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Lens transcriptome profile during cataract development in *Mip*-null mice

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Abstract

Major intrinsic protein or aquaporin-0 (MIP/AQP0) functions as a water channel and a cell-junction molecule in the vertebrate eye lens. Loss of MIP function in the lens leads to degraded optical quality and cataract formation by pathogenic mechanisms that are unclear. Here we have used microarray-hybridization analysis to detect lens transcriptome changes during cataract formation in mice that are functionally null for MIP (*Mip*^{-/-}). In newborn *Mip*^{-/-} lenses (P1) 11 genes were up-regulated and 18 were down-regulated (>2-fold, $p < 0.05$) and a similar number of genes was differentially regulated at P7. The most up-regulated genes (>6-fold) in the *Mip*^{-/-} lens at P1 included those coding for a mitochondrial translocase (*Timm8c1*), a matrix metalloproteinase (*Mmp2*), a Rho GTPase-interacting protein (*Ubx11*) and a transcription factor (*Twist2*). Apart from *Mip*, the most down-regulated genes (>4-fold) in the *Mip*^{-/-} lens at P1 included those coding for a proteasome sub-unit (*Psm8*), a ribonuclease (*Pop4*), and a heat-shock protein (*Hspb1*). Lens fiber cell degeneration in the *Mip*^{-/-} lens was associated with increased numbers of TUNEL-positive cell nuclei and dramatically elevated levels of calpain-mediated proteolysis of α II-spectrin. However red-ox status, measured by glutathione and free-radical levels, was similar to that of wild-type. These data suggest that while relatively few genes (~1.5% of the transcriptome) were differentially regulated >2-fold in the *Mip*^{-/-} lens, calpain hyper-activation acts as a terminal pathogenic event during lens fiber cell death and cataract formation.

Keywords

lens; cataract; aquaporin-0; transcriptome; calpain; *Hspb1*

1. Introduction

The major intrinsic protein of the mammalian ocular lens is an evolutionarily conserved member of the aquaporin family of water channels also known as aquaporin-0 (AQP0) [1]. In the mouse lens, MIP accounts for over 40% of the lens membrane proteome [2] and,

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despite relatively modest aquaporin activity, facilitates approximately 80% of water transport across fiber cell membranes [3–6]. In addition to water transport, MIP has been implicated in the formation of junctional micro-domains between the tightly packed concentric layers of fiber cells that makeup the crystalline mass of the lens and, in particular, MIP is enriched at interlocking protrusions decorating the vertices of fiber cell membranes – suggesting a role in cell-cell junction formation and adhesion [7–10]. The combined water-transport and cell-adhesion functions of MIP have been proposed to play a synergistic role in establishing the refractive index gradient and biomechanical properties of the lens including its fine-focusing power or accommodation reflex [11–13].

Mutations in the human MIP gene (*MIP*) mostly resulting in missense substitutions have been linked with autosomal dominant forms of cataract that present during infancy or childhood exhibiting wide variability in size, shape, and density of lens opacities [14,15] [<http://cat-map.wustl.edu/>]. Similarly, mutations in the rodent MIP gene (*Mip*) have been associated with semi-dominant cataract in the Shrivelled/Fraser (*Svl/Fr*), lens opacity (*Lop*), hydropic fibers (*Hff*), and Tohoku (*Tohm*) mouse strains and with recessive cataract in the Kyoto Fancy Rat Stock 4 (*kfrs4*) strain [16–20]. In addition to these mutant strains, mice functionally lacking (null) or deficient (hemizygous) in MIP also develop fully penetrant cataract [4].

