregulation of HMGCS1 in neurons in a concentration-dependent manner. At 10 μ M 25-HC HMGCS was downregulated by 75 %. In astrocytoma cells downregulation was less prominent, and no concentration-dependence was detected (Figure 42).

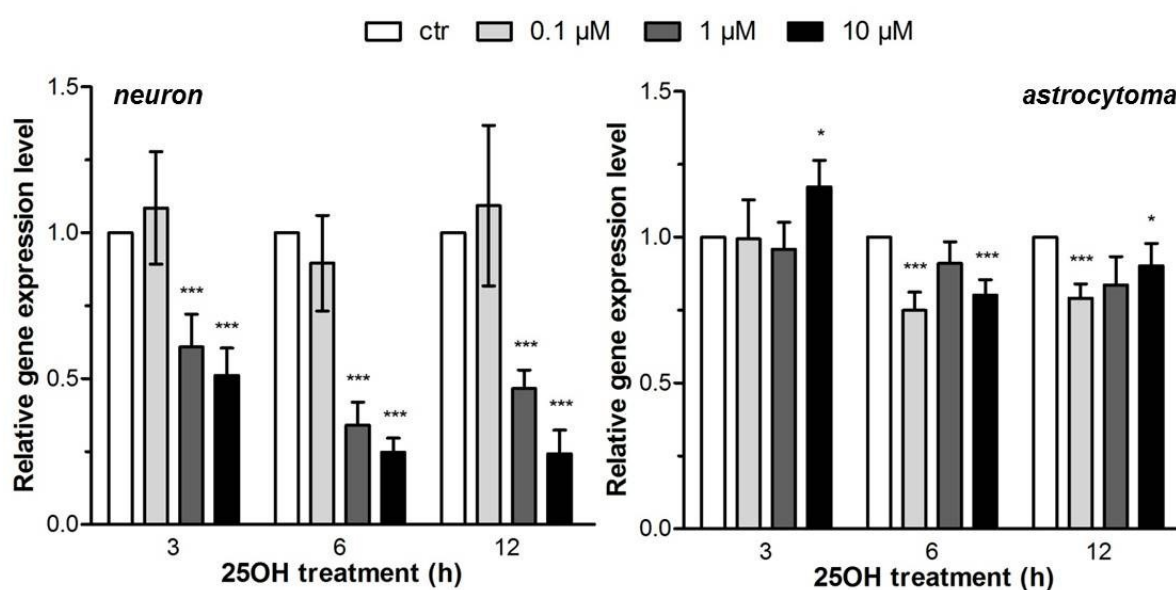


Figure 42: HMGCS gene expression in neurons and astrocytoma cells *in vitro*. At the indicated time points mRNA was isolated and reverse transcribed. cDNA levels of HMGCS were normalized to β -actin as a reference gene and expressed relative to gene expression in vehicle controls. Values represent means of triplicates $\pm$ SEM. Statistics were calculated with REST program. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Three-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) is the key regulator enzyme of cholesterol biosynthesis, as it catalyzes the rate-limiting step of cholesterol biosynthesis from HMG-CoA to mevalonate (Goldstein and Brown 1990). Treatment of cells with 25-HC led to a decreased expression of HMGCR in neurons as well as astrocytoma cells (Figure 43). The change in expression was much more pronounced in neurons, with a decrease to 0.4-fold of the control at 1 μ M and 10 μ M concentrations. At a concentration of 0.1 μ M, expression of HMGCR slightly increased. In astrocytoma cells only a slight decrease of expression was detected.

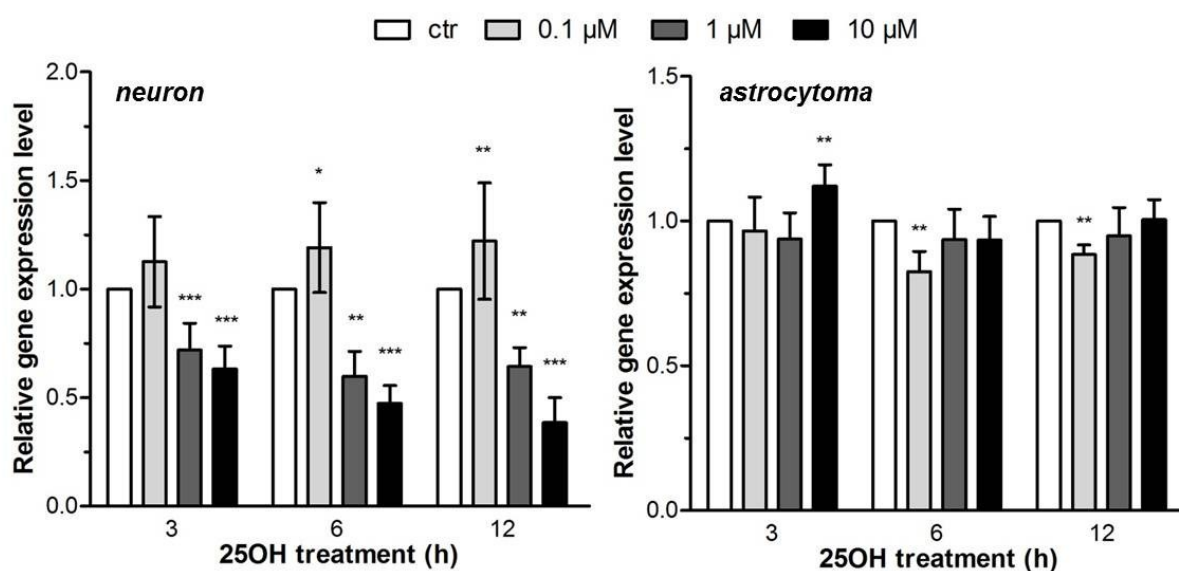


Figure 43: HMGCR gene expression in neurons and astrocytoma cells *in vitro*. At the indicated time points mRNA was isolated and reverse transcribed. cDNA levels of HMGCR were normalized to β -actin as a reference gene and expressed relative to gene expression in vehicle controls. Values represent means of triplicates $\pm$ SEM. Statistics were calculated with REST program. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Next we measured the expression of the key regulator gene of cholesterol biosynthesis, sterol regulatory element binding protein 2 (SREBP2). The results indicate that 25-HC regulates the transcription of SREBP2 in neurons and astrocytoma cells. A prominent decrease in transcription was seen in neurons, where reduction was concentration-dependent and reached half of control values. Similar results were seen in astrocytoma cells, where reduction was most prominent at 12 hours post treatment with a decrease to 0.7 of the control (Figure 44).

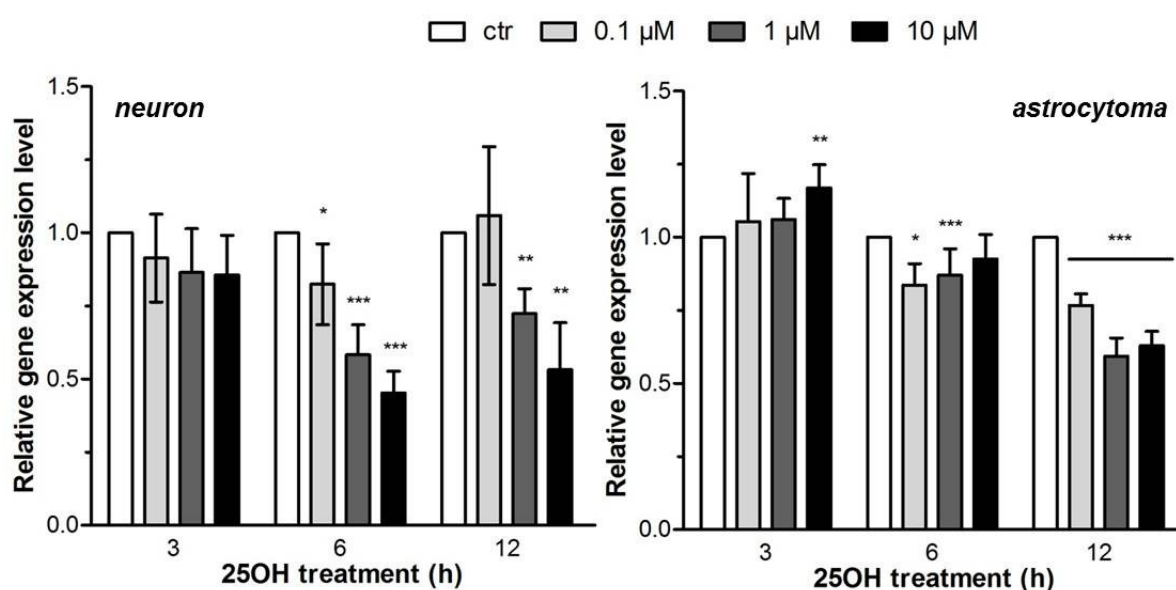


Figure 44: SREBP2 gene expression in neurons and astrocytoma cells *in vitro*. At the indicated time points mRNA was isolated and reverse transcribed. cDNA levels of SREBP2 were normalized to β -actin as a reference gene and expressed relative to gene expression in vehicle controls. Values represent means of triplicates $\pm$ SEM. Statistics were calculated with REST program. *P<0.05, **P<0.01, ***P<0.001.

4.15 SREBP2 neuronal expression is regulated by 25-hydroxycholesterol

So far I could demonstrate that neuronal cholesterol biosynthesis (using [^{14}C]-acetate as precursor) is downregulated by 25-HC. Furthermore, decreased expression of genes regulating cholesterol biosynthesis was observed. Transcript levels of SREBP2, a key transcription factor in cholesterol biosynthesis were decreased. Therefore, it was important to test whether 25-HC affects protein expression and/or processing of SREBP2 in neuronal cells. Before measuring the impact of 25-HC on SREBP2 processing, a time course for optimum maturation of SREBP2 was assessed. We show here that SREBP2 maturation is inhibited in fed cells. At 60 min fasting, mature SREBP2 can be detected (Figure 45). Therefore, we chose 4 h as the optimal time point for measuring the impact of different concentrations of 25-HC on SREBP2 processing.

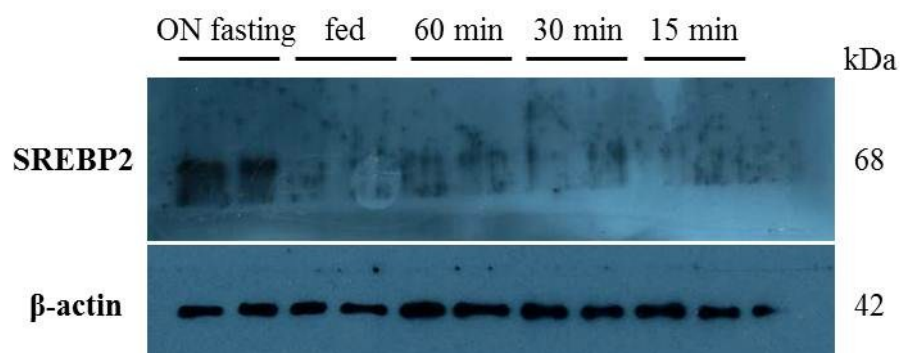


Figure 44: Time course for optimum maturation of SREBP2. CATH.a mouse neurons were serum-starved in the presence of 5 % LPDS, 50 μM mevalonate and 10 μM compactin ON (ON fasting), or for 60, 30 and 15 minutes, respectively. Whole protein was isolated and mature SREBP2 was detected with Western blotting. β -actin was used as a reference gene. This experiment was done by Jay Patankar.

For measurement of the impact of 25-HC on SREBP2 processing, CATH.a cells grown ON were serum-starved for 4 h in the presence of various concentrations of 25-HC and LPDS, mevalonate and compactin (Figure 46). Mevalonate and compactin were used at the described concentrations to repress endogenous cholesterol biosynthesis and promote SREBP2 processing (DeBose-Boyd, Ou et al. 2001). The results in Figure 45 indicate a significant reduction in SREBP2 maturation in CATH.a neurons in a 25-HC concentration-dependent manner. A significant downregulation was already observed at 100 nM 25-HC, with no detectable mature SREBP2 protein at 1 μ M 25-HC. This experiment was done by Jay Patankar.

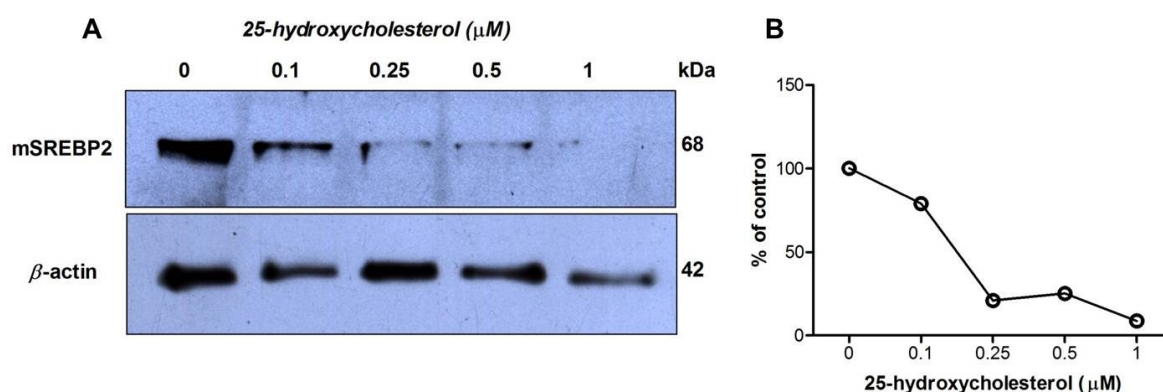


Figure 45: SREBP2 protein processing is regulated by 25-HC. CATH.a mouse neurons were serum-starved in the presence of 5 % LPDS, 50 μ M mevalonate and 10 μ M compactin for 4 h. During this time, cells were treated with 25-HC (0.1-1 μ M). Whole protein was isolated and mature SREBP2 was detected with Western blotting (A). β -actin was used as a reference gene. (B) Band intensity (%) relative to control, normalized to β -actin. This experiment was done by Jay Patankar.

5 DISCUSSION

Neuroinflammation is involved in the progression of several neurodegenerative diseases, like Alzheimer's disease (AD), Parkinson's disease (PD) and Multiple sclerosis (MS). In the present study, a murine model of systemic inflammation and concomitant neuroinflammation was used to characterize transcriptional changes that underlie the physiological and pathophysiological effects of neuroinflammation. A single systemic LPS injection drives a chain reaction leading to elevation of TNF α in the periphery through a TNF α -receptor dependent signal transduction cascade at the blood-brain barrier (BBB) to a consequent and long-lasting upregulation of TNF α in the brain (Qin, Wu et al. 2007). This shows that one inflammatory event starting in the periphery might already be sufficient to drive a persistent and self-perpetuating neuroinflammation, accompanied by a progressive loss of dopaminergic neurons in the substantia nigra (Liu, Qin et al. 2008).

Establishing a mouse model of neuroinflammation

The first task of this study was to establish a mouse model of neuroinflammation. For this, we chose to identify transcriptional changes of the proinflammatory markers MPO, TNF α , iNOS and COX2 in mouse brains after peripheral administration of LPS (Figure 16). We found, that MPO mRNA increased in a bimodal fashion, with the first peak of induction (higher) at 6 h post treatment and the second peak (lower) at 72 h post treatment. Since the study was terminated at 72 h, the question whether MPO mRNA expression reaches another peak (e.g. due to neutrophil/monocyte diapedesis) remains unanswered. Although the reason for this biphasic regulation was not investigated, it could be speculated that the transcriptional regulation is dependent on the specific interactions of inhibiting and activating co-factors, which occur in a time-dependent manner. Similarly, it has been shown that the transcription factor NF- κ B is activated by proinflammatory cytokines in a biphasic manner involving two different assembling factors (MEKK2 and MEKK3) leading to a rapid/transient and a delayed/persistent NF- κ B activation and gene expression (Schmidt, Peng et al. 2003). The proinflammatory cytokines TNF α , iNOS and COX2 were shown to peak at 6 h post LPS treatment. COX2 was inversely regulated at 72 h post treatment. It is well known that events that ensue soon after a severe inflammatory stimulus already set into motion the pathways that eventually lead to the resolution of inflammation. This

has been documented by decreased expression levels of several inflammatory markers at later time points after an acute initial surge in expression. It might be assumed that such feedback pathways control the transcription of COX2 at later time points to prevent overt effects of persistent inflammation. A similar feedback-mechanism was shown in differentiated macrophage-like U937 cells, where the prostaglandin D2 metabolite 15-deoxy- $\Delta^{12,14}$ PGJ2 was shown to negatively regulate COX2 expression via peroxisome proliferator-activated receptor- γ (PPAR γ) signaling (Inoue, Tanabe et al. 2000).

Next, the severity of the inflammatory response elicited by LPS treatment was compared in brain and blood. For this, mice were injected i.p. with LPS and timepoints between 48 h and 360 h were chosen for detection of the above mentioned proinflammatory markers (Figure 17). We saw a strong upregulation of MPO in the blood, which peaked at 96 h post treatment. The upregulation in the brain also peaked at 96 h, but was much weaker compared to the blood. The regulation of TNF α was lower in blood compared to brain. Additionally, it must be mentioned that TNF α reached almost basal levels at 360 h post treatment. This is in line with published results, as it was shown that a single LPS dose can lead to an upregulation of TNF α protein for months, although mRNA levels drop to baseline levels already 9 h post treatment (Qin, Wu et al. 2007). iNOS level peaked at 48 h post injection in blood and brain, although was downregulated at a later time point (192 h) in the brain, similar to COX2. NO, the enzymatic endproduct of iNOS was shown to inhibit transcription of iNOS by inhibiting binding of NF- κ B to iNOS promoter (Park, Lin et al. 1997). COX2 was negatively regulated in the blood with a negative peak at 96 h post treatment, whereas it was slightly upregulated at 48 h and downregulated at 360 h in the brain.

Our next aim was to detect MPO positive cells in brains of mice suffering from neuroinflammation, as the source of the detected mRNA was not yet known. We detected MPO-positive cells in the vasculature of the brain (Figure 18), especially at the meninges, but no staining was detected in the brain itself. These results together with the lower transcriptional upregulation in brain compared to blood led us to the conclusion that in this rodent model of neuroinflammation MPO is not expressed in the brain, but rather in blood-cells, such as invading neutrophils or monocytes. Similar findings were shown by Ji *et al.* (Ji, Yang et al. 2007), who injected LPS

directly into the SNpc and found that microglia died 6 h after injection, whereas neutrophils and monocytes infiltrated within 12 and 24 h, respectively. Furthermore, no upregulation of MPO mRNA in a mouse and human microglial cell line was detected upon LPS treatment (data not shown). Nevertheless, a chlorinative attack due to MPO activation in close vicinity to the blood-brain barrier might disrupt it, subsequently leading to an increase in paracellular permeability of harmful substances, thereby participating in the progression of neuroinflammation.

Mice injected with LPS showed pathophysiological symptoms of LPS induction, such as diarrhea, fever, shivering and social withdrawal and these mice refused to eat and drink during the acute phase of inflammation. These changes in feeding behavior could be explained by differential expression of genes known to regulate food intake and satiety. Surprisingly, we have observed decreased transcript levels of dipeptidylpeptidase (Dpp) 6 and 8 (0.189 and 0.539, respectively) at 3 h post LPS injection. Dpps are serine proteases which are known to proteolytically cleave regulatory factors including chemokines, growth factors and neuroendocrine hormones regulating satiety such as GIP and GLP-1 (Kieffer, McIntosh et al. 1995; Conarello, Li et al. 2003; Yazbeck, Howarth et al. 2009; Calabrese 2011). We also observed a significant body weight loss after LPS treatment. The reason for this reduction might be increased water excretion and an increased metabolic flux, both of which are reported in cases of systemic inflammation (Liang, Liu et al. 2005).

Microarray analysis

Next we explored transcriptional changes occurring in the brain due to neuroinflammation. Here we identified the overall transcriptional changes under neuroinflammatory conditions at two distinct time points (3 h and 48 h) allowing us to compare temporal effects of neuroinflammation. Previous studies have shown the transcriptional changes upon systemic (i.p.) (Godbout, Chen et al. 2005; Mastronardi, Whelan et al. 2007) and central (lateral ventricle) (Lund, Christensen et al. 2006; Bonow, Aid et al. 2009) LPS injection. Godbout *et al.* reported the distinct effects in aged mice and Mastronardi *et al.* reported effects upon induction of a mild transient inflammation by injecting a low dose of LPS (0.33 mg/kg). The present study focused on the effects of a rather severe neuroinflammation on lipid metabolism, barrier function and inflammatory gene expression in the brain. These analyses revealed that 1,218 transcripts at 3 h and 504 transcripts at 48 h were regulated.

Several pathways were regulated post LPS treatment. Here I discuss the 4 most affected pathways (Figure 21):

1. VEGF signaling: The top common pathway affected in brains of LPS treated mice at 3 h as well as 48 h after treatment is the vascular endothelial growth factor (VEGF) signaling pathway via vascular endothelial growth factor receptor 2 (VEGFR2). VEGF plays a key role in the CNS. In higher organisms, VEGF is involved in the vascularization of the neural tube, which develops into brain and spinal cord. During development of the cerebellum, VEGF is expressed by astrocytes and Purkinje cells while VEGFR's are expressed by endothelial cells. VEGF is also involved in controlling cell migration in the CNS, like the migration of facial motor neurons in the developing hindbrain. It is not only important for the developing, but also for the adult CNS. VEGF stimulates endothelial cells, which release factors that induce differentiation of neuronal progenitors. Thereby, VEGF indirectly promotes neuronal cell proliferation. Furthermore, VEGF is suggested to be involved in synaptic plasticity. In cultured hippocampal neurons it increases protein synthesis in a long-lasting manner. It also has a neuroprotective role on postmitotic neuronal cell types (cortical, dopaminergic, hippocampal, cerebellar and retinal neurons) and induces proliferation of microglia through VEGFR-1. VEGF is also involved in neurodegenerative diseases. In amyotrophic lateral sclerosis, reduced VEGF triggers the degeneration of motor neurons. In patients suffering from Alzheimer's disease an increase of VEGF in plasma and CSF was found. In Parkinson's disease VEGF has a neuroprotective effect on dopaminergic neurons (Ruiz de Almodovar, Lambrechts et al. 2009).
2. WNT signaling: The WNT signaling pathway was found to be the second most affected pathway. WNT signaling in microglia is associated with a proinflammatory morphogenic transformation under pathogenic conditions (Halleskog, Mulder et al. 2011). Furthermore, WNT signaling is supposed to have a neuroprotective role. Wnt/ β -catenin signaling is inhibited by amyloid- β peptide, which is one of the major inducers of neurotoxicity in AD (Inestrosa and Toledo 2008).
3. Retinoid signaling: Retinoids are regulators of gene transcription in several cells and tissues. They bind to nuclear retinoid receptor proteins, which act as transcription factors. Retinoic receptor proteins were found to be expressed in

the developing brain as well as the adult brain, although the distribution was different. Therefore, it is likely that retinoid signaling has a physiological role in the adult hippocampus, hypothalamus, cortex, amygdala and striatum. Several neuronal specific genes are directly regulated by retinoids. In a rodent model, the disruption of the retinoid signaling pathway resulted in a deregulation of synaptic plasticity and associated memory and learning behaviors. Furthermore, evidence shows a link between retinoid signaling pathways and AD, schizophrenia and depression (Lane and Bailey 2005).

4. ASK1 signaling: Apoptosis signal-regulating kinase 1 (ASK1) plays important roles in stress and immune responses. It is required for LPS-induced activation of p38, which is a crucial determinant for the production of pro-inflammatory cytokines via TLR4 in endotoxemia (Noh, Park et al. 2011). Guo *et al.* showed that ASK1 deficiency attenuates neuroinflammation in a model of EAE (Guo, Harada et al. 2010).

The 4 most affected process networks commonly regulated post LPS treatment described in the results are discussed here:

1. Neurogenesis: Neurogenesis was the most affected network. There is evidence, that LPS induced neuroinflammation leads to a decreased proliferation, survival and differentiation of hippocampal progenitor cells (Russo, Amornphimoltham et al. 2011). Furthermore, an upregulation of cell cycle proteins occurs in mitotic as well as postmitotic neural cells after a central nervous system injury. In the case of microglia and astrocytes, this leads to induced proliferation whereas in postmitotic neurons it initiates apoptosis (Cernak, Stoica et al. 2005).
2. Synaptogenesis: Synaptogenesis, the process in which new synapses are formed between neurons is impaired in LPS treated mice. Glutamate has been shown to participate in regulating synaptogenesis, neurogenesis, neurite outgrowth and neuron survival in the developing and adult nervous system (Mattson, Lee et al. 1988). In our study, the expressions of receptors and transporters involved in glutamate signaling (SLC1A3, GRIK4, GRM7 and GRIND2D) are downregulated.

3. Protein folding: It is known that in neurodegenerative diseases like AD, PD or HD accumulation and aggregation of misfolded proteins play an important role in the progress of the disease. The presence of misfolded proteins leads to cellular responses, including an ER stress response, which aims in protecting the cells against a build-up of misfolded proteins. Furthermore, the ER response also aims in reducing protein synthesis and translation, and in augmenting protein-folding capacity (Rao and Bredesen 2004).
4. Cadherins: Several types of cadherins are expressed in the CNS. They play a key role in development, like proliferation, morphogenesis, neurite outgrowth and synaptogenesis. N-cadherin (CDH2) is important for cell-cell interaction as well as adhesion in the development of the CNS. It promotes neuronal survival and synaptic plasticity (Suzuki, Suzuki et al. 2004). β -catenin connects cadherins with the actin cytoskeleton, modulates cadherin-based adhesion and regulates neuronal differentiation and synaptic structure (Yu and Malenka 2003; Otero, Fu et al. 2004; Chen, Zacharek et al. 2006). In our microarray study, the cadherin-associated protein δ -catenin was regulated (CTNND1). δ -catenin is known to stabilize receptors, like the GluR2-containing AMPA receptor at the surface of the synapse (Arikath and Reichardt 2008).

