

# Involvement of TGF- $\beta$ Receptor- and Integrin-Mediated Signaling Pathways in the Pathogenesis of Granular Corneal Dystrophy II

Seung-il Choi,<sup>1</sup> Yeong-Min Yoo,<sup>2</sup> Bong-Yoon Kim,<sup>1</sup> Tae-im Kim,<sup>1</sup> Hyun-ju Cho,<sup>1</sup> So-yoen Ahn,<sup>1</sup> Hyung Keun Lee,<sup>1</sup> Hyun-Soo Cho,<sup>3</sup> and Eung Kweon Kim<sup>1</sup>

**PURPOSE.** The purpose of this study was to elucidate the pathophysiological process in primary cultured corneal fibroblasts (PCFs) from normal subjects and granular corneal dystrophy (GCD) II patients, by using cDNA microarrays.

**METHODS.** PCFs were isolated from the corneas of normal subjects and GCD II patients who were heterozygous and homozygous for the *TGFBI* R124H mutation. RNA was isolated from each sample, and gene expression profiles were analyzed with a cDNA microarray consisting of approximately 29,000 genes. Cell adhesion assays were performed to confirm the functionality of the detected gene expression profiles.

**RESULTS.** Twofold differences were detected in the expression of 555 genes between wild-type and homozygous GCD II PCFs. Of these, 319 genes were upregulated, and 236 genes were downregulated in the homozygous GCD II PCFs. The most abundant and consistent changes were observed in gene families encoding signal transduction pathways involving the TGF- $\beta$  receptor- and integrin-mediated signaling, cell differentiation and proliferation, immune responses, cell adhesion, extracellular matrix (ECM) proteolytic enzymes, cell cycle, cytoskeletal organization, mitochondrial energy metabolism, collagen catabolism, response to wounding, response to oxidative stress, and the ubiquitin-mediated proteasomal degradation pathway. Cell adhesion assays demonstrated that heterozygous and homozygous GCD II PCFs strongly attached to collagen-I, collagen-IV, fibronectin, and laminin, compared with wild-type cells.

**CONCLUSIONS.** Alterations in the TGF- $\beta$  receptor- and integrin-mediated signaling pathway may play a key role in GCD II pathophysiology. If the novel factors identified in this study are involved in GCD II pathogenesis, they could assist in designing further studies to elucidate specific mechanisms of this disease.

(*Invest Ophthalmol Vis Sci.* 2010;51:1832-1847) DOI: 10.1167/iovs.09-4149

Granular corneal dystrophy II (GCD II) is a disorder characterized by age-dependent progressive accumulation of protein deposits in the corneal epithelia and stroma, followed by disruption of corneal transparency. GCD II is an autosomal dominant disorder caused by a point mutation (R124H) in the transforming growth factor- $\beta$ -induced gene (*TGFBI*) on chromosome 5, region q31.<sup>1,2</sup> *TGFBI* encodes a highly conserved 683 amino acid protein (TGFBIp) that contains a secretory signal sequence and an Arg-Gly-Asp (RGD) motif that serves as a ligand recognition site for integrins.<sup>1</sup> TGFBIp is a component of extracellular matrix (ECM) that mediates cell adhesion and migration by interacting with integrins.<sup>3-5</sup> More recently, it has been shown that loss of TGFBIp induces cell proliferation and spontaneous tumor development in *TGFBI*-knockout mice.<sup>6</sup>

Thirty-eight different mutations in *TGFBI* are involved in corneal dystrophies. Remarkably, different mutations cause unique corneal dystrophy phenotypes, such as R124H in GCD II (also called Avellino corneal dystrophy), R124C in lattice corneal dystrophy type I, R555W in granular corneal dystrophy type I, and R555Q in Thiel-Behnke corneal dystrophy.<sup>7</sup>

The cornea is an avascular tissue located in the anterior part of the eye and consists of five layers of tissue that serve as a barrier to infection. The principal cell types of the cornea include corneal epithelial cells in the outer region, keratocytes in the middle region, and endothelial cells in the inner region. Keratocytes, also known as corneal fibroblasts, have a dendritic morphology and produce keratan sulfate proteoglycans that are necessary for the maintenance of the corneal structure and physiology, particularly for the maintenance of corneal transparency.<sup>8,9</sup> These cells are also responsible for the synthesis of collagen fibrils and the ECM and for stromal repair after injury or infection.

TGFBIp plays a significant role in the health of the cornea. For example, increased production of TGFBIp by corneal fibroblasts has been detected in areas of corneal injury,<sup>10</sup> and ablation of the corneal stroma by laser in situ keratomileusis (LASIK) in GCD II patients accelerates TGFBIp deposition.<sup>11,12</sup> Overexpression of mutant *TGFBI* induces apoptotic cell death in human corneal epithelial cells,<sup>13</sup> and age-dependent retinal degeneration has been observed in transgenic mice that express a mutant form of the human *TGFBI*.<sup>14</sup> More recently, we have reported that PCFs are most susceptible to oxidative stress.<sup>15</sup> However, the pathophysiological process underlying GCD II has yet to be fully elucidated. To understand the pathophysiology of GCD II, we used cDNA microarray analysis to identify differential gene expression profiles between homozygous GCD II and wild-type PCFs. We also tested the ability of these cells to adhere to various ECM proteins to confirm the functional relevance of the gene expression results.

From the <sup>1</sup>Corneal Dystrophy Research Institute, Department of Ophthalmology, College of Medicine, and the <sup>3</sup>Department of Biology, College of Life Science and Biotechnology, Yonsei University, Seoul, Korea; and the <sup>2</sup>Laboratory of Veterinary Biochemistry and Molecular Biology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Korea.

Supported by Grant A080320 from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare, and Family Affairs, Republic of Korea.

Submitted for publication June 15, 2009; revised November 7, 2009; accepted November 11, 2009.

Disclosure: **S. Choi**, None; **Y.-M. Yoo**, None; **B.-Y. Kim**, None; **T. Kim**, None; **H. Cho**, None; **S. Ahn**, None; **H.K. Lee**, None; **H.-S. Cho**, None; **E.K. Kim**, None

Corresponding author: Eung Kweon Kim, Severance Hospital, Yonsei University, College of Medicine, Department of Ophthalmology, 134 Shinchon-dong, Seodaemun-ku, 120-752, Seoul, Korea; eungkkim@yuhs.ac.

TABLE 1. Pairs of GCD II and Normal Samples

| Pair | Normal Cornea Sex/Age | Mean Age | Heterozygous Sex/Age | Mean Age | Homozygous Sex/Age | Mean Age | Use of Samples  |
|------|-----------------------|----------|----------------------|----------|--------------------|----------|---|
| 1    | F/20                  |          | F/37                 |          | F/13               |          | Microarray<br>RT-PCR<br>Western blot<br>Cell-adhesion assay |
| 2    | M/10                  | 25.3     | F/20                 | 35.3     | M/10               | 16.6     | Microarray<br>RT-PCR<br>Cell-adhesion assay                 |
| 3    | M/45                  |          | F/49                 |          | F/27               |          | Microarray<br>Cell-adhesion assay                           |

## MATERIALS AND METHODS

### Isolation and Culture of Primary Corneal Fibroblasts

Wild-type ( $n = 3$ ), heterozygous ( $n = 3$ ), and homozygous ( $n = 3$ ) primary human corneal fibroblasts were prepared by using a published method.<sup>15</sup> Donor confidentiality was maintained according to the Declaration of Helsinki, and the protocol was approved by Severance Hospital IRB Committee (CR04124), Yonsei University. GCD II was diagnosed by DNA sequencing analysis of *TGFBI* mutations. Age, sex, and diagnosis in the GCD II cases are shown in Table 1. After removal of the corneal button for penetrating keratoplasty, the remaining corneal rims were harvested for culture of the normal corneal fibroblast. The medical records of the donors from the eye bank of Yonsei University Severance Hospital did not show any genetic or systemic metabolic disease. The fibroblasts grown from the pieces of corneal rims were treated as normal ones. Genetic normality of the *BIGH3* gene in normal primary corneal fibroblasts was determined by DNA sequencing analysis. Table 1 presents information regarding the corneal fibroblasts used in these studies. For cDNA microarray analysis, six samples (normal sample pairs 1, 2, and 3; and homozygous sample pairs 1, 2, and 3) were analyzed, excluding the heterozygous sample. For RT-PCR studies, the samples were prepared by pooling total RNA from two samples (Table 1, pairs 1 and 2). The sample pairs 1 and 2 were used for Western blot analysis. For the cell adhesion assay, nine samples (normal, heterozygous, and homozygous sample pairs 1, 2, and 3) were analyzed.

### RNA Extraction and Gene Expression Profiling

Transcriptional profiles were evaluated in three independent cell preparations, by using a cDNA microarray (GeneChip Human Gene 1.0 ST Array, GeneChip; Affymetrix, Santa Clara, CA) containing approximately 29,000 genes. To determine a variation and average changes ( $\alpha$ -fold) in the expression of different genes in each sample and to compare these samples, we analyzed six samples (normal sample pairs 1, 2 and 3, and homozygous samples pair 1, 2, and 3) for new microarray analysis without total RNA pooling. Total RNA was extracted from the PCFs (TRIzol; Invitrogen, Carlsbad, CA) followed by purification (RNeasy kit; Qiagen, Valencia, CA) to remove residual

DNA. The concentration of total RNA was determined by UV spectrophotometry (ND-1000 UV-Vis Spectrophotometer; Nanodrop Technologies, Wilmington, DE). Two quality controls were used for each RNA sample: (1) an  $A_{260}/A_{280}$  ratio between 1.7 and 2.3; and (2) an electropherogram showing two distinct ribosomal peaks corresponding to either 18S and 28S for eukaryotic RNA bands at a ratio of 28S/18S of  $>0.5$  with minimal or no degradation. The arrays were scanned (GeneChip Scanner 3000 7G; Affymetrix), raw signal intensities were normalized (GeneChip Operating Software [GCOS] algorithm; Affymetrix), and the data were analyzed (Gene Chip DNA Analysis Software [GDAS], ver. 2.0 according to the Affymetrix GeneChip Expression Analysis Technical Manual; <http://www.affymetrix.com>). We detected a twofold change in differential gene expression between the normal and homozygous samples. For statistical data analysis, we used a two-tailed unpaired Student's *t*-test ( $P < 0.05$ ), to assess significant differences between the two cell types.

### Reverse Transcription-Polymerase Chain Reaction

Two micrograms of total RNA was reverse transcribed into cDNA (Superscript II reverse transcriptase; Invitrogen, Carlsbad, CA) and an Oligo (dT) primer (Invitrogen). The cDNA was amplified by using primers derived from the sequence of the selected differentially expressed genes, and  $\beta$ -actin expression was used as the control. Amplification products were visualized by electrophoresis in 1.2% agarose gels containing ethidium bromide. Specific RT-PCR primer sets and annealing temperatures are listed in Table 2.