The pathogenetic mechanisms underlying MIP-related cataract are only partly understood. Ectopic expression of several human MIP mutants in *Xenopus* oocytes or cultured cells results in abnormal intracellular accumulation and impaired water transport across the plasma membrane [14, 21–23]. In lenses of *Mip*-mutant mice, the mutant forms of MIP have been shown to accumulate within intracellular endoplasmic reticulum (ER)-like membranes rather than plasma membranes of lens fiber cells [16–19]. Further, in the case of the heterozygous *Lop* mutant (*Lop/+*) intracellular accumulation of mutant MIP (p.Ala51Pro) was associated with ER-stress and activation of the unfolded response coupled with oxidative stress and calpain hyper-activation [24]. However, in contrast to mutant forms of MIP that underlie autosomal dominant forms of cataract, less is known about the pathogenic mechanisms associated with ‘recessive’ cataract resulting from MIP loss-of-function. Here, we have undertaken a transcriptome profiling approach to detect potential pathogenic mechanisms associated with cataract development in *Mip*-null (*Mip*^{−/−}) lenses.

2. Materials and methods

2.1. Mice and lenses

Mice functionally null for MIP (*Mip*^{−/−}) were generated by gene-trap technology on a hybrid 129/B6J background as described [4] then backcrossed (>6 generations) to C57BL/6J (B6J, Jackson Lab, Bar Harbor ME; stock # 000664) and genotyped as described [24]. Heterozygous (*Mip*^{+/-}) mice were bred to generate Mendelian ratios of homozygous and wild-type (*+/+*) littermates for lens transcript profiling on postnatal day 1 (P1) and P7. Following euthanasia, eyes were removed and fixed or lenses were dissected as described [24]. All mouse studies were approved by the Washington University Animal Studies Committee in compliance with the guidelines published by the Institute of Laboratory

Animal Resources (ILAR) and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Microarray profiling and statistical analysis

Lens RNA was extracted using the RNeasy Plus Kit (Qiagen, Valencia, CA) and subjected to microarray analysis using the MouseRef-8 v2.0 (1883 genes) Expression BeadChip Kit (Illumina, San Diego, CA) according to the manufacturers' instructions and data were extracted, normalized, and summarized using the GenomeStudio Gene Expression (GX) Module (Illumina) as described [24]. The raw data were quality assessed by box-plot analysis (Supplementary Fig. 1) and the quantile normalized log₂ signal data analyzed using R packages 'lima' and 'gage' with Bonferroni correction of *p*-values as described [24].

2.3. Immunofluorescence microscopy

Immunofluorescent labeling of formaldehyde-fixed-paraffin-embedded mouse eye sections with aquaporin-0 antibody (AB3071, EMD Millipore, Billerica, MA) was imaged by confocal microscopy (Fluoview1000 MPE; Olympus, Center Valley, PA) as described [24].

2.4. TUNEL assay

DNA double-strand breaks (3'-OH ends) were detected in mouse lens sections (P7) using the Click-iT Plus TUNEL assay according to the manufacturer's protocol (ThermoFisher Scientific, Waltham, MA) as described [24].

2.5. Glutathione (GSH) assay

Free GSH concentration was measured in mouse lens extracts using Ellman's reagent, 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB, Sigma-Aldrich, St. Louis, MO) as described [24].

2.6. Reactive oxygen species (ROS) assay

Intracellular ROS levels in live mouse lenses were measured by vital staining with the cell-permeant, free-radical sensor Dihydrorhodamine 123 (DHR123, Sigma) essentially as described [24].

2.7. Immunoblotting and calpain assay

Lens proteins were subject to immunoblot analysis (Odyssey Infrared Imaging System Li-Cor, Lincoln, NE) as described [24] using anti-HSP27 (SC1049, Santa Cruz Biotechnology, Dallas, TX). Lens calpain activity was measured by immunoblot analysis of α II-spectrin cleavage products using anti-spectrin (MAB1622, EMD Millipore) and anti- β -actin (#3700, Cell Signaling Technology, CST, Danvers, MA) to control for sample loading as described [24].

3. Results

3.1. *Mip*^{-/-} lens phenotype

Dark-field microscopy revealed that the *Mip*^{-/-} lens *ex vivo* (P7) exhibited a translucent grey central opacity with a clear cortex and was smaller in size to that of wild-type (Fig. 1b).