Next, the three most affected pathways will be discussed:

1. HMGB1/TLR signaling: We found that the HMGB1/TLR-signaling pathway was predominantly affected in LPS treated mice at 3 h post treatment. It is well known, that TLRs recognize a wide array of pathogen-associated molecular patterns, including the endotoxin LPS. The microarray data showed that LPS leads to a significant upregulation of TLR2, which is expressed in microglia in response to systemic and peripheral LPS treatment (Yang, Mark et al. 1998).
2. Interleukin-1 signaling: The IL-1 pathway was affected in LPS treated mice. IL1 α and IL1 β are potent proinflammatory cytokines that are expressed at low levels in the healthy CNS and are induced rapidly in response to local or peripheral insults. The consequences include an augmentation of T-cell responses to mitogens and increased expression of vascular adhesion molecules which could further enhance diapedesis of circulating inflammatory

cells across the BBB. It has been shown, that elevation of IL-1 is involved in numerous neurodegenerative diseases (Shafteel, Griffin et al. 2008).

3. Oxidative stress signaling: Next, we found that the oxidative stress pathway was affected in LPS treated mice. Oxidative stress occurs in the tissue, when the antioxidant mechanisms are overwhelmed by reactive oxygen species (ROS). This happens, if the innate immune system, comprising of neutrophils, dendritic cells, macrophages and microglia, is activated, thereby producing ROS which are potentially toxic to invading organisms, but also harmful to “self”. ROS include singlet oxygen ($^1\text{O}_2$), superoxide (O_2^-), hydroxyl radical ($\text{OH}^\cdot$), peroxy radical ($\text{ROO}^\cdot$) and hydrogen peroxide (H_2O_2), as well as reactive nitrogen species and reactive chlorine species. The source of ROS can be the NADPH oxidase complex, mitochondrial respiratory chain or an uncontrolled arachidonic acid cascade. Oxidative stress, as a major cause of neuronal injury, is implicated in a wide variety of neurological diseases, including PD and AD (Mosley, Benner et al. 2006).
4. During the microarray analyses performed during the present study thioredoxin (TRX) and related genes were regulated. TRX, together with related molecules maintain a reducing environment in the cell, by catalyzing the reduction of protein disulfide bonds, thereby having protective effects against oxidative stress. It was shown that thioredoxins are upregulated in the brain in response to oxidative stress (Patenaude, Murthy et al. 2005). In the microarray data we saw a downregulation of the cytosolic thioredoxin reductase 1 (TXNRD1). It was shown that NS specific TXNRD1 null mice are smaller than their wildtype littermates and show ataxia and tremor. Furthermore, they show a patterned cerebellar hypoplasia, which resulted from decreased proliferation of granule cell precursors within the EGL (Soerensen, Jakupoglu et al. 2008). A selective thioredoxin reductase inhibitor, 1-chloro-2,4-dinitrobenzene (CDNB) causes apoptosis in neuronal cells due to early increase of ROS and a rapid activation of stress-inducible kinases (Seyfried and Wullner 2007).
5. Furthermore, glycogen synthase kinase 3 (GSK3), a kinase which is particularly abundant in the brain is downregulated in these mice at both time points. GSK3 participates in several signaling pathways, such as insulin or wnt. An increased level of GSK3 was reported to be linked with HD, AD and

schizophrenia. With regard to GSK3 and neurodegeneration, elevated GSK3 activity has been shown to result in neuronal apoptosis as well as GSK3 inhibitors have been reported to exert antiapoptotic and neuroprotective effects in various cell and mouse models (Fuster-Matanzo, Llorens-Martin et al. 2011).

Next the process networks defined via the KEGG pathway, found to be regulated in the LPS mouse brain microarray study will be discussed:

Blood-brain barrier: The blood-brain barrier protects the brain from harmful substances by restricting paracellular diffusion. This is mediated by a combination of membrane transport systems and tight junctions (Hawkins and Davis 2005; Loscher and Potschka 2005). In our microarray study several genes involved in the formation of junctional complexes were downregulated due to LPS treatment. These include the junctional adhesion molecules occluding and claudin 5 (CLD5), as well as par-3 partitioning defective 3 homolog (PARD3), which directly associates with the junctional adhesion molecule (JAM) and is involved in the establishment of cell polarity at the tight junctions (Ebnet, Suzuki et al. 2001). Furthermore, cortactin, a protein which is involved in regulation of interactions between proteins of adherens junctions was downregulated. Interestingly, most of these changes were encountered at the early phase (3 h) after induction of neuroinflammation. However, most of the genes in this pathway regulated at the early phase were normalized at the late phase (48 h). This implies that an acute peripheral inflammatory load rapidly translates to a defective barrier which is subject to a temporal resolution mechanism at the late phase. This is reinforced by the fact that inflammatory markers are still highly upregulated at 48 h post induction.

Sphingolipid metabolism: Sphingosine kinase 1 (SPHK1) is involved in the synthesis of sphingosine-1-phosphate (S1P). We found an upregulation in the levels of SPHK1 in the brains of LPS treated mice. It is known that LPS activates the SPHK1/S1P signaling pathway in microglia (Nayak, Huo et al. 2010), macrophages (Hammad, Crellin et al. 2008) and also in astrocytes (Fischer, Alliod et al. 2011). The activation leads to a translocation of SPHK1 from the cytoplasm to the plasma membrane, where its substrate sphingosine is converted to the bioactive sphingolipid S1P (Pitson, Moretti et al. 2003). S1P is involved in a

variety of cellular responses, including inflammation. In the CNS, S1P is involved in astrocyte proliferation, migration and survival (Miron, Schubart et al. 2008). It is known that levels of S1P are increased in the CSF of MS patients (Kulakowska, Zendzian-Piotrowska et al. 2010). Other enzymes regulated during neuroinflammatory conditions are $\delta(4)$ -desaturase sphingolipid 2 (DEGS2), which converts ceramide from dihydroceramide, and galactose-3-O-sulfotransferase 1 (GAL3ST1) which is involved in the synthesis of sulfatide and digalactosylceramidesulfate.

Glycerophospholipid metabolism: The expression of phosphatidyl-ethanolamine N-methyl-transferase (PEMT) a key enzyme involved in the biosynthesis of phosphatidylcholine was decreased in LPS treated mice. It was shown previously, that the activity of this enzyme is decreased in the frontal cortex of AD patients (Guan, Wang et al. 1999). Furthermore, 1-acylglycerol-3-phosphate O-acyltransferase 6 (AGPAT6) was downregulated in LPS treated mice. This enzyme catalyzes the conversion of lysophosphatidic acid to phosphatidic acid by transferring an acyl-residue to phosphatidylcholine to generate 1-acyl-sn-glycero-3-phosphocholine. Next, phospholipase A2 (PLA2G6), which catalyzes the release of fatty acids from phospholipids, was downregulated in LPS treated mice. It is known, that several phospholipases A2 are regulated due to inflammation. Thereby, they produce polyunsaturated fatty acids (PUFA) as well as lysophospholipids. The hydrolysis products lead to a change in membrane fluidity and structure, which may change the properties of ion channels (Shuttleworth 1996) as well as the responses mediated through G protein-coupled receptors (Kiselyov and Muallem 1999). Furthermore, it was shown recently that expression of PLA2 is increased in LPS stimulated astrocytes *in vitro* (Strokin, Sergeeva et al. 2011). Other enzymes in the glycerophospholipid pathway regulated during LPS treatment in mouse brains were diacylglycerol kinase zeta (DGKZ), which generates glycerol-3-phosphate from diacylglycerol, phosphatidylserine decarboxylase (PISD), which converts phosphatidylserine to phosphatidylethanolamine and mitochondrial glycerol-3-phosphate acyltransferase (GPAM), which converts glycerol-3-phosphate to acyl-glycerol-3-phosphate. The pathophysiological impact these changes have on inflammatory and post inflammatory mechanisms needs to be evaluated.

Plasmalogen metabolism: Plasmalogens, which are major constituents of the neural tissue, are essential for a physiologic function of the CNS. Studies using ether lipid deficient mice as a model for Zellweger syndrome showed severe phenotypic alterations in CNS myelination (Gorgas, Teigler et al. 2006). Furthermore, it has been shown that plasmalogens are susceptible to modification by MPO-derived HOCl (Ullen, Fauler et al. 2010). In our study, the expression of platelet-activating factor acetylhydrolase 1b, regulatory subunit 1 (PAFAH1B1), which is responsible for the generation of 1-alkyl-sn-glycero-3-phosphocholine was decreased. It was shown that an *in vitro* knock down of PAFAH1B1 inhibits endocytic membrane trafficking and the formation of membrane tubules and delays the process of transferrin recycling (Bechler, Doody et al. 2011). The enzyme PAFAH1B1 has crucial roles in the localization of mitotic spindle machinery via direct regulation of the dynein cargo-binding and motor activities (Tai, Dujardin et al. 2002). The protein is also known to play an important role in regulating downstream targets of Wnt signaling via mediating the recruitment of an unknown ubiquitin ligase to phosphorylate β -catenin, thus influencing self-renewal and pluripotency (Wnt Signaling Pathway and Pluripotency, KEGG pathways). Interestingly, we also find in our microarray analysis Wnt signaling pathway as the second most affected pathway at both time points involved in neuronal development and self-renewal.

Upregulation of cholesterol-25 hydroxylase

Microarray analyses revealed a potent, 45-fold upregulation of CH25H in brains of mice suffering from neuroinflammation. Recently, CH25H and its regulation came to limelight, due to findings that bone marrow derived mouse macrophages treated with LPS showed transcriptional upregulation of CH25H. This was shown to be dependent on TLR signaling, but independent of myeloid differentiation protein 88 (MyD88) signaling. Furthermore, this group demonstrated that healthy volunteers which were intravenously injected with LPS showed an increase in plasma 25-HC concentration (Diczfalusy, Olofsson et al. 2009). However, regulatory properties of CH25H in the brain are enigmatic. We found a time- and concentration dependent upregulation of CH25H post i.p. LPS injection in brains and livers of mice (Figure 28). This elevation was paralleled by an upregulation of the proinflammatory marker TNF α .

Li-Hawkins *et al.* investigated the effects of 7 α hydroxylase (CYP7B1) knockdown in mice, an enzyme which metabolizes 25-HC and 27-HC in the liver to synthesize bile acids. Although, a lack of this enzyme led to an increase of 25-HC in the plasma, no alterations on inflammatory pathways were reported. Furthermore, no changes in overall plasma cholesterol and triglyceride levels were found (Li-Hawkins, Lund *et al.* 2000). However, several other studies have shown an increase in proinflammatory cytokine release due to treatment with 25-HC in several cellular systems (Liu, Hulten *et al.* 1997; Bai, Yamamoto *et al.* 2005; Lemaire-Ewing, Prunet *et al.* 2005; Prunet, Montange *et al.* 2006; Joffre, Leclerc *et al.* 2007; Lemaire-Ewing, Berthier *et al.* 2009). In recent years, Bauman *et al.* reported an inhibition of B cell proliferation and class switch recombination to immunoglobulin A (IgA) by 25-HC *in vitro*. Furthermore, *in vivo* findings showed increased levels of IgA in the plasma of CH25H knockout mice (Bauman, Bitmansour *et al.* 2009).

In this study, I show that protein expression of CH25H increases in brains of mice post LPS treatment, but at the same time slightly decreases in the livers of these mice (Figure 30). Furthermore, preliminary experiments suggest that protein expression is equal in distinct brain regions (Figure 32). It might be speculated that processes involved in the resolution of inflammation may have direct or indirect effects on CH25H transcripts as well as protein levels. Several posttranscriptional mechanisms might be involved, as has been shown for HuR, an RNA-binding protein which selectively binds AREs (AU-rich elements) and stabilizes ARE-containing mRNAs (Brennan and Steitz 2001). The primary sites of inflammation in our model are systemic, and hence we expect that resolution mechanisms initiate earlier in systemic organs such as the liver. Central inflammation ensues thereafter and hence we expect a longer persistence of inflammation. This also implies that attenuation in protein expression of CH25H seen at 48 h in the liver might be a reflection of resolution mechanisms whereas the persistence of its protein expression in the brain, at the same time point, could be due to secondary/late central effects.

Additionally to an increase of CH25H mRNA and protein levels the enzymatic end product 25-HC was increased (Figure 31). This is in line with previous reports, where 25-HC levels in the lungs and serum of mice as well as in the plasma of healthy volunteers increased upon injection of an TLR4 agonist (Bauman, Bitmansour *et al.*

2009; Diczfalusy, Olofsson et al. 2009). These findings taken together implicate a role for 25-HC in various organs during inflammation.

Impact of 25-hydroxycholesterol on brain capillary endothelial cells

In the present study I used an *in vitro* model of the BBB to evaluate the effects of 25-HC on cerebrovascular permeability and cell integrity. These experiments revealed that permeability and cell integrity decreased in a time-dependent manner. A comparison with the end product of cholesterol elimination in the brain, 24-HC demonstrated that both oxysterols affect permeability and cell integrity of pBMVECs to a similar extent (Figure 35). To rule out the possibility of a cytotoxic effect of 25-HC, we investigated the tolerance of pBMVEC for 25-HC by measuring cell survival at different time points and concentrations (Figure 34). Only at the longest incubation time (24 h) and concentration ≥ 100 μ M 25-HC a cytotoxic effect on pBMVEC was observed. The average concentration of 25-HC is assumed to be at least 10-fold lower, hence, we speculate that 25-HC does not affect BBB integrity at *in vivo* concentrations.

Impact of 25-HC on cholesterol and lipid metabolism

In the CNS cholesterol plays a central role as it is the major architectural component of compact myelin. There is solid evidence, that the majority if not all cholesterol required in the brain is produced *in situ* rather than imported from the periphery. The reason might be that lipoproteins, the cholesterol shuttles, cannot pass the blood-brain barrier (Pfrieger 2003). Therefore, cholesterol metabolism has to be tightly regulated. Several groups reported that oxysterols act as ligands for LXR, thereby controlling cholesterol biosynthesis. To study the influence of oxysterols on LXR signaling, Chen *et al.* generated triple-knockout mice deficient in CYP46, CH25H and CYP27. They reported a reduction of endogenous levels of the respective oxysterols to below detection limits. LXR responses in these mice were altered, with LPL, ABCG5 and ABCG8 being affected, SREBP-1c being impaired and CYP7A1 being unaffected in the mouse liver. However, no phenotypic data in the context of neuroinflammation are available in these mice (Chen, Chen et al. 2007). Janowski *et al.* reported an intermediate ability of 25-HC as well as 27-HC to activate LXR α *in vitro*. 24-HC was also shown to be a more potent activator (Janowski, Willy et al. 1996). Furthermore, it was reported that cholesterol interacts with SCAP directly by

inducing it to bind to INSIGs, whereas 25-HC works indirectly through a putative 25-HC sensor protein that elicits SCAP-INSIG binding (Adams, Reitz et al. 2004).

We found that neuroinflammation mediated a strong upregulation of the transcript and protein level of 25-HC. Furthermore, we found a transcriptional upregulation of the cholesterol metabolic genes ABCA1 and ApoE in mouse brains post LPS treatment (Figure 33). We therefore investigated the downstream effects of 25-HC on LXR mediated transcriptional regulation, SREBP2 maturation and cholesterol metabolism. Most of the cholesterol biosynthesis in the mature healthy mammalian brain occurs in astrocytes although data on the site of brain cholesterol biosynthesis under patho-physiological conditions are limited. We therefore employed a murine neuronal cell line CATH.a and a human astrocytoma cell line CCF-STTG1 in an *in vitro* study to investigate the effects of 25-HC on cholesterol metabolism. Transcript levels of well-known LXR target genes in neuronal as well as astrocyte cell lines were co-ordinately regulated leading to increased transcripts of cholesterol dissipating pathways and reduced transcripts of cholesterol biosynthetic pathways. Consistent with previous reports in other tissues, we also found that in the neuronal cell line CATH.a, 25-HC led to reduced maturation of the SREBP2 protein (Adams, Reitz et al. 2004; Radhakrishnan, Ikeda et al. 2007). Adams *et al.* speculate that the yet unknown 25-HC sensor protein could be the oxysterol binding protein 1 (OSBP1) which has a potent 25-HC binding ability. OSBP overexpression and silencing influence neither cholesterol metabolism nor the ability of 25-HC to inhibit SREBP2 processing (Adams, Reitz et al. 2004; Nhek, Ngo et al. 2010). Interestingly, in the presence of 25-HC Golgi trafficking of OSBP is enhanced since it was shown to be phosphorylated via protein kinase D (PKD) in the presence of oxysterols or depletion of cholesterol, both of which indicate a low sterol status. This occurs via phosphorylation and inactivation of the pleckstrin homology (PH) domain of OSBP, retaining it in the ER. Other accessory proteins which influence ceramide export to the Golgi and hence reduce sphingomyelin (SM) and diacylglycerol synthesis such as the ceramide transfer protein (CERT) are also susceptible to this regulation and their respective Golgi import is inhibited upon 25-HC treatment *in vitro* (Nhek, Ngo et al. 2010). 25-HC might mediate a regulatory cross-talk influencing other lipid metabolic pathways apart from cholesterol biosynthesis. All these lipid metabolic pathways and their regulation are central for normal neuronal physiology where 25-HC seems to play a poorly explored yet crucial role.

6 ABBREVIATIONS

24-HC	24-hydroxycholesterol
25-HC	25-hydroxycholesterol
AA	arachidonic acid
ABCA1	ATP-binding cassette sub-family A member 1
ACN	acetonitrile
AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
Apo	apolipoprotein
APS	ammonium persulfate
ASK1	Apoptosis signal-regulating kinase 1
ATP	adenosine triphosphate
A β	Amyloid beta
BBB	blood-brain barrier
BC	brain capillaries
BMVEC	brain microvascular endothelial cells
BSA	bovine serum albumin
cAMP	cyclic adenosine monophosphate
CD	Cluster of differentiation
CH25H	Cholesterol-25 hydroxylase
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
CNS	central nervous system
COX2	cyclooxygenase 2
CPM	Counts per minute
CSF	cerebrospinal fluid
CSR	Class switch recombination
CTX	Cerebrotendinous xanthomatosis
Da	Dalton
DAG	Diacylglycerol
dNTP	desoxyribonucleosidetriphosphate
DTT	dithiothreitol
EB	equilibration buffer
ECL	enhanced chemiluminescence
ECM	extracellular matrix
EDTA	ethylene diamine tetraacetic acid
ER	Endoplasmic reticulum
ESI	electrospray ionization
EtOH	ethanol
FASN	fatty acid synthase
FC	Fold change
FDA	food and drug administration
FDFT1	farnesyl-diphosphate farnesyltransferase 1
GABA	γ -amino butyric acid
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GFAP	glial fibrillary acidic protein
GIT	gastrointestinal tract

GLUT1	glucose transporter 1
GPCR	G-protein coupled receptor
GTPase	guanidine triphosphatase
H ₂ O ₂	hydrogen peroxide
HAS	human serum albumin
HC	hydrocortisone
HD	Huntington's disease
HDL	high density lipoprotein
HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase
HMGCS	3-hydroxy-3-methylglutaryl-CoA synthase 1
HPLC	high performance liquid chromatography
HRP	horse radish peroxidase
i.p.	intraperitoneal
IFN	interferon
IgA	Immunoglobulin A
IL-1 β	interleukin 1 β
iNOS	inducible nitric oxide synthase (NOS2)
INSIG2	insulin induced gene 2
JAM	junctional adhesion molecule
kDa	kilodalton
LDL	low density lipoprotein
L-DOPA	L-3,4-Dihydroxyphenylalanine
LPDS	lipoprotein deficient serum
LPL	lipoprotein lipase
LPS	lipopolysaccharide
LSM	laser scanning microscope
LXR	Liver X receptor
MAb	monoclonal antibody
MAC	myelin associated glycoprotein
MALDI	matrix-assisted laser desorption ionization
MHC	major histocompatibility complex
MPO	myeloperoxidase
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MS	Multiple sclerosis
MTT	3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide
MW	molecular weight
NADPH	nicotinamide adenine dinucleotide phosphate
Net	neutrophil extracellular traps
NO	nitric oxide
NOO ⁻	peroxynitrite
NOS2	nitric oxide synthase 2
NP-40	non-ionic detergent for protein extraction
NSAID	nonsteroidal anti-inflammatory drug
O ₂ ⁻	superoxide
ON	over night
P/S	Penicillin/Streptomycin
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PD	Parkinson's disease
PECAM1	platelet endothelial cellular adhesion molecule 1

PGE2	prostaglandin E 2
PKC	protein kinase C
PL	phospholipid
PMN	polymorphonuclear neutrophils
PMSF	phenylmethanesulfonyl fluoride
PNS	peripheral nervous system
PVDF	polyvinylidene difluoride
qPCR	quantitative polymerase chain reaction
RIPA	radioimmunoprecipitation assay
ROS	reactive oxygen species
RPM	rotations per minute
RT	room temperature
RTK	receptor tyrosine kinase
S1P	sphingosine 1 phosphate
SCAP	SREBP-cleavage-activating protein
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SHH	sonic hedgehog
SM	sphingomyelin
SNpc	substantia nigra pars compacta
SR-B1	scavenger receptor class B, type 1
SREBP	sterol regulatory binding protein
TAE	tris-acetate-EDTA
TBP1	tata binding protein 1
TBS	tris-buffered saline
TCA	trichloroacetic acid
TGF	tumour growth factor
TJ	tight junction
TNF α	tumor necrosis factor- α
TRIS	tris(hydroxymethyl)-aminomethan
VE-cadherin	vascular endothelial cadherin
VEGF	vascular endothelial growth factor
VEGFR2	vascular endothelial growth factor receptor 2
VIP	vasoactive intestinal peptide
VLDL	very low density lipoprotein
WNT	wingless
ZMF	Zentrum für medizinische Forschung, Meduni Graz
ZO	zonula occludens protein

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APPENDIX

Appendix I: Whole list of genes regulated at 3 h post LPS treatment in mouse brains.