### Preparation of Cell Lysates and Western Blot Analysis

Cell lysates were prepared in radio immunoprecipitation assay (RIPA) buffer (pH 7.4; containing a Complete Mini Protease Inhibitor Tablet; Roche Diagnostics, Indianapolis, IN). Crude cell lysates were centrifuged at 10,000g for 10 minutes at 4°C, to remove nuclear fragments and tissue debris. A portion of the supernatant was used to determine the total protein concentration with a bicinchoninic acid protein assay (BCA; Kit; Pierce, Rockford, IL), and equal amounts of each sample were analyzed by Western blot, as described previously.<sup>15</sup> The following antibodies were used: anti-integrin  $\alpha_2$  (1:1000; Chemicon, Te-

TABLE 2. PCR Primer Pairs

| Genes                           | Accession Number | Forward Primers            | Reverse Primers            | Product Size (bp) | Annealing Temp. (°C) |
|---------------------------------|------------------|----------------------------|----------------------------|-------------------|----------------------|
| <i>FMOD</i>                     | NM_002023        | 5'-GGAAGAGGGGATCTTTGGAC-3' | 5'-CCACCCTCATGCTTTTCCT-3'  | 199               | 60                   |
| <i>SOD-2</i>                    | NM_001024466     | 5'-CGTCACCGAGGAGAAGTACC-3' | 5'-CTGATTTGGACAAGCAGCAA-3' | 196               | 59                   |
| <i>TGF-<math>\beta</math>1</i>  | NM_000660        | 5'-GGGACTATCCACCTGCAAGA-3' | 5'-CCTCCTTGGCGTAGTAGTCG-3' | 239               | 60                   |
| <i>TGF-<math>\beta</math>2</i>  | NM_001135599     | 5'-CCGGAGGTGATTTCCATCTA-3' | 5'-CTCCATTGCTGAGACGTCAA-3' | 287               | 58                   |
| <i>TGF-<math>\beta</math>3</i>  | NM_003239        | 5'-GAGTCAGAGCCCAGCAAAAC-3' | 5'-AGAAGGAGGGAGGAAAACCA-3' | 246               | 58                   |
| <i>TGFB1</i>                    | NM_000358        | 5'-GTGTGTGCTGTGCAGAAGGT-3' | 5'-TTGAGAGTGGTAGGGGTGCT-3' | 172               | 58                   |
| <i><math>\beta</math>-Actin</i> | NM_001101        | 5'-GGACTTCGAGCAAGAGATGG-3' | 5'-AGCACTGTGTTGGCGTACAG-3' | 234               | 58                   |

mecula, CA); anti-TGF- $\beta$  receptors I, II, and III (1:200, 1:200, and 1:100, respectively; Santa Cruz Biotechnology, Santa Cruz, CA); and anti-fibronectin (1  $\mu$ g/mL; Calbiochem, San Diego, CA). Horseradish peroxidase (HRP)-conjugated anti-mouse IgG or anti-rabbit IgG at 1:5000 were used as secondary antibodies (GE Healthcare, Piscataway, NJ). Immunoblots were developed using enhanced chemiluminescence (ECL) as described by the manufacturer (Pierce, Rockford, IL). Each immunoreactive protein band was image scanned, and optical densities were quantified (ImageJ software, version 1.37; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at <http://rsb.info.nih.gov/ij/index.html>) and were corrected by background subtraction and normalized to the intensity of the corresponding  $\beta$ -actin protein bands.

### Cell-ECM Adhesion Profile Assays

Cell adhesion assays were performed using the ECM cell adhesion array kit (Cytomatrix; Chemicon International) according to the manufacturer's instructions. Nine samples (three samples for each of normal, heterozygous, and homozygous corneal fibroblast) were analyzed, and each experiment was repeated three times.

### Statistical Analysis

Results were evaluated for significance ( $P < 0.05$ ) with one-way ANOVA followed by Newman-Keuls multiple comparison tests. Results are expressed as the mean  $\pm$  SD (Prism ver. 4.0; Graph Pad Software Inc, San Diego, CA).

## RESULTS

### Gene Expression Profiles in Wild-Type and Homozygous GCD II PCFs

The GeneChip Human Gene 1.0 ST Array (Affymetrix) is a whole-transcript analysis chip composed of approximately 29,000 genes. To identify specific genes involved in the pathogenesis of GCD II, we compared the gene expression profiles between primary cultured wild-type and homozygous GCD II corneal fibroblasts. We detected twofold differences in the expression of 555 genes between these two cell types. Of these, 319 of these genes were upregulated and 236 were downregulated in the homozygous GCD II PCFs compared with wild-type (Table 3). Characterization of these genes into ontology groups suggests that GCD II-specific changes largely relate to changes in signal transduction (17%), cell cycle (13%), immune response (13%), cell adhesion (9%), cell differentiation (9%), cytoskeleton (9%), cell proliferation (6%), extracellular space (6%), cell-cell signaling (5%), response to wounding (5%), mitochondrion (2%), ubiquitin cycle (2%), endopeptidase activity (2%), TGF- $\beta$  receptor signaling pathway (1%), integrin-mediated signaling pathway (1%), collagen (1%), and response to oxidative stress (1%) (Fig. 1). Specific genes that were markedly upregulated were *IFI44L* (43.5-fold,  $P < 0.001777$ ), *IF16* (17.0-fold,  $P < 0.0002129$ ), *SFRP4* (16.5-fold,  $P < 0.0000311$ ), *PDEPDC6* (15.2-fold,  $P < 0.0000081$ ), *JARID1D* C3 (15.2-fold,  $P < 0.0000078$ ), *OAS2* (12.9-fold,  $P < 0.0006065$ ), *XAF1* (14.1-fold,  $P < 0.0005192$ ), *STEA2P* (10.2-fold,  $P < 0.0000087$ ), and *CLDN1* (10.2-fold,  $P < 0.0002109$ ) (Table 4). The most downregulated genes were *DDX3Y* (-59.9-fold,  $P < 0.0002855$ ), *FBN2* (-47.8-fold,  $P < 0.0000903$ ), *RPS4Y1* (-27.2-fold,  $P < 0.0000016$ ), *EIF1AY* (-19.5-fold,  $P < 0.0014353$ ), *RELN* (-17.4-fold,  $P < 0.0001692$ ), *USP9Y* (-16.3-fold,  $P < 0.0000009$ ), *KRT34* (-12.1-fold,  $P < 0.0000017$ ), *NLGN4Y* (-11.8-fold,  $P < 0.0001143$ ), *UTY* (-11.2-fold,  $P < 0.0000027$ ), *CYorf15B* (-11.1-fold,  $P < 0.0000012$ ), and *CDH6* (-10.2-fold,  $P < 0.0000005$ ) (Table 5). We next confirmed the differential expression of genes identified by the microarray using RT-PCR.

We tested the expression patterns of 10 representative genes in wild-type and homozygous GCD II PCFs and found that their relative expression levels were similar to those identified by the microarray (Figs. 2A, 2B). For example, expression of *FMOD* and *SOD2* was increased in homozygous GCD II PCFs as opposed to that in wild-type cells. Expression of *TGFBI*, *TGFB1*, *TGFB2*, and *TGFB3* was the same in heterozygous GCD II, homozygous GCD II, and wild-type PCFs.

### Differential Expression of ECM-Associated Genes

To confirm the ECM affinity data, we next tested the expression of proteins involved in ECM remodeling by Western blot (Figs. 2C, 2D), as we expected that expression levels between wild-type, heterozygous, and homozygous GCD II PCFs would be different. We first investigated the expression patterns of collagen metabolism-related matrix metalloprotease (MMP)-1 and -2, because microarray assay profiles have shown a differential expression of MMP-1 and -2. The protein levels of MMP-1 and -2 increased in heterozygous and homozygous GCD II compared with wild-type PCFs (Figs. 2C, 2D). Expression level of integrin  $\alpha_2$ , which is involved in collagen metabolism and cell adhesion, was also increased in heterozygous and homozygous GCD II PCFs. Furthermore, significantly increased protein levels of TGF- $\beta$  receptors I, II, and III were observed in heterozygous and homozygous GCD II PCFs (Figs. 2C, 2D). These results suggest that the disturbance of ECM metabolism, especially collagen metabolism, is a major factor leading to TGF $\beta$  deposition in corneal ECM of GCD II.

### Increased Affinities for ECM Substrates of GCD II PCFs

We detected altered expression of several ECM component genes (Table 3) in wild-type versus GCD II PCFs. As such, we hypothesized that the adhesive properties of GCD II PCFs may be different from those of wild-type PCFs. To test this, we investigated ECM adhesive properties of GCD II PCFs using an adhesion assay (Cytomatrix; Chemicon International), as described in Materials and Methods. The heterozygous and homozygous GCD II PCFs were more adhesive to collagen-I, collagen-IV, fibronectin, and lamine, than were the wild-type cells (Fig. 3).

## DISCUSSION

In this study, cDNA microarray technology was used to compare the gene expression profiles of homozygous GCD II PCFs to that of wild-type cells, in an attempt to better understand a potential mechanism of GCD II disease. We detected at least a twofold change in expression of 555 genes and confirmed the relative expression levels of a select number of genes by RT-PCR and Western blot analysis. Finally, differences in the cell adhesion properties between GCD II and wild-type PCFs were detected, suggesting that disturbances in ECM-cell adhesion may play an important role in this disease.

### Expression Levels of Genes Involved in the TGF- $\beta$ Signaling Pathway

The TGF- $\beta$  signaling pathway has been implicated as a regulator in numerous cellular and physiological processes including ECM homeostasis.<sup>16</sup> The expression of TGF- $\beta$  in corneal fibroblast cultures also suggests that it plays a role in regulating ECM metabolism through the TGF- $\beta$  signaling pathway.<sup>17-19</sup> TGF- $\beta$  initiates signaling through a complex made up of TGF- $\beta$  receptor (T $\beta$ R) I and T $\beta$ R II<sup>20</sup> and signals to the nucleus through the Smads protein.<sup>21</sup> Our results show that T $\beta$ R I, -II, and -III were upregulated in heterozygous and homozygous GCD II PCFs