Histochemical staining of *Mip*^{-/-} lens sections (P7) revealed severe degeneration of fiber cells within the central core region flanked by many displaced cell nuclei from the disturbed bow-regions and the equatorial axis appeared misshapen compared with wild-type (Fig. 1d). Immunofluorescent staining with MIP-antibody also confirmed that the intense labeling of fiber cell membranes in wild-type lenses was absent from *Mip*^{-/-} lenses (Fig. 1f) [4]. Overall, these observations are consistent with central fiber cell death in the *Mip*^{-/-} lens.

3.2. *Mip*^{-/-} lens microarray analysis

At P1 in the *Mip*^{-/-} lens, 29 genes were significantly, differentially regulated (>2-fold, p=0.05) with 11 genes up-regulated and 18 genes down-regulated (Supplementary Table 1). Similarly at P7, 28 genes were differentially regulated (>2-fold) with 11 genes up-regulated and 17 down regulated (Supplementary Table 2). Eight of the most up-regulated genes at P1 were similarly up-regulated at P7 including; *Timmdc1*, *Twist2*, *Ubxn11*, *Mmp2*, *Prx*, *Cat*, *Gfer*, and *Fchsd1* (Table 1). However, three of the genes up-regulated at P1 (*Igfbp5*, *Atp10d*, *Rab4a*) were replaced by *Raet1b*, *Chac1*, and *Cd3eap* at P7 (Supplementary Tables 1 and 2).

As expected, *Mip* was significantly down-regulated (>15-fold) in the *Mip*^{-/-} lens at P1 and P7. Besides *Mip*, 11 of the most down-regulated genes in the *Mip*^{-/-} lens at P1 were similarly down-regulated at P7 including, *Psmd8*, *Pop4*, *Fcgr*, and *Ddr1* (Table 1). However, seven of the genes down-regulated at P1 (*Hspb1*, *Sppl2b*, *Xbp1*, *Smco3*, *Spryd4*, *Tsc2*, *Rshl2a*) were replaced by six others at P7 (*Clic5*, *Clcn7*, *Clasrp*, *Zfp420*, *Ankrd46*, and *Ooep*) (Supplementary Tables 1 and 2).

A Pathway analysis using the Gene Ontology (GO) Biological Process database (<http://geneontology.org/>) suggested that the most differentially regulated genes in the *Mip*^{-/-} lens at P1 were associated with multiple biosynthetic and metabolic processes including the oxidation-reduction process (GO:0055114, p=0.002) and at P7 with regulation of ion and water transport/homeostasis (e.g. GO:0010959, p=0.02; GO:0030104, p=0.03) and, to a lesser extent, response to oxidative stress (GO:0006979, p=0.04) and ROS (GO:0000302, p=0.04). (Supplementary Tables 3 and 4).

3.3. *Mip*^{-/-} lens cell death

In order to gain further insights about cell-death mechanisms in the *Mip*^{-/-} lens we assayed several molecular markers that were strongly associated with fiber cell-death in the *Lop*^{+/+} lens [24]. First, we used the TUNEL assay to detect DNA-fragmentation *in situ* that is often associated with apoptotic cell death [25]. In both *Mip*^{-/-} and wild-type lenses (P7) TUNEL-positive nuclei were absent from equatorial epithelial cells and nascent fiber cells at the periphery (Fig. 2). However, a modest increase in TUNEL positive nuclei was detected in degenerating central fiber cells of the *Mip*^{-/-} lens when compared with the central region of wild-type lenses. These observations suggest that while apoptosis is not activated in equatorial lens epithelial cells or nascent fiber cells of *Mip*^{-/-} lenses, it may be active within the central zone of degenerating fiber cells. Since TUNEL is specific for the 3'-OH ends of fragmented DNA rather than apoptosis, however, it is also possible that the observed DNA-fragmentation may result from a cell-death pathway other than classical apoptosis (e.g. necrosis). Second, we assessed oxidative-stress in the *Mip*^{-/-} lens by measuring 1) the