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
0.000	517.40	Cxcl10	NM_021274.1	chemokine (C-X-C motif) ligand 10
0.000	264.00	Lcn2	NM_008491.1	lipocalin 2
0.000	146.20	Cxcl2	NM_009140.1	chemokine (C-X-C motif) ligand 2
0.003	118.80	Ccl2	NM_011333.1	chemokine (C-C motif) ligand 2
0.001	105.30	Rsad2	NM_021384.2	radical S-adenosyl methionine domain containing 2
0.001	98.24	Ccl4	NM_013652.1	chemokine (C-C motif) ligand 4
0.001	77.22	Cxcl1	NM_008176.1	chemokine (C-X-C motif) ligand 1
0.010	75.39	Ccl7	NM_013654.2	chemokine (C-C motif) ligand 7
0.000	66.20	Cxcl9	NM_008599.1	chemokine (C-X-C motif) ligand 9
0.004	54.56	Ccl12	NM_011331.1	chemokine (C-C motif) ligand 12
0.001	53.42	Oasl1	NM_145209.2	2'-5' oligoadenylate synthetase-like 1
0.000	48.61	Ifit1	NM_008331.1	interferon-induced protein with tetratricopeptide repeats 1
0.000	48.01	Cxcl11	NM_019494.1	chemokine (C-X-C motif) ligand 11
0.002	47.13	Csf3	NM_009971.1	colony stimulating factor 3 (granulocyte)
0.000	44.61	Ch25h	NM_009890.1	cholesterol 25-hydroxylase
0.006	41.18	Ccl5	NM_013653.1	chemokine (C-C motif) ligand 5
0.000	40.49	Cd274	NM_021893.2	CD274 antigen
0.005	36.94	Saa3	NM_011315.2	serum amyloid A 3
0.003	35.72	Ccl3	null	chemokine (C-C motif) ligand 3
0.000	35.24	Parp14	null	poly (ADP-ribose) polymerase family, member 14
0.001	31.12	Ifi47	NM_008330.1	interferon gamma inducible protein 47
0.001	30.69	Serpina3g	null	serine (or cysteine) peptidase inhibitor, clade A, member 3G
0.001	28.84	Gbp2	NM_010260.1	guanylate nucleotide binding protein 2
0.005	25.66	Cd14	NM_009841.2	CD14 antigen
0.002	25.60	Il6	NM_031168.1	interleukin 6
0.008	24.86	Timp1	null	tissue inhibitor of metalloproteinase 1
0.014	24.28	Ilgp1	NM_021792.3	interferon inducible GTPase 1
0.000	23.92	Ccl2	NM_017466.3	chemokine (C-C motif) receptor-like 2
0.000	23.65	Socs3	NM_007707.2	suppressor of cytokine signaling 3
0.002	23.48	Tgtp	NM_011579.2	T-cell specific GTPase
0.006	22.04	Il1a	NM_010554.3	interleukin 1 alpha
0.016	22.00	Birc3	NM_007464.1	baculoviral IAP repeat-containing 3
0.006	21.64	Il1b	NM_008361.2	interleukin 1 beta
0.000	21.47	Saa3	null	serum amyloid A 3
0.000	20.32	Gpr84	NM_030720.1	G protein-coupled receptor 84
0.032	18.97	Casp11	NM_007609.1	caspase 11, apoptosis-related cysteine peptidase
0.004	17.68	Zfp36	NM_011756.3	zinc finger protein 36
0.019	17.19	Tlr2	NM_011905.2	toll-like receptor 2
0.027	16.18	Nfkbiz	NM_030612.1	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta
0.002	16.09	Mx2	NM_013606.1	myxovirus (influenza virus) resistance 2
0.000	16.08	Irg1	null	immuno-responsive gene 1
0.019	15.44	Ifi44	null	interferon-induced protein 44
0.000	14.57	Maff	NM_010755.2	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein F (avian)

P	FC	Gene Name	Genbank	Description
0.002	13.18	Isg20	NM_020583.4	interferon-stimulated protein
0.004	12.71	Gbp1	null	guanylate nucleotide binding protein 1
0.004	11.83	Zbp1	null	Z-DNA binding protein 1
0.002	11.44	Gbp4	NM_018734.2	guanylate nucleotide binding protein 4
0.001	11.42	Atf3	NM_007498.2	activating transcription factor 3
0.004	11.36	Usp18	NM_011909.1	ubiquitin specific peptidase 18
0.009	11.29	Map3k8	NM_007746.1	mitogen activated protein kinase kinase kinase 8
0.003	10.36	Sele	NM_011345.1	selectin, endothelial cell
0.047	10.32	Trim30	NM_009099.1	tripartite motif protein 30
0.014	10.24	Trim30	null	tripartite motif protein 30
0.002	10.21	Irgm	NM_008326.1	immunity-related GTPase family, M
0.008	9.94	Tnfaip3	NM_009397.2	tumor necrosis factor, alpha-induced protein 3
0.001	9.92	Ptgs2	null	prostaglandin-endoperoxide synthase 2
0.008	9.81	Fas	NM_007987.1	Fas (TNF receptor superfamily member)
0.000	9.76	Gvin1	NM_029000.2	GTPase, very large interferon inducible 1
0.000	9.70	Ifit2	NM_008332.2	interferon-induced protein with tetratricopeptide repeats 2
0.011	9.69	Ptx3	null	pentraxin related gene
0.009	9.35	Samsn1	NM_023380.1	SAM domain, SH3 domain and nuclear localisation signals, 1
0.005	9.34	Tnf	NM_013693.1	tumor necrosis factor
0.000	9.27	Cebpd	NM_007679.2	CCAAT/enhancer binding protein (C/EBP), delta
0.002	9.24	Gm1075	null	gene model 1075, (NCBI)
0.019	8.97	Stx11	null	syntaxin 11
0.001	8.94	Serpine1	NM_008871.1	serine (or cysteine) peptidase inhibitor, clade E, member 1
0.000	8.93	G1p2	NM_015783.1	interferon, alpha-inducible protein
0.011	8.51	Bcl2a1c d a	NM_009742.2	B-cell leukemia/lymphoma 2 related protein A1c A1d A1a
0.001	8.40	Zc3h12a	NM_153159.1	zinc finger CCCH type containing 12A
0.003	8.37	Ifit3	NM_00100585.1	interferon-induced protein with tetratricopeptide repeats 3
0.013	8.28	Ifi204	NM_008329.1	interferon activated gene 204
0.000	8.19	Ccl19	NM_011888.2	chemokine (C-C motif) ligand 19
0.003	8.10	S3-12	NM_020568.1	plasma membrane associated protein, S3-12
0.020	7.84	Ilgp2	NM_019440.2	interferon inducible GTPase 2
0.014	7.65	Slc10a6	NM_029415.1	solute carrier family 10 (sodium/bile acid cotransporter family), member 6
0.001	7.45	Irf1	NM_008390.1	interferon regulatory factor 1
0.039	7.26	Samd9l	NM_177590.1	sterile alpha motif domain containing 9-like
0.014	7.21	Bcl3	NM_033601.1	B-cell leukemia/lymphoma 3
0.028	7.15	Gp49a Lilrb4	NM_013532.1	glycoprotein 49 A leukocyte immunoglobulin-like receptor, subfamily B, member 4
0.007	7.14	Tnfsf10	null	tumor necrosis factor (ligand) superfamily, member 10
0.014	7.11	Tubb6	NM_026473.2	tubulin, beta 6
0.034	6.99	Ms4a6d	NM_026835.1	membrane-spanning 4-domains, subfamily A, member 6D
0.008	6.89	Bcl2a1b d	NM_007534.1	B-cell leukemia/lymphoma 2 related protein A1b A1d
0.034	6.77	Ccl11	NM_011330.1	small chemokine (C-C motif) ligand 11
0.003	6.74	Apobec3	null	apolipoprotein B editing complex 3
0.024	6.73	Ifi204	null	interferon activated gene 204
0.004	6.70	Steap4	NM_054098.2	STEAP family member 4
0.036	6.68	S100a8	NM_013650.1	S100 calcium binding protein A8 (calgranulin A)
0.010	6.62	Mnda Ifi205	NM_172648.2	myeloid cell nuclear differentiation

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
				antigen interferon activated gene 205
0.005	6.58	Slfn4	NM_011410.1	schlafen 4
0.010	6.49	Mx1	null	myxovirus (influenza virus) resistance 1
0.000	6.36	Ier3	NM_133662.1	immediate early response 3
				nuclear factor of kappa light chain gene enhancer
0.000	6.24	Nfkbia	null	in B-cells inhibitor, alpha
				a disintegrin-like and metallopeptidase with
0.007	6.19	Adamts1	NM_009621.3	thrombospondin type 1 motif, 1
0.027	5.93	C5r1	NM_007577.2	complement component 5, receptor 1
0.020	5.90	Oasl2	NM_011854.1	2'-5' oligoadenylate synthetase-like 2
0.001	5.83	Akap12	NM_031185.1	A kinase (PRKA) anchor protein (gravin) 12
				membrane-spanning 4-domains, subfamily A,
0.009	5.77	Ms4a6c	NM_028595.1	member 6C
				nuclear factor of kappa light polypeptide gene
0.000	5.76	Nfkb2	NM_019408.1	enhancer in B-cells 2, p49/p100
0.000	5.73	Plscr1	NM_011636.1	phospholipid scramblase 1
0.000	5.67	Igtp	NM_018738.2	interferon gamma induced GTPase
0.007	5.63	Ifi203	null	interferon activated gene 203
0.048	5.44	Stno	NM_183426.1	strawberry notch homolog (Drosophila)
0.001	5.33	Ddx58	null	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58
0.000	5.18	Pglyrp1	NM_009402.1	peptidoglycan recognition protein 1
0.022	5.02	Emp1	NM_010128.3	epithelial membrane protein 1
0.002	4.95	Gch1	NM_008102.2	GTP cyclohydrolase 1
0.004	4.66	Tyki	NM_020557.3	thymidylate kinase family LPS-inducible member
0.001	4.66	Robo4	NM_028783.2	roundabout homolog 4 (Drosophila)
0.045	4.60	Il15	NM_008357.1	interleukin 15
0.045	4.47	Irf7	NM_016850.1	interferon regulatory factor 7
0.010	4.45	Ddx58	null	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58
				phosphatidylinositol 3-kinase, C2 domain
0.030	4.38	Pik3c2a	NM_011083.1	containing, alpha polypeptide
0.008	4.35	Lgals9	null	lectin, galactose binding, soluble 9
0.025	4.35	Cd40	NM_170703.1	CD40 antigen
0.021	4.01	Asah3l	NM_139306.1	N-acylsphingosine amidohydrolase 3-like
0.007	4.00	Cyr61	NM_010516.1	cysteine rich protein 61
0.001	4.00	Sp100	NM_013673.1	nuclear antigen Sp100
				proteasome (prosome) subunit, beta type 8 (large
0.013	3.96	Psmb8	NM_010724.1	multifunctional peptidase 7)
0.002	3.87	T2bp	NM_145133.2	Traf2 binding protein
				transporter 1, ATP-binding cassette, sub-family B
0.010	3.87	Tap1	NM_013683.1	(MDR/TAP)
0.001	3.69	Errfi1	NM_133753.1	ERBB receptor feedback inhibitor 1
0.036	3.65	Fos	NM_010234.2	FBJ osteosarcoma oncogene
0.004	3.62	Fkbp5	NM_010220.2	FK506 binding protein 5
0.018	3.61	Ctla2b	null	cytotoxic T lymphocyte-associated protein 2 beta
0.034	3.60	Vipr2	NM_009511.1	vasoactive intestinal peptide receptor 2
0.025	3.58	Plscr2	NM_008880.2	phospholipid scramblase 2
0.004	3.55	Ifih1	NM_027835.1	interferon induced with helicase C domain 1
0.004	3.51	Timeless	NM_011589.1	timeless homolog (Drosophila)
0.015	3.50	Apobec3	NM_030255.1	apolipoprotein B editing complex 3
0.002	3.48	Mt1	NM_013602.2	metallothionein 1
0.005	3.46	Cp	NM_007752.1	ceruloplasmin
0.004	3.45	Il2rg	NM_013563.2	interleukin 2 receptor, gamma chain
0.000	3.37	Sgk	NM_011361.1	serum/glucocorticoid regulated kinase
0.002	3.37	Ifitm3	NM_025378.1	interferon induced transmembrane protein 3
0.007	3.37	Tlr3	NM_126166.2	toll-like receptor 3

P	FC	Gene Name	Genbank	Description
0.009	3.35	Samhd1	NM_018851.2	SAM domain and HD domain, 1
0.008	3.30	Ccl9	NM_011338.1	chemokine (C-C motif) ligand 9
0.044	3.29	Gimap6	NM_153175.3	GTPase, IMAP family member 6
0.002	3.14	Ifitm2	NM_030694.1	interferon induced transmembrane protein 2
0.007	3.10	Tm4sf1	NM_008536.2	transmembrane 4 superfamily member 1
0.005	3.06	Trex1	NM_011637.6	three prime repair exonuclease 1
0.003	3.05	Vcam1	NM_011693.2	vascular cell adhesion molecule 1
0.000	3.05	Arrdc2	NM_027560.1	arrestin domain containing 2
0.002	3.02	Dtx3l	null	deltex 3-like (Drosophila)
0.004	3.01	Rasd1	NM_009026.1	RAS, dexamethasone-induced 1 protein phosphatase 1, regulatory (inhibitor)
0.000	3.00	Ppp1r3g	null	subunit 3G
0.001	2.99	Nmi	NM_019401.1	N-myc (and STAT) interactor
0.000	2.99	Ifitm1	NM_026820.2	interferon induced transmembrane protein 1
0.037	2.96	Cdc73	null	Vcell division cycle 73, Paf1
0.000	2.96	Cebpb	NM_009883.1	CCAAT/enhancer binding protein (C/EBP), beta
0.041	2.95	Gas5	null	growth arrest specific 5
0.009	2.93	H2-Q1	null	histocompatibility 2, Q region locus 1
0.001	2.92	Tiparp	NM_178892.3	TCDD-inducible poly(ADP-ribose) polymerase
0.001	2.90	H2-Q1	null	histocompatibility 2, Q region locus 1
0.007	2.83	Mt2	null	metallothionein 2
0.004	2.83	Pmaip1	NM_021451.1	phorbol-12-myristate-13-acetate-induced protein 1 v-maf musculoaponeurotic fibrosarcoma
0.011	2.81	Mafk	NM_010757.1	oncogene family, protein K (avian)
0.002	2.80	Prg1	NM_011157.1	proteoglycan 1, secretory granule development and differentiation enhancing factor-
0.045	2.76	Ddefl1	null	like 1
0.000	2.73	Xdh	NM_011723.1	xanthine dehydrogenase
0.000	2.73	Gadd45g	NM_011817.1	growth arrest and DNA-damage-inducible 45 gamma
0.003	2.72	Polr3e	NM_025298.2	polymerase (RNA) III (DNA directed) polypeptide E
0.006	2.71	Nfkb1	NM_008689.1	nuclear factor of kappa light chain gene enhancer in B-cells 1, p105
0.004	2.68	Tor3a	NM_023141.1	torsin family 3, member A
0.008	2.63	Fbxo39	null	F-box protein 39
0.004	2.62	Tuba6	NM_009448.2	tubulin, alpha 6
0.001	2.62	Kirrel2	NM_172898.1	kin of IRRE like 2 (Drosophila)
0.015	2.61	Btg2	NM_007570.1	B-cell translocation gene 2, anti-proliferative ankyrin repeat and SOCS box-containing protein
0.010	2.60	Asb4	NM_023048.4	4
0.018	2.60	Rnf125	null	ring finger protein 125
0.030	2.59	Txnip	NM_023719.1	thioredoxin interacting protein sulfotransferase family 1A, phenol-preferring,
0.008	2.59	Sult1a1	null	member 1
0.002	2.56	Sphk1	NM_011451.1	sphingosine kinase 1
0.005	2.55	Trim56	NM_201373.1	tripartite motif-containing 56
0.038	2.51	Slfn8 Slfn9	NM_172796.1	schlafen 8 schlafen 9
0.020	2.51	Ifi35	NM_027320.1	interferon-induced protein 35
0.014	2.48	Zfp189	NM_145547.1	zinc finger protein 189
0.003	2.45	Olfr132	NM_00100548 1.1	olfactory receptor 132
0.000	2.42	Stra6	NM_009291.1	stimulated by retinoic acid gene 6
0.010	2.42	Pkp2	NM_026163.1	plakophilin 2
0.001	2.39	Arfl4	NM_025404.1	ADP-ribosylation factor 4-like
0.013	2.38	Ier2	NM_010499.2	immediate early response 2

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
0.002	2.37	Plekhf1	NM_024413.1	pleckstrin homology domain containing, family F (with FYVE domain) member 1
0.029	2.36	Crispld2	NM_030209.1	cysteine-rich secretory protein LCCL domain containing 2
0.001	2.33	Arfl4	null	ADP-ribosylation factor 4-like
0.002	2.32	Dppa5	NM_025274.1	developmental pluripotency associated 5
0.001	2.31	Fcgr3	NM_010188.2	Fc receptor, IgG, low affinity III
0.011	2.28	Plekhg2	NM_138752.1	pleckstrin homology domain containing, family G (with RhoGef domain) member 2
0.004	2.28	P2ry6	NM_183168.1	pyrimidinergic receptor P2Y, G-protein coupled, 6
0.031	2.27	Saa2	NM_011314.1	serum amyloid A 2
0.002	2.26	Nfkbie	NM_008690.2	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon
0.001	2.25	Imp2l	NM_053122.2	IMP2 inner mitochondrial membrane peptidase-like (S. cerevisiae)
0.000	2.25	Trfr2	NM_015799.2	transferrin receptor 2
0.018	2.20	Tlr7	NM_133211.2	toll-like receptor 7
0.006	2.20	Ncf4	NM_008677.1	neutrophil cytosolic factor 4
0.023	2.20	Dcakd	null	dephospho-CoA kinase domain containing
0.003	2.20	Mt1	NM_013602.2	metallothionein 1
0.009	2.20	Rasip1	null	Ras interacting protein 1
0.050	2.19	Ly6i	NM_020498.1	lymphocyte antigen 6 complex, locus I
0.007	2.19	Hspb1	null	heat shock protein 1
0.033	2.16	Trim21	NM_009277.2	tripartite motif protein 21
0.025	2.15	Rbms2	null	RNA binding motif, single stranded interacting protein 2
0.000	2.14	Vps4b	null	vacuolar protein sorting 4b (yeast)
0.005	2.14	Aldh1l2	NM_153543.1	aldehyde dehydrogenase 1 family, member L2
0.048	2.14	Apobec1	NM_031159.2	apolipoprotein B editing complex 1
0.004	2.11	Hspca	null	heat shock protein 1, alpha
0.002	2.11	Akap2	NM_009649.1	A kinase (PRKA) anchor protein 2
0.013	2.10	Lrrc33	NM_146069.2	leucine rich repeat containing 33
0.012	2.08	Aatf	null	apoptosis antagonizing transcription factor
0.007	2.08	Traf3ip2	NM_134000.2	Traf3 interacting protein 2
0.032	2.08	Slit3	null	slit homolog 3 (Drosophila)
0.032	2.06	H2-Q8	NM_023124.2	histocompatibility 2, Q region locus 8
0.031	2.02	Mertk	NM_008587.1	c-mer proto-oncogene tyrosine kinase
0.003	2.01	Cd83	NM_009856.1	CD83 antigen
0.001	2.00	Aff1	null	AF4/FMR2 family, member 1
0.000	2.00	Gadd45b	NM_008655.1	growth arrest and DNA-damage-inducible 45 beta
0.009	2.00	Ifrd1	NM_013562.1	interferon-related developmental regulator 1
0.013	1.99	Npas3	NM_013780.1	neuronal PAS domain protein 3
0.035	1.98	Ift74	null	intraflagellar transport 74 homolog (Chlamydomonas)
0.043	1.98	Rela	NM_009045.2	v-rel reticuloendotheliosis viral oncogene homolog A (avian)
0.017	1.98	Pms1	NM_153556.1	postmeiotic segregation increased 1 (S. cerevisiae)
0.004	1.98	Lfng	null	lunatic fringe gene homolog (Drosophila)
0.003	1.97	Fv4	NM_010245.1	Friend virus susceptibility 4
0.005	1.97	Myg1	null	melanocyte proliferating gene 1
0.024	1.95	Hdc	NM_008230.4	histidine decarboxylase
0.015	1.95	Col5a2	NM_007737.1	procollagen, type V, alpha 2
0.005	1.94	Anub1	null	AN1, ubiquitin-like, homolog (Xenopus laevis)
0.011	1.94	Pnpla2	NM_025802.1	patatin-like phospholipase domain containing 2
0.023	1.92	H2-K1	NM_019909.1	histocompatibility 2, K1, K region

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
0.005	1.91	Uaca	NM_028283.1	uveal autoantigen with coiled-coil domains and ankyrin repeats
0.048	1.90	Map2k7	null	mitogen activated protein kinase kinase 7
0.004	1.89	Trp53inp1	NM_021897.1	transformation related protein 53 inducible nuclear protein 1
0.008	1.89	Atoh7	null	atonal homolog 7 (Drosophila)
0.020	1.87	Gdap10	NM_010268.1	ganglioside-induced differentiation-associated-protein 10
0.035	1.86	Clec2l	null	C-type lectin domain family, member I
0.030	1.86	Tinagl	NM_023476.1	tubulointerstitial nephritis antigen-like DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked
0.049	1.84	Ddx3y	NM_012008.1	elastin microfibril interfacer 2
0.005	1.84	Emilin2	NM_145158.2	major facilitator superfamily domain containing 2
0.001	1.84	Mfsd2	NM_029662.1	serum amyloid A 2[serum amyloid A 1
0.016	1.82	Saa2 Saa1	NM_011314.1	brain expressed myelocytomatosis oncogene
0.006	1.80	Bmyc	null	alpha-2-macroglobulin
0.007	1.80	A2m	NM_175628.2	E74-like factor 1
0.037	1.80	Elf1	NM_007920.2	olfactory receptor 810
0.034	1.80	Olf1810	NM_146550.1	myeloid differentiation primary response gene 88
0.031	1.80	Myd88	null	suppressor of cytokine signaling 2
0.004	1.80	Socs2	null	glutamic pyruvate transaminase (alanine aminotransferase) 2
0.027	1.79	Gpt2	NM_173866.1	gap junction membrane channel protein beta 6
0.021	1.77	Gjb6	NM_008128.3	retinol dehydrogenase 10 (all-trans)
0.005	1.76	Rdh10	NM_133832.2	transmembrane and coiled coil domains 3
0.034	1.76	Tmcc3	NM_172051.1	poly (ADP-ribose) polymerase family, member 12
0.043	1.75	Parp12	NM_172893.1	ribosomal protein S27
0.026	1.74	Rps27	null	Ras and Rab interactor 2
0.015	1.72	Rin2	NM_028724.2	ubiquitin specific peptidase 53
0.001	1.71	Usp53	NM_133857.1	AT rich interactive domain 5A (Mrf1 like)
0.014	1.70	Arid5a	NM_145996.2	cell division cycle 7 (S. cerevisiae)
0.004	1.70	Cdc7	NM_009863.1	solute carrier family 25 (mitochondrial carrier), member 13
0.040	1.70	Slc25a13	NM_015829.1	centrosomal protein 152
0.045	1.70	Cep152	null	leucine rich repeat and fibronectin type III domain containing 2
0.039	1.69	Lfn2	NM_027452.1	RAB20, member RAS oncogene family
0.020	1.69	Rab20	NM_011227.1	zinc finger protein 422, related sequence 1
0.010	1.68	Zfp422-rs1	NM_029952.2	tropomyosin 4
0.003	1.67	Tpm4	null	5'-nucleotidase, cytosolic III
0.001	1.67	Nt5c3	NM_026004.1	intraflagellar transport 74 homolog (Chlamydomonas)
0.027	1.67	Ift74	NM_026319.1	CWF19-like 2, cell cycle control (S. pombe)
0.009	1.67	Cwf19l2	NM_027545.1	interferon alpha responsive gene torsin A
0.045	1.66	lfrg15 Tor1ai	NM_144791.1	interacting protein 1
0.019	1.66	p1	NM_144791.1	triple functional domain (PTPRF interacting)
0.019	1.66	Trio	null	phosphodiesterase 4B, cAMP specific
0.016	1.66	Pde4b	NM_019840.1	CASP8 and FADD-like apoptosis regulator
0.016	1.65	Cflar	null	CKLF-like MARVEL transmembrane domain containing 3
0.000	1.65	Cmtm3	NM_024217.2	insulin-like growth factor binding protein 7
0.011	1.65	Igfbp7	NM_008048.1	zinc finger protein 709
0.015	1.64	Zfp709	NM_145624.2	ras homolog gene family, member U
0.006	1.63	Rhou	NM_133955.1	adiponectin receptor 2
0.026	1.62	Adipor2	NM_197985.2	Down syndrome critical region homolog 1
0.025	1.60	Dscr1	NM_019466.2	

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
				(human)
0.024	1.60	Crtam	NM_019465.1	cytotoxic and regulatory T cell molecule
0.024	1.59	Bach1	null	BTB and CNC homology 1
				LON peptidase N-terminal domain and ring finger 3
0.037	1.59	Lonrf3	null	
0.014	1.59	Plat	NM_008872.1	plasminogen activator, tissue
0.036	1.58	Tns3	null	tensin 3
				growth arrest and DNA-damage-inducible 45 alpha
0.005	1.58	Gadd45a	NM_007836.1	
0.016	1.58	F3	NM_010171.2	coagulation factor III
0.014	1.58	Bcor	NM_175044.1	Bcl6 interacting corepressor
				proteasome (prosome, macropain) activator subunit 4
0.027	1.58	Psme4	null	
			NM_00100842	
0.006	1.57	Cdh12	0.1	cadherin 12
0.050	1.56	Pdk4	NM_013743.1	pyruvate dehydrogenase kinase, isoenzyme 4
0.002	1.56	Brcc3	NM_145956.2	BRCA1/BRCA2-containing complex, subunit 3
0.033	1.56	Lypd1	NM_145100.2	Ly6/Plaur domain containing 1
0.029	1.56	Spata13	null	spermatogenesis associated 13
0.015	1.55	Pcolce2	NM_029620.1	procollagen C-endopeptidase enhancer 2
0.006	1.55	Irs2	null	insulin receptor substrate 2
0.045	1.54	Bzrp	NM_009775.2	benzodiazepine receptor, peripheral
0.014	1.53	Gca	null	granulocyte colony-stimulating factor receptor
				tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase
0.033	1.51	Trmu	NM_028063.1	
0.042	1.51	Olfr514	NM_146726.1	olfactory receptor 514
0.012	1.51	Egfr	null	epidermal growth factor receptor
				dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3
0.014	1.51	Dyrk3	NM_145508.1	
0.018	1.50	Slitl2	NM_139307.2	Slit-like 2 (Drosophila)
0.016	1.49	Rhob	NM_007483.2	ras homolog gene family, member B
0.021	1.49	Eif1a	NM_010120.3	eukaryotic translation initiation factor 1A
0.031	1.49	Vil2	NM_009510.1	villin 2
0.025	1.48	Tmem2	NM_031997.2	transmembrane protein 2
0.035	1.47	Rock1	NM_009071.1	Rho-associated coiled-coil forming kinase 1
0.029	1.47	Phxr5	NM_008836.2	per-hexamer repeat gene 5
0.007	1.47	Ctdp1	null	CTD phosphatase, subunit 1
0.037	1.47	Fmnl2	null	formin-like 2
				leucine rich repeat and coiled-coil domain containing 1
0.011	1.46	Lrrcc1	NM_028915.1	
0.000	1.46	Tapbpl	NM_145391.1	TAP binding protein-like
				solute carrier family 7 (cationic amino acid transporter, y system), member 11
0.026	1.44	Slc7a11	null	
0.010	1.43	Phactr3	NM_028806.1	phosphatase and actin regulator 3
0.010	1.43	Irf2	null	interferon regulatory factor 2
				UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 5
0.026	1.42	B3galt5	NM_033149.2	
0.042	1.42	Atad3a	NM_179203.1	ATPase family, AAA domain containing 3A
				leucine-rich repeats and calponin homology (CH) domain containing 1
0.047	1.42	Lrch1	null	
0.003	1.41	Usp34	null	ubiquitin specific peptidase 34
				thyroid hormone responsive SPOT14 homolog (Rattus)
0.033	1.40	Thrsp	NM_009381.2	
0.049	1.39	Jun	NM_010591.1	Jun oncogene
0.010	1.39	Sav1	NM_022028.1	salvador homolog 1 (Drosophila)
0.015	1.36	Ccrn4l	NM_009834.1	CCR4 carbon catabolite repression 4-like (S.

