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**Identification of Genes involved in Gentamicin induced Nephrotoxicity in rats- a  
Toxicogenomic investigation**

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**ABSTRACT**

For the application of microarray technology as an additional endpoint in toxicological studies, there is a need to understand associations between pathological processes and gene expression alterations. In the current study we investigated gentamicin as a nephrotoxic model compound. Gene expression changes of the kidney in response to a dose of 80 mg/kg gentamicin were analyzed by using DNA microarray technology and alterations in gene expression were associated with results from conventional histopathological investigations and with the described pathomechanisms of gentamicin. Under the conditions of our experiment, the mRNA level of 211 genes were found to be deregulated by gentamicin. The gentamicin-induced affection of proximal convoluted tubules was associated with a strong up-regulation of mRNAs encoding for proteins which are used as nephrotoxicity markers in urine and plasma such as Kim-1, Osteopontin and TIMP1. Candidate marker genes for nephrotoxicity such as GATM were deregulated. Gentamicin-induced lysosomal phospholipidosis was indicated by deregulation of lysosomal located gene products such as ATP6V1D, a subunit of the lysosomal H<sup>+</sup> transporting ATPase. Effects on glucose transport and metabolism were indicated by the down-regulation on SGLT-2 and glucose-6-phosphatase. Renal cell apoptosis was indicated by up-regulated genes as TP53 and BAX. The role of oxidative stress in gentamicin toxicity was reflected by deregulation of transferrin receptor and heme oxygenase. The results of the study show the potential of microarray technology to study a complex mechanism of toxicity in a single study.

**Keywords:** Toxicogenomics, Gentamicin, Nephrotoxicity, Kidney, Rat, Microarray, Kim-1, ATP6V1D, GATM, transferrin receptor

## Introduction

Microarray technology makes it possible to study genome-wide expression profiles in response to the administration of pharmaceutical compounds and xenobiotics. The description of altered gene expression offers the possibility to understand the mode of action and to gain new insight into side effects at the molecular level. There is also the potential to study complex mechanisms of toxicity within a single animal study. Bridging between previous knowledge generated by classical biochemical or pathological work and gene expression analysis is needed to understand associations between gene expression alterations and described pathological processes. In the current study we have chosen gentamicin as nephrotoxic model compound. The pathomechanism of this compound was studied in detail in many studies but only a limited number of transcriptional gene expression changes after gentamicin administration has been published (Amin et al., 2004; Kramer et al., 2004; Nagy et al., 2004).

Gentamicin was chosen as a nephrotoxic model compound as this compound was the object of many mechanistic investigations. Its tissue specific toxicity is considered to be caused by a selective accumulation of this drug in the tubular epithelial cells in renal cortex, where its concentration is several times higher than in the plasma (Nagai et al., 2004; Goodman et al., 2001; Mingeot-Leclercq and Tulkens, 1999). On the cellular level an intense binding of the drug to the brush border of the proximal tubule could be demonstrated (Moestrup et al., 1995). After binding, gentamicin is taken into the cell by endocytosis where it inhibits the activities of lysosomal phospholipases and sphingomyelinase (Laurent et al., 1982). Therefore, gentamicin also inhibits the degradation of phospholipid-rich cell membranes which leads to an accumulation of phospholipids. This phenomenon is reflected ultrastructurally by the appearance of myeloid or lamellar bodies implicating the

accumulation of cell organelles like lysosomes and endosomes. The overload of phospholipid membranes may result in compromised lysosomal membrane integrity which might lead to occurrence of lysosomal enzyme leakage. This may explain the tubular necrosis seen after administration of gentamicin (Bennett et al., 1988). Within one week a replicative rate increase in the proximal tubule is observed. In addition, apoptosis plays an important role in gentamicin toxicity which could also contribute to clear off damaged cells and to control the compensatory proliferative response (Servais et al., 2006). Finally, there is evidence for oxidative stress in gentamicin-induced nephrotoxicity.

In the current study a dose of 80 mg/kg gentamicin was given daily to male rats for one week. Selected parameters of clinical pathology were measured and the kidney were evaluated by histopathology. Affymetrix GeneChip® microarrays were used for gene expression analysis of the kidneys.

## MATERIALS AND METHODS

### Chemicals

The study performed at Boehringer Ingelheim used gentamicin obtained from Sigma (Steinheim, Germany).

### Animal Treatment and sample collection

The gentamicin study was performed by Boehringer Ingelheim Pharma GmbH & Co. KG. Male Han Wistar rats (CrI:GLX(Br)Han:WI) were purchased from Charles River Laboratories (Sulzfeld, Germany). At administration start the rats were about 12 week old and weighed approximately 200 g. The animals were dosed intramuscularly in the right hind limb once daily with 80 mg/kg gentamicin (solved in 0.9% saline) for 7 days. Corresponding control rats were dosed with equivalent volumes of vehicle (0.9% saline). The animals were assigned to groups (3 rats/group) by weight using weight stratification-based computer software. The animals received a food ration in pellet form (Kliba No. 3438.0.25, Provimi Kliba SA (Kaiseraugst, Switzerland)) ad libitum but it was withdrawn in the afternoon before necropsy. Municipal tap water (Stadtwerke Biberach; Germany) was available ad libitum via drinking bottles. The animals were kept under controlled temperature ( $20 \pm 2^{\circ}\text{C}$ ), humidity (45-75%), and lighting (12 h light/dark cycle) and were acclimatized for a minimum of 11 days. The animals were kept in groups of 3 per cage (Noryl, type V). The study has been performed in accordance with OECD Principles of Good Laboratory Practice (GLP; as revised in 1997, ENV/MC/CHEM(98)17) and the “Chemikaliengesetz” (Chemicals Act) of the Federal Republic of Germany. Standard operating procedures (SOPs) covering techniques, conditions and items involved in this study were available. The animal facilities of Boehringer Ingelheim are accredited by AAALAC International (Association for Assessment and Accreditation of Laboratory Animal Care International).

At necropsy the kidneys were removed and hemi-dissected. One half of the kidney were preserved in 10% buffered formaldehyde for histological examinations, the other half were frozen in liquid nitrogen for RNA isolation.

### **Clinical chemistry**

For clinical chemistry 550µl blood per animal were collected at necropsy. Plasma creatinine and Blood Urea Nitrogen (BUN) were determined with a Hitachi 917 (405657) and Roche Diagnostics Kits (Roche Diagnostics GmbH, Mannheim).

### **Histopathology**

Representative sections of the kidneys were processed and embedded in paraffin, sectioned at a thickness of approximately 4 µm and stained with hematoxylin-eosin (H&E), Periodic Acid Schiff reagent, and immunohistochemically for the Proliferating Cell Nuclear Antigen (PCNA) by application of the streptavidin-biotin method and diaminobenzidine as substrate.

### **RNA Isolation**

Total RNA was isolated individually from harvested snap frozen hemi-dissected left and right kidneys using QIAGEN RNeasy kits (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. The RNA concentration was determined spectrophotometrically (BioPhotometer, Eppendorf, Wesseling-Berzdorf). The integrity of the RNA was checked by gel analysis using RNA 6000 Nano chips on an Agilent 2100 Bioanalyzer from Agilent Technologies (Waldbronn, Germany).