levels of free GSH, an important anti-oxidant molecule in the lens [26], with the sulphydryl assay reagent DTNB and 2) free-radical levels by vital staining with the cell-permeant dye DHR123 that when oxidized by ROS emits fluorescence. Neither GSH levels nor ROS production in the *Mip*^{-/-} lens (P8) appeared significantly different from wild-type (Supplementary Fig. 2 and 3). However, we cannot rule out the possibility that increased oxidative-stress manifests later during *Mip*^{-/-} lens development. Third, immunoblot analysis of the *Mip*^{-/-} lens (P1) detected about a 3-fold decrease in *Hspb1* protein levels compared with wild-type (Supplementary Fig. 4) consistent with the approximately 4-fold decrease in *Hspb1* transcript levels detected on the microarray platform (Supplementary Table 1). Finally, we measured the activity of cytosolic, calcium (Ca²⁺) activated, cysteine proteases or calpains that are involved in remodeling of membrane cytoskeleton proteins, including α II-spectrin (α -fodrin), during mouse lens fiber cell differentiation [27]. Immunoblot analysis revealed that the level of α II-spectrin cleavage to several specific breakdown products, including C-terminal fragments of 150 kDa and 145 kDa, was greatly increased consistent with strong post-translational activation of calpains and, possibly, other proteases in the *Mip*^{-/-} lens (Fig. 3).

4. Discussion

In this study we have monitored transcriptome changes during cataract development in neonatal lenses from mice functionally null for MIP. Overall, relatively few genes (29/1883, ~1.5% of transcriptome) were differentially regulated >2-fold in the *Mip*^{-/-} lens at P1 and P7 with more genes down-regulated (~18) than up-regulated. Pathway analysis suggested that while water transport and/or homeostasis were impaired in the *Mip*^{-/-} lens at P7 no specific stress pathway was strongly associated with cataract formation at P1. These data contrasted somewhat with transcriptome changes during cataract development in the *Lop*^{+/+} lens, which harbors a heterozygous missense mutation in *Mip* (p.A51P) that causes intracellular accumulation of the mutant protein [24]. While a similar number of genes (32/1883, ~1.7% of transcriptome) was differentially regulated at P1 in both *Mip*^{-/-} and *Lop*^{+/+} lenses many more genes (174/1883, ~9% of transcriptome) were differentially regulated at P7 in the *Lop*^{+/+} lens with more genes up-regulated (99) than down regulated. Further, pathway analysis of the *Lop*^{+/+} lens identified the lysosome pathway at P1 progressing at P7 to ER-stress and activation of the unfolded protein response (UPR) coupled with severe oxidative stress [24]. These observations suggest that the presence and accumulation of mutant MIP has more significant and progressive effects on gene transcription than loss of MIP function in the lens.

The most up-regulated genes in the *Mip*^{-/-} lens at both P1 and P7 included *Timmdc1*, *Mmp2*, *Twist2*, *Ubxn11*, *Prx*, *Cat*, *Gfer*, and *Fchsd1*. *Mmp2* codes for a secreted gelatinase that cleaves type IV collagen, a major component of the lens basement membrane or capsule, and up-regulation of *Mmp2* in lens epithelial cells has been associated with fibrotic stress and posterior capsule opacification [28]. *Prx* encodes a double PDZ-domain containing protein that is required for the hexagonal cross-sectional geometry and membrane organization of lens fiber cells [29] suggesting that up-regulation of *Prx* in the *Mip*^{-/-} lens may reflect a compensatory mechanism to help stabilize fiber cell membranes in the absence of MIP. *Cat* encodes catalase, an anti-oxidant heme enzyme found in peroxisomes,