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
				cerevisiae)
0.048	1.36	Ppp1r3c	NM_016854.1	protein phosphatase 1, regulatory (inhibitor)
0.013	1.34	Ktn1	NM_008477.1	subunit 3C
0.008	1.34	Il17r	NM_008359.1	kinectin 1
0.017	1.32	Ranbp6	NM_177721.3	interleukin 17 receptor
0.013	1.31	Klf6	NM_011803.1	RAN binding protein 6
0.021	1.31	Ly6c	NM_010741.2	Kruppel-like factor 6
0.028	1.29	Skil	null	lymphocyte antigen 6 complex, locus C
0.035	0.79	Lrp1	NM_008512.1	SKI-like
				low density lipoprotein receptor-related protein 1
0.012	0.78	Agt	NM_007428.2	angiotensinogen (serpin peptidase inhibitor, clade
0.025	0.76	Plip	NM_026385.3	A, member 8)
0.018	0.76	Stx3	NM_011502.1	plasma membrane proteolipid
0.001	0.75	Tcta	NM_133986.1	syntaxin 3
				T-cell leukemia translocation altered gene
0.008	0.75	Lgr4	null	leucine-rich repeat-containing G protein-coupled
0.008	0.75	Sdad1	NM_172713.1	receptor 4
0.025	0.75	Lemd3	null	SDA1 domain containing 1
0.019	0.75	Fa2h	NM_178086.2	LEM domain containing 3
0.047	0.74	Rab5b	NM_011229.1	fatty acid 2-hydroxylase
				RAB5B, member RAS oncogene family
0.048	0.74	Slc23a3	NM_194333.2	solute carrier family 23 (nucleobase transporters),
				member 3
0.010	0.74	Mid1ip1	NM_026524.2	Mid1 interacting protein 1 (gastrulation specific
0.027	0.74	Mobp	null	G12-like (zebrafish))
0.001	0.73	Limk2	NM_010718.1	myelin-associated oligodendrocytic basic protein
0.007	0.73	Pitpna	NM_008850.1	LIM motif-containing protein kinase 2
0.025	0.73	Mod1	NM_008615.1	phosphatidylinositol transfer protein, alpha
0.026	0.73	Oprl1	NM_011012.2	malic enzyme, supernatant
				opioid receptor-like 1
0.031	0.73	Galnt1	NM_013814.2	UDP-N-acetyl-alpha-D-galactosamine:polypeptide
				N-acetylgalactosaminyltransferase 1
0.016	0.73	Edg2	NM_010336.1	endothelial differentiation, lysophosphatidic acid
0.000	0.72	Cdh2	NM_007664.1	G-protein-coupled receptor, 2
0.010	0.72	Pgbd5	null	cadherin 2
				piggyBac transposable element derived 5
0.008	0.72	B4galt6	NM_019737.1	UDP-Gal:betaGlcNAc beta 1,4-
0.016	0.72	Usp29	NM_021323.1	galactosyltransferase, polypeptide 6
0.015	0.71	Dcbld1	NM_025705.1	ubiquitin specific peptidase 29
0.024	0.71	Zfp655	NM_028298.2	discoidin, CUB and LCCL domain containing 1
				zinc finger protein 655
				solute carrier family 24
				(sodium/potassium/calcium exchanger), member
0.019	0.71	Slc24a2	NM_172426.1	2
0.001	0.71	Gtf2a1	NM_175335.1	general transcription factor II A, 1
0.011	0.71	Gnb5	NM_010313.1	guanine nucleotide binding protein, beta 5
0.000	0.71	Trap1	NM_026508.1	TNF receptor-associated protein 1
0.000	0.70	Hnrph3	null	heterogeneous nuclear ribonucleoprotein H3
0.010	0.70	Olf390	NM_146347.1	olfactory receptor 390
				solute carrier family 6 (neurotransmitter
0.008	0.70	Slc6a11	NM_172890.1	transporter, GABA), member 11
0.042	0.70	Ptpa	NM_008980.1	protein tyrosine phosphatase, receptor type, A
0.002	0.70	Rab15	null	RAB, member of RAS oncogene family-like 5
0.034	0.70	Raf1	NM_178264.2	v-raf-leukemia viral oncogene 1
0.013	0.70	Gdap111	NM_144891.1	ganglioside-induced differentiation-associated
				protein 1-like 1

P	FC	Gene Name	Genbank	Description
0.031	0.69	Hlrc1	null	HEAT-like (PBS lyase) repeat containing 1
0.001	0.69	Aplp2	null	amyloid beta (A4) precursor-like protein 2
0.002	0.69	Kcnv1	NM_026200.1	potassium channel, subfamily V, member 1
0.015	0.69	Slmap	NM_032008.3	sarcolemma associated protein
0.049	0.69	Ugt8a	null	UDP galactosyltransferase 8A
0.042	0.69	Zfp98	NM_016793.1	zinc finger protein 98
0.045	0.69	Cnn2	NM_007725.1	calponin 2
0.007	0.69	Cul4a	NM_146207.1	cullin 4A
0.007	0.69	Phf10	NM_024250.3	PHD finger protein 10
0.001	0.69	Trappc3	NM_013718.2	trafficking protein particle complex 3 vitamin K epoxide reductase complex, subunit 1- like 1
0.004	0.68	Vkorc111	null	eukaryotic translation initiation factor 3, subunit 10 (theta)
0.004	0.68	Eif3s10	NM_010123.2	
0.047	0.68	Fstl4	NM_177059.3	folliculin-like 4
0.039	0.68	Ccdc21	NM_144527.2	coiled-coil domain containing 21 ATP-binding cassette, sub-family C (CFTR/MRP), member 3
0.007	0.68	Abcc3	null	
0.029	0.67	Neto2	null	neuropilin (NRP) and tolloid (TLL)-like 2 proteasome (prosome, macropain) 26S subunit, non-ATPase, 5
0.016	0.67	Psmc5	NM_080554.1	
0.008	0.67	Pdia4	NM_009787.1	protein disulfide isomerase associated 4
0.007	0.67	Vps4a	NM_126165.1	vacuolar protein sorting 4a (yeast)
0.039	0.67	Cpsf3l	NM_028020.2	cleavage and polyadenylation specific factor 3-like UDP-Gal:betaGlcNAc beta 1,3- galactosyltransferase, polypeptide 1
0.037	0.67	B3galt1	NM_020283.1	
0.019	0.67	Gltscr2	NM_133831.1	glioma tumor suppressor candidate region gene 2
0.002	0.67	Nudcd3	NM_173748.2	NudC domain containing 3
0.002	0.66	Wdr54	NM_023790.1	WD repeat domain 54 thioredoxin domain containing 4 (endoplasmic reticulum)
0.013	0.66	Txndc4	NM_029572.1	microtubule-associated protein 1 light chain 3 alpha
0.003	0.66	Map1lc3a	NM_025735.1	
0.015	0.66	Ypel1	NM_023249.2	yippee-like 1 (Drosophila)
0.005	0.66	Ilf2	NM_026374.2	interleukin enhancer binding factor 2
0.019	0.66	Sema5a	null	sema domain, 5A
0.002	0.66	Atp2c1	NM_175025.2	ATPase, Casequestering
0.001	0.66	Srp68	NM_146032.3	signal recognition particle 68
0.005	0.66	Kif3c	NM_008445.1	kinesin family member 3C ATP synthase mitochondrial F1 complex assembly factor 2
0.015	0.66	Atpaf2	NM_145427.1	
0.013	0.65	Smbp	NM_178146.2	SM-11044 binding protein
0.010	0.65	Yif1b	null	Yip1 interacting factor homolog B (S. cerevisiae)
0.027	0.65	Nphp3	null	nephronophthisis 3 (adolescent)
0.031	0.65	Pqlc2	NM_145384.1	PQ loop repeat containing 2 cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)
0.037	0.65	Cdkn2b	NM_007670.2	
0.009	0.65	Thumpd1	NM_145585.1	THUMP domain containing 1
0.005	0.65	Lhx2	NM_010710.2	LIM homeobox protein 2
0.001	0.65	Chrm3	NM_033269.2	cholinergic receptor, muscarinic 3, cardiac
0.007	0.65	Eif4a1	NM_144958.2	eukaryotic translation initiation factor 4A1
0.021	0.65	Mtap4	NM_008633.1	microtubule-associated protein 4
0.004	0.65	Ppie	null	peptidylprolyl isomerase E (cyclophilin E) growth factor receptor bound protein 2-associated protein 1
0.001	0.64	Gab1	NM_021356.2	
0.044	0.64	Ngfb	NM_013609.1	nerve growth factor, beta

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
0.019	0.64	Vdac1	NM_011694.1	voltage-dependent anion channel 1
0.014	0.64	Baalc	NM_080640.3	brain and acute leukemia, cytoplasmic
0.023	0.64	Vamp5	NM_016872.1	vesicle-associated membrane protein 5
0.020	0.64	Gstm2	null	glutathione S-transferase, mu 2
0.016	0.64	Stmn4	NM_019675.2	stathmin-like 4
0.017	0.64	Jph1	NM_020604.1	junctionophilin 1
0.008	0.63	Tln1	NM_011602.3	talin 1
0.017	0.63	Las1l	NM_152822.1	LAS1-like (S. cerevisiae)
0.040	0.63	Olf982	NM_146854.1	olfactory receptor 982
0.033	0.63	Krtcap2	null	keratinocyte associated protein 2
0.009	0.63	Wdr5	NM_080848.1	WD repeat domain 5
0.014	0.63	Mtmr12	NM_172958.2	Vmyotubularin related protein 12
0.003	0.63	Traf2	NM_009422.1	Tnf receptor-associated factor 2
0.048	0.63	Hist2h2be	NM_175666.1	histone 2, H2be
0.005	0.63	Dvl2	NM_007888.1	dishevelled 2, dsh homolog (Drosophila)
0.046	0.63	Scrn1	NM_027268.1	secernin 1
0.044	0.63	Pak2	NM_177326.2	p21 (CDKN1A)-activated kinase 2
0.010	0.63	Psmd13	NM_011875.2	proteasome (prosome, macropain) 26S subunit, non-ATPase, 13
0.005	0.62	Mkrn3	NM_011746.1	makorin, ring finger protein, 3
0.016	0.62	Pycl1	NM_025412.1	pyrroline-5-carboxylate reductase-like
0.008	0.62	Thbs3	NM_013691.1	thrombospondin 3
0.043	0.62	Scmh1	NM_013883.1	sex comb on midleg homolog 1
0.014	0.62	Pafah1b1	NM_013625.1	platelet-activating factor acetylhydrolase, isoform 1b, beta1 subunit
0.038	0.62	Wrip1	null	Werner helicase interacting protein 1
0.015	0.62	Faah	NM_010173.2	fatty acid amide hydrolase
0.045	0.62	Slc38a5	NM_172479.1	solute carrier family 38, member 5
0.006	0.62	Hnrpr	NM_028871.1	heterogeneous nuclear ribonucleoprotein R
0.001	0.62	Klra6	NM_008464.1	killer cell lectin-like receptor, subfamily A, member 6
0.040	0.62	Ptrf	NM_008986.1	polymerase I and transcript release factor
0.030	0.62	Mtx1	null	metaxin 1
0.042	0.61	Abca5	NM_147219.1	ATP-binding cassette, sub-family A (ABC1), member 5
0.020	0.61	Rab3c	NM_023852.2	RAB3C, member RAS oncogene family
0.040	0.61	Xkr5	NM_176951.2	X Kell blood group precursor-related family, member 5
0.018	0.61	Mfhas1	null	malignant fibrous histiocytoma amplified sequence 1
0.009	0.61	Polr2a	NM_009089.1	polymerase (RNA) II (DNA directed) polypeptide A
0.027	0.61	Rgl1	NM_016846.2	ral guanine nucleotide dissociation stimulator,-like 1
0.009	0.61	Foxj3	NM_172699.2	forkhead box J3
0.044	0.61	Ccnd1	NM_007631.1	cyclin D1
0.044	0.61	Dhdds	NM_026144.2	dehydrodolichyl diphosphate synthase
0.011	0.61	Fbxo28	NM_175127.1	F-box protein 28
0.002	0.60	Tmod1	NM_021883.1	tropomodulin 1
0.027	0.60	Ccnj	NM_172839.1	cyclin J
0.020	0.60	Pecam1	NM_008816.1	platelet/endothelial cell adhesion molecule 1
0.020	0.60	Map2k5	NM_011840.1	mitogen activated protein kinase kinase 5
0.011	0.60	Syt11	NM_018804.2	synaptotagmin XI
0.005	0.60	Gm672	NM_201354.1	gene model 672, (NCBI)
0.001	0.60	Gmpr	NM_025508.1	guanosine monophosphate reductase
0.032	0.60	Ltbp4	NM_175641.1	latent transforming growth factor beta binding protein 4

P	FC	Gene Name	Genbank	Description
0.029	0.60	Eng	NM_007932.1	endoglin
0.015	0.60	Gnao1	NM_010308.2	guanine nucleotide binding protein, alpha o
0.008	0.60	Sh3rf2	NM_172966.1	SH3 domain containing ring finger 2
0.007	0.60	Copz1	NM_019817.1	coatamer protein complex, subunit zeta 1
0.036	0.60	Wfdc1	NM_023395.1	WAP four-disulfide core domain 1
0.018	0.60	Ivns1abp	NM_028582.2	influenza virus NS1A binding protein MOB1, Mps One Binder kinase activator-like 2A (yeast)
0.001	0.60	Mobkl2a	NM_172457.1	
0.023	0.60	Rab5c	NM_024456.1	RAB5C, member RAS oncogene family
0.035	0.60	Ppan	NM_145610.1	peter pan homolog (Drosophila)
0.016	0.59	Asrgl1	null	asparaginase like 1
0.003	0.59	Actb	NM_007393.1	actin, beta, cytoplasmic
0.043	0.59	Atf7	NM_146065.1	activating transcription factor 7
0.018	0.59	Aup1	NM_007517.1	ancient ubiquitous protein
0.017	0.59	Dhx34	NM_027883.2	DEAH (Asp-Glu-Ala-His) box polypeptide 34
0.005	0.59	Loxl3	NM_013586.2	lysyl oxidase-like 3
0.001	0.59	Skp2	NM_013787.1	S-phase kinase-associated protein 2 (p45)
0.024	0.59	Rasal2	NM_177644.2	RAS protein activator like 2
0.002	0.59	Dhx57	NM_198942.1	DEAH (Asp-Glu-Ala-Asp/His) box polypeptide 57 potassium voltage-gated channel, shaker-related subfamily, beta member 1
0.007	0.59	Kcnab1	NM_010597.2	
0.012	0.59	Casp3	NM_009810.1	caspase 3
0.000	0.59	St6galnac4	NM_011373.2	ST6
0.041	0.59	Eml3	NM_144872.1	echinoderm microtubule associated protein like 3
0.038	0.59	Dnmbp	null	dynamamin binding protein
0.007	0.59	Tmem24	NM_027909.1	transmembrane protein 24
0.001	0.59	Rkhd3	NM_175366.2	ring finger and KH domain containing 3
0.001	0.59	Acaca	null	acetyl-Coenzyme A carboxylase alpha
0.011	0.59	Sox2	NM_011443.2	SRY-box containing gene 2
0.004	0.59	Bcl9l	NM_030256.1	B-cell CLL/lymphoma 9-like
0.001	0.59	Cdc42se1	NM_172395.1	CDC42 small effector 1
0.002	0.59	Ahcy11	NM_145542.2	S-adenosylhomocysteine hydrolase-like 1
0.016	0.58	Pltp	NM_011125.1	phospholipid transfer protein
0.002	0.58	Vps37c	NM_181403.2	vacuolar protein sorting 37C (yeast)
0.026	0.58	Lss	null	lanosterol synthase
0.024	0.58	Dscr11l	NM_207649.1	Down syndrome critical region gene 1-like 1
0.037	0.58	Lrrtm4	NM_178731.2	leucine rich repeat transmembrane neuronal 4
0.024	0.58	Glr1a	NM_020492.2	glycine receptor, alpha 1 subunit
0.007	0.58	Cntn4	NM_173004.1	contactin 4
0.005	0.58	Galk2	NM_175154.2	galactokinase 2
0.004	0.58	Pigs	NM_201406.1	phosphatidylinositol glycan, class S
0.020	0.58	Sema3g	null	sema domain
0.008	0.58	Osbpl6	NM_145525.1	oxysterol binding protein-like 6 STAR-related lipid transfer (START) domain containing 4
0.023	0.58	Stard4	NM_133774.2	
0.021	0.58	Frs3	NM_144939.1	fibroblast growth factor receptor substrate 3
0.020	0.58	Ttl	NM_027192.1	tubulin tyrosine ligase
0.011	0.58	Gm2a	NM_010299.2	GM2 ganglioside activator protein
0.046	0.58	Krtap5-2	null	keratin associated protein 5-2
0.013	0.58	Aplp1	NM_007467.1	amyloid beta (A4) precursor-like protein 1
0.000	0.58	Sephs2	NM_009266.1	selenophosphate synthetase 2
0.018	0.58	Uck1	NM_011675.1	uridine-cytidine kinase 1
0.017	0.58	Zswim3	NM_178375.2	zinc finger, SWIM domain containing 3
0.049	0.58	Ngfa	NM_010915.1	nerve growth factor, alpha
0.010	0.58	Slc16a1	NM_009196.2	solute carrier family 16 (monocarboxylic acid

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
				transporters), member 1
0.020	0.57	Man1b1	null	mannosidase, alpha, class 1B, member 1
0.012	0.57	H2afz	null	H2A histone family, member Z
0.037	0.57	Rtn4ip1	NM_130892.2	reticulon 4 interacting protein 1
0.005	0.57	Dapk3	NM_007828.1	death-associated kinase 3
				acyl-Coenzyme A dehydrogenase family, member 10
0.049	0.57	Acad10	null	pleckstrin homology domain containing, family H (with MyTH4 domain) member 1
0.039	0.57	Plekhh1	null	leucine-rich repeats and IQ motif containing 2
0.035	0.57	Lrriq2	NM_028815.3	UDP-glucuronate decarboxylase 1
0.023	0.57	Uxs1	NM_026430.1	H1 histone family, member 0
0.003	0.57	H1f0	NM_008197.2	SRY-box containing gene 10
0.022	0.57	Sox10	null	protein kinase C, nu
0.019	0.57	Prkcn	NM_029239.2	neurofilament 3, medium
0.031	0.57	Nef3	NM_008691.1	GA repeat binding protein, alpha
0.022	0.57	Gabpa	NM_008065.2	shadow of prion protein
0.002	0.57	Sprn	NM_183147.2	serine/threonine kinase 24 (STE20 homolog, yeast)
0.004	0.56	Stk24	null	eukaryotic translation elongation factor 1 alpha 2
0.009	0.56	Eef1a2	NM_007906.2	5-hydroxytryptamine (serotonin) receptor 2C
0.030	0.56	Htr2c	NM_008312.2	endosulfine alpha
0.010	0.56	Ensa	null	GRIP1 associated protein 1
0.008	0.56	Gripap1	NM_207670.1	ankyrin repeat and MYND domain containing 2
0.037	0.56	Ankmy2	NM_146033.2	glucocorticoid induced transcript 1
0.036	0.56	Glcci1	NM_133236.1	ATP-binding cassette, sub-family F (GCN20), member 2
0.002	0.56	Abcf2	null	kinesin family member 3B
0.000	0.56	Kif3b	NM_008444.2	translocase of inner mitochondrial membrane 17b
0.012	0.56	Timm17b	NM_011591.2	catenin (cadherin associated protein), delta 1
0.005	0.56	Ctnnd1	NM_007615.1	zinc finger protein 316
0.039	0.56	Zfp316	NM_017467.2	solute carrier family 40 (iron-regulated transporter), member 1
0.026	0.56	Slc40a1	NM_016917.1	protein tyrosine phosphatase, receptor type, B
0.010	0.56	Ptprb	NM_029928.1	mitogen activated protein kinase kinase kinase 3
0.001	0.56	Map3k3	NM_011947.1	homeo box B4
0.029	0.56	Hoxb4	null	coronin 7
0.008	0.56	Coro7	NM_030205.2	deoxycytidine kinase
0.002	0.56	Dck	NM_007832.3	CUB and Sushi multiple domains 1
0.029	0.55	Csmd1	NM_053171.1	downstream of Stk11
0.018	0.55	Dos	null	EGF-like domain 7
0.018	0.55	Egfl7	NM_198725.1	calneuron 1
0.004	0.55	Caln1	NM_021371.1	fibrillarin
0.008	0.55	Fbl	null	tweety homolog 1 (Drosophila)
0.005	0.55	Ttyh1	NM_021324.3	SH3 domain binding glutamic acid-rich protein like 2
0.032	0.55	Sh3bgrl2	NM_172507.2	rhopilin, Rho GTPase binding protein 1
0.040	0.55	Rhpn1	NM_008164.1	lymphoid enhancer binding factor 1
0.009	0.55	Lef1	NM_010703.2	prepronociceptin
0.004	0.55	Pnoc	NM_010932.1	DnaJ (Hsp40) homolog, subfamily C, member 6
0.002	0.55	Dnajc6	NM_198412.1	zinc finger protein 667
0.003	0.55	Zfp667	null	olfactory receptor 544
0.025	0.55	Olf544	NM_020289.1	PDZ domain containing 4
0.015	0.55	Pdzk4	null	retinoblastoma binding protein 7
0.027	0.54	Rbbp7	NM_009031.2	lymphocyte antigen 6 complex, locus E
0.002	0.54	Ly6e	NM_008529.2	

P	FC	Gene Name	Genbank	Description
0.007	0.54	Stim2	null	stromal interaction molecule 2
0.009	0.54	Usp21	NM_013919.2	ubiquitin specific peptidase 21
0.008	0.54	Hist1h4d	NM_175654.1	histone 1, H4d
0.005	0.54	Hsd17b4	NM_008292.2	hydroxysteroid (17-beta) dehydrogenase 4
0.004	0.54	H2-DMb2 1	NM_010388.1	histocompatibility 2, class II, locus Mb2
0.001	0.54	P2ry12	NM_027571.2	purinergic receptor P2Y, G-protein coupled 12
0.042	0.54	Gosr2	NM_019650.2	golgi SNAP receptor complex member 2
0.033	0.54	Dpp8	NM_028906.2	dipeptidylpeptidase 8
0.007	0.54	Gpatc2	NM_026367.3	G patch domain containing 2
0.030	0.54	Rcn3	NM_026555.1	reticulocalbin 3, EF-hand calcium binding domain
0.027	0.54	Cables2	NM_145851.1	Cdk5 and Abl enzyme substrate 2
0.014	0.54	Lrrc28	null	leucine rich repeat containing 28
				arsA (bacterial) arsenite transporter, ATP-binding, homolog 1
0.004	0.54	Asna1	NM_019652.1	
0.000	0.54	Sort1	NM_019972.2	sortilin 1
0.004	0.54	Klhdc1	NM_178253.2	kelch domain containing 1
0.004	0.54	Opa1	NM_133752.1	optic atrophy 1 homolog (human)
0.001	0.54	Sep06	NM_019942.2	sepin 6
0.038	0.54	Tpr	NM_133780.2	translocated promoter region
0.001	0.54	Ece2	NM_139293.1	endothelin converting enzyme 2
0.002	0.53	Gramd1b	NM_172768.1	GRAM domain containing 1B
0.035	0.53	Scamp4	NM_019575.4	secretory carrier membrane protein 4
0.017	0.53	Mef2a	NM_013597.2	myocyte enhancer factor 2A
0.011	0.53	Rbm11	NM_198302.1	RNA binding motif protein 11
0.001	0.53	Numa1	NM_133947.2	nuclear mitotic apparatus protein 1
0.009	0.53	Ptrf	null	polymerase I and transcript release factor
0.020	0.53	Tbc1d4	NM_173380.1	TBC1 domain family, member 4
				KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2
0.009	0.53	Kdelr2	NM_025841.1	
0.007	0.53	Rtn3	null	reticulon 3
				vomeronal 2, receptor, 5 vomeronal 2, receptor, 4
0.022	0.53	V2r5 V2r4	NM_009493.1	
0.018	0.53	Psap	NM_011179.2	prosaposin
0.002	0.53	Syt16	NM_172804.1	synaptotagmin XVI
				vesicle transport through interaction with t-SNAREs homolog 1A (yeast)
0.044	0.53	Vti1a	NM_016862.2	
0.043	0.53	Ubie	NM_199477.1	UbiE-YGHL1 fusion protein
				discs, large (Drosophila) homolog-associated protein 1
0.017	0.53	Dlgap1	NM_027712.2	human immunodeficiency virus type I enhancer binding protein 3
0.010	0.53	Hivep3	NM_010657.2	
0.005	0.53	Cdc42se2	NM_178626.1	CDC42 small effector 2
0.040	0.52	Gda	NM_010266.1	guanine deaminase
0.016	0.52	Ltc4s	null	leukotriene C4 synthase
0.005	0.52	Sox21	NM_145464.1	SRY-box containing gene 21
0.005	0.52	Arpc4	NM_026552.1	actin related protein 2/3 complex, subunit 4
0.004	0.52	Klhdc3	NM_027910.1	kelch domain containing 3
0.044	0.52	Pcgf3	NM_172716.2	polycomb group ring finger 3
0.014	0.52	Cul7	NM_177189.3	cullin 7
0.008	0.52	Akap6	null	A kinase (PRKA) anchor protein 6
0.014	0.52	Hk1	NM_010438.1	hexokinase 1
				GCN5 general control of amino acid synthesis-like 2 (yeast)
0.021	0.52	Gcn5l2	NM_020004.1	
0.012	0.52	Snrpa	NM_015782.2	small nuclear ribonucleoprotein polypeptide A
0.010	0.52	Vwa1	NM_147776.2	von Willebrand factor A domain containing 1