### **Hybridization of cRNA to Oligonucleotide Arrays**

Biotin-labeled cRNA samples for hybridization on Affymetrix GeneChip® RG\_U34A arrays



were prepared according to the GeneChip Expression Analysis Technical Manual from Affymetrix Inc. (Santa Clara, CA). Briefly, 5 µg total RNA was used for cDNA synthesis with the SuperScript Choice System from Invitrogen Life Technologies, (Invitrogen GmbH, Karlsruhe, Germany) employing a modified T7 primer with 24 thymidines at the 5' end. After spin column purification, biotin-labeled cRNA was synthesized from the cDNA using the ENZO RNA Transcript Labeling Kit (Affymetrix Inc., Santa Clara, CA). Spin column-purified cRNA was quality controlled using an Agilent 2100 Bioanalyzer and spectrophotometrically quantified. The cRNA (15 µg) was then fragmented in buffer supplied with the GeneChip Sample Cleanup Module and hybridized for 16 h at 45°C. The microarrays were washed and stained with streptavidin-phycoerythrin (Dianova, Hamburg, Germany) on the Affymetrix Fluidics Station 400. Fluorescent images were read using the Affymetrix GeneChip Scanner. The raw data image files (DAT) were converted into CEL files using the Affymetrix Microarray Suite 5.0.

### Gene Expression Profiles

Each animal was analyzed individually on a Affymetrix RG\_U34A Gene Chip. This chip contains 8740 probe sets corresponding to about 7000 annotated rat genes and 1740 expressed sequence tags (ESTs). For comparison between the gentamicin-treated group and the vehicle-treated group, the Wilcoxon test was applied using the software Expressionist Analyst (Version 5.1.2, Genedata AG, 4016 Basel, Switzerland). To identify the genes that had a significant increase or decrease in intensity values a significance level of  $\alpha = 0.05$  was chosen. Due to the fact that a two sided Wilcoxon rank test was used, the p-value cutoff was set at  $p = 0.099$  which correspond to a p-value cutoff at 0.05 for a one sided Wilcoxon rank test. Median fold changes in intensity values across the statistically significant genes were required to be  $\geq 1.7$ -fold or  $\leq -1.7$ -fold. This approach using a combination of a p-value cut-off with a

fold change cut-off was chosen based on the results of the Micro Array Quality Control Consortium (Shi et al., 2006). The probe sets were annotated through the use of Ingenuity Pathways Analysis (Ingenuity Systems, Redwood City, CA).

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## RESULTS

### Clinical Chemistry

The clinical chemistry results of the study displayed a statistically significant increase of creatinine in blood plasma after gentamicin treatment with 80 mg/kg for 7 days. BUN levels were not affected (Table 1).

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## Histopathology

Gentamicin-induced effects on the kidneys were generally minimal to moderate in severity and were degenerative, regenerative, and inflammatory in nature. Degenerative changes were most prominent in proximal convoluted tubules and consisted of vacuolization, cytoplasmic granularity and cell death by necrosis and apoptosis with cell desquamation (Figure 1). The PAS-negative cytoplasmic granularity was considered to represent the histological correlate of exaggerated lysosomal phospholipids storage. The epithelium of convoluted tubules was slightly hypercellular with increased basophilia, indicating regenerative proliferation. Hyaline tubular casts occurred occasionally. The interstitial tissue adjacent to prominently affected tubules was minimally to slightly infiltrated by lymphocytes. Affected tubules were unevenly distributed indicating that some, but not all nephrons were affected.

Correlating to the regenerative proliferation identified in H&E stained slides, PCNA immunostaining revealed more labeled nuclei in proximal convoluted tubules of animals treated at 80 mg/kg for one week when compared to controls. An increased labeling was also evident in distal convoluted tubules (Figure 1).

## Gene Expression Analysis

Based on chosen selection criteria  $n=313$  regulated probe sets were identified to be deregulated by gentamicin. Among these probe sets, 211 genes were assigned to their molecular functions, pathways and biological processes. The functions are outlined in Table 2 and are described in more detail in the following discussion part.

## DISCUSSION

### *Vesicles, endocytotic pathways and lysosomes*

Gentamicin is described to be internalized by proximal tubule cells via receptor-mediated endocytosis. In our study we identified five statistically significantly deregulated genes which encode for proteins which are involved in intracellular vesicle movement (Myo1G, ARF6, RSC1A1, EXOC3, PAFAH1B1). The observed transcriptional changes of these genes are therefore considered to reflect disturbances in vesicle movement caused by the gentamicin. One strongly up-regulated gene of this group was Myo1G (Myosin IG), which is involved in multiple types of vesicle motility (Allan et al., 2002). ARF6 (ADP-ribosylation factor 6), which regulates clathrin-mediated endocytosis (Wennerberg et al., 2005) and endosome movement (Taunton, 2001) was also deregulated. It was described to be localized to specialized apical endosomes in kidney proximal tubules (Maranda et al., 2001). Further deregulated genes of this “vesicle”-group were RSC1A1 (regulatory solute carrier protein, family 1, member 1, also known as PLEKHM2), which is involved in the transport of vesicles and in maintaining the structure and position of the Golgi apparatus (Boucrot et al., 2005; Goldstein et al., 1999; Clarke et al., 2002; Allan et al., 2002), EXOC3 (exocyst complex component 3), which is part of the exocyst complex that mediates targeting and tethering of post-Golgi secretory vesicles (Hsu et al., 2004) and PAFAH1B1 (platelet-activating factor acetylhydrolase, isoform Ib/ alpha subunit 45kDa), which plays a role in the retrograde movement of cytoplasmic dynein (Smith et al., 2000). This is of interest as gentamicin is transported retrograde through the secretory pathway to the Golgi complex and to the endoplasmatic reticulum (Sandoval and Molitoris, 2003). Deregulation of the megalin receptor which is described to play an important role in receptor mediated endocytosis of gentamicin (Sandoval et al., 2004; Moestrup et al., 1995) were not represented on the RG\_U34A Gene Chip and could not be analyzed.

The mRNA of five genes encoding proteins which are localised in the lysosomal and endosomal compartment was found to be deregulated, among them the TFRC (transferrin receptor) gene. As TFRC is a marker for early endosomes (Kurz et al., 2007) the observed up-regulation is considered to reflect a gentamicin effect on endocytosis. The change of TFRC mRNA might also indicate a gentamicin effect on intracellular iron-homeostasis. A protective effect of iron chelators on gentamicin toxicity was previously reported (Walker and Shah, 1988; Piruska and Schacht, 1995; Song and Schacht, 1996; Shah and Schacht, 1996). Interestingly, gentamicin chelates mitochondrial iron in hair cells of the inner ear, forming a very oxidant Fe(II)-gentamicin complex capable of causing cell death (Song et al., 1997). Effects of gentamicin on lysosomal function were indicated by up-regulation of CTSL2 (cathepsin L2) and CTSD (cathepsin D), which encode lysosomal cysteine proteinases (Ishidoh and Kominami, 1998; Zaidi et al., 2008). The enzymatic activity of Cathepsin L enzyme was previously reported to be inhibited in S1 and S2 proximal tubule cells after gentamicin administration to rats (Olbricht et al., 1991). Therefore the up-regulation of CTSL2 may indicate a counter-regulatory effect on mRNA level. Furthermore, the membrane transporter SLC9A3 (solute carrier family 9 (sodium/hydrogen exchanger), member 3, also known as NHE-3) was strongly downregulated on mRNA level by gentamicin. This antiporter is present in renal endosomes and plays a role in pH regulation, Na<sup>+</sup> homeostasis and endosomal trafficking (D'Souza et al., 1998). This gentamicin effect was previously described by Sassen et al. (2006) on protein level in the renal cortex of gentamicin treated rats (80mg/kg). Furthermore, we observed an up-regulation of ATP6V1D (ATPase, H<sup>+</sup> transporting, lysosomal 34kDa, V1 subunit D), a subunit of a key enzyme for early endosomal fusion and acidification of intracellular organelles (Hammond et al., 1998; Sun-Wada et al., 2003). An association between the observed up-regulation of this gene and gentamicin induced lysosomal phospholipidosis is likely as the acidic pH in the lysosomes is essential for