suggesting that its up-regulation in the *Mip*^{-/-} lens was indicative of oxidative-stress. However, we did not detect increased ROS production in the *Mip*^{-/-} lens (Supplementary Fig. 2) and catalase-deficiency in the mouse lens was not associated with increased sensitivity to photochemically-induced oxidative-stress [30]. The remaining up-regulated genes at P1 and P7 encoded an assembly factor for the mitochondrial complex-I (C1) of the electron transport chain (*Timmdc1*), a Rho GTPase-interacting protein that functions in disassembly of actin stress fibers (*Ubxn1l*), an F-BAR and double SH3 domain protein implicated in F-actin polymerization (*Fchsdl*), a basic helix-loop-helix (bHLH) transcription factor implicated in cancer-associated epithelial-mesenchymal transition (*Twist2*), and an anti-apoptotic factor with anti-oxidant properties (*Gfer*) [31–35]. However, the precise roles of these genes in lens biology and cataractogenesis remains to be determined.

Besides *Mip*, the most down-regulated genes in the *Mip*^{-/-} lens at P1 and P7 coded for a proteasome sub-unit (*Psmd8*), a ribonuclease (*Pop4*), a receptor tyrosine kinase (*Ddr1*), an IgG-receptor (*Fcgr4*), a small ribosomal sub-unit (*Cmss1*), a Golgi protein (*1190002N15Rik*), a transcription factor interacting protein (*Atf7ip*), a death-effector domain protein (*Dedd2*), a pleiotropic regulator (*Plrg1*), and a mitochondrial dehydrogenase (*Bckdhh*). Again however, the role of these genes in lens biology and cataractogenesis remains enigmatic.

When compared with differentially regulated genes in the *Lop*^{+/+} lens only four genes (*Chac1*, *1190002N15Rik*, *Xbp1* and *Hspb1*) matched with those in the *Mip*^{-/-} lens at P1 and/or P7 (Supplementary Table 5) [24]. First, *Chac1* was mildly up-regulated (~2.5-fold) in the *Mip*^{-/-} lens at P7 and strongly up-regulated in the *Lop*^{+/+} lens at P7 (~80-fold). *Chac1* encodes a γ glutamyl cyclo-transferase that catalyzes the degradation of the tri-peptide thiol-antioxidant, GSH [36]. While GSH levels in the *Lop*^{+/+} lens were significantly decreased, GSH levels in the *Mip*^{-/-} lens were similar to wild-type. However, we cannot exclude the possibility that *Chac1* continues to increase after P7 in the *Mip*^{-/-} lens resulting in subsequent GSH depletion and oxidative-stress. Second *Xbp1*, which encodes a key UPR transcription factor [37], was down-regulated in the *Mip*^{-/-} lens at P1 and progressively up-regulated in the *Lop*^{+/+} lens at P1 and P7, consistent with UPR activation in the latter but not the former. Third, *1190002N15Rik* was the only gene down-regulated in the *Mip*^{-/-} lens and the *Lop*^{+/+} lens at both P1 and P7 [24]. *1190002N15Rik* encodes a secreted Golgi protein (GoPro49), also known as deleted in autism protein 1 homolog (DIA1) or hypoxia and Akt-induced stem cell factor (HASF), that possesses cytoprotective and anti-apoptotic properties; however, its role in the lens secretory pathway is unclear [38–40]. Finally, *Hspb1* was moderately down-regulated in the *Mip*^{-/-} lens at P1 and strongly down-regulated in the *Lop*^{+/+} lens at P1 and P7. *Hspb1* encodes a member of the small heat-shock proteins (25–27kDa) that is abundant in the lens and is known to possess robust molecular chaperone and anti-apoptotic properties [41]. While we cannot distinguish between cause and effect, such down-regulation of *Hspb1* is likely to increase susceptibility to cell stress and/or cell death pathways in the lens.