P	FC	Gene Name	Genbank	Description
0.030	0.52	Arfgef2	null	ADP-ribosylation factor guanine nucleotide-exchange factor 2
0.015	0.52	Gsk3b	NM_019827.3	glycogen synthase kinase 3 beta
0.033	0.52	Stil	null	Scf/Tal1 interrupting locus
0.022	0.52	Snx12	NM_018875.1	sorting nexin 12
0.040	0.52	Bivm	NM_144558.2	basic, immunoglobulin-like variable motif containing
0.038	0.52	Glrx2	null	glutaredoxin 2 (thioltransferase)
0.015	0.51	Acsl1	NM_007981.2	acyl-CoA synthetase long-chain family member 1
0.006	0.51	Stx6	NM_021433.2	syntaxin 6
0.033	0.51	Cntnap1	NM_016782.1	contactin associated protein 1
0.007	0.51	Ldlrap1	NM_145554.1	low density lipoprotein receptor adaptor protein 1
0.004	0.51	Dyrk1a	NM_007890.1	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1a
0.020	0.51	Cpne7	NM_170684.1	copine VII
0.019	0.51	Myadm	NM_016969.1	myeloid-associated differentiation marker
0.012	0.51	Elovl7	NM_029001.2	ELOVL family member 7, elongation of long chain fatty acids (yeast)
0.007	0.51	Anapc5	NM_021505.1	anaphase-promoting complex subunit 5
0.003	0.51	Yipf3	NM_145353.1	Yip1 domain family, member 3
0.023	0.51	Fkbp8	NM_010223.1	FK506 binding protein 8
0.007	0.51	Dtna	NM_207650.1	dystrobrevin alpha
0.006	0.51	Syng3	NM_011522.2	synaptogyrin 3
0.001	0.51	Foxc1	NM_008592.2	forkhead box C1
0.008	0.51	Adrm1	NM_019822.2	adhesion regulating molecule 1
0.004	0.51	Sult4a1	NM_013873.3	sulfotransferase family 4A, member 1
0.024	0.51	Mapk8ip2	NM_021921.2	mitogen-activated protein kinase 8 interacting protein 2
0.014	0.51	Arrb2	NM_145429.1	arrestin, beta 2
0.013	0.51	Wdr1	NM_011715.1	WD repeat domain 1
0.042	0.50	Mfng	NM_008595.1	manic fringe homolog (Drosophila)
0.027	0.50	P2ry1	NM_008772.2	purinergic receptor P2Y, G-protein coupled 1
0.018	0.50	Ampd2	NM_028779.3	adenosine monophosphate deaminase 2 (isoform L)
0.002	0.50	Serf2	null	small EDRK-rich factor 2
0.050	0.50	Commd2	NM_175095.2	COMM domain containing 2
0.004	0.50	Pla2g6	NM_016915.2	phospholipase A2, group VI
0.000	0.50	Agpat6	NM_018743.2	1-acylglycerol-3-phosphate O-acyltransferase 6
0.015	0.50	Itn2c	NM_022417.1	integral membrane protein 2C
0.002	0.50	Sppl3	null	signal peptide peptidase 3
0.001	0.50	Rbbp5	NM_172517.1	retinoblastoma binding protein 5
0.022	0.50	Apcdd1	NM_133237.1	adenomatosis polyposis coli down-regulated 1
0.020	0.50	Gpr62	null	G protein-coupled receptor 62
0.009	0.50	Zfp114	null	zinc finger protein 114
0.009	0.50	Dgkz	NM_138306.1	diacylglycerol kinase zeta
0.008	0.50	Aloxe3	NM_011786.1	arachidonate lipoxygenase 3
0.025	0.50	Lef1	null	lymphoid enhancer binding factor 1
0.022	0.50	Cd2bp2	NM_027353.2	CD2 antigen (cytoplasmic tail) binding protein 2
0.007	0.50	Cav1	NM_007616.2	caveolin, caveolae protein 1
0.008	0.50	Thra	NM_178060.2	thyroid hormone receptor alpha
0.001	0.50	Metap1	null	methionyl aminopeptidase 1
0.043	0.50	Zfp106	NM_011743.1	zinc finger protein 106
0.030	0.50	Galc	NM_008079.2	galactosylceramidase
0.004	0.50	Hmg20b	NM_010440.1	high mobility group 20 B
0.029	0.50	Ilf3	NM_010561.1	interleukin enhancer binding factor 3

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
0.015	0.50	Ubl4	NM_145405.1	ubiquitin-like 4
0.012	0.50	Eno1	null	enolase 1, alpha non-neuron
0.009	0.50	Slc38a2	NM_175121.3	solute carrier family 38, member 2
0.007	0.50	Atp9a	NM_015731.2	ATPase, class II, type 9A
0.029	0.50	Sh3md2	NM_021506.1	SH3 multiple domains 2
0.014	0.50	Pou3f1	NM_011141.1	POU domain, class 3, transcription factor 1
0.005	0.50	Syt3	NM_016663.2	synaptotagmin III
0.003	0.50	Eif4g2	NM_013507.2	eukaryotic translation initiation factor 4, gamma 2 general transcription factor IIIC, polypeptide 2, beta
0.006	0.49	Gtf3c2	NM_027901.1	
0.002	0.49	Fip1l1	NM_024183.3	FIP1 like 1 (<i>S. cerevisiae</i>)
0.023	0.49	Lgi3	NM_145219.2	leucine-rich repeat LGI family, member 3
0.015	0.49	Ihpk2	NM_029634.1	inositol hexaphosphate kinase 2
0.014	0.49	Tnks1bp1	null	tankyrase 1 binding protein 1 spastic paraplegia 20, spartin (Troyer syndrome)
0.005	0.49	Spg20	NM_144895.1	homolog (human)
0.016	0.49	Srpk1	NM_016795.2	serine/arginine-rich protein specific kinase 1 proteasome (prosome, macropain) 26S subunit, non-ATPase, 11
0.008	0.49	Psmd11	null	
0.001	0.49	Bmp1	NM_009755.2	bone morphogenetic protein 1
0.006	0.49	Itgam	NM_008401.1	integrin alpha M
0.001	0.49	Leng4	NM_029934.2	leukocyte receptor cluster (LRC) member 4
0.033	0.49	Fgd1	NM_008001.2	FYVE, RhoGEF and PH domain containing 1
0.038	0.49	Pde8b	NM_172263.1	phosphodiesterase 8B
0.021	0.49	C1qtnf7	NM_175425.2	C1q and tumor necrosis factor related protein 7 solute carrier family 7 (cationic amino acid transporter, y system), member 8
0.016	0.49	Slc7a8	NM_016972.2	
0.012	0.49	Prrt1	NM_030890.1	proline-rich transmembrane protein 1
0.009	0.49	Clic5	NM_172621.1	chloride intracellular channel 5
0.043	0.48	Pon3	null	paraoxonase 3 U2 small nuclear ribonucleoprotein auxiliary factor (U2AF) 1
0.020	0.48	U2af1	null	
0.009	0.48	Slc20a2	NM_011394.1	solute carrier family 20, member 2
0.000	0.48	Klhl18	NM_177771.3	kelch-like 18 (<i>Drosophila</i>)
0.038	0.48	Cyt1	null	cytokine like 1
0.006	0.48	Ocln	NM_008756.2	occludin
0.008	0.48	Bai2	NM_173071.1	brain-specific angiogenesis inhibitor 2
0.020	0.48	Htr7	NM_008315.1	5-hydroxytryptamine (serotonin) receptor 7 a disintegrin and metallopeptidase domain 9 (meltrin gamma)
0.016	0.48	Adam9	NM_007404.1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2
0.012	0.48	Hmgcs2	NM_008256.2	
0.005	0.48	Gpr175	NM_011906.1	G protein-coupled receptor 175 potassium voltage-gated channel, shaker-related subfamily, member 2
0.004	0.48	Kcna2	NM_008417.2	src homology 2 domain-containing transforming protein C2
0.016	0.48	Shc2	null	
0.015	0.48	Arhgap1	NM_146124.2	Rho GTPase activating protein 1 tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein
0.010	0.48	Ywhab	NM_018753.3	
0.043	0.48	Opn3	NM_010098.1	opsin (encephalopsin) matrix associated, actin dependent regulator of chromatin, subfamily a, member 4
0.031	0.48	Smarca4	NM_011417.1	
0.005	0.48	Jub	null	ajuba
0.024	0.48	Acot8	NM_133240.1	acyl-CoA thioesterase 8
0.016	0.48	Rtkn	NM_009106.1	rhotekin

P	FC	Gene Name	Genbank	Description
0.016	0.47	Tmcc2	NM_178874.2	transmembrane and coiled-coil domains 2
0.013	0.47	Sympk	null	symplesin
0.002	0.47	Mpp2	NM_016695.1	membrane protein, palmitoylated 2 (MAGUK p55 subfamily member 2)
0.020	0.47	Fath2	null	fat tumor suppressor homolog 2 (Drosophila)
0.011	0.47	Blcap	NM_016916.2	bladder cancer associated protein homolog (human)
0.005	0.47	Gja12	NM_175452.2	gap junction membrane channel protein alpha 12
0.049	0.47	Pold4	NM_027196.1	polymerase (DNA-directed), delta 4
0.031	0.47	Satb2	NM_139146.1	special AT-rich sequence binding protein 2
0.022	0.47	Cdc27	null	cell division cycle 27 homolog (S. cerevisiae)
0.012	0.47	Zdhhc8	null	zinc finger, DHHC domain containing 8
0.017	0.47	Usp29	NM_021323.1	ubiquitin specific peptidase 29
0.031	0.47	Zfp524	NM_025324.1	zinc finger protein 524
0.018	0.47	Rhobtb3	NM_028493.1	Rho-related BTB domain containing 3
0.011	0.47	Pcbp4	NM_021567.2	poly(rC) binding protein 4
0.007	0.47	Chd4	NM_145979.1	chromodomain helicase DNA binding protein 4
0.030	0.47	Gdf11	null	growth differentiation factor 11
0.014	0.47	Gtlf3a	NM_025293.1	gene trap locus F3a
0.009	0.47	Aadacl1	NM_178772.2	arylacetamide deacetylase-like 1
0.005	0.47	Msl31	NM_010832.2	male-specific lethal-3 homolog 1 (Drosophila)
0.037	0.47	Cbx4	NM_007625.1	chromobox homolog 4 (Drosophila Pc class)
0.017	0.47	Ncoa2	NM_008678.1	nuclear receptor coactivator 2
0.001	0.47	Rgs4	NM_009062.2	regulator of G-protein signaling 4
0.013	0.46	Pou3f2	NM_008899.1	POU domain, class 3, transcription factor 2
0.006	0.46	Bcl9	NM_029933.3	B-cell CLL/lymphoma 9
0.016	0.46	Pgd	NM_025801.1	phosphogluconate dehydrogenase
0.014	0.46	Orm1 2 3	NM_011016.1	orosomucoid 1 orosomucoid 2 orosomucoid 3
0.043	0.46	Rxra	null	retinoid X receptor alpha
0.015	0.46	Gpr153	NM_178406.1	G protein-coupled receptor 153
0.034	0.46	Pou3f3	NM_008900.1	POU domain, class 3, transcription factor 3
0.007	0.46	Rnf141	NM_025999.1	ring finger protein 141
0.042	0.45	Fntb	NM_145927.1	farnesyltransferase, CAAX box, beta
0.031	0.45	Accn2	NM_009597.1	amiloride-sensitive cation channel 2, neuronal
0.020	0.45	Scotin	NM_025858.1	scotin gene
0.019	0.45	Stat3	NM_213659.1	signal transducer and activator of transcription 3
0.048	0.45	Clic6	NM_172469.1	chloride intracellular channel 6
0.015	0.45	Prpsap1	null	phosphoribosyl pyrophosphate synthetase-associated protein 1
0.036	0.45	Prss19	NM_008940.1	protease, serine, 19 (neuropsin)
0.007	0.45	Atp13a2	NM_029097.1	ATPase type 13A2
0.022	0.45	Usp12	NM_011669.2	ubiquitin specific peptidase 12
0.007	0.45	Cdc42se2	NM_178626.1	CDC42 small effector 2
0.024	0.45	Sh2bp1	NM_009431.1	SH2 domain binding protein 1 (tetra-ricopeptide repeat containing)
0.012	0.44	Rad23b	NM_009011.2	RAD23b homolog (S. cerevisiae)
0.002	0.44	Ddx46	null	DEAD (Asp-Glu-Ala-Asp) box polypeptide 46
0.000	0.44	Dcc	NM_007831.1	deleted in colorectal carcinoma
0.027	0.44	zfp507	null	zinc finger protein 507
0.016	0.44	Pitpnm1	NM_008851.1	phosphatidylinositol membrane-associated 1
0.014	0.44	Tspsy13	NM_198617.1	TSPY-like 3
0.007	0.44	Ppp3r1	NM_024459.1	protein phosphatase 3, regulatory subunit B, alpha isoform (calcineurin B, type I)
0.001	0.44	Sult5a1	NM_020564.1	sulfotransferase family 5A, member 1
0.014	0.44	Snx11	NM_028965.2	sorting nexin 11

P	FC	Gene Name	Genbank	Description
0.002	0.44	Hip1	NM_146001.1	huntingtin interacting protein 1
0.036	0.44	Gtf3c4	NM_172977.1	general transcription factor IIIC, polypeptide 4
0.010	0.44	Acp2	NM_007387.1	acid phosphatase 2, lysosomal
0.037	0.43	Wnt6	NM_009526.2	wingless-related MMTV integration site 6
0.016	0.43	Vangl1	NM_177545.2	vang, van gogh-like 1 (Drosophila)
0.015	0.43	Syt1	NM_009306.2	synaptotagmin I
0.005	0.43	Pqlc1	NM_025861.1	PQ loop repeat containing 1 myeloid ecotropic viral integration site-related gene 2
0.034	0.43	Mrg2	NM_008627.1	golgi reassembly stacking protein 2
0.019	0.43	Gorasp2	NM_027352.2	kit ligand
0.010	0.43	Kitl	NM_013598.1	Bcl-associated death promoter
0.009	0.43	Bad	NM_007522.1	SH3-domain GRB2-like 2
0.005	0.43	Sh3gl2	NM_019535.2	calsyntenin 1
0.024	0.43	Clstn1	NM_023051.1	polymerase (DNA directed), lambda potassium voltage-gated channel, subfamily F, member 1
0.001	0.43	Poll	NM_020032.1	splicing factor, arginine/serine-rich 1 (ASF/SF2)
0.044	0.43	Kcnf1	NM_201531.1	EPS8-like 1
0.024	0.43	Sfrs1	NM_173374.2	nudix (nucleoside diphosphate linked moiety X)- type motif 21
0.010	0.43	Eps8l1	null	FYVE, RhoGEF and PH domain containing 5
0.002	0.43	Nudt21	NM_026623.1	hydroxyacyl-Coenzyme A dehydrogenase
0.033	0.43	Fgd5	NM_172731.2	signal recognition particle receptor, B subunit
0.032	0.43	Hadha	NM_178878.1	FK506 binding protein 11
0.007	0.43	Srprb	NM_009275.3	macrophage erythroblast attacher
0.010	0.43	Fkbp11	NM_024169.2	nitric oxide synthase 3, endothelial cell
0.008	0.43	Maea	NM_021500.1	transmembrane protein 20
0.006	0.43	Nos3	NM_008713.2	lymphocyte antigen 9
0.044	0.42	Tmem20	NM_175507.2	DiGeorge syndrome critical region gene 8
0.008	0.42	Ly9	NM_008534.1	XPA binding protein 2
0.028	0.42	Dgcr8	NM_033324.2	four jointed box 1 (Drosophila)
0.014	0.42	Xab2	NM_026156.1	transcriptional adaptor 3 (NGG1 homolog, yeast)- like
0.011	0.42	Fjx1	NM_010218.2	SET binding protein 1
0.048	0.42	Tada3l	NM_133932.1	proprotein convertase subtilisin/kexin type 6
0.038	0.42	Setbp1	NM_053099.1	aortic preferentially expressed gene 1
0.030	0.42	Pcsk6	null	unc-5 homolog C (C. elegans)
0.026	0.42	Apeg1	NM_007463.2	cellular repressor of E1A-stimulated genes 2
0.019	0.42	Unc5c	NM_009472.1	meningioma expressed antigen 5 (hyaluronidase)
0.006	0.42	Creg2	NM_170597.3	adaptor protein complex AP-1, gamma 1 subunit
0.001	0.42	Mgea5	NM_023799.2	leukotriene A4 hydrolase
0.008	0.42	Ap1g1	NM_009677.3	lipolysis stimulated lipoprotein receptor
0.007	0.42	Lta4h	NM_008517.1	GRAM domain containing 1A
0.004	0.42	Lsr	NM_017405.1	v-raf-leukemia viral oncogene 1
0.029	0.42	Gramd1a	NM_027898.2	coronin, actin binding protein 1C
0.010	0.42	Raf1	NM_029780.1	defensin, alpha 1
0.005	0.42	Coro1c	NM_011779.2	
0.048	0.42	Defa1	NM_010031.2	
0.009	0.42	Eif4g1	NM_00100533	eukaryotic translation initiation factor 4, gamma 1
0.033	0.42	Dusp7	NM_153459.1	dual specificity phosphatase 7
0.002	0.41	Pcdh18	NM_130448.2	protocadherin 18
0.039	0.41	Mmrn2	NM_153127.2	multimerin 2
0.004	0.41	Prkrir	null	protein-kinase
0.003	0.41	Eif5a2	NM_177586.4	eukaryotic translation initiation factor 5A2
0.020	0.41	Sncb	NM_033610.2	synuclein, beta

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
0.006	0.41	Zfp690	NM_178889.2	zinc finger protein 690
0.026	0.41	Kcnk4	NM_008431.1	potassium channel, subfamily K, member 4
0.022	0.41	Ptptr	NM_021464.1	protein tyrosine phosphatase, receptor type, T
0.008	0.41	Gpr61	NM_175470.2	G protein-coupled receptor 61
0.033	0.41	Mcm7	null	minichromosome maintenance deficient 7 (S. cerevisiae)
0.009	0.41	Lphn3	NM_198702.1	latrophilin 3
0.008	0.41	Cmtm8	NM_027294.1	CKLF-like MARVEL transmembrane domain containing 8
0.021	0.41	Pygb	NM_153781.1	brain glycogen phosphorylase
0.009	0.41	Clptm1	NM_019649.1	cleft lip and palate associated transmembrane protein 1
0.044	0.41	Foxq1	NM_008239.3	forkhead box Q1
0.023	0.41	Xpr1	NM_011273.1	xenotropic and polytropic retrovirus receptor 1
0.021	0.41	Grm7	null	glutamate receptor, metabotropic 7
0.008	0.41	Pex2	NM_021483.1	peroxin 2
0.044	0.40	Rilp	null	Rab interacting lysosomal protein
0.039	0.40	Wdr22	NM_177267.3	WD repeat domain 22
0.019	0.40	Rhbd1	NM_144816.1	rhomboid, veinlet-like 1 (Drosophila)
0.008	0.40	Trak2	NM_172406.2	trafficking protein, kinesin binding 1
0.010	0.40	Mgat1	NM_010794.2	mannoside acetylglucosaminyltransferase 1
0.021	0.40	Mapre2	NM_153058.2	microtubule-associated protein, RP/EB family, member 2
0.002	0.40	Pvr	NM_009310.1	poliovirus receptor
0.042	0.40	Tmem106b	NM_027992.1	transmembrane protein 106B
0.023	0.40	Ppp2r1a	NM_016891.2	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), alpha isoform
0.017	0.40	Lrig2	null	leucine-rich repeats and immunoglobulin-like domains 2
0.018	0.40	Skb1	null	SKB1 homolog (S. pombe)
0.015	0.40	Zfp289	NM_023854.1	zinc finger protein 289
0.041	0.40	Foxf2	NM_010225.1	forkhead box F2
0.018	0.40	Fto	NM_011936.1	fatso
0.018	0.40	Gabbr1	null	gamma-aminobutyric acid (GABA-B) receptor, 1
0.007	0.40	Fbxw5	NM_013908.1	F-box and WD-40 domain protein 5
0.016	0.40	Rybp	NM_019743.2	RING1 and YY1 binding protein
0.045	0.39	Accn5	NM_021370.1	amiloride-sensitive cation channel 5, intestinal
0.003	0.39	Lrrtm1	NM_028880.2	leucine rich repeat transmembrane neuronal 1
0.040	0.39	Pdzk3	null	PDZ domain containing 3
0.005	0.39	Xpo7	NM_023045.1	exportin 7
0.002	0.39	Aggf1	NM_025630.2	angiogenic factor with G patch and FHA domains 1
0.017	0.39	Igsf4c	NM_153112.1	immunoglobulin superfamily, member 4C
0.017	0.39	Dynl12	NM_026556.2	dynein light chain LC8-type 2
0.012	0.39	Zdhc3	NM_026917.4	zinc finger, DHHC domain containing 3
0.000	0.39	Nkx2-2	NM_010919.1	NK2 transcription factor related, locus 2 (Drosophila)
0.026	0.39	Tmem74	NM_175502.2	transmembrane protein 74
0.015	0.39	Cxx1a Cxx1b	NM_024170.1	CAAX box 1 homolog A (human) CAAX box 1 homolog B (human)
0.014	0.39	Spred3	NM_182927.2	sprouty-related, EVH1 domain containing 3
0.040	0.39	Ap2a2	NM_007459.2	adaptor protein complex AP-2, alpha 2 subunit
0.021	0.39	Slc25a1	NM_153150.1	solute carrier family 25 (mitochondrial carrier, citrate transporter), member 1
0.010	0.39	Whdc1	NM_001004185.2	WAS protein homology region 2 domain containing 1

P	FC	Gene Name	Genbank	Description
0.008	0.39	Ikbkg	NM_178590.2	inhibitor of kappaB kinase gamma
0.046	0.39	Eif4ebp2	NM_010124.1	eukaryotic translation initiation factor 4E binding protein 2
0.010	0.39	Cdc2l6	NM_198164.1	cell division cycle 2-like 6 (CDK8-like)
0.025	0.39	Saa1	null	serum amyloid A 1
0.023	0.39	Nupl2	NM_153092.1	nucleoporin like 2
0.015	0.39	Mtch1	NM_019880.2	mitochondrial carrier homolog 1 (C. elegans)
0.009	0.39	Fcgrt	null	Fc receptor, IgG, alpha chain transporter
0.003	0.39	Slc35b4	NM_021435.1	solute carrier family 35, member B4
0.003	0.39	Cd97	NM_011925.1	CD97 antigen
0.036	0.39	Rims2	NM_053271.1	regulating synaptic membrane exocytosis 2
0.021	0.39	Ankrd13b	NM_172945.1	ankyrin repeat domain 13b
0.016	0.39	Myo1b	NM_010863.1	myosin IB
0.007	0.39	Plekha2	NM_031257.2	pleckstrin homology domain-containing, family A member 2
0.002	0.38	Hes5	NM_010419.2	hairy and enhancer of split 5 (Drosophila)
0.002	0.38	Cx3cr1	null	chemokine (C-X3-C) receptor 1
0.042	0.38	Ngb	NM_022414.2	neuroglobin
0.039	0.38	Nvl	NM_026171.1	nuclear VCP-like
0.025	0.38	Kcnb1	NM_008420.3	potassium voltage gated channel, Shab-related subfamily, member 1
0.023	0.38	Gcn1l1	null	GCN1 general control of amino-acid synthesis 1-like 1 (yeast)
0.006	0.38	Ephb4	NM_010144.4	Eph receptor B4
0.031	0.38	Lmtk2	null	lemur tyrosine kinase 2
0.030	0.38	H13	NM_010376.2	histocompatibility 13
0.025	0.38	Vps11	NM_027889.1	vacuolar protein sorting 11 (yeast)
0.020	0.38	Hic1	NM_010430.1	hypermethylated in cancer 1
0.018	0.38	Rb1	NM_009029.1	retinoblastoma 1
0.012	0.38	Smug1	NM_027885.2	single-strand selective monofunctional uracil DNA glycosylase
0.023	0.38	Myrip	NM_144557.2	myosin VIIA and Rab interacting protein
0.050	0.38	Vac14	NM_146216.2	Vac14 homolog (S. cerevisiae)
0.045	0.38	Pcsk5	null	proprotein convertase subtilisin/kexin type 5
0.035	0.38	Rgs5	NM_009063.2	regulator of G-protein signaling 5
0.030	0.38	Nova1	null	neuro-oncological ventral antigen 1
0.005	0.37	Atp6v1b2	NM_007509.2	ATPase, H transporting, V1 subunit B, isoform 2
0.024	0.37	Adh1	NM_007409.2	alcohol dehydrogenase 1 (class I)
0.018	0.37	Aldoa	NM_007438.3	aldolase 1, A isoform
0.002	0.37	Prcp	NM_028243.1	prolylcarboxypeptidase (angiotensinase C)
0.005	0.37	Eif4g1	NM_00100533.1	eukaryotic translation initiation factor 4, gamma 1 gene model 462, (NCBI) gene model 1073, (NCBI)
0.044	0.37	Gm462 1073	null	(NCBI)
0.035	0.37	Txnl4b	NM_175646.2	thioredoxin-like 4B
0.010	0.37	Aes	NM_010347.1	amino-terminal enhancer of split
0.008	0.37	Nfic	NM_026756.1	nuclear factor I/C
0.002	0.37	Cd4	NM_013488.1	CD4 antigen
0.038	0.37	Lst1	null	leukocyte specific transcript 1
0.035	0.37	Ptprcap	NM_016933.2	protein tyrosine phosphatase, receptor type
0.029	0.37	Fzd7	NM_008057.2	frizzled homolog 7 (Drosophila)
0.017	0.36	Rnf185	NM_145355.2	ring finger protein 185
0.004	0.36	Itpk1	NM_172584.1	inositol 1,3,4-triphosphate 5/6 kinase
0.019	0.36	Plin	NM_175640.1	perilipin
0.015	0.36	Cnih2	NM_009920.1	cornichon homolog 2 (Drosophila)