the degradation of phospholipids by lysosomal hydrolases. Interestingly this ATPase is co-localized in proximal tubule cells with ARF6, which was also deregulated on mRNA level in our study.

### ***Membrane transporters, amino acid/glucose metabolism***

The mRNA expression of several genes encoding transporter proteins was significantly altered after gentamicin application in our study (Table 1). The majority of these genes were downregulated, which is interpreted as a sign of decreased kidney function. Among them two amino acid transporter, SLC6A18 (solute carrier family 6, member 18) and SLC6A19 (solute carrier family 6 (neutral amino acid transporter), member 19), were down-regulated which might reflect the observed aminoaciduria after gentamicin treatment (Suhl et al., 1997). The observed deregulation of amino acid metabolism genes (please refer to Table 1, Amino acid metabolism), might be a secondary effect in this context.

An effect on water reabsorption was indicated by the down-regulation of AQP2 (aquaporin 2). The AQP2 gene encodes a water channel protein expressed only in the principal cells of renal collecting duct (Agre et al., 1996) with important functions at water reabsorption. An effect on AQP2 on protein level in the tubulointerstitial structures of the rat renal cortex was previously described after gentamicin application (Lee et al., 2001). The observed deregulation might contribute to the observation that gentamicin reduces the urinary concentration ability of the kidney (Miura et al. 1985). An additional down-regulated transporter was SGLT-2 (= SLC5A2; solute carrier family 5, sodium/glucose cotransporter, member 2), indicating an effect of gentamicin on renal glucose reabsorption. An effect on glucose metabolism was indicated by the observed down-regulation of G6PC (glucose 6-phosphatase), a key gluconeogenic enzyme, which is expressed in the proximale tubule, but not in other segments of the nephron (Marsenic, 2009).

### ***Fatty acid metabolism***

Several genes with functions in fatty acid metabolism, like ACAA1 (acetyl-Coenzyme A acyltransferase 1), ACADM (acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain), ACADSB (acyl-Coenzyme A dehydrogenase, short/branched chain) and ACSL3 (acyl-CoA synthetase long-chain family member 3) were found to be down-regulated ("Fatty acid metabolism", Ingenuity Pathways Analysis (Ingenuity® Systems, [www.ingenuity.com](http://www.ingenuity.com)). The observed down-regulation of these genes is considered to reflect a disturbance in lipid degradation by gentamicin. Whether these changes in lipid-metabolism are connected with the gentamicin-related inhibition of phospholipid-degradation or are due to other toxic effects of this compound on the kidney cells remains open.

### ***Translation***

The expression level of 9 genes involved in the regulation and initiation of translation were found to be up-regulated (please refer to Table 1). This is of interest as gentamicin exerts its pharmacological activity mainly by inhibition of protein synthesis by binding to the prokaryotic 30S ribosomal subunit to prevent the formation of an initiation complex with messenger RNA. However, at high doses the translation of eukaryotic tissues is also affected. It is reported that gentamicin induces translational readthrough of stop codons of a messenger RNA leading to the production of nonsense peptides (Howard et al., 2000) and that aminoglycosides produce mistranslation in eukaryotic tissues including the kidneys (Manuvakhova et al., 2000; Bennett et al., 1988; Buss and Piatt, 1985; Palmer and Wilhelm 1978). The expression changes of the 9 genes involved in translation in our study might be therefore considered to indicate a counter-regulatory mechanism induced by effects of a nephrotoxic dose of gentamicin on translation in the kidneys.



### ***Proliferation***

High doses of gentamicin were described to cause tubular necrosis followed by a replicative rate increase in the proximal tubule within one week, an effect which was also seen histopathologically and immunohistochemically (PCNA-staining) in our study. In line with this effect we observed a deregulation of a number of genes which play a role in the proliferation processes such as Jun (v-jun sarcoma virus 17 oncogene), JunB (jun b proto-oncogene) and MYC (v-myc myelocytomatosis viral oncogene) (Karin and Gallagher, 2005; Shaulian and Karin, 2002; Cowling and Cole, 2006; Sakisaka et al., 2007). In addition, NRG1 (neuregulin 1), with a function in regenerative processes, and NUDC (nuclear distribution gene C homolog), which is described to be up-regulated in proliferating cells (Zheng et al., 1999; Castagnino et al., 2000; Gocke et al., 2000) were deregulated. PCNA was not statistically significant up-regulated on mRNA level in our study but there was a clear trend to higher mRNA expression in the gentamicin treated animals.

### ***Cell death***

Cell death by apoptosis and necrosis were evident in proximal convoluted tubules of our study, in line with the well documented fact that apoptosis plays a central role in the gentamicin induced nephrotoxicity. As possible mechanisms, destabilization of the lysosomal membrane with the subsequent release of cathepsins but also effects of cytosolic gentamicin on mitochondria or the impairment of Bax (BCL2-associated X protein) proteosomal degradation were suggested (Chwieralski et al. 2006; Servais et al., 2008). In our study we found an up-regulation of CTSL2 (Cathepsin L2) which might reflect the activation of apoptosis. The observed up-regulation of Bax mRNA could be interpreted as a transcriptional response to the described impairment of the Bax proteasomal degradation. In addition, we observed a deregulation of TP53 (tumor protein p53) and several downstream targets of TP53

(e.g. CCNG1 (cyclin G1), BTG2 (BTG family, member 2), MDM2 (Mdm2 p53 binding protein homolog (mouse))), indicating that this apoptotic pathway is also affected by gentamicin. As proliferation pathways are closely linked to apoptotic pathways a clear separation of “proliferation-genes” and “apoptosis-genes” were not possible and, therefore, these genes were listed together in Table 1.