TABLE 3. GO Analysis of Genes Up- and Downregulated in GCD II CFBS

| GO ID      | GO Category  | Genes  |  | n  |
|------------|--|--|--|----|
|            |  | Up   | Down   |    |
| GO:0007165 | Signal transduction  | <i>SFRP4, C3, GRP, TLR3, CXCL5, CXCL6, AHR, DTNA, CCL26, TNFAIP6, F2RL2, TAS2R43, GDF15, LPHN2, TNFRSF10A, TLR4, ANGPT1, TGFBR3, PTGER4, RASSF2, TLR1, ANK2, CLIC2, TNC, MAPK10, PBEF1, RAPH1, IRAK3</i>   | <i>ARHGAP118, FGL2, PTGER2, ARHGAP11A, CAMK4, GPSM2, IGFBP5, CXCL12, TRHDE</i>   | 37 |
| GO:0007267 | Cell-cell signaling  | <i>IL8, MME, STC1, CXCL5, CXCL6, CCL2, CCL26, TNFAIP6, GDF15, EFNA5, LIF, PBEF1</i>  | <i>INHBA, CXCL12, DLG7, TRHDE</i>  | 15 |
| GO:0007229 | Integrin-mediated signaling pathway                                    | <i>ITGB3</i>   | <i>ITGA8</i>   | 2  |
| GO:0005615 | Extracellular space  | <i>SFRP4, SCG2, GRP, APOL1, IGFBP2, IL8, STC1, CXCL5, CXCL1, CXCL6, CCL2, CFH, CCL26, AKRIB1, CFHR1, MMP3, SULF2, APOD, CXCL2, LGALS3BP, CSF1, GDF15, GREM2, CLU, EFNA5, PLA2R1, WFDC1, LIF, PAPPA, TNFAIP2</i>  | <i>BRCA2, GREM1, PSG7, CXCL12, MMP1, PSG5, RELN</i>  | 37 |
| GO:0030574 | Collagen catabolism  | <i>MMP3</i>  | <i>MMP1</i>  | 2  |
| O:0001306  | Response to oxidative stress   | <i>SOD2, OSGIN2</i>  |  | 2  |
| GO:0007179 | Transforming growth factor-beta receptor signaling pathway             | <i>GDF15, TGFBR3</i>   |  | 2  |
| GO:0007155 | Cell adhesion  | <i>CLDN1, PCDHB3, CCL2, TNFAIP6, LGALS3BP, ROBO1, CNTNAP2, ITGB3, COL8A1, SRPX, ADAM23, RASSF2, HMCN1, TNC, LAMC2, CASK, THBS3, PCDHB13, NFASC</i>   | <i>AEBP1, PCDH18, TROAP, DSG2, HAPLN1, CDH18, CXCL12, NLGN1, EDIL3, CDH6, NLGN4Y, RELN</i>   | 31 |
| GO:0007156 | Homophilic cell adhesion   | <i>PCDHB3, ROBO1, PCDHB2, PCDHB4, PCDHB13</i>  | <i>PCDH18, CDH10, DSG2, CDH18, CDH6</i>  | 10 |
| GO:0030155 | Regulation of cell adhesion  | <i>ICAM1, IL8, LAMA3</i>   |  | 3  |
| GO:0006955 | Immune response  | <i>IFI16, OAS2, OAS1, IFI27, IFIT3, HLA-DPA1, IL8, CXCL5, CXCL1, CXCL6, DPP4, CCL26, IGJ, IFI30, CXCL2, PSMB9, ILIR1, TAP1, PTGER4, HLA-DPBI, MR1, LIF, HLA-E, HLA-C, MICA, IRF1, HLA-E, HLA-C, IFIT5, IFITM3, HLA-B, PSMB8, MICA, HLA-B, HLA-DPB1</i> | <i>EXO1, CXCL12, GBP3</i>  | 37 |
| GO:0006954 | Inflammatory response  | <i>C3, SCG2, IL8, TLR3, CXCL5, CXCL1, CXCL6, CCL2, CCL26, TNFAIP6, CXCL2, ILIR1, AOX1, TLR4, EPHX2, TLR1</i>   | <i>CXCL12</i>  | 17 |
| GO:0006958 | Complement activation, classic pathway                                 | <i>C3, C1S, SERPING1, CF1, CLU</i>   |  | 5  |
| GO:0006956 | Complement activation, classic pathway                                 | <i>CFHR1, CFB</i>  |  | 2  |
| GO:0002474 | Antigen processing and presentation of peptide antigen via MHC class I | <i>HLA-E, HLA-C, MICA, HLA-B, MICA,</i>  |  | 5  |
| GO:0019885 | Antigen processing and presentation of endogenous                      | <i>ARTS-1</i>  | <i>LRAP</i>  | 2  |
| GO:0030154 | Cell differentiation   | <i>SFRP4, FRZB, CSF1, TTLL7, DUSP6, EFNA5, FRK, PAPPA, DCLK1</i>   | <i>SEMA3D, BEX1, CENPF, STMN1, ITGA8, PEG10</i>  | 15 |
| GO:0008283 | Cell proliferation   | <i>CREG1</i>   | <i>KIF2C, KIF15, TPX2</i>  | 8  |
| GO:0008285 | Negative regulation of cell proliferation                              | <i>IL8, CXCL1, RARRES3, FRK, GPNMB</i>   |  | 5  |
| GO:0005856 | Cytoskeleton   | <i>FRMD4B, PLEKHH2, FLG, EPB4IL5, MAP9, RAPH1</i>  | <i>PKP2, DSG2, SHROOM3, ACTC1</i>  | 10 |
| GO:0005739 | Mitochondrion  | <i>IFI6, OAS2, OAS1, SOD2, AK3L1, ACSL5, PDK3, DMGDH, LACTB, BDH2, BNIP3, SQRDL</i>  | <i>KIAA0101</i>  | 13 |
| GO:0007049 | Cell cycle   | <i>PDPN, RASSF2, MAP9, MAPK13</i>  | <i>SGOL2, FANCI, CDC2, CLSPN, C14orf106, UBE2C, CIT, SMC4, RACGAP1, UHRF1, CKS2, CCNF, CDCA8, NCAPD2, WEE1, FBXO5, CENPE, ERCC6L, KIFC1, ESCO2, KIF23, SGOL1, KIFC1, CDCA3, PRC1, NDC80, NUSAP1, BUBIB, PLK1, CDCA2, NCAPG, FAM64A, SPC25, CCNB2, CEP55, CDC20, ASPM, ANLN, FOXM1, SPAG5, NUF2, DLG7</i> | 46 |

(continues)

TABLE 3 (continued). GO Analysis of Genes Up- and Downregulated in GCD II PCFBs

| GO ID      | GO Category   | Genes                            |   | n |
|------------|---|----------------------------------|---|---|
|            |   | Up                               | Down  |   |
| GO:0051318 | G <sub>1</sub> phase  | <i>PRUNE2, PRUNE2</i>            |   | 2 |
| GO:0007601 | Visual perception   | <i>GLRB, HMCN1</i>               | <i>GJA7</i>                                     | 3 |
| GO:0004252 | Serine-type endopeptidase activity  | <i>CFB, CORIN, PRSS23, CF1</i>   | <i>RELN</i>                                     | 5 |
| GO:0004867 | Serine-type endopeptidase inhibitor activity                                      | <i>SERPING1, SERPIN11, WFDC1</i> | <i>SERPINB7</i>                                 | 4 |
| GO:0004190 | Aspartic-type endopeptidase activity  |                                  | <i>PEG10</i>                                    | 1 |
| GO:0004222 | Metalloendopeptidase activity   | <i>ADAM23</i>                    |   | 1 |
| GO:0030574 | Collagen catabolic process  | <i>MMP3</i>                      | <i>MMP1</i>                                     | 2 |
| GO:0030199 | Collagen fibril organization  | <i>COL14A1</i>                   |   | 1 |
| GO:0005581 | Collagen  | <i>COL14A1</i>                   |   | 1 |
| GO:0004232 | Interstitial collagenase activity   |                                  | <i>MMP1</i>                                     | 1 |
| GO:0007181 | Transforming growth factor beta receptor complex assembly                         | <i>FMOD</i>                      |   | 1 |
| GO:0007179 | Transforming growth factor-beta receptor signaling pathway                        | <i>GDF15, TGFBR3</i>             |   | 2 |
| GO:0030512 | Negative regulation of transforming growth factor beta receptor signaling pathway |                                  | <i>PEG10</i>                                    | 1 |
| GO:0006512 | Ubiquitin cycle   | <i>FBXO32, FBXO16, FBXL2</i>     | <i>UBE2C, UHRF1, FBXO5, CDCA3, CDC20, USP9Y</i> | 9 |
| GO:0004842 | Ubiquitin-protein ligase activity   | <i>FBXL2</i>                     | <i>UBE2C</i>                                    | 2 |
| GO:0009611 | Response to wounding  | <i>F2RL2, GAP43</i>              |   | 2 |
| GO:0006979 | Response to oxidative stress  | <i>SOD2, PRNP</i>                |   | 2 |
| GO:0004364 | Glutathione S-transferase activity  | <i>GSTM1</i>                     |   | 1 |

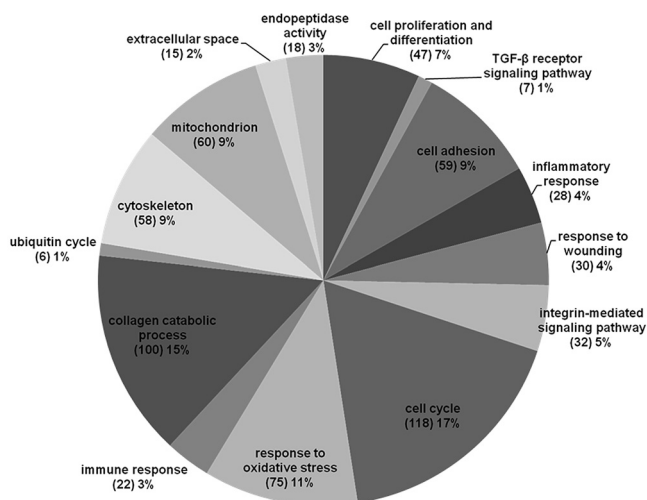


FIGURE 1. Gene ontological classification of differentially regulated genes in homozygous GCD II PCFBs. Genes that were differentially expressed at a minimum of twofold were included in the analysis. Numbers in parentheses represent the number of genes.

compared with wild-type. Moreover, we identified different gene expression levels of the T $\beta$ R complex assembly gene (*FMOD*; 3.2-fold increased,  $P < 0.0000113$ ) in homozygous GCD II PCFBs compared with wild-type PCFBs (Table 4). This suggests that TGF $\beta$ I $\beta$  expression may occur more rapidly in GCD II than in wild-type PCFBs under various physiological conditions and could result in accelerated TGF $\beta$ I $\beta$  deposition and ultimate GCD II disease. Also, because overexpression of misfolded or mutant proteins could induce protein aggregation or deposition, increased T $\beta$ R expressions may play an important role in exacerbating GCD II pathogenesis. This idea is supported by previous studies in which GCD II was aggravated by TGF- $\beta$  after LASIK surgery.<sup>11,12,22</sup> However, the specific mechanism(s) regarding the increase in T $\beta$ R expression in GCD II corneal fibroblasts remains unknown.