Histochemical analysis of the *Mip*^{-/-} lens revealed central fiber cell degeneration similar, if less severe, to that of the *Lop*^{+/+} lens (Fig. 1) [24]. There was also a more modest increase in TUNEL positive nuclei associated with degeneration in the *Mip*^{-/-} lens (Fig. 2) compared

with that in the *Lop/+* lens [24]. Further, the free radical overproduction in the *Lop/+* lens [24] was not observed in the *Mip-/-* lens (Supplementary Fig. 3). By contrast, calpain-mediated cleavage of α II-spectrin was strongly increased in the *Mip-/-* lens (Fig. 3) similar to that observed in the *Lop/+* lens [24]. While we cannot exclude caspase-dependent apoptosis in either lens these observations suggest that calpain-mediated cell-death, associated with impaired de-nucleation of fiber cells, predominated in both the *Mip-/-* and *Lop/+* lenses. Chemically induced activation of calpains in the rodent lens, particularly calpain-2 and calpain-3 (Lp82), is an established cause of crystallin and cytoskeleton proteolysis resulting in cataractogenesis [42]. In the *Mip-/-* lens it is likely that absence of MIP function and, consequently, loss of fiber cell membrane integrity disrupts the normal microcirculation of ions and water throughout the lens [43] permitting uncontrolled Ca^{2+} influx to randomly hyper-activate calpain-mediated proteolysis and cataractogenesis. In summary, our data suggest that *Hspb1* depletion, increased TUNEL-positive nuclei, and calpain hyper-activation are common signatures of lens fiber cell death and cataract development in mouse models of MIP-related cataract in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

MIP	major intrinsic protein
AQP0	aquaporin-0
TUNEL	terminal deoxynucleotidyl transferase [TdT]-mediated dUTP nick-end labeling
ROS	reactive oxygen species
GSH	glutathione
DTNB	5,5'-dithio-bis-(2-nitrobenzoic acid)
DHR123	Dihydrorhodamine 123
H&E	hematoxylin and eosin

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Highlights

- Microarray analysis detected 29 genes regulated >2-fold in the *Mip-null* lens (P1).
- 18 genes were down-regulated (e.g. *Hspb1*) and 11 were up-regulated (e.g. *Mmp2*).
- TUNEL-positive nuclei were increased but GSH and ROS levels were not.
- Calpain hyper-activation was associated with lens fiber cell death and cataract.

Fig. 1.

Lens phenotype of the *Mip*^{-/-} mouse. (A–B) Dark-field images of the wild-type (A) and *Mip*^{-/-} (B) lenses (P7) showing dense polymorphic central ‘grey’ opacification and reduced lens size (microphakia) of the latter. NB: The P7 wild-type lens exhibits a central, spherical, white opacity or ‘cold cataract’ resulting from phase-separation of crystallin proteins [44] that can be readily reversed by gentle warming (~30°C) of the media. (C–D) Sagittal sections of lenses from wild-type (C) and *Mip*^{-/-} (D) mice stained with H&E showing degeneration of central fiber cells flanked by disturbed nuclei near the equator of the latter. (E–F) Immunofluorescent labeling of wild-type (E) and *Mip*^{-/-} (F) lenses showing absence of intense anti-MIP staining of fiber cell membranes in the latter. Scale bar: 300 μm (A,B), 100 μm (C,D), 200 μm (E,F).

Fig. 2. TUNEL analysis of DNA-fragmentation in the *Mip*^{-/-} lens (P7). Sagittal sections of wild-type (**A, B**) and *Mip*^{-/-} (**C, D**) lenses (P7) showing an increase in TUNEL positive nuclei within the central zone of fiber cell degeneration in the *Mip*^{-/-} lens (**C**) versus only trace levels in wild-type (**A**). (**B, D**) merged images of TUNEL positive nuclei (red) and DAPI stained nuclei (green) in wild-type (**B**) and *Mip*^{-/-} (**D**) lenses. Scale bar: 200 μ m.

Fig. 3.