### ***Oxidative stress/ DNA repair mechanisms/ Stress response***

Reactive oxygen species (ROS) are also discussed to participate in gentamicin-induced apoptosis (Lesniak et al 2005). In our study we observed the deregulation of a number of genes associated with the protection against oxidative injury. HMOX1 (heme oxygenase 1) which was unregulated was of specific interest as it was previously reported to be induced in kidney following toxic and ischemic insults (Nath, 2006; Takahashi et al., 2004). APEX1 (APEX nuclease 1), a multifunctional DNA repair enzyme, and MGMT (O-6-methylguanine-DNA methyltransferase), which repairs alkylated guanine in DNA, were up-regulated in our study. In addition, we observed the up-regulation of several genes, which are involved in stress response pathways, like the transcription factor DDIT3 (DNA-damage-inducible transcript 3), several heatshock proteins (HSPA5, HSP90AA1, HSP90B1, HSPB1) and CHKA (Choline kinase alpha) (Welch, 1992; Wang et al., 1996; Chen et al., 1998; Aoyama et al., 2004; van de Water et al. 2006). Taken together these gene expression changes are judged to reflect a rather unspecific stress response.

### ***Nephrotoxicity***

Gentamicin induced in our study the expected tubulopathy with particular affection of proximal convoluted tubules. This was associated with a strong deregulation of several mRNAs encoding for kidney proteins which are considered to be nephrotoxicity markers. The

most prominent gene with an up-regulation of 38-fold after gentamicin administration was the Kim-1 (Kidney injury molecule-1, also known as HAVCR1) gene. This type I cell membrane glycoprotein serves as an earlier urinary diagnostic marker for proximal tubule injury (Zhou et al., 2008; Han et al., 2002, Vaidya et al., 2006). In addition SPP1 (Osteopontin), ATF3 (activating transcription factor 3), Lipocalin (LCN2) and TIMP1 (TIMP metalloproteinase inhibitor 1) (Hai et al., 1999, Thukral et al., 2005, Yuen et al., 2006, Sieber et al., 2009) which were described to be affected after kidney injury were found to be strongly up-regulated on mRNA level in our study. The finding of the EGF (epidermal growth factor) down-regulation was in line with the report of Verstrepen et al. (1993) and was of interest as EGF down-regulation is frequently observed after acute renal failure (Perco et al., 2006).

We also observed a strong deregulation of several genes that were previously associated with nephrotoxicity of gentamicin. The down-regulation of KLK1 (Kallikrein 1) after gentamicin administration was previously described by Amin et al. (2004). In addition gentamicin-treated rats were shown to have strongly reduced levels of urinary Kallikrein (Higa et al., 1985). In addition, a renal protective effect of Kallikrein against gentamicin-induced nephrotoxicity was reported (Murakami et al., 1998; Bledsoe et al., 2008). The observed changes of ICAM1 (intercellular adhesion molecule 1 (CD54)) might also be associated with nephrotoxicity. The deregulation of this gene was previously observed in gentamicin treated rats via RT-PCR (Bledsoe et al. 2008) and was also reported to be up-regulated after cisplatin induced acute renal failure and in experimental tubulointerstitial nephritis (Tang et al., 1994; Kelly et al., 1999; Perco et al., 2006; Safirstein, 2004).

Furthermore we observed a reduced mRNA level of GATM (L-arginine:glycine amidinotransferase). A connection to nephrotoxicity is suggested by the fact that a decreased expression of GATM was also detected in the kidney of cisplatin treated rats (Amin et al., 2004). GATM catalyzes the transfer of a guanido group from arginine to glycine, forming

guanidinoacetic acid, the immediate precursor of creatine, which is the rate-limiting step of creatine biosynthesis in kidney (Wyss and Kaddurah-Daouk, 2000). Interestingly, a decrease in urinary excretion of guanidinoacetic acid was reported as a sensitive marker of gentamicin nephrotoxicity in rats (Kiyatake et al., 2006; Kiyatake et al., 2004; Takeda et al., 1995). A possible interrelation between the reduced GATM mRNA level and the observed increased plasma concentration of creatinine, which was measured as known marker for renal function in our study, must be discussed as well. There is increasing evidence that increased plasma creatinine levels are associated with increased plasma creatine levels and it was shown that the serum concentration of creatine is increased in many patients with chronic renal failure (Jones and Burnet 1974; Mitch et al., 1980; Wyss and Kaddurah-Daouk, 2000). In rats increased plasma creatine levels resulted in a decrease of GATM mRNA, protein expression and enzymatic activity. This effect was described as a pretranslational feedback repression (Guthmiller et al., 1994; van Pilsum et al., 1992). Therefore the observed down-regulation of GATM after treatment with gentamicin on mRNA expression level might be explained by a feedback repression of GATM by creatine. In conclusion this suggests that GATM and the above discussed reaction-product guanidinoacetic acid are interesting candidate markers for nephrotoxicity.

GSTM1 (glutathione S-transferase M1) gene encodes GST Yb1 protein which was described as urinary leakage biomarker for distal tubule damage (Kilty et al., 2007). Interestingly the GSTM1 mRNA level was down-regulated after gentamicin treatment in this study. Down-regulation of GSTM1 mRNA was also described in a study comparing gene expression patterns of GSTs in kidney and liver of iron-overloaded mice (Desmots et al., 2001) which indicates a relation between GSTM1 mRNA down-regulation and general oxidative stress mechanisms in kidney.

In contrast CALB1 (calbindin 1, 28kDa), considered to detect specifically distal tubular or

collecting duct damage (Steiner et al., 1996; Takashi et al., 1996) was not deregulated in our study. Albumin, Cystatin C or  $\beta$ -microglobulin which are used as urinary markers for nephrotoxicity due to increased glomerular filtration or decreased reabsorption were not expected and not found to be deregulated in the kidney after gentamicin administration.

It is worth discussing that a mRNA based analysis with microarrays has some limitations. Several potential events could arise between the mRNA and the final protein, e.g. translational regulation and differences in protein in vivo half-lives (Greenbaum et al., 2003; Beyer et al., 2004). Furthermore changes on phosphorylation cascades as well as posttranslational modifications and metabolites can not be detected. Nevertheless up to now microarray analysis is the tool of choice for mechanistic analysis as the number of annotated molecules is very high when compared to other techniques. With genomics it is possible to gain an overall picture of intracellular processes. The high number of measured endpoints has the advantage that hypothesis are not generated based on only one or a minimal number of gene or protein deregulation. Rather it is possible to strengthen the value of the gained information by verifying a hypothesis with a big group of analyzed molecules.

In summary, we could identify 211 genes which showed significant changes at the mRNA level in response to a nephrotoxic dose of gentamicin. The majority of the described genes are correlated for the first time with gentamicin toxicity. Based on the mechanistic knowledge of gentamicin toxicity from the literature, it was possible to hypothesize a potential damage sequence in accordance with histopathological observations. Deregulation of lysosomal genes or genes involved in vesicle trafficking could reflect accumulation of gentamicin in lysosomes of proximal tubule epithelial cells which might be a primary effect of gentamicin. Several of the genes involved here were previously reported to be expressed in proximal tubule cells (e.g. CTSL2, ARF6 and ATP6V1D). An acute stress on proximal tubule cells was also

indicated by stress responses and likely real damage responses further downstream of the damage cascade, i.e. by deregulation of genes associated with cell death together with proliferation genes. Which effects like the deregulation of nephrotoxicity marker genes as well as fatty acid metabolism genes are primary, secondary or even tertiary responses to direct gentamicin influence would need a time response investigation. Furthermore, additional studies are needed to validate these associations and to use toxicogenomics for safety evaluation of new compounds. An example for this type of analysis is the study of Wang et al. (2008), where a subset of genes was investigated after administration of several nephrotoxins including gentamicin.