### Differential Expression of Proteolytic Enzymes Involved in ECM Metabolism

T $\beta$ R-mediated signaling stimulates matrix deposition by promoting the expression of components of the ECM such as collagen and suppresses and/or activates proteolytic enzymes such as MMPs, which also degrade collagen.<sup>23,24</sup> Previous studies have shown that expression of abnormal proteoglycan filaments and disruption in collagen organization were present

TABLE 4. Upregulated Homozygous GCD II-Related Genes

| No. | Gene Accession  | Gene Symbol       | Gene Description   | Change | P         |
|-----|-----------------|-------------------|--|--------|-----------|
| 1   | NM_006820       | <i>IFI44L</i>     | Interferon-induced protein 44-like   | 43.5   | 0.0017777 |
| 2   | NM_022872       | <i>IFI6</i>       | Interferon, alpha-inducible protein 6  | 17.1   | 0.0002129 |
| 3   | NM_003014       | <i>SFRP4</i>      | Secreted frizzled-related protein 4  | 16.5   | 0.0000311 |
| 4   | NM_022783       | <i>DEPDC6</i>     | DEP domain containing 6  | 15.2   | 0.0000081 |
| 5   | NM_000064       | <i>C3</i>         | Complement component 3   | 15.2   | 0.0000078 |
| 6   | NM_002535       | <i>OAS2</i>       | 2'-5'-Oligoadenylate synthetase 2, 69/71kDa  | 12.9   | 0.0006065 |
| 7   | NM_017523       | <i>XAF1</i>       | XIAP associated factor-1   | 11.4   | 0.0005192 |
| 8   | NM_152999       | <i>STEAP2</i>     | Six transmembrane epithelial antigen of the prostate 2   | 10.2   | 0.0000087 |
| 9   | NM_021101       | <i>CLDN1</i>      | Claudin I  | 10.2   | 0.0002109 |
| 10  | NM_016816       | <i>OAS1</i>       | 2',5'-Oligoadenylate synthetase 1, 40/46kDa  | 9.7    | 0.0035803 |
| 11  | ENST00000260184 | <i>FLJ20035</i>   | Hypothetical protein FLJ20035 (FLJ20035), mRNA   | 9.7    | 0.0013714 |
| 12  | NM_005532       | <i>IFI27</i>      | Interferon, alpha-inducible protein 27   | 9.1    | 0.0003262 |
| 13  | NM_003469       | <i>SCG2</i>       | Secretogranin II (chromogranin C)  | 8.4    | 0.0000092 |
| 14  | NM_002091       | <i>GRP</i>        | Gastrin-releasing peptide  | 8.3    | 0.0000031 |
| 15  | NM_001031683    | <i>IFIT3</i>      | Interferon-induced protein with tetratricopeptide repeats 3  | 7.6    | 0.0061816 |
| 16  | uc002feh.1      | <i>CHST6</i>      | Carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 6  | 7.6    | 0.0000052 |
| 17  | NM_139072       | <i>DNER</i>       | Delta/notch-like EGF repeat containing   | 7.5    | 0.0000055 |
| 18  | NM_080284       | <i>ABCA6</i>      | ATP-binding cassette, sub-family A (ABC1), member 6  | 7.3    | 0.0000006 |
| 19  | NM_203349       | <i>SHC4</i>       | SHC (Src homology 2 domain containing) family, member 4  | 7      | 0.0000941 |
| 20  | NM_020299       | <i>AKRIB10</i>    | Aldo-keto reductase family 1, member B10 (aldose reductase)  | 6.9    | 0.0000183 |
| 21  | NM_007168       | <i>ABCA8</i>      | ATP-binding cassette, sub-family A (ABC1), member 8  | 6.7    | 0.0001116 |
| 22  | NM_006417       | <i>IFI44</i>      | Interferon-induced protein 44  | 6.7    | 0.002165  |
| 23  | NM_033554       | <i>HLA-DPA1</i>   | Major histocompatibility complex, class II, DP alpha 1   | 6.6    | 0.0000318 |
| 24  | NM_024574       | <i>C4orf31</i>    | Chromosome 4 open reading frame 31   | 6.6    | 0.0000578 |
| 25  | NM_145343       | <i>APOL1</i>      | Apolipoprotein L, 1  | 6.6    | 0.0001028 |
| 26  | NM_033554       | <i>HLA-DPA1</i>   | Major histocompatibility complex, class II, DP alpha 1   | 6.5    | 0.0002743 |
| 27  | NM_033554       | <i>HLA-DPA1</i>   | Major histocompatibility complex, class II, DP alpha 1   | 6.5    | 0.0002743 |
| 28  | NR_003198       | <i>SNORD114-6</i> | Small nucleolar RNA, C/D box 114-6   | 6.5    | 0.0000037 |
| 29  | NM_000597       | <i>IGFBP2</i>     | Insulin-like growth factor binding protein 2, 36kDa  | 6.2    | 0.0005975 |
| 30  | NM_004163       | <i>RAB27B</i>     | RAB27B, member RAS oncogene family   | 6.1    | 0.0001163 |
| 32  | NM_000201       | <i>ICAM1</i>      | Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor  | 6      | 0.0000264 |
| 33  | NM_000584       | <i>IL8</i>        | Interleukin 8  | 5.9    | 0.0001314 |
| 34  | NM_021110       | <i>COL14A1</i>    | Collagen, type XIV, alpha I (undulin)  | 5.5    | 0.0000128 |
| 35  | NM_001024465    | <i>SOD2</i>       | Superoxide dismutase 2, mitochondrial  | 5.5    | 0.0004007 |
| 38  | NM_003265       | <i>TLR3</i>       | Toll-like receptor 3   | 5.3    | 0.0012359 |
| 39  | NM_152703       | <i>SAMD9L</i>     | Sterile alpha motif domain containing 9-like   | 5.1    | 0.0005941 |
| 40  | NM_007288       | <i>MME</i>        | Membrane metallo-endopeptidase   | 4.8    | 0.0000029 |
| 41  | NM_005907       | <i>MAN1A1</i>     | Mannosidase, alpha, class IA, member 1   | 4.7    | 0.0000526 |
| 42  | NM_003155       | <i>STC1</i>       | Stanniocalcin 1  | 4.7    | 0.0003264 |
| 43  | NM_022154       | <i>SLC39A8</i>    | Solute carrier family 39 (zinc transporter), member 8  | 4.7    | 0.0034818 |
| 44  | NM_139248       | <i>LIPH</i>       | Lipase, member H   | 4.6    | 0.0000636 |
| 45  | NM_001002264    | <i>EPSTI1</i>     | Epithelial stromal interaction I (breast)  | 4.6    | 0.0019874 |
| 46  | BC022571        | <i>PRUNE2</i>     | Prune homolog 2 (Drosophila)   | 4.5    | 0.0006296 |
| 47  | NM_002994       | <i>CXCL5</i>      | Chemokine (C-X-C motif) ligand 5   | 4.5    | 0.001963  |
| 48  | NM_001511       | <i>CXCL1</i>      | Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)   | 4.5    | 0.006012  |
| 49  | NM_002538       | <i>OCLN</i>       | Occludin   | 4.4    | 0.00084   |
| 50  | NM_018937       | <i>PCDH3</i>      | Protocadherin beta 3   | 4.4    | 0.0001115 |
| 51  | NM_145172       | <i>WDR63</i>      | WD repeat domain 63  | 4.3    | 0.0000206 |
| 52  | NM_004585       | <i>RARRES3</i>    | Retinoic acid receptor responder (tazarotene induced) 3  | 4.3    | 0.0002066 |
| 53  | NM_003739       | <i>AKR1C3</i>     | Aldo-keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II)  | 4.3    | 0.0000081 |
| 54  | NM_002993       | <i>CXCL6</i>      | Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)   | 4.3    | 0.0004347 |
| 55  | NM_016352       | <i>CPA4</i>       | Carboxypeptidase A4  | 4.3    | 0.0000333 |
| 56  | NM_017554       | <i>PARP14</i>     | Poly (ADP-ribose) polymerase family, member 14   | 4.2    | 0.0019238 |
| 57  | NM_002982       | <i>CCL2</i>       | Chemokine (C-C motif) ligand 2   | 4.2    | 0.0000148 |
| 58  | NM_205845       | <i>AKR1C2</i>     | Aldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid binding protein; 3-alpha hydroxysteroid dehydrogenase, type III) | 4.2    | 0.0000279 |
| 59  | NM_032812       | <i>PLXDC2</i>     | Plexin domain containing 2   | 4.1    | 0.0000003 |
| 60  | NM_138818       | <i>PRUNE2</i>     | Prune homolog 2 (Drosophila)   | 4      | 0.0000703 |
| 61  | NM_001935       | <i>DPP4</i>       | Dipeptidyl-peptidase 4 (CD26, adenosine deaminase complexing protein 2)  | 4      | 0.0000083 |
| 62  | NM_001013442    | <i>EPGN</i>       | Epithelial mitogen homolog (mouse)   | 3.9    | 0.0000298 |
| 63  | NM_000186       | <i>CFH</i>        | Complement factor H  | 3.9    | 0.0000126 |
| 64  | NM_014314       | <i>DDX58</i>      | DEAD (Asp-Glu-Ala-Asp) box polypeptide 58  | 3.8    | 0.0033267 |
| 65  | NM_182767       | <i>SLC6A15</i>    | Solute carrier family 6, member 15   | 3.8    | 0.000131  |
| 66  | NM_080671       | <i>KCNE4</i>      | Potassium voltage-gated channel, Isk-related family, member 4  | 3.7    | 0.0004738 |
| 67  | NM_001390       | <i>DTNA</i>       | Dystrobrevin, alpha  | 3.7    | 0.0000589 |
| 68  | NM_175861       | <i>TMTC1</i>      | Transmembrane and tetratricopeptide repeat containing 1  | 3.7    | 0.0000083 |
| 69  | NM_006072       | <i>CCL26</i>      | Chemokine (C-C motif) ligand 26  | 3.6    | 0.0000234 |
| 70  | NM_144646       | <i>IGJ</i>        | Immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides  | 3.6    | 0.0011411 |

(continues)