P	FC	Gene Name	Genbank	Description
0.031	0.36	Fbs1	null	fibrosin 1
0.030	0.36	Zc3h5	NM_172569.1	zinc finger CCCH type containing 5
0.024	0.36	Nab2	NM_008668.1	Ngfi-A binding protein 2
0.015	0.36	C1qtnf2	null	C1q and tumor necrosis factor related protein 2
0.013	0.36	Csl	NM_027945.1	citrate synthase like
0.007	0.36	Bmp7	NM_007557.2	bone morphogenetic protein 7
0.016	0.36	Plxnd1	null	plexin D1
0.020	0.36	Prox1	NM_008937.2	prospero-related homeobox 1
0.015	0.36	Smc1l1	NM_019710.1	SMC (structural maintenance of chromosomes 1)-like 1 (S. cerevisiae)
0.004	0.36	Klhl14	null	kelch-like 14 (Drosophila)
0.049	0.36	Folr2	NM_008035.1	folate receptor 2 (fetal)
0.011	0.35	Dlst	NM_030225.3	dihydrolipoamide S-succinyltransferase
0.007	0.35	Parp6	null	poly (ADP-ribose) polymerase family, member 6
0.004	0.35	Loxl1	NM_010729.2	lysyl oxidase-like 1
0.028	0.35	Wdr18	NM_175450.2	WD repeat domain 18
0.027	0.35	Kcnab1	null	potassium voltage-gated channel, shaker-related subfamily, beta member 1
0.018	0.35	Snapc2	null	small nuclear RNA activating complex, polypeptide 2
0.007	0.35	B4galt3	NM_020579.1	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 3
0.024	0.35	Zfp9	NM_011763.1	zinc finger protein 9
0.019	0.35	Ccdc27	null	coiled-coil domain containing 27
0.016	0.35	Sf3b2	NM_030109.1	splicing factor 3b, subunit 2
0.016	0.35	Cnr1	NM_007726.2	cannabinoid receptor 1 (brain)
0.035	0.35	Smyd5	NM_144918.1	SET and MYND domain containing 5
0.007	0.35	Tbl1x	NM_020601.1	transducin (beta)-like 1 X-linked solute carrier family 24 (sodium/potassium/calcium exchanger), member 6
0.032	0.35	Slc24a6	NM_133221.1	6
0.032	0.35	Zfp710	NM_175433.4	zinc finger protein 710
0.043	0.35	Sdccag3	NM_030155.1	serologically defined colon cancer antigen 3
0.036	0.35	Dbpht1	NM_019416.1	DNA binding protein with his-thr domain
0.016	0.35	Lysmd1	NM_028134.1	LysM, putative peptidoglycan-binding, domain containing 1
0.001	0.35	Zxda	null	zinc finger, X-linked, duplicated A
0.024	0.34	Apbb1	NM_009685.1	amyloid beta (A4) precursor protein-binding, family B, member 1
0.008	0.34	Gpi1	null	glucose phosphate isomerase 1
0.036	0.34	Zdhhc17	NM_172554.1	zinc finger, DHHC domain containing 17
0.004	0.34	Slc2a8	NM_019488.2	solute carrier family 2, (facilitated glucose transporter), member 8
0.028	0.34	Txnrd1	NM_015762.1	thioredoxin reductase 1
0.009	0.34	Atg7	NM_028835.1	autophagy-related 7 (yeast)
0.048	0.34	Tsga10ip	null	testis specific 10 interacting protein
0.029	0.34	Smarcal1	NM_018817.1	actin dependent regulator of chromatin, subfamily a-like 1
0.022	0.34	Fgfr1	NM_010206.1	fibroblast growth factor receptor 1
0.008	0.34	Vapb	NM_019806.3	vesicle-associated membrane protein, associated protein B and C
0.008	0.34	Mink1	NM_016713.1	misshapen-like kinase 1 (zebrafish)
0.023	0.34	Prkcz	null	protein kinase C, zeta
0.015	0.34	Pomgnt1	NM_026651.1	protein O-linked mannose beta1,2-N-acetylglucosaminyltransferase
0.008	0.34	Hipk3	NM_010434.1	homeodomain interacting protein kinase 3

P	FC	Gene Name	Genbank	Description
0.005	0.34	Tom1	NM_011622.1	target of myb1 homolog (chicken)
0.017	0.34	Cyp7a1	NM_007824.2	cytochrome P450, family 7, subfamily a, polypeptide 1
0.008	0.34	Hyal2	null	hyaluronidase 2
0.007	0.34	Zic2	NM_009574.2	Zic finger protein of the cerebellum 2
0.000	0.34	Gpr34	NM_011823.2	G protein-coupled receptor 34
0.018	0.34	Ankrd25	NM_145611.2	ankyrin repeat domain 25
0.027	0.34	Efna1	NM_010107.2	ephrin A1
0.003	0.34	Ash1l	NM_138679.3	ash1 (absent, small, or homeotic)-like (Drosophila)
0.026	0.33	Cpne2	NM_153507.2	copine II
0.014	0.33	Eif2s3x	NM_012010.2	eukaryotic translation initiation factor 2, subunit 3, structural gene X-linked
0.007	0.33	Brwd2	NM_172255.1	bromodomain and WD repeat domain containing 2
0.009	0.33	Slc26a6	NM_134420.2	solute carrier family 26, member 6
0.039	0.33	Usp35	null	ubiquitin specific peptidase 35
0.010	0.33	Pea15	NM_011063.1	phosphoprotein enriched in astrocytes 15
0.014	0.33	Arpp21	NM_033264.1	cyclic AMP-regulated phosphoprotein, 21
0.002	0.33	Lims2	NM_144862.1	LIM and senescent cell antigen like domains 2
0.030	0.33	Mttr3	NM_028860.1	myotubularin related protein 3
0.031	0.33	Map3k7ip2	NM_138667.1	mitogen-activated protein kinase kinase kinase 7 interacting protein 2
0.000	0.33	Cxcl12	NM_013655.3	chemokine (C-X-C motif) ligand 12
0.007	0.33	Ixl	NM_026042.2	intersex-like (Drosophila)
0.026	0.33	Bcl2	NM_009741.2	B-cell leukemia/lymphoma 2
0.021	0.33	Rfx5	NM_017395.1	regulatory factor X, 5 (influences HLA class II expression)
0.036	0.33	Ushbp1	NM_181418.2	Usher syndrome 1C binding protein 1
0.009	0.33	Zbtb7b	NM_009565.3	Vzinc finger and BTB domain containing 7B
0.008	0.33	Clcn4-2	NM_011334.2	chloride channel 4-2
0.017	0.32	Tuba7	NM_009449.2	tubulin, alpha 7
0.037	0.32	Spry4	NM_011898.2	sprouty homolog 4 (Drosophila)
0.001	0.32	Slco1a4	NM_030687.1	solute carrier organic anion transporter family, member 1a4
0.022	0.32	Cul7	null	cullin 7
0.018	0.32	Scn1b	NM_011322.2	sodium channel, voltage-gated, type I, beta
0.018	0.32	Nsmaf	NM_010945.1	neutral sphingomyelinase (N-SMase) activation associated factor
0.042	0.32	Kifc3	NM_010631.1	kinesin family member C3
0.030	0.32	Rhbd17	NM_146002.1	rhomboid, veinlet-like 7 (Drosophila)
0.032	0.32	Nrp2	NM_010939.1	neuropilin 2
0.029	0.32	Top3b	NM_011624.2	topoisomerase (DNA) III beta
0.011	0.32	Spata2	NM_170756.1	spermatogenesis associated 2
0.009	0.32	L1cam	NM_008478.2	L1 cell adhesion molecule
0.038	0.32	Nhlh2	NM_178777.2	nescient helix loop helix 2
0.010	0.32	Itsn1	NM_010587.1	intersectin 1 (SH3 domain protein 1A)
0.002	0.32	Hip1r	NM_145070.2	huntingtin interacting protein 1 related
0.043	0.32	Abtb1	NM_030251.1	ankyrin repeat and BTB (POZ) domain containing 1
0.039	0.32	Rbm3	NM_016809.2	RNA binding motif protein 3
0.017	0.32	Cpeb3	NM_198300.1	cytoplasmic polyadenylation element binding protein 3
0.035	0.32	Prkrip1	null	Prkr interacting protein 1 (IL11 inducible)
0.021	0.32	Sdc1	NM_011519.1	syndecan 1
0.019	0.31	Hipk1	NM_010432.1	homeodomain interacting protein kinase 1

P	FC	Gene Name	Genbank	Description
0.001	0.31	Grik4	NM_175481.2	glutamate receptor, ionotropic, kainate 4
0.021	0.31	Hcls1	NM_008225.1	hematopoietic cell specific Lyn substrate 1
0.009	0.31	Usf2	NM_011680.1	upstream transcription factor 2
0.038	0.31	Ahcy	null	S-adenosylhomocysteine hydrolase
0.010	0.31	Epb4.1l3	NM_013813.1	erythrocyte protein band 4.1-like 3
0.031	0.31	Acvrl1	NM_009612.1	activin A receptor, type II-like 1
				potassium voltage-gated channel, shaker-related
0.029	0.31	Kcnab3	NM_010599.2	subfamily, beta member 3
0.014	0.31	Gpr89	null	G protein-coupled receptor 89
0.004	0.31	Tollip	NM_023764.2	toll interacting protein
0.034	0.31	Myo10	NM_019472.1	myosin X
0.032	0.31	Osbp2	null	oxysterol binding protein 2
0.003	0.31	Zfp462	NM_172867.1	zinc finger protein 462
0.029	0.31	Tmc7	NM_172476.2	transmembrane channel-like gene family 7
0.024	0.31	Rage	null	renal tumor antigen
0.039	0.31	Gpr165	null	G protein-coupled receptor 165
0.037	0.31	Jarid1d	NM_011419.1	jumonji, AT rich interactive domain 1D (Rbp2 like)
0.037	0.31	Sash1	NM_175155.3	SAM and SH3 domain containing 1
0.039	0.30	Pak6	null	p21 (CDKN1A)-activated kinase 6
0.039	0.30	Sgk	null	serum/glucocorticoid regulated kinase
0.025	0.30	Exoc4	NM_009148.1	exocyst complex component 4
0.017	0.30	Rab5b	NM_177411.2	RAB5B, member RAS oncogene family
0.024	0.30	Peg10	NM_130877.1	paternally expressed 10
0.004	0.30	Cd82	NM_007656.1	CD82 antigen
0.005	0.30	Rasa3	NM_009025.1	RAS p21 protein activator 3
0.025	0.30	Spfh2	NM_153592.1	SPFH domain family, member 2
0.022	0.30	Parva	NM_020606.4	parvin, alpha
0.007	0.30	Trim44	null	tripartite motif-containing 44
0.007	0.30	Vps52	null	vacuolar protein sorting 52 (yeast)
0.014	0.30	Nptx2	NM_016789.2	neuronal pentraxin 2
0.048	0.30	Olf1013	NM_146762.1	olfactory receptor 1013
0.009	0.30	Ube2s	null	ubiquitin-conjugating enzyme E2S
0.017	0.30	Jph3	NM_020605.1	junctional protein 3
0.005	0.30	Eif5a	null	eukaryotic translation initiation factor 5A
0.046	0.29	Akt2	NM_007434.2	thymoma viral proto-oncogene 2
0.017	0.29	Gpx3	NM_008161.1	glutathione peroxidase 3
0.011	0.29	Ccdc50	NM_026202.1	coiled-coil domain containing 50
				ADP-ribosylation factor GTPase activating protein
0.001	0.29	Arfgap1	null	1
0.025	0.29	Tspan5	NM_019571.1	tetraspanin 5
0.027	0.29	Htra3	NM_030127.1	HtrA serine peptidase 3
0.017	0.29	Nrf1	NM_010938.2	nuclear respiratory factor 1
0.039	0.29	Olf870	NM_146904.1	olfactory receptor 870
0.025	0.29	Celsr2	NM_017392.2	cadherin EGF LAG seven-pass G-type receptor 2
0.025	0.29	Katnal1	NM_153572.1	katanin p60 subunit A-like 1
0.000	0.29	Hyou1	NM_021395.2	hypoxia up-regulated 1
0.041	0.29	Mtmr9	null	myotubularin related protein 9
0.012	0.29	Gpr27	NM_008158.1	G protein-coupled receptor 27
0.009	0.29	Vil1	NM_009509.1	villin 1
				ATP-binding cassette, sub-family G (WHITE),
0.038	0.28	Abcg4	NM_138955.2	member 4
0.049	0.28	Opa1	NM_133752.1	optic atrophy 1 homolog (human)
				solute carrier family 6 (neurotransmitter
0.021	0.28	Slc6a8	NM_133987.1	transporter, creatine), member 8
0.026	0.28	Stim1	NM_009287.2	stromal interaction molecule 1

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
0.010	0.28	Cacna1d	NM_028981.1	calcium channel, voltage-dependent, L type, alpha 1D subunit
0.049	0.28	Kdr	NM_010612.2	kinase insert domain protein receptor
0.005	0.28	Jup	NM_010593.1	junction plakoglobin
0.001	0.28	Tec	NM_013689.2	cytoplasmic tyrosine kinase, Dscr28C related (Drosophila)
0.004	0.28	Repin1	NM_175099.2	replication initiator 1
0.036	0.28	Cdyl	NM_009881.1	chromodomain protein, Y chromosome-like protein phosphatase 1, regulatory (inhibitor)
0.050	0.28	Ppp1r14b	null	subunit 14B
0.026	0.28	Oaz2	null	ornithine decarboxylase antizyme 2
0.014	0.28	Tek	NM_013690.1	endothelial-specific receptor tyrosine kinase
0.012	0.28	Fzd6	NM_008056.2	frizzled homolog 6 (Drosophila)
0.002	0.28	Gata2	NM_008090.3	GATA binding protein 2
0.003	0.28	Stard4	NM_133774.2	StAR-related lipid transfer (START) domain containing 4
0.032	0.27	Ap1s1	NM_007457.1	adaptor protein complex AP-1, sigma 1
0.025	0.27	Vamp1	NM_009496.2	vesicle-associated membrane protein 1
0.022	0.27	Cacng4	NM_019431.1	calcium channel, voltage-dependent, gamma subunit 4
0.014	0.27	Scarb1	NM_016741.1	scavenger receptor class B, member 1
0.014	0.27	Oaz2	NM_010952.2	ornithine decarboxylase antizyme 2
0.023	0.27	Vps39	NM_178851.1	vacuolar protein sorting 39 (yeast)
0.013	0.27	Zfp336	NM_028986.1	zinc finger protein 336
0.042	0.27	Csmd1	null	CUB and Sushi multiple domains 1
0.008	0.27	Nfkbil1	NM_010909.1	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1
0.041	0.27	Unc45a	NM_133952.1	unc-45 homolog A (C. elegans)
0.017	0.27	Slc27a4	NM_011989.1	solute carrier family 27 (fatty acid transporter), member 4
0.008	0.27	Mapk14	NM_011951.1	mitogen activated protein kinase 14
0.035	0.27	Map3k4	NM_011948.1	mitogen activated protein kinase kinase kinase 4
0.015	0.27	Ret	NM_009050.1	ret proto-oncogene
0.016	0.27	Btbd2	null	BTB (POZ) domain containing 2
0.038	0.27	Slc16a11	NM_153081.1	solute carrier family 16 (monocarboxylic acid transporters), member 11
0.031	0.27	Cdk9	NM_130860.1	cyclin-dependent kinase 9 (CDC2-related kinase)
0.023	0.27	Paqr7	NM_027995.1	progesterone and adipoQ receptor family member VII
0.021	0.27	Ube2v1	NM_023230.1	ubiquitin-conjugating enzyme E2 variant 1
0.013	0.26	Ddn	null	dendrin
0.012	0.26	Scamp2	NM_022813.2	secretory carrier membrane protein 2
0.012	0.26	Vps37b	NM_177876.2	vacuolar protein sorting 37B (yeast)
0.037	0.26	Pkd1	NM_013630.1	polycystic kidney disease 1 homolog
0.037	0.26	Ckap5	null	cytoskeleton associated protein 5
0.016	0.26	Wasf3	NM_145155.1	WAS protein family, member 3
0.013	0.26	Eif5a	NM_181582.2	eukaryotic translation initiation factor 5A
0.017	0.26	Rere	null	arginine glutamic acid dipeptide (RE) repeats
0.023	0.26	Nadk	NM_138671.1	NAD kinase
0.041	0.26	Vars2	NM_011690.2	valyl-tRNA synthetase 2
0.008	0.26	Ust	NM_177387.2	uronyl-2-sulfotransferase
0.031	0.26	Ifnb1	NM_010510.1	interferon beta 1, fibroblast
0.024	0.25	Slc1a3	NM_148938.2	solute carrier family 1 (glial high affinity glutamate transporter), member 3
0.004	0.25	Pigt	NM_133779.1	phosphatidylinositol glycan, class T
0.021	0.25	Apbb1ip	NM_019456.1	amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein

P	FC	Gene Name	Genbank	Description
0.004	0.25	Scnn1b	NM_011325.1	sodium channel, nonvoltage-gated 1 beta
0.011	0.25	Mapk8ip1	NM_011162.2	mitogen activated protein kinase 8 interacting protein 1
0.040	0.25	Hs3st2	NM_177575.2	heparan sulfate (glucosamine) 3-O-sulfotransferase 2
0.018	0.25	Nrxn3	null	neurexin III
0.041	0.24	Ids	NM_010498.1	iduronate 2-sulfatase
0.037	0.24	Sgsh	NM_018822.2	N-sulfoglucosamine sulfohydrolase (sulfamidase)
0.010	0.24	Mapre3	NM_133350.1	microtubule-associated protein, RP/EB family, member 3
0.009	0.24	Slc25a19	null	solute carrier family 25 (mitochondrial deoxynucleotide carrier), member 19
0.006	0.24	Gas7	NM_008088.1	growth arrest specific 7
0.007	0.24	Sf3b4	NM_153053.3	splicing factor 3b, subunit 4
0.022	0.24	Parn	NM_028761.1	poly(A)-specific ribonuclease (deadenylation nuclease)
0.007	0.24	Pard3	NM_033620.1	par-3 (partitioning defective 3) homolog (C. elegans)
0.020	0.24	Baiap3	null	BAI1-associated protein 3
0.021	0.24	Pik3r2	NM_008841.1	phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 2 (p85 beta)
0.039	0.24	Mjd	NM_029705.1	Machado-Joseph disease homolog (human)
0.034	0.24	Clstn3	NM_153508.2	calsyntenin 3
0.024	0.24	Clasp1	null	CLIP associating protein 1
0.030	0.23	Myst2	NM_177619.2	MYST histone acetyltransferase 2
0.020	0.23	Pfkm	NM_021514.2	phosphofructokinase, muscle
0.014	0.23	Mmab	NM_029956.2	methylmalonic aciduria (cobalamin deficiency) type B homolog (human)
0.041	0.23	Plk3	NM_013807.1	polo-like kinase 3 (Drosophila)
0.021	0.23	Abca2	NM_007379.1	ATP-binding cassette, sub-family A (ABC1), member 2
0.019	0.23	Kcnc4	NM_145922.1	potassium voltage gated channel, Shaw-related subfamily, member 4
0.017	0.23	Pex5	NM_008995.1	peroxisome biogenesis factor 5
0.041	0.23	Wdfy1	NM_027057.1	WD repeat and FYVE domain containing 1
0.030	0.23	Pcbp1	NM_011865.3	poly(rC) binding protein 1
0.028	0.23	Pabpc1	NM_008774.2	poly A binding protein, cytoplasmic 1
0.026	0.23	Ly6h	NM_011837.1	lymphocyte antigen 6 complex, locus H
0.028	0.23	Larp5	NM_172585.1	La ribonucleoprotein domain family, member 5
0.014	0.23	Gtf2i	NM_010365.1	general transcription factor II I
0.020	0.23	Cd2bp2	NM_027353.2	CD2 antigen (cytoplasmic tail) binding protein 2
0.049	0.22	Hp1bp3	NM_010470.1	heterochromatin protein 1, binding protein 3
0.024	0.22	Tnfrsf19l	NM_177073.3	tumor necrosis factor receptor superfamily, member 19-like
0.003	0.22	Camsap1	null	calmodulin regulated spectrin-associated protein 1
0.045	0.22	Ecel1	NM_021306.2	endothelin converting enzyme-like 1
0.028	0.22	Kcnj4	NM_008427.2	potassium inwardly-rectifying channel, subfamily J, member 4
0.023	0.22	Edn3	NM_007903.2	endothelin 3
0.038	0.22	Smad5	NM_008541.2	MAD homolog 5 (Drosophila)
0.029	0.22	Dao1	NM_010018.1	D-amino acid oxidase 1
0.012	0.22	Tgm4	NM_177911.2	transglutaminase 4 (prostate)
0.030	0.22	Vps33a	NM_029929.2	vacuolar protein sorting 33A (yeast)
0.016	0.22	Ptpn2	NM_011215.1	protein tyrosine phosphatase, receptor type, N polypeptide 2
0.025	0.22	Tbc1d14	NM_133910.2	TBC1 domain family, member 14
0.034	0.22	Rph3a	NM_011286.2	rabphilin 3A

P	FC	Gene Name	Genbank	Description
0.019	0.22	Lrrc4b	NM_198250.1	leucine rich repeat containing 4B
0.021	0.22	Hoxb3	NM_010458.1	homeo box B3
0.019	0.21	Ptk2	NM_007982.1	PTK2 protein tyrosine kinase 2
0.023	0.21	Ptprs	null	protein tyrosine phosphatase, receptor type, S
0.040	0.21	Itgb5	NM_010580.1	integrin beta 5
0.022	0.21	Sf4	NM_027481.1	splicing factor 4
0.018	0.21	Prkaca	NM_008854.3	protein kinase, cAMP dependent, catalytic, alpha potassium channel tetramerisation domain
0.016	0.21	Kctd17	null	containing 17
0.011	0.21	Sbf2	null	SET binding factor 2
				UDP-N-acetyl-alpha-D-galactosamine:polypeptide
0.021	0.21	Galnt6	NM_172451.1	N-acetylgalactosaminyltransferase 6
0.043	0.21	Acvr1b	null	activin A receptor, type 1B
0.045	0.21	Ap2a1	NM_007458.1	adaptor protein complex AP-2, alpha 1 subunit
0.014	0.21	Enc1	NM_007930.3	ectodermal-neural cortex 1
0.029	0.20	Ctnn	NM_007803.1	cortactin
				protein phosphatase 2 (formerly 2A), regulatory subunit B, alpha
0.019	0.20	Ppp2r3a	null	
0.030	0.20	Cnp1	NM_009923.1	cyclic nucleotide phosphodiesterase 1
0.022	0.20	Axin2	NM_015732.3	axin2
0.006	0.20	Cldn5	NM_013805.2	claudin 5
				kelch repeat and BTB (POZ) domain containing 11
0.019	0.20	Kbtbd11	null	
0.033	0.20	Ldb1	NM_010697.1	LIM domain binding 1
0.039	0.19	Itpkb	null	inositol 1,4,5-trisphosphate 3-kinase B
				signal transducing adaptor molecule (SH3 domain and ITAM motif) 2
0.015	0.19	Stam2	NM_019667.2	
0.008	0.19	Gm691	NM_198627.1	gene model 691, (NCBI)
				CDK2 (cyclin-dependent kinase 2)-associated protein 1
0.031	0.19	Cdk2ap1	null	
0.002	0.19	Nr3c2	null	nuclear receptor subfamily 3, group C, member 2
0.019	0.19	Arhgef16	null	Rho guanine nucleotide exchange factor (GEF) 16
0.020	0.19	Dpp6	NM_010075.1	dipeptidylpeptidase 6
0.032	0.19	Lzf	NM_133185.1	leucine zipper domain protein
0.049	0.19	Rtn4r	NM_022982.1	reticulon 4 receptor
0.045	0.19	Fthl17	NM_031261.1	ferritin, heavy polypeptide-like 17
0.027	0.19	Mgrn1	NM_029657.2	mahogunin, ring finger 1
0.006	0.19	Pisd	NM_177298.2	phosphatidylserine decarboxylase
0.030	0.19	Syt6	NM_018800.1	synaptotagmin VI
				guanine nucleotide binding protein, related sequence 1
0.036	0.18	Gna-rs1	NM_008136.1	
0.010	0.18	Gtf2h4	NM_010364.1	general transcription factor II H, polypeptide 4
0.035	0.18	Tie1	NM_011587.1	tyrosine kinase receptor 1
0.002	0.18	Glce	NM_033320.2	glucuronyl C5-epimerase
0.045	0.18	Sox3	NM_009237.1	SRY-box containing gene 3
				phosphatidylinositol (4,5) bisphosphate 5-phosphatase, A
0.009	0.18	Pib5pa	NM_172439.1	
0.030	0.18	ORF34	null	open reading frame 34
0.016	0.18	Usp20	NM_028846.2	ubiquitin specific peptidase 20
				achaete-scute complex homolog-like 2 (Drosophila)
0.041	0.18	Ascl2	NM_008554.1	ADP-ribosylation factor GTPase activating protein 1
0.033	0.18	Arfgap1	NM_145760.2	
0.026	0.18	Rasl10b	null	RAS-like, family 10, member B
0.032	0.17	Etv5	NM_023794.2	ets variant gene 5
0.020	0.17	Akt1	NM_009652.1	thymoma viral proto-oncogene 1