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Table 1 Clinical chemistry results for plasma creatinine and blood urea nitrogen (BUN) levels

Dose [0mg/kg]			Dose [80mg/kg]			$\Delta\%$	
Animal Nr.	BUN [mmol/L]	Creatinine [ $\mu$ mol/L]	Animal Nr.	BUN [mmol/L]	Creatinine [ $\mu$ mol/L]	BUN	Creatinine
111	9.29	59.2	311	10.99	72.7		
113	9.61	57.4	312	10.22	73.7		
114	10.08	60.5	313	8.9	68.8		
Mean	9.66	59.03	Mean	10.04	71.73*	3.90%	21.51%

\* statistical significant ( $p < 0.01$ )

**Table 2 Classification of significant gene expression changes in the rat kidney induced by gentamicin (80 mg/kg/day). Platform: Affymetrix RG\_U34A. Deregulated genes with an expression change  $\geq 1.7$ -fold and  $\leq -1.7$  respectively and a  $p < 0.01$  (Wilcoxon rank test) are shown.**

Gene Symbol	Entrez Gene Name	Affymetrix ID	FC
<b>Amino acid metabolism</b>			
ABAT	4-aminobutyrate aminotransferase	D87839_g_at	-1.70
ALDH1A1	aldehyde dehydrogenase 1 family, member A1	AF001898_at	-1.74
ASL	argininosuccinate lyase	D13978_s_at	2.10
ASL	argininosuccinate lyase	AB016536_s_at	1.81
ASNSD1	asparagine synthetase domain containing 1	rc_AA799531_at	1.75
CBS	cystathionine-beta-synthase	M88347_s_at	-1.79
CDO1	cysteine dioxygenase, type I	E03229cds_s_at	-2.23
DDC	dopa decarboxylase (aromatic L-amino acid decarboxylase)	M84648mRNA_s_at	-2.63
GNMT	glycine N-methyltransferase	rc_AA893219_at	-1.84
GNMT	glycine N-methyltransferase	X06150cds_at	-1.72
GNMT	glycine N-methyltransferase	X06150cds_g_at	-1.99
HSD17B4	hydroxysteroid (17-beta) dehydrogenase 4	rc_AI101743_s_at	-1.79
KMO	kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	AF056031_at	-2.30
KYNU	kynureninase (L-kynurenine hydrolase)	U68168_at	-2.34
MPST	mercaptopyruvate sulfurtransferase	D50564_at	-1.78
NEDD4	neural precursor cell expressed, developmentally down-regulated 4	U50842_at	1.73
PHGDH	phosphoglycerate dehydrogenase	X97772_g_at	-1.79
PSAT1	phosphoserine aminotransferase 1	rc_AI102868_at	-2.45
PSAT1	phosphoserine aminotransferase 1	rc_AI102868_g_at	-2.79
<b>Cell death/ Proliferation</b>			
AGTR1	angiotensin II receptor, type I	M86912exon_g_at	-2.34
AGTR1	angiotensin II receptor, type I	M74054_s_at	-2.26
AGTR1	angiotensin II receptor, type I	M86912exon_at	-2.38
BAX	BCL2-associated X protein	S76511_s_at	1.86
BAX	BCL2-associated X protein	U59184_at	1.83
BTG2	BTG family, member 2	rc_AA944156_s_at	3.96
BTG2	BTG family, member 2	M60921_at	2.08
BTG2	BTG family, member 2	M60921_g_at	5.24
BTG3	BTG family, member 3	AF087037_g_at	2.66
BTG3	BTG family, member 3	AF087037_at	1.97
CCNG1	cyclin G1	X70871_at	5.09
CDC2L5	cell division cycle 2-like 5 (cholinesterase-related cell division controller)	rc_AA875127_g_at	2.00
CDC2L5	cell division cycle 2-like 5 (cholinesterase-related cell division controller)	rc_AA875127_at	1.92

CDC34	cell division cycle 34 homolog (S. cerevisiae)	L38482_g_at	1.87
CDC34	cell division cycle 34 homolog (S. cerevisiae)	rc_AA799788_s_at	2.09
CSRP2	cysteine and glycine-rich protein 2	U44948_at	-2.08
DNASE1	deoxyribonuclease I	U76635mRNA_at	-1.74
EGR1	early growth response 1	AF023087_s_at	11.25
EGR1	early growth response 1	M18416_at	11.91
EGR1	early growth response 1	U75397UTR#1_s_at	5.97
GAS5	growth arrest-specific 5 (non-protein coding)	U77829mRNA_i_at	4.04
GAS5	growth arrest-specific 5 (non-protein coding)	U77829mRNA_s_at	5.61
GHR	growth hormone receptor	Z83757mRNA_g_at	-1.71
GNL3	guanine nucleotide binding protein-like 3 (nucleolar)	rc_AA892598_g_at	3.10
GNL3	guanine nucleotide binding protein-like 3 (nucleolar)	rc_AA892598_at	3.52
IGFBP3	insulin-like growth factor binding protein 3	rc_AI009405_s_at	-2.97
JUN	jun oncogene	rc_AI175959_at	2.81
JUN	jun oncogene	rc_AA945867_at	5.95
JUN	jun oncogene	X17163cds_s_at	2.65
JUNB	jun B proto-oncogene	rc_AA891041_at	2.30
KLF6	Kruppel-like factor 6	AF001417_s_at	11.29
MCM6	minichromosome maintenance complex component 6	U17565_g_at	1.83
MDM2	Mdm2 p53 binding protein homolog (mouse)	AA875509_at	1.71
MDM2	Mdm2 p53 binding protein homolog (mouse)	rc_AI639488_at	4.48
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	Y00396mRNA_at	14.20
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	Y00396mRNA_g_at	18.93
NME1	non-metastatic cells 1, protein (NM23A) expressed in	rc_AI233173_at	2.26
NRG1	neuregulin 1	U02315_g_at	2.75
NRG1	neuregulin 1	U02320_s_at	4.17
NRG1	neuregulin 1	U02322_s_at	3.60
NUDC	nuclear distribution gene C homolog (A. nidulans)	X82445_at	1.94
PELO	pelota homolog (Drosophila)	rc_AA799330_at	3.35
PNN	pinin, desmosome associated protein	rc_AI639151_at	1.75
PPP1R10	protein phosphatase 1, regulatory (inhibitor) subunit 10	AF040954_at	3.07
PPP1R15A	protein phosphatase 1, regulatory (inhibitor) subunit 15A	AF020618_g_at	4.03
PPP1R1A	protein phosphatase 1, regulatory (inhibitor) subunit 1A	J05592_at	-2.08
PPP1R1A	protein phosphatase 1, regulatory (inhibitor) subunit 1A	J05592_g_at	-1.89
PTP4A1	protein tyrosine phosphatase type IVA, member 1	L27843_s_at	1.90
PTPN1	protein tyrosine phosphatase, non-receptor type 1	rc_AI113289_s_at	1.84
RUVBL1	RuvB-like 1 (E. coli)	AB002406_at	2.35
TP53	tumor protein p53	X13058_at	3.16