TABLE 4 (continued). Upregulated Homozygous GCD II-Related Genes

| No. | Gene Accession  | Gene Symbol       | Gene Description  | Change | P         |
|-----|-----------------|-------------------|---|--------|-----------|
| 71  | NM_003851       | <i>CREG1</i>      | Cellular repressor of E1A-stimulated genes 1  | 3.6    | 0.0000007 |
| 72  | NM_153703       | <i>PODN</i>       | Podocan   | 3.6    | 0.0000006 |
| 73  | NM_007115       | <i>TNFAIP6</i>    | Tumor necrosis factor, alpha-induced protein 6  | 3.6    | 0.0009063 |
| 74  | NM_001628       | <i>AKR1B1</i>     | Aldo-keto reductase family 1, member B1 (aldose reductase)                                | 3.5    | 0.000003  |
| 75  | NM_016848       | <i>SHC3</i>       | SHC (Src homology 2 domain containing) transforming protein 3                             | 3.5    | 0.000021  |
| 76  | NM_018349       | <i>MCTP2</i>      | Multiple C2 domains, transmembrane 2  | 3.5    | 0.000013  |
| 77  | NM_015123       | <i>FRMD4B</i>     | FERM domain containing 4B   | 3.5    | 0.0000545 |
| 78  | NM_015529       | <i>MOXD1</i>      | Monoxygenase, DBH-like 1  | 3.5    | 0.0000004 |
| 79  | NM_198129       | <i>LAMA3</i>      | Laminin, alpha 3  | 3.5    | 0.0000003 |
| 80  | NM_080283       | <i>ABCA9</i>      | ATP-binding cassette, sub-family A (ABC1), member 9                                       | 3.4    | 0.0002608 |
| 81  | NM_000313       | <i>PROS1</i>      | Protein S (alpha)   | 3.4    | 0.0000002 |
| 82  | NM_015028       | <i>TNIK</i>       | TRAF2 and NCK interacting kinase  | 3.4    | 0.0016728 |
| 83  | NM_004101       | <i>F2RL2</i>      | Coagulation factor II (thrombin) receptor-like 2  | 3.4    | 0.0000043 |
| 84  | NM_017439       | <i>Icag7.1314</i> | Hypothetical protein LOC54103   | 3.3    | 0.0007954 |
| 85  | NM_005824       | <i>LRRC17</i>     | Leucine rich repeat containing 17   | 3.3    | 0.0000081 |
| 86  | NM_001001924    | <i>MTUS1</i>      | Mitochondrial tumor suppressor 1  | 3.3    | 0.0002024 |
| 87  | NM_006393       | <i>NEBL</i>       | Nebulette   | 3.3    | 0.0000077 |
| 88  | NM_176884       | <i>TAS2R43</i>    | Taste receptor, type 2, member 43   | 3.3    | 0.0005163 |
| 89  | NM_000115       | <i>EDNRB</i>      | Endothelin receptor type B  | 3.3    | 0.0010275 |
| 90  | NM_153366       | <i>SVEP1</i>      | Sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1                | 3.2    | 0.0000004 |
| 91  | NM_016246       | <i>HSD17B14</i>   | Hydroxysteroid (17-beta) dehydrogenase 14   | 3.2    | 0.0000521 |
| 92  | NM_002023       | <i>FMOD</i>       | Fibromodulin  | 3.2    | 0.0000113 |
| 93  | NM_002113       | <i>CFHR1</i>      | Complement factor H-related 1   | 3.2    | 0.0004559 |
| 94  | NM_024636       | <i>STEAP4</i>     | STEAP family member 4   | 3.2    | 0.0013693 |
| 95  | NM_002422       | <i>MMP3</i>       | Matrix metalloproteinase 3 (stromelysin 1, progelatinase)                                 | 3.1    | 0.0000206 |
| 96  | NM_018837       | <i>SULF2</i>      | Sulfatase 2   | 3.1    | 0.0000052 |
| 97  | NM_017614       | <i>BHMT2</i>      | Betaine-homocysteine methyltransferase 2  | 3.1    | 0.0000017 |
| 98  | NM_006332       | <i>IFI30</i>      | Interferon, gamma-inducible protein 30  | 3.1    | 0.0000761 |
| 99  | NM_001463       | <i>FRZB</i>       | Frizzled-related protein  | 3.1    | 0.0012231 |
| 100 | NM_201442       | <i>C1S</i>        | Complement component 1, s subcomponent  | 3.1    | 0.0000036 |
| 101 | NM_058229       | <i>FBXO32</i>     | F-box protein 32  | 3.1    | 0.0000464 |
| 102 | NM_001710       | <i>CFB</i>        | Complement factor B   | 3.1    | 0.0011518 |
| 103 | NM_173567       | <i>ABHD7</i>      | Abhydrolase domain containing 7   | 3.1    | 0.0016296 |
| 104 | NM_001647       | <i>APOD</i>       | Apolipoprotein D  | 3.0    | 0.0048695 |
| 105 | NR_003578       | <i>ZNF702</i>     | Zinc finger protein 702   | 3.0    | 0.0003033 |
| 106 | NM_006474       | <i>PDPN</i>       | Podoplanin  | 3.0    | 0.0000074 |
| 107 | NM_002089       | <i>CXCL2</i>      | Chemokine (C-X-C motif) ligand 2  | 3.0    | 0.0071753 |
| 108 | NM_198520       | <i>C12orf63</i>   | Chromosome 12 open reading frame 63   | 3.0    | 0.0016097 |
| 109 | NM_001012967    | <i>FLJ31033</i>   | Hypothetical protein FLJ31033   | 3.0    | 0.0010071 |
| 110 | NM_018355       | <i>ZNF415</i>     | Zinc finger protein 415   | 3.0    | 0.0001087 |
| 111 | NM_005103       | <i>FEZ1</i>       | Fasciculation and elongation protein zeta 1 (zygin 1)                                     | 2.9    | 0.0013669 |
| 112 | NM_001030060    | <i>SAMD5</i>      | Sterile alpha motif domain containing 5   | 2.9    | 0.0003825 |
| 113 | NM_000062       | <i>SERPING1</i>   | Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary)    | 2.9    | 0.0017786 |
| 114 | ENST00000326754 | <i>FLJ25801</i>   | Hypothetical protein FLJ25801 (FLJ25801), mRNA  | 2.9    | 0.00033   |
| 115 | NM_001038628    | <i>B3GALNT1</i>   | Beta-1,3-N-acetylgalactosaminyltransferase 1 (globoside blood group)                      | 2.9    | 0.000004  |
| 116 | NM_005567       | <i>LGALS3BP</i>   | Lectin, galactoside-binding, soluble, 3 binding protein                                   | 2.9    | 0.0004468 |
| 117 | NM_000689       | <i>ALDH1A1</i>    | Aldehyde dehydrogenase 1 family, member A1  | 2.9    | 0.0019248 |
| 118 | NM_000757       | <i>CSF1</i>       | Colony stimulating factor 1 (macrophage)  | 2.9    | 0.0000119 |
| 119 | NM_004864       | <i>GDF15</i>      | Growth differentiation factor 15  | 2.9    | 0.0001212 |
| 120 | NM_001710       | <i>CFB</i>        | Complement factor B   | 2.9    | 0.0013733 |
| 121 | NM_001013732    | <i>C6orf138</i>   | Chromosome 6 open reading frame 138   | 2.9    | 0.0005379 |
| 122 | NM_012302       | <i>LPHN2</i>      | Latrophilin 2   | 2.8    | 0.0001229 |
| 123 | NM_183376       | <i>ARRDC4</i>     | Arrestin domain containing 4  | 2.8    | 0.0000015 |
| 124 | NM_007257       | <i>PNMA2</i>      | Paraneoplastic antigen MA2  | 2.8    | 0.0052895 |
| 125 | ENST00000296529 | <i>TMEM144</i>    | Transmembrane protein 144 (TMEM144), mRNA   | 2.8    | 0.0056544 |
| 126 | NM_052831       | <i>C6orf192</i>   | Chromosome 6 open reading frame 192   | 2.8    | 0.0000583 |
| 127 | NM_004675       | <i>DIRAS3</i>     | DIRAS family, GTP-binding RAS-like 3  | 2.8    | 0.0000624 |
| 128 | NM_172069       | <i>PLEKHH2</i>    | Pleckstrin homology domain containing, family H (with MyTH4 domain) member 2              | 2.8    | 0.0001397 |
| 129 | NM_182969       | <i>XRRA1</i>      | X-ray radiation resistance associated 1   | 2.8    | 0.0000341 |
| 130 | NM_018242       | <i>SLC47A1</i>    | Solute carrier family 47, member 1  | 2.8    | 0.0001539 |
| 131 | NM_017549       | <i>EPDR1</i>      | Ependymin related protein 1 (zebrafish)   | 2.8    | 0.0000008 |
| 132 | NM_203464       | <i>AK3L1</i>      | Adenylate kinase 3-like 1   | 2.8    | 0.0001709 |
| 133 | NM_004827       | <i>ABCG2</i>      | ATP-binding cassette, sub-family G (WHITE), member 2                                      | 2.7    | 0.0000899 |
| 134 | NM_003844       | <i>TNFRSF10A</i>  | Tumor necrosis factor receptor superfamily, member 10a                                    | 2.7    | 0.0004671 |
| 135 | NM_002222       | <i>ITPR1</i>      | Inositol 1,4,5-triphosphate receptor, type 1  | 2.7    | 0.0000039 |
| 136 | NM_003543       | <i>HIST1H4H</i>   | Histone cluster 1, H4h  | 2.7    | 0.0001016 |
| 137 | NM_148954       | <i>PSMB9</i>      | Proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2) | 2.7    | 0.003365  |

(continues)