P	FC	Gene Name	Genbank	Description
0.041	0.17	Fgf22	null	fibroblast growth factor 22
0.038	0.17	Rfx1	NM_009055.2	regulatory factor X, 1 (influences HLA class II expression)
0.015	0.17	Apba2	NM_007461.1	amyloid beta (A4) precursor protein-binding, family A, member 2
0.034	0.17	Dnmt3a	NM_007872.2	DNA methyltransferase 3A
0.006	0.17	Accn4	NM_183022.1	amiloride-sensitive cation channel 4, pituitary
0.033	0.17	Mdm4	NM_008575.2	transformed mouse 3T3 cell double minute 4
0.024	0.16	Dctn1	NM_007835.1	dynactin 1
0.046	0.16	Scn8a	NM_011323.1	sodium channel, voltage-gated, type VIII, alpha degenerative spermatocyte homolog 2
0.023	0.16	Degs2	NM_027299.2	(Drosophila), lipid desaturase
0.022	0.16	Slc38a3	NM_023805.2	solute carrier family 38, member 3
0.021	0.16	Ankrd45	null	ankyrin repeat domain 45
0.009	0.16	Plekhg6	NM_198604.1	pleckstrin homology domain containing, family G member 6
0.016	0.15	Pcyox1	NM_025823.3	prenylcysteine oxidase 1
0.029	0.15	Gpr162	NM_013533.2	G protein-coupled receptor 162
0.022	0.15	Ppme1	NM_028292.1	protein phosphatase methylesterase 1
0.014	0.15	Mybbp1a	NM_016776.2	MYB binding protein (P160) 1a
0.012	0.15	Foxc2	NM_013519.1	forkhead box C2
0.006	0.15	Sox18	NM_009236.1	SRY-box containing gene 18
0.042	0.14	Nfe2l1	NM_008686.2	nuclear factor, erythroid derived 2,-like 1
0.032	0.14	Acox1	NM_015729.1	acyl-Coenzyme A oxidase 1, palmitoyl
0.017	0.14	Sbf1	null	SET binding factor 1
0.006	0.14	Hspa12b	NM_028306.2	heat shock protein 12B
0.035	0.14	Ppp5c	NM_011155.1	protein phosphatase 5, catalytic subunit
0.033	0.14	Phgdh	NM_016966.3	3-phosphoglycerate dehydrogenase
0.032	0.13	Ulk1	NM_009469.3	Unc-51 like kinase 1 (C. elegans)
0.037	0.12	Gm1052	null	gene model 1052, (NCBI)
0.046	0.12	Centa1	NM_172723.1	centaurin, alpha 1
0.021	0.12	Csf1r	null	colony stimulating factor 1 receptor
0.040	0.12	Sez6	NM_021286.2	seizure related gene 6
0.026	0.11	Gabbr1	NM_019439.2	gamma-aminobutyric acid (GABA-B) receptor, 1
0.018	0.11	Nr1d1	NM_145434.1	nuclear receptor subfamily 1, group D, member 1
0.046	0.11	Rfng	NM_009053.2	radical fringe gene homolog (Drosophila)
0.048	0.11	Man2b1	NM_010764.1	mannosidase 2, alpha B1
0.025	0.11	Gpam	NM_008149.2	glycerol-3-phosphate acyltransferase, mitochondrial
0.019	0.10	Gprasp1	NM_00100538	G protein-coupled receptor associated sorting protein 1
0.031	0.10	Wbp2	NM_016852.1	WW domain binding protein 2
0.050	0.09	Syp	NM_009305.1	synaptophysin
0.035	0.09	Prkce	NM_011104.1	protein kinase C, epsilon
0.022	0.09	Vamp2	NM_009497.2	vesicle-associated membrane protein 2
0.013	0.09	Elmo2	NM_080287.2	engulfment and cell motility 2, ced-12 homolog (C. elegans)
0.033	0.09	Brsk1	NM_00100392	BR serine/threonine kinase 1
0.040	0.08	Apba1	0.1	amyloid beta (A4) precursor protein binding, family A, member 1
0.029	0.08	Cacnb3	NM_177034.2	calcium channel, voltage-dependent, beta 3 subunit
0.023	0.08	Tyro3	null	TYRO3 protein tyrosine kinase 3
0.032	0.08	Nelf	NM_019392.1	nasal embryonic LHRH factor
0.029	0.07	Ppp2r4	NM_020276.2	protein phosphatase 2A, regulatory subunit B (PR
			NM_138748.2	

<i>P</i>	FC	Gene Name	Genbank	Description
0.019	0.06	Atp1a2	NM_178405.2	ATPase, Na transporting, alpha 2 polypeptide
0.031	0.04	Mfn2	NM_133201.1	mitofusin 2

P=p value; FC=fold change

Appendix II: Whole list of genes regulated at 48 h post LPS treatment in mouse brains.

<i>P</i>	FC	Gene Name	Genbank	Description
0.050	132.00	Saa3	NM_011315.2	serum amyloid A 3
0.037	4.63	Zc3h12d	NM_172785.1	zinc finger CCCH-type containing 12D
0.037	3.21	Ang4	NM_177544.2	angiogenin, ribonuclease A family, member 4
0.002	3.09	Nfic	NM_008688.2	nuclear factor I/C
0.035	3.01	Slp C4	NM_009780.1	sex-limited protein complement component 4 (within H-2S)
0.020	3.01	Abi3	NM_025659.1	ABI gene family, member 3
0.045	3.00	Tbl3	NM_145396.3	transducin (beta)-like 3
0.002	2.95	Rpl18a	NM_029751.1	Ribosomal protein L18A
0.024	2.94	Id1	NM_010495.1	inhibitor of DNA binding 1
0.016	2.67	Pycard	NM_023258.3	PYD and CARD domain containing
0.011	2.64	Trim47	NM_172570.2	tripartite motif protein 47
0.044	2.52	Ptpn6	NM_013545.1	protein tyrosine phosphatase, non-receptor type 6
0.049	2.48	Fzd2	null	frizzled homolog 2 (Drosophila)
0.024	2.46	D10Bwg136 4e	NM_145421.1	DNA segment, Chr 10, Brigham & Women's Genetics 1364 expressed
0.013	2.44	Msln	NM_018857.1	mesothelin
0.023	2.44	Ier3	NM_133662.1	immediate early response 3
0.033	2.43	Slc7a4	NM_144852.1	solute carrier family 7 (cationic amino acid transporter, y+ system), member 4
0.005	2.43	Rpl13	null	ribosomal protein L13
0.002	2.43	Syne1	NM_022027.1	synaptic nuclear envelope 1
0.018	2.39	Nudt16l1	NM_025839.1	nudix (nucleoside diphosphate linked moiety X)-type motif 16-like 1
0.009	2.37	Abca3	null	ATP-binding cassette, sub-family A (ABC1), member 3
0.049	2.36	Cdc73	null	Vcell division cycle 73, Paf1/RNA polymerase II complex component, homolog
0.008	2.35	Rpsa	null	ribosomal protein SA
0.014	2.33	Evc2	NM_145920.2	Ellis van Creveld syndrome 2 homolog (human)
0.048	2.31	Ddx55	NM_026409.1	DEAD (Asp-Glu-Ala-Asp) box polypeptide 55
0.041	2.31	Rasgef1c	null	RasGEF domain family, member 1C
0.014	2.30	Kif19a	null	kinesin family member 19A
0.013	2.30	Cxx1c	NM_028375.2	CAAX box 1 homolog C (human)
0.033	2.26	Cxcl9	NM_008599.1	chemokine (C-X-C motif) ligand 9
0.024	2.24	Alg5	NM_025442.1	asparagine-linked glycosylation 5 homolog
0.009	2.23	Pes1	NM_022889.2	pescadillo homolog 1, containing BRCT domain (zebrafish)
0.004	2.23	Comm4	NM_025417.1	COMM domain containing 4
0.038	2.22	Emp3	NM_010129.1	epithelial membrane protein 3
0.005	2.21	H2-Q8	NM_023124.2	histocompatibility 2, Q region locus 8
0.014	2.20	Id4	NM_031166.1	inhibitor of DNA binding 4
0.022	2.20	Trex1	NM_011637.6	three prime repair exonuclease 1
0.004	2.20	Rag1ap1	NM_009057.1	recombination activating gene 1 activating protein 1
0.041	2.19	Egfl9	NM_207666.1	EGF-like-domain, multiple 9
0.012	2.19	Cep2	null	centrosomal protein 2

P	FC	Gene Name	Genbank	Description
0.001	2.19	Drd1ip	NM_026769.2	dopamine receptor D1 interacting protein
0.010	2.17	Ccl27	NM_011336.1	chemokine (C-C motif) ligand 27
0.047	2.17	Sla	NM_009192.1	src-like adaptor
0.027	2.16	Pigp	null	phosphatidylinositol glycan, class P
0.039	2.15	Fcgr3	NM_010188.2	Fc receptor, IgG, low affinity III
0.032	2.15	Scyl1	NM_023912.1	SCY1-like 1 (<i>S. cerevisiae</i>)
0.003	2.13	Hspb1	null	heat shock protein 1
0.041	2.11	Rab3b	NM_023537.4	RAB3B, member RAS oncogene family
0.030	2.10	H2-K1	NM_019909.1	histocompatibility 2, K1, K region
0.006	2.08	Plid4	NM_178911.2	phospholipase D family, member 4
0.043	2.08	Rps27	null	ribosomal protein S27
0.050	2.08	Oas3	NM_145226.1	2'-5' oligoadenylate synthetase 3
0.013	2.07	BolA1	NM_026975.1	bolA-like 1 (<i>E. coli</i>)
0.007	2.05	Hmgb2	null	high mobility group box 2
0.006	2.04	Rpsa	null	ribosomal protein SA
0.016	2.03	Slit3	null	slit homolog 3 (<i>Drosophila</i>)
0.012	2.03	D11Lgp1e	NM_031871.1	DNA segment, Chr 11, Lothar Hennighausen 1, expressed
0.013	2.03	D2Wsu81e	NM_172660.1	DNA segment, Chr 2, Wayne State University 81, expressed
0.018	2.01	Smn1	NM_011420.1	survival motor neuron 1
0.047	2.01	Timeless	NM_011589.1	timeless homolog (<i>Drosophila</i>)
0.019	2.00	Slc25a28	NM_145156.1	solute carrier family 25, member 28
0.024	2.00	Dennd3	null	DENN/MADD domain containing 3
0.025	1.99	D14Ert668e	NM_199015.1	DNA segment, Chr 14, ERATO Doi 668, expressed
0.038	1.96	Unc45b	NM_178680.3	unc-45 homolog B (<i>C. elegans</i>)
0.038	1.96	Tmem86b	null	transmembrane protein 86B
0.025	1.96	Arfp2	NM_029802.2	ADP-ribosylation factor interacting protein 2
0.024	1.95	Atp5j	NM_016755.2	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit F
0.008	1.95	Avpi1	NM_027106.1	arginine vasopressin-induced 1
0.030	1.93	Lrp10	NM_022993.3	low-density lipoprotein receptor-related protein 10
0.043	1.93	Snrp70	NM_009224.2	U1 small nuclear ribonucleoprotein polypeptide A
0.007	1.93	Chchd2	NM_183088.1	coiled-coil-helix-coiled-coil-helix domain containing 2
0.023	1.93	Malat1	null	metastasis associated lung adenocarcinoma transcript 1
0.017	1.92	Fkbp1a	NM_008019.1	FK506 binding protein 1a
0.045	1.89	Clec2l	null	C-type lectin domain family, member I
0.002	1.87	Cd9	NM_007657.3	CD9 antigen
0.011	1.86	Camta2	null	calmodulin binding transcription activator 2
0.033	1.86	Ccl9	NM_011338.1	chemokine (C-C motif) ligand 9
0.045	1.86	Slc26a8	NM_146076.1	solute carrier family 26, member 8
0.003	1.86	Pik3cd	null	phosphatidylinositol 3-kinase catalytic delta polypeptide
0.020	1.85	Robo4	NM_028783.2	roundabout homolog 4 (<i>Drosophila</i>)
0.003	1.84	Ndufb10	NM_026684.1	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10
0.016	1.84	Adck4	NM_133770.1	aarF domain containing kinase 4
0.023	1.84	Slc9a3r2	NM_023449.1	solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulator 2
0.000	1.84	Eya1	NM_010164.1	eyes absent 1 homolog (<i>Drosophila</i>)
0.011	1.83	Ndufs7	NM_029272.1	NADH dehydrogenase (ubiquinone) Fe-S protein 7
0.011	1.83	Dak	NM_145496.1	dihydroxyacetone kinase 2 homolog (yeast)

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
0.043	1.82	Rras	NM_009101.1	Harvey rat sarcoma oncogene, subgroup R
0.028	1.82	Isyna1	NM_023627.1	myo-inositol 1-phosphate synthase A1
0.019	1.82	Sult2b1	NM_017465.1	sulfotransferase family, cytosolic, 2B, member 1
0.033	1.81	Crispld1	NM_031402.1	cysteine-rich secretory protein LCCL domain containing 1
0.026	1.81	Mafb	NM_010658.2	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B (avian)
0.021	1.80	Prr6	null	proline-rich polypeptide 6
0.049	1.80	Safb2	null	scaffold attachment factor B2
0.002	1.80	Atp5g2	NM_026468.1	ATP synthase, mitochondrial F0 complex, subunit c (subunit 9), isoform 2
0.017	1.79	Apcdd1	NM_133237.1	adenomatosis polyposis coli down-regulated 1
0.029	1.79	Npas3	NM_013780.1	neuronal PAS domain protein 3
0.040	1.79	Ppic	NM_008908.1	peptidylprolyl isomerase C
0.011	1.78	Hpcal1	NM_016677.1	hippocalcin-like 1
0.037	1.78	Gpatc3	NM_172876.1	G patch domain containing 3
0.015	1.78	Nfix	NM_010906.1	nuclear factor I/X
0.035	1.78	Prodh	NM_011172.1	proline dehydrogenase
0.023	1.78	Rpl37a	NM_009084.2	ribosomal protein L37a
0.047	1.77	Akp2	NM_007431.1	alkaline phosphatase 2, liver
0.047	1.77	Tyrobp	null	TYRO protein tyrosine kinase binding protein
0.045	1.77	Caskin2	NM_080643.2	cask-interacting protein 2
0.016	1.77	H2-D4	NM_008200.1	histocompatibility 2, D region locus 4
0.050	1.77	Sfrp5	NM_018780.2	secreted frizzled-related sequence protein 5
0.012	1.76	Tmu	NM_028063.1	tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase
0.008	1.74	Itga6	NM_008397.2	integrin alpha 6
0.006	1.74	Gm101	null	gene model 101, (NCBI)
0.012	1.74	Ifrg15 Tor1a ip1	NM_144791.1	interferon alpha responsive gene torsin A interacting protein 1
0.025	1.74	D10Wsu52e	NM_145422.1	DNA segment, Chr 10, Wayne State University 52, expressed
0.031	1.74	Itih2	NM_010582.1	inter-alpha trypsin inhibitor, heavy chain 2
0.017	1.73	Sparc	NM_009242.1	secreted acidic cysteine rich glycoprotein
0.016	1.72	Ttyh3	NM_175274.2	tweety homolog 3 (Drosophila)
0.039	1.72	Pfn1	null	profilin 1
0.024	1.71	Gal3st1	NM_016922.2	galactose-3-O-sulfotransferase 1
0.006	1.70	Kars	NM_053092.1	lysyl-tRNA synthetase
0.025	1.70	Tex264	NM_011573.1	testis expressed gene 264
0.007	1.69	Blmk	NM_008528.3	B-cell linker
0.001	1.69	Brd3	NM_023336.2	bromodomain containing 3
0.025	1.69	Eng	NM_007932.1	endoglin
0.008	1.68	Naprt1	NM_172607.2	nicotinate phosphoribosyltransferase domain containing 1
0.047	1.68	Phkg1	NM_011079.1	phosphorylase kinase gamma 1
0.005	1.67	D15Erd785 e	NM_025795.2	DNA segment, Chr 15, ERATO Doi 785, expressed
0.023	1.67	Ephb4	NM_010144.4	Eph receptor B4
0.022	1.67	Mrpl12	null	mitochondrial ribosomal protein L12
0.030	1.67	Spint2	NM_011464.1	serine protease inhibitor, Kunitz type 2
0.029	1.66	Txn14	NM_025299.1	thioredoxin-like 4
0.046	1.65	Atp6v0c	null	ATPase, H+ transporting, V0 subunit C
0.045	1.65	Aatf	null	apoptosis antagonizing transcription factor
0.046	1.64	Nudt9	null	nudix (nucleoside diphosphate linked moiety X)-type motif 9
0.006	1.64	Psme4	null	proteasome (prosome, macropain) activator

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
				subunit 4
0.023	1.63	Ube1l	NM_023738.2	ubiquitin-activating enzyme E1-like
0.004	1.62	Abhd8	NM_022419.1	abhydrolase domain containing 8
0.020	1.62	Pgk1	null	phosphoglycerate kinase 1
0.006	1.62	Atp6v0c	null	ATPase, H ⁺ transporting, V0 subunit C
0.024	1.61	Bmyc	null	brain expressed myelocytomatosis oncogene
0.019	1.60	Por	NM_008898.1	P450 (cytochrome) oxidoreductase
0.005	1.59	Ccnt1	NM_009833.1	cyclin T1
0.031	1.59	Rps29	NM_009093.1	ribosomal protein S29
				pleckstrin homology, Sec7 and coiled/coil domains 4
0.047	1.59	Pscd4	null	myeloid cell nuclear differentiation antigen interferon activated gene 205
0.047	1.56	Mnda Ifi205	NM_172648.2	aldolase 1, A isoform, pseudogene 2
0.039	1.56	Aldoa-ps2	null	thyroid hormone receptor associated protein 4
0.043	1.56	Thrap4	NM_011869.1	isocitrate dehydrogenase 2 (NADP+), mitochondrial
0.041	1.55	Idh2	NM_173011.1	HEAT-like (PBS lyase) repeat containing 1
0.004	1.54	Hlrc1	NM_133964.1	solute carrier family 30 (zinc transporter), member 7
0.010	1.53	Slc30a7	NM_023214.3	plexin B1
0.034	1.52	Plxnb1	NM_172775.1	sphingosine kinase 1
0.008	1.52	Sphk1	NM_011451.1	protocadherin 1
0.016	1.51	Pcdh1	NM_029357.2	cathepsin S
0.045	1.51	Ctss	NM_021281.1	WD repeat domain 77
0.012	1.49	Wdr77	NM_027432.3	symplesin
0.019	1.49	Sympk	null	pleckstrin homology domain containing, family F member 1
0.035	1.48	Plekhf1	NM_024413.1	ATP-binding cassette, sub-family C (CFTR/MRP), member 3
0.045	1.47	Abcc3	null	solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 25
0.015	1.46	Slc25a25	NM_146118.2	c-src tyrosine kinase
0.047	1.45	Csk	NM_007783.2	low density lipoprotein receptor-related protein 1
0.040	1.44	Lrp1	NM_008512.1	ankyrin repeat domain 35
0.018	1.43	Ankrd35	null	G protein-coupled receptor 124
0.035	1.43	Gpr124	NM_054044.1	acyl-Coenzyme A binding domain containing 3
0.022	1.42	Acbd3	NM_133225.1	SRY-box containing gene 9
0.022	1.42	Sox9	NM_011448.2	opioid receptor-like 1
0.004	1.35	Oprl1	NM_011012.2	telomerase associated protein 1
0.043	1.33	Tep1	NM_009351.1	deltex 3-like (Drosophila)
0.035	1.31	Dtx3l	null	glioma tumor suppressor candidate region gene 2
0.046	1.30	Gltscr2	NM_133831.1	zinc finger, matrin type 5
0.010	1.13	Zmat5	NM_026015.1	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 5
0.043	0.83	B3galt5	NM_033149.2	RAN binding protein 6
0.036	0.83	Ranbp6	NM_177721.3	5-hydroxytryptamine (serotonin) receptor 2C
0.018	0.76	Htr2c	NM_008312.2	BTB and CNC homology 1
0.038	0.76	Bach1	null	hydroxysteroid (17-beta) dehydrogenase 4
0.023	0.75	Hsd17b4	NM_008292.2	thioredoxin domain containing 4 (endoplasmic reticulum)
0.048	0.75	Txndc4	NM_029572.1	CCR4 carbon catabolite repression 4-like (S. cerevisiae)
0.020	0.74	Ccrn4l	NM_009834.1	PHD finger protein 10
0.045	0.74	Phf10	NM_024250.3	acetyl-Coenzyme A carboxylase alpha
0.029	0.73	Acaca	null	WD repeat domain 54
0.029	0.72	Wdr54	NM_023790.1	

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
0.021	0.71	Zfp516	NM_183033.1	zinc finger protein 516
0.039	0.71	Galk2	NM_175154.2	galactokinase 2
0.031	0.71	Pdia4	NM_009787.1	protein disulfide isomerase associated 4
0.030	0.71	St6galnac4	NM_011373.2	ST6
0.047	0.70	Zfp108	NM_018791.1	zinc finger protein 108
0.025	0.70	Thbs3	NM_013691.1	thrombospondin 3
0.039	0.70	Mod1	NM_008615.1	malic enzyme, supernatant
0.026	0.70	Atad3a	NM_179203.1	ATPase family, AAA domain containing 3A
0.048	0.69	Klhdc3	NM_027910.1	kelch domain containing 3
0.022	0.69	Trim59	NM_025863.2	tripartite motif-containing 59
0.008	0.68	Stx3	NM_011502.1	syntaxin 3
0.009	0.68	Plekhh1	null	pleckstrin homology domain containing, family H member 1
0.021	0.68	Stk24	null	serine/threonine kinase 24 (STE20 homolog, yeast)
0.037	0.68	Apbb1ip	NM_019456.1	amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein
0.031	0.68	H3f3b	NM_008211.2	H3 histone, family 3B
0.024	0.68	Pgbd5	null	piggyBac transposable element derived 5
0.050	0.68	Pak2	NM_177326.2	p21 (CDKN1A)-activated kinase 2
0.040	0.67	Ilf2	NM_026374.2	interleukin enhancer binding factor 2
0.034	0.67	Kdelr2	NM_025841.1	KDEL endoplasmic reticulum protein retention receptor 2
0.028	0.67	Cdc42se2	NM_178626.1	CDC42 small effector 2
0.006	0.67	Elovl7	NM_029001.2	ELOVL family member 7, elongation of long chain fatty acids (yeast)
0.018	0.67	Rab15	null	RAB, member of RAS oncogene family-like 5
0.048	0.67	Cdkn2b	NM_007670.2	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)
0.041	0.67	Traf2	NM_009422.1	Tnf receptor-associated factor 2
0.046	0.66	Gm2a	NM_010299.2	GM2 ganglioside activator protein
0.009	0.66	Pitpna	NM_008850.1	phosphatidylinositol transfer protein, alpha
0.039	0.66	Sav1	NM_022028.1	salvador homolog 1 (Drosophila)
0.014	0.66	Slmap	NM_032008.3	sarcolemma associated protein
0.036	0.66	Gpr62	null	G protein-coupled receptor 62
0.020	0.66	Gm672	NM_201354.1	gene model 672, (NCBI)
0.007	0.66	Lhx2	NM_010710.2	LIM homeobox protein 2
0.006	0.66	Chchd2	null	coiled-coil-helix-coiled-coil-helix domain containing 2
0.021	0.65	Sema5a	null	sema domain, seven thrombospondin repeats, 5A
0.004	0.65	Cul4a	NM_146207.1	cullin 4A
0.038	0.65	Hist2h2be	NM_175666.1	histone 2, H2be
0.041	0.65	Agbl2	null	ATP/GTP binding protein-like 2
0.021	0.65	Gm740	null	gene model 740, (NCBI)
0.014	0.64	Baalc	NM_080640.3	brain and acute leukemia, cytoplasmic cleavage and polyadenylation specific factor 3-like
0.021	0.64	Cpsf3l	NM_028020.2	SDA1 domain containing 1
0.033	0.64	Sdad1	NM_172713.1	SDA1 domain containing 1
0.040	0.64	Zfp654	NM_028059.1	zinc finger protein 654
0.027	0.64	Chac1	NM_026929.2	ChaC, cation transport regulator-like 1 (E. coli)
0.007	0.64	Trap1	NM_026508.1	TNF receptor-associated protein 1
0.041	0.63	Rab11fip1	NM_029423.1	RAB11 family interacting protein 1 (class I)
0.003	0.63	Nudcd3	NM_173748.2	NudC domain containing 3
0.028	0.63	Pqlc2	NM_145384.1	PQ loop repeat containing 2
0.008	0.63	Rxra	null	retinoid X receptor alpha
0.031	0.63	Syt11	NM_018804.2	synaptotagmin XI