ZNF622	zinc finger protein 622	rc_AA894086_g_at	2.70
<b>Cytoskeleton</b>			
CAPZA1	capping protein (actin filament) muscle Z-line, alpha 1	rc_AA891917_at	1.76
GJB2	gap junction protein, beta 2, 26kDa	X51615_at	-1.94
GJB2	gap junction protein, beta 2, 26kDa	X51615_g_at	-1.72
TPM3	tropomyosin 3	rc_AA859305_s_at	1.75
TUBB	tubulin, beta	rc_AA860030_s_at	2.08
<b>DNA repair</b>			
APEX1	APEX nuclease (multifunctional DNA repair enzyme) 1	D44495_s_at	2.92
LIG4	ligase IV, DNA, ATP-dependent	rc_AI639510_at	1.87
MGMT	O-6-methylguanine-DNA methyltransferase	M76704_s_at	1.96
<b>Endocrine control</b>			
GUCA2A	guanylate cyclase activator 2A (guanylin)	M95493_at	2.45
PTH1R	parathyroid hormone 1 receptor	AB012944cds_s_at	-2.92
PTH1R	parathyroid hormone 1 receptor	L31394exon_s_at	-2.51
<b>Fatty acid metabolism</b>			
ACAA1	acetyl-Coenzyme A acyltransferase 1	J02749_at	-2.82
ACAA1	acetyl-Coenzyme A acyltransferase 1	J02749_g_at	-1.80
ACADM	acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain	J02791_at	-1.73
ACADSB	acyl-Coenzyme A dehydrogenase, short/branched chain	U64451_at	-1.72
ACSL3	acyl-CoA synthetase long-chain family member 3	D30666_at	-1.81
ACSM3	acyl-CoA synthetase medium-chain family member 3	S62516_s_at	-4.17
ACSM3	acyl-CoA synthetase medium-chain family member 3	AF027188UTR#1_at	-2.89
ADFP	adipose differentiation-related protein	rc_AA893280_at	1.99
DECR1	2,4-dienoyl CoA reductase 1, mitochondrial	D00569_at	-1.81
DECR1	2,4-dienoyl CoA reductase 1, mitochondrial	D00569_g_at	-1.78
DECR2	2,4-dienoyl CoA reductase 2, peroxisomal	AF044574_g_at	-1.86
HACL1	2-hydroxyacyl-CoA lyase 1	rc_AA893239_at	-1.99
PXMP2	peroxisomal membrane protein 2, 22kDa	X70223_at	-1.80
<b>Genes association with secretory pathways and vesicles</b>			
ARF6	ADP-ribosylation factor 6	rc_AA944324_at	1.79
ATP6V1D	ATPase, H <sup>+</sup> transporting, lysosomal 34kDa, V1 subunit D	rc_AA893246_at	1.71
CTSD	cathepsin D	X54467_at	1.82
CTSD	cathepsin D	rc_AI235585_s_at	1.97
CTSL2	cathepsin L2	S85184_at	1.77
CTSL2	cathepsin L2	S85184_g_at	2.02
EXOC3	exocyst complex component 3	U32575_at	1.97
MYO1G	myosin IG	rc_AA875126_at	75.95

MYO1G	myosin 1G	rc_AA875126_g_at	113.71
PAFAH1B1	platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45kDa	AA801441_at	1.76
RSC1A1	regulatory solute carrier protein, family 1, member 1	rc_AA874927_g_at	2.11
SLC9A3	solute carrier family 9 (sodium/hydrogen exchanger), member 3	M85300_at	-2.51
TFRC	transferrin receptor (p90, CD71)	M58040_at	2.79
<b>Glucose metabolism</b>			
G6PC	glucose-6-phosphatase, catalytic subunit	L37333_s_at	-3.90
PC	pyruvate carboxylase	U32314_at	-2.82
PC	pyruvate carboxylase	U32314_g_at	-2.13
SLC2A2	solute carrier family 2 (facilitated glucose transporter), member 2	L28135_at	3.69
<b>Histon related</b>			
DNAJA2	DnaJ (Hsp40) homolog, subfamily A, member 2	rc_AI170685_at	1.72
DNAJA2	DnaJ (Hsp40) homolog, subfamily A, member 2	rc_AI170685_g_at	1.87
EAF1	ELL associated factor 1	rc_AI639008_at	1.81
H2AFX	H2A histone family, member X	rc_AA875257_at	1.77
H3F3B	H3 histone, family 3B (H3.3B)	rc_AI177503_at	1.87
HIST1H4F	histone cluster 1, H4f	rc_AA946439_at	2.74
MYBBP1A	MYB binding protein (P160) 1a	rc_AI229637_at	2.83
MYBBP1A	MYB binding protein (P160) 1a	rc_AI237258_at	1.82
<b>Immune response / Inflammation</b>			
APCS	amyloid P component, serum	rc_AA945608_at	-4.42
C4B	complement component 4B (Chido blood group)	U42719_at	1.72
CEBPB	CCAAT/enhancer binding protein (C/EBP), beta	S77528cds_s_at	2.10
CEBPB	CCAAT/enhancer binding protein (C/EBP), beta	X60769mRNA_at	2.78
CXCL10	chemokine (C-X-C motif) ligand 10	U17035_s_at	5.18
IRF1	interferon regulatory factor 1	M34253_g_at	3.84
IRF7	interferon regulatory factor 7	rc_AA799861_at	2.01
IRF7	interferon regulatory factor 7	rc_AA799861_g_at	2.36
RBP4	retinol binding protein 4, plasma	M10934_s_at	-1.71
SPP2	secreted phosphoprotein 2, 24kDa	U19485_at	-2.10
SPP2	secreted phosphoprotein 2, 24kDa	rc_AA858573_s_at	-1.75
<b>Nephrotoxicity</b>			
ATF3	activating transcription factor 3	M63282_at	6.92
EGF	epidermal growth factor (beta-urogastrone)	U04842_at	-1.80
EGF	epidermal growth factor (beta-urogastrone)	X12748cds_s_at	-2.41
GATM	glycine amidinotransferase (L-arginine:glycine amidinotransferase)	U07971_at	-2.63
GSTM1	glutathione S-transferase mu 1	J03914cds_s_at	-1.83
HAVCR1	hepatitis A virus cellular receptor 1 (KIM-1)	AF035963_at	38.21