Immunoblot analysis of calpain activity in the *Mip*^{-/-} lens (P7). α II-spectrin cleavage was greatly increased in the *Mip*^{-/-} lens compared with wild-type. Asterisks indicate cleavage products of 150 kDa and 145 kDa. When normalized to β -actin levels, the signal intensity of the 150 kDa product was over 6-fold greater than that of wild-type. Other cleavage products <100 kDa were also clearly detected in the *Mip*^{-/-} lens. M, molecular weight markers.

Table 1

Differentially regulated genes (>2-fold) in the *Mip*^{-/-} lens at P1 and P7 detected by microarray analysis.

Gene symbol	P1 fold-change	P1 adjusted p-value	P7 fold-change	P7 adjusted p-value	Mus musculus mRNA transcript	GENE ID	CYTOBAND
<i>Timm8c1</i>	17.02166195	1.47903E-07	15.01687796	2.50239E-08	Translocase of inner mitochondrial membrane domain containing 1	76916	16qB4
<i>Twist2</i>	5.247062592	3.19474E-07	6.179799365	0.000963147	Twist basic helix-loop-helix transcription factor 2	22160	12qB-C1
<i>Ubxn11</i>	4.632859723	1.47334E-05	6.204766847	0.000973355	UBX domain protein 11	67586	4qD3
<i>Mmp2</i>	3.926535662	1.7121E-05	8.267837307	4.36964E-06	Matrix metalloproteinase 2	17390	8qC5
<i>Prx</i>	2.994966778	3.00074E-06	2.615892038	3.57482E-05	Periactin	19153	7qA3
<i>Cat</i>	2.472910455	1.05715E-05	2.213505564	0.000248867	Catalase	12359	2qE2
<i>Gfer</i>	2.464793871	2.11979E-06	2.309158176	3.57482E-05	Growth factor, erv1 (<i>S. cerevisiae</i>)-like (augmenter of liver regeneration)	11692	17qA3.3
<i>Fchsdl</i>	2.119269409	0.000391052	3.544855943	0.003532385	FCH and double SH3 domains 1	319262	18qB3
<i>Beckdhb</i>	-2.095547175	0.001415678	-3.205812393	0.00023145	Branched chain ketoacid dehydrogenase E1, beta polypeptide (mitochondrial)	12040	9qE2
<i>Plg1</i>	-2.221539818	0.000258117	-2.04622246	4.33416E-05	Pleiotropic regulator 1, PRL1 homolog (<i>Arabidopsis</i>)	53317	3qE3
<i>Dedd2</i>	-2.371159974	0.000731086	-2.204487047	6.03092E-05	Death effector domain-containing DNA binding protein 2	67379	7qA3
<i>Att7ip</i>	-2.883531934	2.11979E-06	-2.42022806	3.34561E-06	Activating transcription factor 7 interacting protein	54343	6qG1
<i>1190002N15Rik</i>	-3.088443632	0.001270686	-2.135164168	0.1585206	RIKEN cDNA 1190002N15 gene	68861	9qE3.3
<i>Cms1</i>	-3.247170188	6.91313E-05	-3.531832687	0.000617299	Cms small ribosomal subunit 1	66497	16qC1.1
<i>Fcgr4</i>	-3.351407232	1.36237E-06	-5.877460752	1.18924E-07	Fc receptor, IgG, low affinity IV	246256	1qH3
<i>Ddr1</i>	-3.997098153	3.63247E-07	-2.404688464	3.57482E-05	Discoidin domain receptor family, member 1	12305	17qB1
<i>Pop4</i>	-4.457061908	1.61061E-08	-4.585407658	2.68116E-08	Processing of precursor 4, ribonuclease P/MRP family, (<i>S. cerevisiae</i>)	66161	7qB2
<i>Mip</i>	-14.73218996	1.01711E-10	-19.64649434	2.79045E-11	Major intrinsic protein of eye lens fiber	17339	10qD3
<i>Psmd8</i>	-21.17700995	5.48003E-12	-23.3181754	4.89024E-11	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 8	57296	7qB1