TABLE 4 (continued). Upregulated Homozygous GCD II-Related Genes

| No. | Gene Accession | Gene Symbol     | Gene Description  | Change | P         |
|-----|----------------|-----------------|---|--------|-----------|
| 138 | NM_148954      | <i>PSMB9</i>    | Proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)                           | 2.7    | 0.003365  |
| 139 | NM_002800      | <i>PSMB9</i>    | Proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)                           | 2.7    | 0.003365  |
| 140 | NM_021244      | <i>RRAGD</i>    | Ras-related GTP binding D   | 2.7    | 0.0004553 |
| 141 | NM_178826      | <i>TMEM16D</i>  | Transmembrane protein 16D   | 2.6    | 0.0000977 |
| 142 | NM_000877      | <i>IL1R1</i>    | Interleukin 1 receptor, type 1  | 2.6    | 0.0000427 |
| 143 | NM_133631      | <i>ROBO1</i>    | Roundabout, axon guidance receptor, homolog 1 (Drosophila)  | 2.6    | 0.000011  |
| 144 | NM_014141      | <i>CNTNAP2</i>  | Contactin associated protein-like 2   | 2.6    | 0.0000317 |
| 145 | NM_001334      | <i>CTSO</i>     | Cathepsin O   | 2.6    | 0.0000504 |
| 146 | NM_001085423   | <i>C17orf60</i> | Chromosome 17 open reading frame 60   | 2.6    | 0.0002676 |
| 147 | NM_002045      | <i>GAP43</i>    | Growth associated protein 43  | 2.6    | 0.0000103 |
| 148 | NM_024686      | <i>TTL7</i>     | Tubulin tyrosine ligase-like family, member 7   | 2.6    | 0.0000499 |
| 149 | NM_005502      | <i>ABCA1</i>    | ATP-binding cassette, sub-family A (ABC1), member 1   | 2.6    | 0.0000177 |
| 150 | NM_000593      | <i>TAP1</i>     | Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)   | 2.6    | 0.0031895 |
| 151 | NM_000593      | <i>TAP1</i>     | Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)   | 2.6    | 0.0031895 |
| 152 | NM_000593      | <i>TAP1</i>     | Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)   | 2.6    | 0.0031895 |
| 153 | NM_005779      | <i>LHFPL2</i>   | Lipoma HMG1C fusion partner-like 2  | 2.6    | 0.0001421 |
| 154 | NM_007047      | <i>BTN3A2</i>   | Butyrophilin, subfamily 3, member A2  | 2.6    | 0.0000841 |
| 155 | NM_012472      | <i>LRRC6</i>    | Leucine rich repeat containing 6  | 2.5    | 0.0006272 |
| 156 | NM_001159      | <i>AOX1</i>     | Aldehyde oxidase 1  | 2.5    | 0.000033  |
| 157 | NM_017734      | <i>PALMD</i>    | Palmdelphin   | 2.5    | 0.0003744 |
| 158 | NM_004170      | <i>SLC1A1</i>   | Solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1             | 2.5    | 0.000006  |
| 159 | NM_000311      | <i>PRNP</i>     | Prion protein (p27-30) (Creutzfeldt-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia) | 2.5    | 0         |
| 160 | NM_005025      | <i>SERPINI1</i> | Serpin peptidase inhibitor, clade 1.(neuroserpin), member 1   | 2.5    | 0.0001759 |
| 161 | NM_001946      | <i>DUSP6</i>    | Dual specificity phosphatase 6  | 2.5    | 0.0000949 |
| 162 | NM_000212      | <i>ITGB3</i>    | Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)   | 2.5    | 0.0001519 |
| 164 | NR_003530      | <i>MEG3</i>     | Maternally expressed 3  | 2.5    | 0.0001135 |
| 165 | NM_015274      | <i>MAN2B2</i>   | Mannosidase, alpha, class 2B, member 2  | 2.5    | 0.0000109 |
| 166 | NM_006207      | <i>PDGFRL</i>   | Platelet-derived growth factor receptor-like  | 2.5    | 0.0001507 |
| 167 | NM_000722      | <i>CACNA2D1</i> | Calcium channel, voltage-dependent, alpha 2/delta subunit 1   | 2.5    | 0.0000006 |
| 168 | NM_001975      | <i>ENO2</i>     | Enolase 2 (gamma, neuronal)   | 2.5    | 0.000141  |
| 169 | NM_002637      | <i>PHKA1</i>    | Phosphorylase kinase, alpha 1 (muscle)  | 2.5    | 0.0000154 |
| 170 | NM_022469      | <i>GREM2</i>    | Gremlin 2, cysteine knot superfamily, homolog (Xenopus laevis)  | 2.5    | 0.0000111 |
| 171 | NM_001040458   | <i>ARTS-1</i>   | Type 1 tumor necrosis factor receptor shedding aminopeptidase regulator   | 2.5    | 0.0000227 |
| 172 | NM_001962      | <i>EFNA5</i>    | Ephrin-A5   | 2.5    | 0.0000425 |
| 173 | NM_001850      | <i>COL8A1</i>   | Collagen, type VIII, alpha 1  | 2.5    | 0.0000884 |
| 174 | NM_014367      | <i>C3orf28</i>  | Chromosome 3 open reading frame 28  | 2.5    | 0.0038544 |
| 175 | NM_024621      | <i>VEPH1</i>    | Ventricular zone expressed PH domain homolog 1 (zebrafish)  | 2.5    | 0.000141  |
| 176 | NM_017901      | <i>TPCN1</i>    | Two pore segment channel 1  | 2.5    | 0.0000426 |
| 177 | NM_003551      | <i>NME5</i>     | Non-metastatic cells 5, protein expressed in (nucleoside-diphosphate kinase)  | 2.5    | 0.0014518 |
| 178 | NM_203380      | <i>ACSL5</i>    | Acyl-CoA synthetase long-chain family member 5  | 2.5    | 0.004572  |
| 179 | NM_007036      | <i>ESM1</i>     | Endothelial cell-specific molecule 1  | 2.5    | 0.0005156 |
| 180 | NM_006587      | <i>CORIN</i>    | Corin, serine peptidase   | 2.5    | 0.000425  |
| 181 | NM_138554      | <i>TLR4</i>     | Toll-like receptor 4  | 2.5    | 0.0000129 |
| 182 | NM_000187      | <i>HGD</i>      | Homogentisate 1,2-dioxygenase (homogentisate oxidase)   | 2.4    | 0.000498  |
| 183 | NM_000187      | <i>HGD</i>      | Homogentisate 1,2-dioxygenase (homogentisate oxidase)   | 2.4    | 0.000498  |
| 184 | NM_005391      | <i>PDK3</i>     | Pyruvate dehydrogenase kinase, isozyme 3  | 2.4    | 0.0017101 |
| 185 | NM_173508      | <i>SLC35F3</i>  | Solute carrier family 35, member F3   | 2.4    | 0.0002497 |
| 186 | NM_000147      | <i>FUCA1</i>    | Fucosidase, alpha-L-1, tissue   | 2.4    | 0.0001624 |
| 187 | NM_181785      | <i>SLC46A3</i>  | Solute carrier family 46, member 3  | 2.4    | 0.0000173 |
| 188 | NM_002031      | <i>FRK</i>      | Fyn-related kinase  | 2.4    | 0.0002699 |
| 189 | NM_206996      | <i>SPAG17</i>   | Sperm associated antigen 17   | 2.4    | 0.0002856 |
| 190 | NM_006307      | <i>SRPX</i>     | Sushi-repeat-containing protein, X-linked   | 2.4    | 0.0003416 |
| 191 | NM_007366      | <i>PLA2R1</i>   | Phospholipase A2 receptor 1, 180kDa   | 2.4    | 0.000002  |
| 192 | NM_020041      | <i>SLC2A9</i>   | Solute carrier family 2 (facilitated glucose transporter), member 9   | 2.4    | 0.0001373 |
| 193 | NM_031419      | <i>NFKBIZ</i>   | Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta                                  | 2.4    | 0.0001795 |
| 194 | NM_000214      | <i>JAG1</i>     | Jagged 1 (Alagille syndrome)  | 2.4    | 0.0004701 |
| 195 | NM_001979      | <i>EPHX2</i>    | Epoxide hydrolase 2, cytoplasmic  | 2.4    | 0.0002466 |
| 196 | NM_006095      | <i>ATP8A1</i>   | ATPase, aminophospholipid transporter (APLT), Class 1, type 8A, member 1  | 2.4    | 0.0000686 |
| 197 | NM_053276      | <i>VIT</i>      | Vitrin  | 2.4    | 0.0000835 |
| 198 | NM_001146      | <i>ANGPT1</i>   | Angiotensin 1   | 2.4    | 0.0003832 |
| 199 | NM_001040457   | <i>RHBDD2</i>   | Rhomboid domain containing 2  | 2.4    | 0.0000221 |
| 200 | NM_138287      | <i>DTX3L</i>    | Deltex 3-like (Drosophila)  | 2.4    | 0.0052558 |
| 201 | NM_003812      | <i>ADAM23</i>   | ADAM metalloproteinase domain 23  | 2.4    | 0.0000041 |
| 202 | NM_006994      | <i>BTN3A3</i>   | Butyrophilin, subfamily 3, member A3  | 2.4    | 0.0003593 |
| 204 | NM_018936      | <i>PCDHB2</i>   | Protocadherin beta 2  | 2.4    | 0.0002569 |

(continues)