ICAM1	intercellular adhesion molecule 1	D00913_at	2.62
ICAM1	intercellular adhesion molecule 1	D00913_g_at	4.35
KLK1	kallikrein 1	M19647_f_at	-3.11
KLK1	kallikrein 1	M22922cds_f_at	-2.62
LCN2	lipocalin 2	rc_AA946503_at	1.71
SPP1	secreted phosphoprotein 1	M14656_at	2.36
TIMP1	TIMP metalloproteinase inhibitor 1	rc_AI169327_g_at	1.80
<b>Nucleotide metabolism</b>			
ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase	D89514_at	1.83
GUK1	guanylate kinase 1	rc_AA800291_at	1.81
PRPS1	phosphoribosyl pyrophosphate synthetase 1	X16554_at	1.95
UPB1	ureidopropionase, beta	M97662_at	-2.39
<b>Oxidative Phosphorylation</b>			
ETFDH	electron-transferring-flavoprotein dehydrogenase	rc_AI237007_at	-1.90
GFER	growth factor, augmentor of liver regeneration	D30735_at	1.84
<b>Oxidative stress</b>			
GDF15	growth differentiation factor 15	AJ011969mRNA_at	9.23
HMOX1	heme oxygenase (decycling) 1	J02722cds_at	2.74
HMOX1	heme oxygenase (decycling) 1	rc_AI179610_at	6.12
SQSTM1	sequestosome 1	Y08355cds#2_at	2.82
<b>Protein Metabolism/ Modification/Degradation</b>			
CAND1	cullin-associated and neddylation-dissociated 1	E12829cds_at	2.31
CAND1	cullin-associated and neddylation-dissociated 1	rc_AI230395_at	1.81
CUL2	cullin 2	rc_AA875598_at	1.85
FN3K	fructosamine 3 kinase	rc_AA891965_at	-3.25
PFDN2	prefoldin subunit 2	rc_AA891049_at	1.70
PGCP	plasma glutamate carboxypeptidase	AF097723_s_at	-1.78
PSMB8	proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional peptidase 7)	D10729_s_at	1.90
UBQLN1	ubiquilin 1	rc_AA875206_at	1.71
<b>Regulation of signal transduction</b>			
MAPK6	mitogen-activated protein kinase 6	M64301_at	1.88
MAPKAPK3	mitogen-activated protein kinase-activated protein kinase 3	rc_AA955477_at	1.97
YWHAH	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide	D17445_g_at	2.02
<b>RNA metabolism, processing and transport</b>			
CPEB1	cytoplasmic polyadenylation element binding protein 1	rc_H31550_at	2.20

CPSF2	cleavage and polyadenylation specific factor 2, 100kDa	rc_AI639474_at	1.80
HNRNPU	heterogeneous nuclear ribonucleoprotein U (scaffold attachment factor A)	D14048_g_at	1.84
NUPL1	nucleoporin like 1	AF000899_at	2.28
NUPL1	nucleoporin like 1	AF000899_g_at	3.52
NUPL1	nucleoporin like 1	AF000901_s_at	2.34
PHAX	phosphorylated adaptor for RNA export	X67877_g_at	1.93
PTBP1	polypyrimidine tract binding protein 1	rc_AA891901_g_at	2.01
SART3	squamous cell carcinoma antigen recognized by T cells 3	rc_AI639476_s_at	1.91
SFPQ	splicing factor proline/glutamine-rich	AF036335_at	3.74
SMN1	survival of motor neuron 1, telomeric	AF044910_at	1.79
SMN1	survival of motor neuron 1, telomeric	AF044910_g_at	1.71
SSB	Sjogren syndrome antigen B (autoantigen La)	S59893_f_at	1.86
ZRANB2	zinc finger, RAN-binding domain containing 2	rc_AI170608_at	2.27
<b>Stress Response</b>			
CHKA	choline kinase alpha	D10262_at	5.44
CHKA	choline kinase alpha	E04239cds_s_at	3.45
DDIT3	DNA-damage-inducible transcript 3	U30186_at	13.35
GADD45A	growth arrest and DNA-damage-inducible, alpha	L32591mRNA_at	2.61
GADD45A	growth arrest and DNA-damage-inducible, alpha	L32591mRNA_g_at	2.81
GADD45A	growth arrest and DNA-damage-inducible, alpha	rc_AI070295_g_at	1.93
HSP90AA1	heat shock protein 90kDa alpha (cytosolic), class A member 1	rc_AA944397_at	2.54
HSP90B1	heat shock protein 90kDa beta (Grp94), member 1	AA685903_at	2.12
HSP90B1	heat shock protein 90kDa beta (Grp94), member 1	S69315_at	1.76
HSP90B1	heat shock protein 90kDa beta (Grp94), member 1	S69316_s_at	2.14
HSPA1A	heat shock 70kDa protein 1A	AA848563_s_at	2.80
HSPA1A	heat shock 70kDa protein 1A	Z75029_s_at	8.64
HSPA1B	heat shock 70kDa protein 1B	L16764_s_at	7.47
HSPA1B	heat shock 70kDa protein 1B	Z27118cds_s_at	5.04
HSPA5	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)	M14050_s_at	2.91
HSPA5	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)	S63521_i_at	2.98
HSPB1	heat shock 27kDa protein 1	rc_AA998683_g_at	1.97
HYOU1	hypoxia up-regulated 1	rc_AI009098_at	1.97
<b>Transcription</b>			
CSDA	cold shock domain protein A	D28557_s_at	1.92
DDX39	DEAD (Asp-Glu-Ala-Asp) box polypeptide 39	AF063447_at	2.59
DDX50	DEAD (Asp-Glu-Ala-Asp) box polypeptide 50	rc_AA799576_at	1.90
EED	embryonic ectoderm development	rc_AA799481_at	1.88
MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)	U56242_at	-1.98

NR1D2	nuclear receptor subfamily 1, group D, member 2	U20796_at	2.63
RNF4	ring finger protein 4	AF022081_at	1.91
TSC22D1	TSC22 domain family, member 1	L25785_at	1.91
ZFP36L1	zinc finger protein 36, C3H type-like 1	rc_AI136891_at	1.90
ZNF354A	zinc finger protein 354A	M96548_at	-3.41

### Transcription factor

HIVEP2	human immunodeficiency virus type 1 enhancer binding protein 2	M65251_s_at	2.20
HNF4A	hepatocyte nuclear factor 4, alpha	X57133mRNA_g_at	-2.02
HNF4A	hepatocyte nuclear factor 4, alpha	D10554_s_at	-2.19
LITAF	lipopolysaccharide-induced TNF factor	rc_AI237535_s_at	1.87
ZFP36L1	zinc finger protein 36, C3H type-like 1	rc_AI112516_at	1.74

### Translation

EEF1A1	eukaryotic translation elongation factor 1 alpha 1	rc_AI008852_at	-1.82
EIF2B2	eukaryotic translation initiation factor 2B, subunit 2 beta, 39kDa	U31880_at	1.88
EIF2B4	eukaryotic translation initiation factor 2B, subunit 4 delta, 67kDa	Z48225_at	1.80
EIF3B	eukaryotic translation initiation factor 3, subunit B	rc_AA875205_g_at	2.22
EIF3B	eukaryotic translation initiation factor 3, subunit B	rc_AA875205_at	1.98
EIF3D	eukaryotic translation initiation factor 3, subunit D	rc_AA891553_at	1.86
MRPL23	mitochondrial ribosomal protein L23	U62635_s_at	-1.74
NCL	nucleolin	M55015cds_s_at	2.56
NCL	nucleolin	M55017exon_s_at	1.86
NPM1	nucleophosmin 1	J03969_at	2.19
NPM1	nucleophosmin 1	J04943_at	4.92
TARS	threonyl-tRNA synthetase	rc_AA893860_at	2.49