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
0.009	0.63	Mid1ip1	NM_026524.2	Mid1 interacting protein 1 (gastrulation specific G12-like (zebrafish))
0.009	0.63	Kif3c	NM_008445.1	kinesin family member 3C
0.003	0.62	Usp6nl	NM_181399.2	USP6 N-terminal like
0.015	0.62	Mtmr12	NM_172958.2	Vmyotubularin related protein 12
0.023	0.62	Cml4	null	camello-like 4
0.026	0.62	Slc16a1	NM_009196.2	solute carrier family 16 (monocarboxylic acid transporters), member 1
0.017	0.62	Gabpa	NM_008065.2	GA repeat binding protein, alpha SH3 domain binding glutamic acid-rich protein like 2
0.036	0.62	Sh3bgrl2	NM_172507.2	
0.038	0.62	Rcn3	NM_026555.1	reticulocalbin 3, EF-hand calcium binding domain
0.020	0.61	Foxj3	NM_172699.2	forkhead box J3
0.040	0.61	Lef1	NM_010703.2	lymphoid enhancer binding factor 1
0.037	0.61	B4galt6	NM_019737.1	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 6
0.025	0.61	Rab5c	NM_024456.1	RAB5C, member RAS oncogene family
0.017	0.61	Snap29	NM_023348.3	synaptosomal-associated protein
0.029	0.61	Vps37c	NM_181403.2	vacuolar protein sorting 37C (yeast)
0.040	0.61	Mdh1	NM_008618.2	malate dehydrogenase 1, NAD (soluble)
0.026	0.61	Galnt1	NM_013814.2	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1
0.033	0.60	Gtf2a1	NM_175335.1	general transcription factor II A, 1
0.035	0.60	Klhdcl1	NM_178253.2	kelch domain containing 1
0.047	0.60	Set	NM_023871.2	SET translocation
0.017	0.59	Atp5k	null	ATP synthase, H ⁺ transporting, mitochondrial F1F0 complex, subunit e
0.027	0.59	Lemd3	null	LEM domain containing 3
0.048	0.59	Ccdc21	NM_144527.2	coiled-coil domain containing 21
0.017	0.59	Pemt	NM_008819.2	phosphatidylethanolamine N-methyltransferase
0.018	0.59	Gsk3b	NM_019827.3	glycogen synthase kinase 3 beta
0.049	0.59	Ptp4a1	NM_011200.1	protein tyrosine phosphatase 4a1
0.005	0.59	Wfdc1	NM_023395.1	WAP four-disulfide core domain 1
0.015	0.59	Ptrf	NM_008986.1	polymerase I and transcript release factor bromodomain and WD repeat domain containing 2
0.017	0.59	Brwd2	NM_172255.1	
0.005	0.58	Fstl4	NM_177059.3	follicle-stimulating-like 4
0.007	0.58	Zfp655	NM_028298.2	zinc finger protein 655
0.033	0.58	Fabp7	NM_021272.2	fatty acid binding protein 7, brain
0.046	0.57	Sms	null	spermine synthase
0.020	0.57	Oas1h	NM_145228.1	2'-5' oligoadenylate synthetase 1H
0.020	0.57	Dand5	NM_201227.2	DAN domain family, member 5
0.003	0.57	Metap1	null	methionyl aminopeptidase 1
0.003	0.57	Lgr4	null	leucine-rich repeat-containing G protein-coupled receptor 4
0.044	0.57	Tspyl3	NM_198617.1	TSPY-like 3
0.044	0.56	Sc4mol	NM_025436.1	sterol-C4-methyl oxidase-like
0.049	0.56	Lonrf1	null	LON peptidase N-terminal domain and ring finger 1
0.009	0.56	Ly6g6c	NM_023463.3	lymphocyte antigen 6 complex, locus G6C
0.038	0.56	Hmgcs1	NM_145942.2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1
0.004	0.56	Tnfaip8l1	NM_025566.2	tumor necrosis factor, alpha-induced protein 8-like 1
0.000	0.55	Psap	NM_011179.2	prosaposin
0.012	0.55	Txndc9	NM_172054.2	thioredoxin domain containing 9

P	FC	Gene Name	Genbank	Description
0.019	0.55	Creg2	NM_170597.3	cellular repressor of E1A-stimulated genes 2
0.011	0.55	Pcdh18	NM_130448.2	protocadherin 18
0.008	0.55	Dll1	NM_007865.2	delta-like 1 (Drosophila)
0.025	0.55	Cd80	NM_009855.1	CD80 antigen
0.031	0.54	Prkcñ	NM_029239.2	protein kinase C, nu
0.033	0.54	Fes	NM_010194.1	feline sarcoma oncogene
0.037	0.54	H2afz	null	H2A histone family, member Z
0.034	0.54	Grpel1	NM_024478.2	GrpE-like 1, mitochondrial
0.033	0.53	Ctps2	NM_018737.2	cytidine 5'-triphosphate synthase 2
0.028	0.53	Chrm3	NM_033269.2	cholinergic receptor, muscarinic 3, cardiac
0.020	0.53	Frs3	NM_144939.1	fibroblast growth factor receptor substrate 3
0.002	0.53	Tcta	NM_133986.1	T-cell leukemia translocation altered gene
0.032	0.53	Gpatc2	NM_026367.3	G patch domain containing 2
0.002	0.53	Klhdcc4	NM_145605.1	kelch domain containing 4
				StAR-related lipid transfer (START) domain
0.015	0.52	Stard4	NM_133774.2	containing 4
0.014	0.52	Sectm1	NM_026907.2	secreted and transmembrane 1
0.002	0.52	Pou3f4	NM_008901.1	POU domain, class 3, transcription factor 4
0.011	0.52	Csmd1	NM_053171.1	CUB and Sushi multiple domains 1
				proteasome (prosome, macropain) 26S subunit,
0.000	0.52	Psmc5	NM_080554.1	non-ATPase, 5
0.014	0.51	Mov10	NM_008619.1	Moloney leukemia virus 10
0.017	0.51	Tph2	NM_173391.1	tryptophan hydroxylase 2
0.008	0.51	Dcbld1	NM_025705.1	discoidin, CUB and LCCL domain containing 1
0.029	0.50	Col5a3	NM_016919.1	procollagen, type V, alpha 3
0.015	0.50	Wdr36	NM_144863.2	WD repeat domain 36
0.037	0.50	Gpr89	null	G protein-coupled receptor 89
			NM_00101232	extracellular matrix protein 2, female organ and
0.027	0.50	Ecm2	4.1	adipocyte specific
0.012	0.50	Lta4h	NM_008517.1	leukotriene A4 hydrolase
0.024	0.49	Alk	NM_007439.1	anaplastic lymphoma kinase
0.023	0.49	Aloxe3	NM_011786.1	arachidonate lipoxygenase 3
0.044	0.49	Ccnj	NM_172839.1	cyclin J
				solute carrier family 23 (nucleobase transporters),
0.031	0.49	Slc23a3	NM_194333.2	member 3
0.025	0.49	Rab5b	NM_011229.1	RAB5B, member RAS oncogene family
0.000	0.49	Fgf20	NM_030610.1	fibroblast growth factor 20
0.002	0.49	H2-M3	NM_013819.1	histocompatibility 2, M region locus 3
0.007	0.49	Clec2d	NM_053109.1	C-type lectin domain family 2, member d
0.003	0.49	Ptptra	NM_008980.1	protein tyrosine phosphatase, receptor type, A
				potassium inwardly-rectifying channel, subfamily
0.046	0.49	Kcnj9	NM_008429.1	J, member 9
0.010	0.48	Ifnb1	NM_010510.1	interferon beta 1, fibroblast
0.050	0.48	Rdh9	NM_153133.1	retinol dehydrogenase 9
			NM_00100180	
0.022	0.48	Zfp36l2	6.1	zinc finger protein 36, C3H type-like 2
0.030	0.48	Rhoq	NM_145491.2	ras homolog gene family, member Q
				NK6 transcription factor related, locus 1
0.026	0.48	Nkx6-1	NM_144955.1	(Drosophila)
0.005	0.47	Bmpr1b	NM_007560.3	bone morphogenetic protein receptor, type 1B
0.000	0.47	Csdc2	NM_145473.1	cold shock domain containing C2, RNA binding
0.028	0.47	Cd5	NM_007650.2	CD5 antigen
0.016	0.46	Dlx1	null	distal-less homeobox 1
0.036	0.46	Prph1	NM_013639.1	peripherin 1
				solute carrier family 22 (organic cation
0.034	0.46	Slc22a4	null	transporter), member 4

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
0.041	0.46	Plk4	NM_011495.1	polo-like kinase 4 (Drosophila)
0.017	0.46	Sh2d1b	NM_012009.2	SH2 domain protein 1B
0.007	0.46	Speer2	null	spermatogenesis associated glutamate (E)-rich protein 2
0.029	0.46	Zfp51	NM_009558.2	zinc finger protein 51
0.003	0.46	Aldh1a7	NM_011921.1	aldehyde dehydrogenase family 1, subfamily A7
0.001	0.45	Rhpn1	NM_008164.1	rhophilin, Rho GTPase binding protein 1
0.006	0.45	Olfr438	NM_146654.1	olfactory receptor 438
0.004	0.44	Mycs	NM_010850.1	myc-like oncogene, s-myc protein
0.030	0.44	Tnnc1	NM_009393.1	troponin C, cardiac/slow skeletal protein (peptidyl-prolyl cis/trans isomerase)
0.030	0.44	Pin1	NM_023371.2	NIMA-interacting 1
0.032	0.43	Plp2	NM_019755.2	proteolipid protein 2
0.027	0.43	Btdb7	NM_172806.2	BTB (POZ) domain containing 7
			NM_00101175	
0.008	0.43	Olfr671 675	5.1	olfactory receptor 671 olfactory receptor 675
0.009	0.43	Ss18	null	synovial sarcoma translocation, Chromosome 18
0.019	0.42	Olfr536	NM_146520.2	olfactory receptor 536
0.009	0.42	Igh-VJ558	null	immunoglobulin heavy chain (J558 family)
0.031	0.42	Mef2d	NM_133665.1	myocyte enhancer factor 2D
0.035	0.42	Hmga2-ps1	null	high mobility group AT-hook 2, pseudogene 1
0.026	0.42	Ptpcrap	NM_016933.2	protein tyrosine phosphatase, C polypeptide-associated protein
0.000	0.41	Zfp98	NM_016793.1	zinc finger protein 98
0.018	0.41	Nr4a3	NM_015743.1	nuclear receptor subfamily 4, group A, member 3
0.005	0.41	Acad10	null	acyl-Coenzyme A dehydrogenase family, member 10
0.026	0.41	Hes2	NM_008236.1	hairy and enhancer of split 2 (Drosophila)
0.036	0.40	Cks1b	null	CDC28 protein kinase 1b
0.027	0.40	Ryr1	NM_009109.1	ryanodine receptor 1, skeletal muscle
0.013	0.40	Crygc	null	crystallin, gamma C
0.012	0.39	Rbbp7	NM_009031.2	retinoblastoma binding protein 7
0.048	0.39	Zfp71-rs1	NM_145622.1	zinc finger protein 71, related sequence 1
			NM_00100542	
0.031	0.39	Gm347	0.1	gene model 347, (NCBI)
0.018	0.39	Krt2-18 25	NM_016879.2	keratin complex 2, basic, gene 18
0.013	0.38	Edn3	NM_007903.2	endothelin 3
0.024	0.38	Ndst1	NM_008306.2	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1
0.019	0.38	Hmgb1l	null	high mobility group box 1-like
0.016	0.38	Slc39a1	NM_013901.1	solute carrier family 39 (zinc transporter), member 1
0.006	0.38	Edg6	NM_010102.1	endothelial differentiation, G-protein-coupled receptor 6
0.004	0.38	Adarb1	NM_130895.1	adenosine deaminase, RNA-specific, B1
0.000	0.38	Iqcd	NM_029408.1	IQ motif containing D
			NM_00101185	
0.045	0.38	Olfr605	4.1	olfactory receptor 605
0.019	0.37	Kif7	null	kinesin family member 7
0.017	0.37	Wwp2	NM_025830.3	WW domain containing E3 ubiquitin protein ligase 2
0.027	0.37	Acp1	NM_021330.2	acid phosphatase 1, soluble
0.021	0.37	Nck1	null	non-catalytic region of tyrosine kinase adaptor protein 1
0.013	0.37	Tpte2	NM_016715.2	transmembrane phosphoinositide 3-phosphatase and tensin homolog 2
0.019	0.37	Myl6	null	myosin, light polypeptide 6, alkali, smooth muscle

<i>P</i>	<i>FC</i>	Gene Name	Genbank	Description
				and non-muscle
0.031	0.37	Snx4	NM_080557.1	sorting nexin 4
0.008	0.36	Oaz3	NM_016901.1	ornithine decarboxylase antizyme 3
0.044	0.36	Cul7	null	cullin 7
0.008	0.36	Hoxa3	NM_010452.1	homeo box A3
0.006	0.36	Nalp1	null	NACHT, leucine rich repeat and PYD containing 1
				cytochrome P450, family 2, subfamily c,
				polypeptide 40
0.005	0.36	Cyp2c40	NM_010004.1	
0.000	0.36	H13	NM_010376.2	histocompatibility 13
0.005	0.35	Ins1	NM_008386.2	insulin I
				myosin light chain 2, precursor lymphocyte-
				specific
0.003	0.35	Mylc2pl	NM_021611.1	leucine-rich repeats and calponin homology (CH)
				domain containing 3
0.041	0.35	Lrch3	null	
0.022	0.35	Mrpl32	null	mitochondrial ribosomal protein L32
0.024	0.35	Ttc22	NM_177667.2	tetratricopeptide repeat domain 22
0.001	0.34	Ngfb	NM_013609.1	nerve growth factor, beta
0.026	0.34	Olfir870	NM_146904.1	olfactory receptor 870
0.003	0.33	Gtlf3a	NM_025293.1	gene trap locus F3a
0.024	0.33	Wnt6	NM_009526.2	wingless-related MMTV integration site 6
				melanoma-derived leucine zipper, extra-nuclear
				factor
0.007	0.33	MIze	NM_031378.1	U2 small nuclear ribonucleoprotein auxiliary factor
				1, related sequence 2
0.011	0.33	U2af1-rs2	null	
0.007	0.33	Pmm2	NM_016881.1	phosphomannomutase 2
0.021	0.33	Mkrn3	NM_011746.1	makorin, ring finger protein, 3
				cytochrome P450, family 2, subfamily j,
				polypeptide 5
0.004	0.32	Cyp2j5	NM_010007.2	
0.001	0.32	Dhx15	NM_007839.1	DEAH (Asp-Glu-Ala-His) box polypeptide 15
0.009	0.32	Saps1	NM_172894.2	SAPS domain family, member 1
0.003	0.32	Anxa4	NM_013471.1	annexin A4
0.021	0.32	MIl2	null	myeloid/lymphoid or mixed-lineage leukemia 2
0.001	0.31	Tpr	NM_133780.2	translocated promoter region
0.006	0.31	Msi1	NM_008629.1	Musashi homolog 1(Drosophila)
0.047	0.31	Ucn3	null	urocortin 3
				myosin, light polypeptide 2, regulatory, cardiac,
				slow
0.000	0.31	Myl2	NM_010861.1	
0.007	0.31	Scnn1b	NM_011325.1	sodium channel, nonvoltage-gated 1 beta
0.018	0.31	Spata1	NM_027617.1	spermatogenesis associated 1
0.003	0.30	Txn1	NM_011660.2	thioredoxin 1
0.004	0.30	C1qtnf7	NM_175425.2	C1q and tumor necrosis factor related protein 7
0.050	0.30	Fmod	NM_021355.2	fibromodulin
0.033	0.30	Zfp568	null	zinc finger protein 568
0.041	0.30	Accn5	NM_021370.1	amiloride-sensitive cation channel 5, intestinal
0.021	0.30	Olfir411	NM_146709.1	olfactory receptor 411
0.046	0.29	Gm424	null	gene model 424, (NCBI)
0.033	0.29	Nrf1	NM_010938.2	nuclear respiratory factor 1
0.008	0.29	Olfir1145	NM_146320.1	olfactory receptor 1145
0.025	0.29	Cbx4	NM_007625.1	chromobox homolog 4 (Drosophila Pc class)
0.009	0.29	Hipk1	NM_010432.1	homeodomain interacting protein kinase 1
0.031	0.29	Olfir390	NM_146347.1	olfactory receptor 390
0.015	0.28	Aim1	null	absent in melanoma 1
0.001	0.28	Rxra	null	retinoid X receptor alpha
0.000	0.28	Hdac10	NM_199198.1	histone deacetylase 10
0.001	0.28	Tmc5	NM_028930.1	transmembrane channel-like gene family 5

P	FC	Gene Name	Genbank	Description
0.004	0.28	Ccdc27	null	coiled-coil domain containing 27
0.016	0.28	Nhlh2	NM_178777.2	nescient helix loop helix 2
0.015	0.28	Olfr154 100	NM_146573.1	olfactory receptor 154 olfactory receptor 1002
0.017	0.27	Nfkbil1	NM_010909.1	NF of kappa light polypeptide gene enhancer in B-cells inh.-like 1
0.002	0.27	Npm2	NM_181345.1	nucleophosmin/nucleoplasmin 2
0.000	0.27	Cflar	NM_009805.1	CASP8 and FADD-like apoptosis regulator
0.015	0.27	Vil1	NM_009509.1	villin 1
0.000	0.27	Lrrc25	NM_153074.2	leucine rich repeat containing 25
0.003	0.27	Pnma2	NM_175498.3	paraneoplastic antigen MA2
0.013	0.27	Sprl9	NM_026335.1	small proline rich-like 9
0.001	0.27	Teddm1	NM_178244.2	transmembrane epididymal protein 1
0.020	0.27	Zfp54	NM_011760.1	zinc finger protein 54
0.039	0.26	Hist1h2aa	NM_175658.1	histone 1, H2aa
0.012	0.26	Col13a1	NM_007731.1	procollagen, type XIII, alpha 1
0.008	0.26	Prlhr	NM_201615.1	prolactin releasing hormone receptor
0.004	0.26	Olfr985	NM_146855.1	olfactory receptor 985
0.026	0.26	Zdhhc9	NM_172465.1	zinc finger, DHHC domain containing 9
0.043	0.26	Alas2	NM_009653.1	aminolevulinic acid synthase 2, erythroid
0.029	0.26	Prrxl1	6.1	paired related homeobox protein-like 1
0.002	0.26	Iqce	NM_028833.2	IQ motif containing E
0.037	0.26	Krt2-10	null	keratin complex 2, basic, gene 10
0.001	0.25	Tmem86b	null	transmembrane protein 86B
0.014	0.25	Fkbp11	NM_024169.2	FK506 binding protein 11
0.017	0.25	Gm1123	null	gene model 1123, (NCBI)
0.044	0.25	Prkip1	null	Prkr interacting protein 1 (IL11 inducible)
0.002	0.25	Des	NM_010043.1	desmin
0.017	0.24	Tec	NM_013689.2	cytoplasmic tyrosine kinase, Dscr28C related (Drosophila)
0.006	0.24	Xkr5	NM_176951.2	X Kell blood group precursor-related family, member 5
0.041	0.24	Olfr1425	null	olfactory receptor 1425
0.007	0.23	Casq2	NM_009814.1	calsequestrin 2
0.000	0.23	Ppih	NM_028677.2	peptidyl prolyl isomerase H
0.002	0.23	Gm608	null	gene model 608, (NCBI)
0.003	0.23	Zfyve9	null	zinc finger, FYVE domain containing 9
0.037	0.23	Capn12	NM_021894.1	calpain 12
0.028	0.22	Oas3	null	2'-5' oligoadenylate synthetase 3
0.033	0.22	Olfr1013	NM_146762.1	olfactory receptor 1013
0.045	0.22	Ccnd3	NM_007632.1	cyclin D3
0.040	0.22	Olfr483	NM_146735.1	olfactory receptor 483
0.000	0.22	Prp2 Prh1	NM_031499.1	proline rich protein 2 proline rich protein Haell subfamily 1
0.027	0.22	Tsga10ip	null	testis specific 10 interacting protein
0.000	0.21	Foxb2	NM_008023.1	forkhead box B2
0.001	0.21	Syt15	null	synaptotagmin XV
0.020	0.21	Tmem1	null	transmembrane protein 1
0.031	0.21	Fkhl18	NM_010226.1	forkhead-like 18 (Drosophila)
0.030	0.21	Sgsh	NM_018822.2	N-sulfoglucosamine sulfohydrolase (sulfamidase)
0.034	0.21	Fgf11	NM_010198.1	fibroblast growth factor 11
0.016	0.20	Olfr601	NM_146314.1	olfactory receptor 601
0.032	0.19	Tspan31	null	tetraspanin 31
0.000	0.19	Sbp	NM_011321.2	spermine binding protein

P	FC	Gene Name	Genbank	Description
0.015	0.19	Plin	NM_175640.1	perilipin
0.007	0.19	Tcl1b5	NM_013776.1	T-cell leukemia/lymphoma 1B, 5
0.013	0.19	Olfr1087	NM_146846.1	olfactory receptor 1087
0.005	0.19	Wdr70	null	WD repeat domain 70
0.004	0.18	Fbxl18	null	F-box and leucine-rich repeat protein 18
0.039	0.18	Nrp2	NM_010939.1	neuropilin 2
				achaete-scute complex homolog-like 2
0.013	0.18	Ascl2	NM_008554.1	(Drosophila)
0.035	0.17	Gsc	null	goosecoid
0.012	0.17	Psg17	NM_007677.1	pregnancy specific glycoprotein 17
				pleckstrin homology domain containing, family G
0.001	0.17	Plekhhg6	NM_198604.1	member 6
				a disintegrin-like and metallopeptidase with
0.022	0.17	Adamts2	NM_175643.1	thrombospondin type 1 motif, 2
0.022	0.17	Nalp5	null	NACHT, leucine rich repeat and PYD containing 5
0.034	0.16	Foxe1	NM_183298.1	forkhead box E1 (thyroid transcription factor 2)
				protein phosphatase 1, regulatory (inhibitor)
0.023	0.16	Ppp1r14b	null	subunit 14B
				glutamate receptor, ionotropic, NMDA2D (epsilon
0.009	0.14	Grin2d	NM_008172.1	4)
0.042	0.14	Hoxb3	NM_010458.1	homeo box B3
0.009	0.14	Olfr91	NM_182714.1	olfactory receptor 91
				Finkel-Biskis-Reilly murine sarcoma virus
0.010	0.14	Fau	null	ubiquitously expressed
0.032	0.13	D4Ert429e	NM_177672.2	DNA segment, Chr 4, ERATO Doi 429, expressed
0.029	0.13	Wisp2	NM_016873.1	WNT1 inducible signaling pathway protein 2
			NM_00100226	
0.023	0.13	Gpr126	8.1	G protein-coupled receptor 126
0.006	0.13	Il28	NM_177396.1	interleukin 28
0.005	0.13	Zfpn1a3	null	zinc finger protein, subfamily 1A, 3
				calcium/calmodulin-dependent protein kinase II,
0.005	0.12	Camk2b	null	beta
0.002	0.12	Trim66	NM_181853.1	tripartite motif-containing 66
0.010	0.12	Rad51ap1	NM_009013.1	RAD51 associated protein 1
0.001	0.12	Epha2	NM_010139.2	Eph receptor A2
0.014	0.11	Bex6	null	brain expressed gene 6
0.014	0.11	Fgf22	null	fibroblast growth factor 22
0.045	0.11	Refbp2	null	RNA and export factor binding protein 2
0.000	0.11	Caml	null	calcium modulating ligand
0.003	0.11	Mcpt6	NM_010781.1	mast cell protease 6
0.017	0.10	Gpbar1	NM_174985.1	G protein-coupled bile acid receptor 1
0.000	0.10	Ptch2	null	patched homolog 2
0.034	0.09	Ros1	NM_011282.1	Ros1 proto-oncogene
0.001	0.09	Gjc1	NM_178596.1	gap junction membrane channel protein chi 1
0.048	0.09	Egr3	NM_018781.1	early growth response 3
0.010	0.07	Fthl17	NM_031261.1	ferritin, heavy polypeptide-like 17
				Rho guanine nucleotide exchange factor (GEF)
0.006	0.07	Arhgef16	null	16
			NM_00101175	
0.001	0.06	Olfr298	1.1	olfactory receptor 298
0.001	0.06	Zfp648	null	zinc finger protein 648
0.002	0.06	Hbq1	NM_175000.1	hemoglobin, theta 1
			NM_00100542	
0.000	0.05	Gm996	4.1	gene model 996, (NCBI)
0.014	0.04	Sbp	null	spermine binding protein
0.000	0.04	Hpd	NM_008277.1	4-hydroxyphenylpyruvic acid dioxygenase

<i>P</i>	FC	Gene Name	Genbank	Description
0.004	0.02	Snap25	NM_011428.2	synaptosomal-associated protein 25

P=p value; FC=fold change

Appendix III: GeneGo MetaCore Pathway Analysis legend.