TABLE 4 (continued). Upregulated Homozygous GCD II-Related Genes

| No. | Gene Accession  | Gene Symbol     | Gene Description  | Change | P         |
|-----|-----------------|-----------------|---|--------|-----------|
| 205 | NM_015194       | <i>MYO1D</i>    | Myosin ID   | 2.4    | 0.0000391 |
| 206 | NM_001086       | <i>AADAC</i>    | Arylacetamide deacetylase (esterase)  | 2.3    | 0.0000226 |
| 207 | NM_014467       | <i>SRPX2</i>    | Sushi-repeat-containing protein, X-linked 2                                       | 2.3    | 0.0000049 |
| 208 | NM_024726       | <i>IQCA</i>     | IQ motif containing with AAA domain   | 2.3    | 0.0001621 |
| 209 | NM_002016       | <i>FLG</i>      | Filaggrin   | 2.3    | 0.002256  |
| 210 | NM_003243       | <i>TGFBR3</i>   | Transforming growth factor, beta receptor III                                     | 2.3    | 0.0000001 |
| 211 | NM_052867       | <i>NALCN</i>    | Sodium leak channel, non-selective  | 2.3    | 0.0001055 |
| 212 | NM_004337       | <i>OSGIN2</i>   | Oxidative stress induced growth inhibitor family member 2                         | 2.3    | 0.0000301 |
| 213 | NM_000958       | <i>PTGER4</i>   | Prostaglandin E receptor 4 (subtype EP4)  | 2.3    | 0.0006466 |
| 214 | NM_001033045    | <i>GPR155</i>   | G protein-coupled receptor 155  | 2.3    | 0.0000117 |
| 215 | NM_032587       | <i>CARD6</i>    | Caspase recruitment domain family, member 6                                       | 2.3    | 0.0007864 |
| 216 | NM_021197       | <i>WFDC1</i>    | WAP four-disulfide core domain 1  | 2.3    | 0.0001014 |
| 217 | NM_018938       | <i>PCDHB4</i>   | Protocadherin beta 4  | 2.3    | 0.0002702 |
| 218 | NM_013391       | <i>DMGDH</i>    | Dimethylglycine dehydrogenase   | 2.3    | 0.0007769 |
| 219 | NM_014505       | <i>KCNMB4</i>   | Potassium large conductance calcium-activated channel, subfamily M, beta member 4 | 2.3    | 0.0000254 |
| 220 | NM_172366       | <i>FBXO16</i>   | F-box protein 16  | 2.3    | 0.0000801 |
| 221 | NM_203403       | <i>C9orf150</i> | Chromosome 9 open reading frame 150   | 2.3    | 0.0009304 |
| 222 | NM_007048       | <i>BTN3A1</i>   | Butyrophilin, subfamily 3, member A1  | 2.3    | 0.0002037 |
| 223 | NM_014737       | <i>RASSF2</i>   | Ras association (RalGDS/AF-6) domain family 2                                     | 2.3    | 0.0002623 |
| 224 | NM_001148       | <i>ANK2</i>     | Ankyrin 2, neuronal   | 2.3    | 0.0003172 |
| 225 | NM_018421       | <i>TBC1D2</i>   | TBC1 domain family, member 2  | 2.3    | 0.002261  |
| 226 | NM_000824       | <i>GLRB</i>     | Glycine receptor, beta  | 2.3    | 0.000148  |
| 227 | NM_002121       | <i>HLA-DPB1</i> | Major histocompatibility complex, class II, DP beta 1                             | 2.3    | 0.0011187 |
| 228 | NM_001289       | <i>CLIC2</i>    | Chloride intracellular channel 2  | 2.3    | 0.0000485 |
| 229 | NM_020848       | <i>KIAA1462</i> | KIAA1462  | 2.3    | 0.0000095 |
| 230 | NM_004065       | <i>CDR1</i>     | Cerebellar degeneration-related protein 1, 34kDa                                  | 2.2    | 0.000323  |
| 231 | NM_031935       | <i>HMCN1</i>    | Hemicentin 1  | 2.2    | 0.0001081 |
| 232 | NM_001621       | <i>AHR</i>      | Aryl hydrocarbon receptor   | 2.2    | 0.0000101 |
| 233 | NM_002231       | <i>CD82</i>     | CD82 molecule   | 2.2    | 0.0000261 |
| 234 | NM_018295       | <i>TMEM140</i>  | Transmembrane protein 140   | 2.2    | 0.0008474 |
| 235 | NM_002970       | <i>SAT1</i>     | Spermidine/spermine N1 acetyltransferase 1  | 2.2    | 0.0000077 |
| 236 | NM_001531       | <i>MR1</i>      | Major histocompatibility complex, class I-related                                 | 2.2    | 0.0000163 |
| 237 | NM_003043       | <i>SLC6A6</i>   | Solute carrier family 6 (neurotransmitter transporter, taurine), member 6         | 2.2    | 0.0001361 |
| 238 | NM_012157       | <i>FBXL2</i>    | F-box and leucine-rich repeat protein 2   | 2.2    | 0.0000359 |
| 239 | NM_002309       | <i>LIF</i>      | Leukemia inhibitory factor (cholinergic differentiation factor)                   | 2.2    | 0.0007509 |
| 240 | NM_024763       | <i>WDR78</i>    | WD repeat domain 78   | 2.2    | 0.0017849 |
| 241 | NM_021945       | <i>C6orf85</i>  | Chromosome 6 open reading frame 85  | 2.2    | 0.0017489 |
| 242 | NM_001218       | <i>CA12</i>     | Carbonic anhydrase XII  | 2.2    | 0.000822  |
| 243 | NM_002160       | <i>TNC</i>      | Tenascin C (hexabrachion)   | 2.2    | 0.0000479 |
| 244 | NM_005516       | <i>HLA-E</i>    | Major histocompatibility complex, class 1, E                                      | 2.2    | 0.0000489 |
| 245 | NM_005516       | <i>HLA-E</i>    | Major histocompatibility complex, class 1, E                                      | 2.2    | 0.0000489 |
| 246 | NM_003263       | <i>TLR1</i>     | Toll-like receptor 1  | 2.2    | 0.0014844 |
| 247 | NM_002117       | <i>HLA-C</i>    | Major histocompatibility complex, class 1, C                                      | 2.2    | 0.0000444 |
| 248 | NM_000247       | <i>MICA</i>     | MHC class I polypeptide-related sequence A  | 2.2    | 0.0001143 |
| 249 | NM_000202       | <i>IDS</i>      | Iduronate 2-sulfatase (Hunter syndrome)   | 2.2    | 0.0000043 |
| 250 | NM_032857       | <i>LACTB</i>    | Lactamase, beta   | 2.2    | 0.0001232 |
| 251 | NM_002198       | <i>IRF1</i>     | Interferon regulatory factor 1  | 2.2    | 0.0018853 |
| 252 | NM_145235       | <i>FANK1</i>    | Fibronectin type III and ankyrin repeat domains 1                                 | 2.2    | 0.0002057 |
| 254 | NM_182943       | <i>PLOD2</i>    | Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2                                | 2.2    | 0.0000188 |
| 255 | NM_005562       | <i>LAMC2</i>    | Laminin, gamma 2  | 2.2    | 0.0012097 |
| 256 | NM_144629       | <i>RFTN2</i>    | Raftlin family member 2   | 2.2    | 0.0015459 |
| 257 | NM_002581       | <i>PAPPA</i>    | Pregnancy-associated plasma protein A, pappalysin 1                               | 2.2    | 0.0000067 |
| 258 | NM_004090       | <i>DUSP3</i>    | Dual specificity phosphatase 3 (vaccinia virus phosphatase VHI-related)           | 2.2    | 0.0000206 |
| 259 | NM_006291       | <i>TNFAIP2</i>  | Tumor necrosis factor, alpha-induced protein 2                                    | 2.2    | 0.0008835 |
| 260 | NM_145176       | <i>SLC2A12</i>  | Solute carrier family 2 (facilitated glucose transporter), member 12              | 2.2    | 0.0003708 |
| 261 | NM_005516       | <i>HLA-E</i>    | Major histocompatibility complex, class 1, E                                      | 2.2    | 0.0000376 |
| 262 | NM_020909       | <i>EPB41L5</i>  | Erythrocyte membrane protein band 4.1 like 5                                      | 2.2    | 0.0000017 |
| 263 | NM_002117       | <i>HLA-C</i>    | Major histocompatibility complex; class 1, C                                      | 2.2    | 0.0000294 |
| 264 | NM_003688       | <i>CASK</i>     | Calcium/calmodulin-dependent serine protein kinase (MAGUK family)                 | 2.2    | 0.0000365 |
| 265 | NM_001005340    | <i>GPNMB</i>    | Glycoprotein (transmembrane) nmb  | 2.2    | 0.0000069 |
| 266 | NM_012420       | <i>IFIT5</i>    | Interferon-induced protein with tetratricopeptide repeats 5                       | 2.2    | 0.0013319 |
| 267 | NM_138980       | <i>MAPK10</i>   | Mitogen-activated protein kinase 10   | 2.2    | 0.0001154 |
| 268 | NM_002350       | <i>LYN</i>      | V-yes-1 Yamaguchi sarcoma viral related oncogene homolog                          | 2.2    | 0.0001236 |
| 269 | NM_007173       | <i>PRSS23</i>   | Protease, serine, 23  | 2.1    | 0.0000224 |
| 270 | ENST00000222553 | <i>PBEF1</i>    | Pre-B-cell colony enhancing factor 1 (PBEF1), mRNA                                | 2.1    | 0.0006919 |
| 271 | NM_014936       | <i>ENPP4</i>    | Ectonucleotide pyrophosphatase/phosphodiesterase 4 (putative function)            | 2.1    | 0.0000373 |
| 272 | NM_021626       | <i>SCPEP1</i>   | Serine carboxypeptidase 1   | 2.1    | 0.0000678 |
| 273 | NM_173505       | <i>ANKRD29</i>  | Ankyrin repeat domain 29  | 2.1    | 0.0000555 |
| 274 | NM_000120       | <i>EPHX1</i>    | Epoxide hydrolase 1, microsomal (xenobiotic)                                      | 2.1    | 0.0000223 |

(continues)

TABLE 4 (continued). Upregulated Homozygous GCD II-Related Genes

| No. | Gene Accession | Gene Symbol | Gene Description   | Change | P         |
|-----|----------------|-------------|--|--------|-----------|
| 275 | NM_001039580   | MAP9        | Microtubule-associated protein 9   | 2.1    | 0.0003664 |
| 276 | NM_005746      | PBEF1       | Pre-B-cell colony enhancing factor 1   | 2.1    | 0.0007562 |
| 277 | NM_000204      | CF1         | Complement factor 1  | 2.1    | 0.0015781 |
| 278 | NM_021034      | IFITM3      | Interferon induced transmembrane protein 3 (I-8U)  | 2.1    | 0.0000542 |
| 279 | NM_004734      | DCLK1       | Doublecortin-like kinase 1   | 2.1    | 0.0005205 |
| 280 | NM_004696      | SLC16A4     | Solute carrier family 16, member 4 (monocarboxylic acid transporter 5)                           | 2.1    | 0.0002086 |
| 281 | NM_213589      | RAPH1       | Ras association (RalGDS/AF-6) and pleckstrin homology domains 1                                  | 2.1    | 0.0000129 |
| 282 | NM_002395      | ME1         | Malic enzyme 1, NADP(+)-dependent, cytosolic   | 2.1    | 0.0000103 |
| 283 | NM_014157      | CCDC113     | Coiled-coil domain containing 113  | 2.1    | 0.0002231 |
| 284 | NR_002157      | OR2A9P      | Olfactory receptor, family 2, subfamily A, member 9 pseudogene                                   | 2.1    | 0.0008517 |
| 285 | NM_007112      | THBS3       | Thrombospondin 3   | 2.1    | 0.0000632 |
| 286 | NM_005514      | HLA-B       | Major histocompatibility complex, class 1, B   | 2.1    | 0.000079  |
| 287 | NM_018933      | PCHDB13     | Protocadherin beta 13  | 2.1    | 0.0006096 |
| 288 | NM_138452      | DHRS1       | Dehydrogenase/reductase (SDR family) member 1  | 2.1    | 0.0014038 |
| 289 | NM_144599      | NIPA1       | Non imprinted in Prader-Willi/Angelman syndrome 1  | 2.1    | 0.0000307 |
| 290 | XR_019525      | LOC390345   | Hypothetical LOC390345   | 2.1    | 0.0045174 |
| 291 | NM_024642      | GALNT12     | UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 12 (GalNAC-T12) | 2.1    | 0.0001604 |
| 292 | NM_020422      | TMEM159     | Transmembrane protein 159  | 2.1    | 0.0006667 |
| 293 | NM_080593      | HIST1H2BK   | Histone cluster 1, H2bk  | 2.1    | 0.0001187 |
| 294 | NM_198503      | KCNT2       | Potassium channel, subfamily T, member 2   | 2.1    | 0.0035322 |
| 295 | NM_007199      | IRAK3       | Interleukin-1 receptor-associated kinase 3   | 2.1    | 0.0030475 |
| 297 | NM_005514      | HLA-B       | Major histocompatibility complex, class 1, B   | 2      | 0.0002145 |
| 298 | NM_003328      | TXK         | TXK tyrosine kinase  | 2      | 0.0010901 |
| 290 | NM_005419      | STAT2       | Signal transducer and activator of transcription 2, 113kDa                                       | 2      | 0.0012848 |
| 300 | NM_001039706   | FLJ21062    | Hypothetical protein FLJ21062  | 2      | 0.001607  |
| 301 | NM_015090      | NFASC       | Neurofascin homolog (chicken)  | 2      | 0.0000349 |
| 302 | NM_004159      | PSMB8       | Proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional peptidase 7)        | 2      | 0.0001761 |
| 303 | NM_004159      | PSMB8       | Proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional peptidase 7)        | 2      | 0.0001761 |
| 304 | NM_004159      | PSMB8       | Proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional peptidase 7)        | 2      | 0.0001761 |
| 305 | NM_000247      | MICA        | MHC class I polypeptide-related sequence A   | 2      | 0.0000572 |
| 306 | NM_002754      | MAPK13      | Mitogen-activated protein kinase 13  | 2      | 0.0009883 |
| 307 | NM_005929      | MF12        | Antigen p97 (melanoma associated) identified by monoclonal antibodies 133.2 and 96.5             | 2      | 0.0001076 |
| 308 | NM_005514      | HLA-B       | Major histocompatibility complex, class 1, B   | 2      | 0.000205  |
| 309 | NM_018050      | MANSC1      | MANSC domain containing 1  | 2      | 0.0000755 |
| 310 | NM_002121      | HLA-DPB1    | Major histocompatibility complex, class II, DP beta 1  | 2      | 0.000209  |
| 311 | NM_018317      | TBC1D19     | TBC1 domain family, member 19  | 2      | 0.0000012 |
| 312 | NR_003322      | SNORD116-7  | Small nucleolar RNA, C/D box 116-7   | 2      | 0.0051387 |
| 313 | NR_003320      | SNORD116-5  | Small nucleolar RNA, C/D box 116-5   | 2      | 0.0051387 |
| 314 | NM_020139      | BDH2        | 3-hydroxybutyrate dehydrogenase, type 2  | 2      | 0.0000086 |
| 315 | NM_004052      | BNIP3       | BCL2/adenovirus E1B 19kDa interacting protein 3  | 2      | 0.0001215 |
| 316 | NM_021199      | SQRDL       | Sulfide quinone reductase-like (yeast)   | 2      | 0.0008101 |
| 317 | NM_153704      | TMEM67      | Transmembrane protein 67   | 2      | 0.0019696 |
| 318 | NM_001831      | CLU         | Clusterin  | 2      | 0.0001778 |
| 319 | NM_014585      | SLC40A1     | Solute carrier family 40 (iron-regulated transporter), member 1                                  | 2      | 0.0002529 |

among stromal TGFBIp deposits.<sup>25</sup> These studies suggest that altered ECM proteolytic enzyme activities affect TGFBIp deposits by degrading ECM molecules, by either scission of covalent bonds or cleavage of mutant TGFBIp. Several investigations suggest that abnormal proteolysis is involved in deposits of TGFBIp in the cornea of the *TGFBI* gene associated with corneal dystrophy.<sup>26</sup> This finding is consistent with the fact that amyloid precursor proteins associated with other amyloid diseases generally undergo proteolysis during amyloid generation. Such proteins include gelsolin in both lattice corneal dystrophy type I and Finnish-type familial amyloidosis,<sup>27,28</sup> amyloid  $\beta$  precursor protein in familial Alzheimer's disease,<sup>29,30</sup> and BRI2 in familial British dementia.<sup>31,32</sup> Our microarray data show differential expression of endopeptidase-related genes such as serine-type endopeptidase activity-related genes, serine-type endopeptidase inhibitor-related genes, aspartic-type endopeptidase activity, and metallo-

dopeptidase activity-related genes. Although ECM proteolytic enzymes such as MMPs for TGFBIp remain unidentified, our data suggest that proteolytic enzymes associated with ECM turnover may be involved in the generation of TGFBIp deposits in GCD II. Further studies are needed to identify specific proteolytic enzymes responsible for TGFBIp deposits in the cornea.