### Transporter

AQP2	aquaporin 2 (collecting duct)	D13906_g_at	-2.01
SLC1A1	solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1	D63772_at	-1.88
SLC22A5	solute carrier family 22 (organic cation/carnitine transporter), member 5	AB017260_s_at	-2.12
SLC5A2	solute carrier family 5 (sodium/glucose cotransporter), member 2	U29881_at	-1.70
SLC6A18	solute carrier family 6, member 18	U12973_at	-1.75
SLC6A19	solute carrier family 6 (neutral amino acid transporter), member 19	rc_AA893060_s_at	-1.87
TAP1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	X57523_g_at	2.34

### Xenobiotic metabolism and transport

ABCB1B	ATP-binding cassette, sub-family B (MDR/TAP), member 1B	M81855_at	31.37
ABCC2	ATP-binding cassette, sub-family C (CFTR/MRP), member 2	D86086_s_at	18.57
CYP1A1	cytochrome P450, family 1, subfamily A, polypeptide 1	E00717UTR#1_s_at	-2.10
CYP2C9	cytochrome P450, family 2, subfamily C, polypeptide 9	M18363cds_s_at	-7.30

CYP2D12	cytochrome P450, family 2, subfamily d, polypeptide 12	J02869mRNA_s_at	-5.39
CYP2D26	cytochrome P450, family 2, subfamily d, polypeptide 26	AB008423_s_at	-2.30
EPHX2	epoxide hydrolase 2, cytoplasmic	X65083cds_at	-2.70
EPHX2	epoxide hydrolase 2, cytoplasmic	rc_AI104882_s_at	-1.76
EPHX2	epoxide hydrolase 2, cytoplasmic	X60328_at	-3.63
EPHX2	epoxide hydrolase 2, cytoplasmic	X60328_g_at	-2.89
FMO1	flavin containing monooxygenase 1	M84719_at	-1.71
SULT1A1	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	L19998_at	-2.42
SULT1A1	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	L19998_g_at	-1.94
UGT8	UDP glycosyltransferase 8	rc_AI228110_s_at	-2.04
<b>Others</b>			
2610528A11	RIKEN cDNA 2610528A11 gene	S74257_g_at	2.00
ALAD	aminolevulinate, delta-, dehydratase	rc_AA800745_at	-2.03
ALCAM	activated leukocyte cell adhesion molecule	AB008538_at	-1.74
ALPL	alkaline phosphatase, liver/bone/kidney	J03572_i_at	-2.46
AZIN1	antizyme inhibitor 1	D89983_at	1.98
C13ORF15	chromosome 13 open reading frame 15	AF036548_g_at	-2.36
C14ORF149	chromosome 14 open reading frame 149	rc_AA891693_at	-2.31
C22ORF25	chromosome 22 open reading frame 25	rc_AA799663_g_at	-1.98
CA4	carbonic anhydrase IV	S68245_g_at	-2.07
CALR	calreticulin	D78308_at	1.73
CCNL1	cyclin L1	AF030091UTR#1_at	2.24
CCNL1	cyclin L1	AF030091UTR#1_g_at	2.60
CD9	CD9 molecule	X76489cds_g_at	-1.74
CHORDC1	cysteine and histidine-rich domain (CHORD)-containing 1	rc_AA892238_at	1.75
CNKSR3	CNKSR family member 3	rc_AA893357_at	2.02
DIO1	deiodinase, iodothyronine, type I	X57999cds_at	-1.87
DPEP1	dipeptidase 1 (renal)	L07315_at	-1.90
DPP4	dipeptidyl-peptidase 4	J04591_at	-1.92
ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2	D28560_g_at	-2.19
HBB	hemoglobin, beta	M94919mRNA_f_at	-1.93
HSD11B2	hydroxysteroid (11-beta) dehydrogenase 2	U22424_at	-1.83
IDH1	isocitrate dehydrogenase 1 (NADP+), soluble	rc_AA944025_at	-1.76
IFRD1	interferon-related developmental regulator 1	rc_AI014163_at	5.49
IGFBP1	insulin-like growth factor binding protein 1	M58634_at	3.10
IGFBP3	insulin-like growth factor binding protein 3	M31837_at	-2.32
KEG1	kidney expressed gene 1	AB019693_at	-2.18
KIAA1737	KIAA1737	rc_AA799726_at	1.90

KTI12	KTI12 homolog, chromatin associated ( <i>S. cerevisiae</i> )	rc_AA800739_at	2.62
LINCR	likely ortholog of mouse lung-inducible Neutralized-related C3HC4 RING domain protein	rc_AI639029_s_at	2.68
LOC292872	kallikrein	M26534_i_at	-1.94
LOC681884	similar to CG14483-PA	rc_AA858572_at	2.36
NARG1	NMDA receptor regulated 1	rc_AI177404_at	3.73
NOP5/NOP58	nucleolar protein NOP5/NOP58	AF069782_at	5.55
NR1D1	nuclear receptor subfamily 1, group D, member 1	M25804_at	6.73
NR1D1	nuclear receptor subfamily 1, group D, member 1	M25804_g_at	6.62
NVL	nuclear VCP-like	rc_AA893753_at	1.73
PDIA4	protein disulfide isomerase family A, member 4	M86870_at	1.74
PLLP	plasma membrane proteolipid (plasmolipin)	Z49858_at	-1.77
PTN	pleiotrophin	rc_AI102795_at	-1.71
PVR	poliovirus receptor	L12025_at	5.53
PZP	pregnancy-zone protein	rc_AA945569_at	-4.00
RESP18	regulated endocrine-specific protein 18	L25633_at	-1.82
RESP18	regulated endocrine-specific protein 18	L25633_g_at	-1.79
RGD1564865	similar to 20-alpha-hydroxysteroid dehydrogenase	rc_AA891831_at	-1.94
RGD1566118	RGD1566118	rc_AA875620_at	3.73
RGD1566118	RGD1566118	rc_AA875620_g_at	3.91
RPS27L	ribosomal protein S27-like	X59375mRNA_at	1.76
S100A1	S100 calcium binding protein A1	rc_AI228548_at	-1.75
S100G	S100 calcium binding protein G	K00994mRNA_s_at	-2.62
SERBP1	SERPINE1 mRNA binding protein 1	U21718mRNA_at	1.74
SLC22A24	solute carrier family 22, member 24	Y09945_at	-2.44
SPINK1	serine peptidase inhibitor, Kazal type 1	M35299_s_at	-3.88
ST6GALNAC3	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetyl-galactosaminide alpha-2,6-sialyltransferase 3	rc_AI231519_at	-1.74
SUOX	sulfite oxidase	rc_AA891738_at	-1.87
THY1	Thy-1 cell surface antigen	rc_AA874848_s_at	-1.99
TMEM120A	transmembrane protein 120A	rc_AI011746_at	1.76
TTC36	tetratricopeptide repeat domain 36	rc_AA891739_at	-2.62
VAT1	vesicle amine transport protein 1 homolog ( <i>T. californica</i> )	rc_AA892520_at	2.44
VAT1	vesicle amine transport protein 1 homolog ( <i>T. californica</i> )	rc_AA892520_g_at	2.09
		rc_AA800549_at	-2.22
		rc_AA892642_at	1.75
		M81183Exon_UTR_g_at	-2.77
		rc_AA799594_at	2.19

rc_AA875032_at	1.71
rc_AA891596_at	-2.09
rc_AI102620_at	1.78
rc_AI230632_at	1.77
U21719mRNA_s at	6.08

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