### Cell Adhesion and Integrin Signaling

TGFBIp contains a domain rich in cysteine residues (EMI domain), four highly conserved fasciclin-like (FAS) domains, and a COOH-terminal Arg-Gly-Asp (RGD) motif. The presence of the FAS domains and the RGD motif suggests that TGFBIp may play a functional role in cell adhesion. More recently, it was found that TGFBIp plays an inhibitory role in the attachment of human scleral fibroblasts to collagen type I through interaction

TABLE 5. Downregulated Homozygous GCD II-Related Genes

| No. | Gene Accession | Gene Symbol          | Gene Description   | Change | P          |
|-----|----------------|----------------------|--|--------|------------|
| 1   | NM_181503      | <i>EXOSC8</i>        | Exosome component 8  | -2.0   | 0.0011677  |
| 2   | NM_152524      | <i>SGOL2</i>         | Shugoshin-like 2 (S. pombe)  | -2.0   | 0.0050817  |
| 3   | NR_002564      | <i>SNORD26</i>       | Small nucleolar RNA, C/D box 26  | -2.0   | 0.0002281  |
| 4   | NM_003534      | <i>HIST1H3G</i>      | Histone cluster 1, H3g   | -2.0   | 0.0002008  |
| 5   | NM_012074      | <i>DPF3</i>          | D4, zinc and double PHD fingers, family 3  | -2.0   | 0.0000953  |
| 6   | NM_018193      | <i>FANCI</i>         | Fanconi anemia, complementation group 1  | -2.0   | 0.0026856  |
| 7   | NM_052917      | <i>GALNT13</i>       | UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 13 (GalNAc-T13) | -2.0   | 0.0008409  |
| 8   | NM_152754      | <i>SEMA3D</i>        | Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3D           | -2.0   | 0.0021798  |
| 9   | NM_032117      | <i>MND1</i>          | Meiotic nuclear divisions 1 homolog (S. cerevisiae)  | -2.0   | 0.0035557  |
| 10  | NM_000059      | <i>BRCA2</i>         | Breast cancer 2, early onset   | -2.0   | 0.0049817  |
| 11  | NM_006079      | <i>CITED2</i>        | Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2                | -2.0   | 0.0016884  |
| 12  | NM_002192      | <i>INHBA</i>         | Inhibin, beta A  | -2.0   | 0.0001218  |
| 13  | NM_033518      | <i>SLC38A5</i>       | Solute carrier family 38, member 5   | -2.0   | 0.0003078  |
| 14  | NM_005239      | <i>ETS2</i>          | Vets erythroblastosis virus E26 oncogene homolog 2 (avian)                                       | -2.0   | 0.0000035  |
| 15  | NM_001039841   | <i>ARHGAP11B</i>     | Rho GTPase activating protein 11B  | -2.1   | 0.0002937  |
| 16  | NM_016426      | <i>GTSE1</i>         | G-2 and S-phase expressed 1  | -2.1   | 0.0008579  |
| 17  | NM_022111      | <i>CLSPN</i>         | Claspin homolog (Xenopus laevis)   | -2.1   | 0.0013778  |
| 18  | NM_021968      | <i>HIST1H4J</i>      | Histone cluster 1, H4j   | -2.1   | 0.0004086  |
| 19  | BC067351       | <i>GUSBL1</i>        | Glucuronidase, beta-like 1   | -2.1   | 0.0000052  |
| 20  | NM_018353      | <i>C14orf106</i>     | Chromosome 14 open reading frame 106   | -2.1   | 0.0021285  |
| 21  | NM_001099293   | <i>KIF4B</i>         | Kinesin family member 4B   | -2.1   | 0.0007975  |
| 22  | NM_181802      | <i>UBE2C</i>         | Ubiquitin-conjugating enzyme E2C   | -2.1   | 0.0016383  |
| 23  | NM_014573      | <i>TMEM97</i>        | Transmembrane protein 97   | -2.1   | 0.0004448  |
| 34  | NM_182909      | <i>FILIP1L</i>       | Filamin A interacting protein 1-like   | -2.1   | 0.0014915  |
| 35  | NM_002823      | <i>PTMA</i>          | Prothymosin, alpha (gene sequence 28)  | -2.1   | 0.0028224  |
| 36  | NM_000956      | <i>PTGER2</i>        | Prostaglandin E receptor 2 (subtype EP2), 53kDa  | -2.1   | 0.0025474  |
| 37  | NM_004817      | <i>TJP2</i>          | Tight junction protein 2 (zona occludens 2)  | -2.1   | 0.0000007  |
| 38  | NM_007317      | <i>KIF22</i>         | Kinesin family member 22   | -2.1   | 0.000031   |
| 39  | NR_002562      | <i>SNORD28</i>       | Small nucleolar RNA, C/D box 28  | -2.1   | 0.001433   |
| 40  | NM_016095      | <i>GINS2</i>         | GINS complex subunit 2 (Psf2 homolog)  | -2.1   | 0.0018093  |
| 41  | NM_007317      | <i>KIF22</i>         | Kinesin family member 22   | -2.1   | 0.0000356  |
| 42  | NM_004731      | <i>SLC16A7</i>       | Solute carrier family 16, member 7 (monocarboxylic acid transporter 2)                           | -2.1   | 0.0000023  |
| 43  | NM_005496      | <i>SMC4</i>          | Structural maintenance of chromosomes 4  | -2.1   | 0.0004444  |
| 44  | NM_144586      | <i>LYPD1</i>         | LY6/PLAUR domain containing 1  | -2.1   | 0.0005818  |
| 45  | NM_001129      | <i>AEBP1</i>         | AE binding protein 1   | -2.1   | 0.0000008  |
| 46  | NM_014783      | <i>ARHGAP11A</i>     | Rho GTPase activating protein 11A  | -2.1   | 0.0009603  |
| 47  | NM_019035      | <i>PCDH18</i>        | Protocadherin 18   | -2.1   | 0.0000004  |
| 48  | NM_013277      | <i>RACGAP1</i>       | Rac GTPase activating protein 1  | -2.1   | 0.0006113  |
| 49  | NM_006739      | <i>MCM5</i>          | Minichromosome maintenance complex component 5   | -2.1   | 0.0002303  |
| 50  | NM_152270      | <i>SLFN11</i>        | Schlafen family member 11  | -2.1   | 0.0011236  |
| 51  | NM_007243      | <i>NRM</i>           | Nurim (nuclear envelope membrane protein)  | -2.2   | 0.000234   |
| 52  | NM_007243      | <i>NRM</i>           | Nurim (nuclear envelope membrane protein)  | -2.2   | 0.000234   |
| 53  | NR_003125      | <i>LOC85391</i>      | RNA, small nucleolar   | -2.2   | 0.0004524  |
| 54  | NM_182751      | <i>MCM10</i>         | Minichromosome maintenance complex component 10  | -2.2   | 0.00041059 |
| 55  | NM_001048201   | <i>UHRF1</i>         | Ubiquitin-like, containing PHD and RING finger domains, 1  | -2.2   | 0.0001459  |
| 56  | NM_014465      | <i>SULT1B1</i>       | Sulfotransferase family, cytosolic, 1B, member 1   | -2.2   | 0.0006522  |
| 57  | NM_002760      | <i>PRKY</i>          | Protein kinase, Y-linked   | -2.2   | 0.0047595  |
| 58  | NM_001827      | <i>CKS2</i>          | CDC28 protein kinase regulatory subunit 2  | -2.2   | 0.0009191  |
| 59  | NM_004772      | <i>C5orf13</i>       | Chromosome 5 open reading frame 13   | -2.2   | 0.0006237  |
| 60  | NM_005497      | <i>GJA7</i>          | Gap junction protein, alpha 7, 45kDa   | -2.2   | 0.0001851  |
| 61  | NM_001744      | <i>CAMK4</i>         | Calcium/calmodulin-dependent protein kinase IV   | -2.2   | 0.0016928  |
| 62  | NM_152495      | <i>CNIH3</i>         | Cornichon homolog 3 (Drosophila)   | -2.2   | 0.0032016  |
| 63  | NM_006963      | <i>ZNF22</i>         | Zinc finger protein 22 (KOX 15)  | -2.2   | 0.0005145  |
| 64  | NM_014363      | <i>SACS</i>          | Spastic ataxia of Charlevoix-Saguenay (sacsin)   | -2.2   | 0.0000886  |
| 65  | NM_018476      | <i>BEX1</i>          | Brain expressed, X-linked 1  | -2.2   | 0.0043276  |
| 66  | NM_006727      | <i>CDH10</i>         | Cadherin 10, type 2 (T2-cadherin)  | -2.2   | 0.0003019  |
| 67  | NM_003545      | <i>HIST1H4E</i>      | Histone cluster 1, H4e   | -2.2   | 0.0001393  |
| 68  | NM_004415      | <i>DSP</i>           | Desmoplakin  | -2.2   | 0.0000256  |
| 69  | NM_001761      | <i>CCNF</i>          | Cyclin F   | -2.2   | 0.0000636  |
| 70  | NM_018101      | <i>CDC48</i>         | Cell division cycle associated 8   | -2.3   | 0.0015208  |
| 71  | NM_021018      | <i>HIST1H3F</i>      | Histone cluster 1, H3f   | -2.3   | 0.0038466  |
| 72  | NM_004572      | <i>PKP2</i>          | Plakophilin 2  | -2.3   | 0.0008219  |
| 73  | NM_003517      | <i>HIST2H2AC</i>     | Histone cluster 2, H2ac  | -2.3   | 0.0003061  |
| 74  | NM_022908      | <i>NT5DC2</i>        | 5'-nucleotidase domain containing 2  | -2.3   | 0.0003366  |
| 75  | NM_018410      | <i>DKFZp762E1312</i> | Hypothetical protein DKFZp762E1312   | -2.3   | 0.0010354  |
| 76  | NM_024908      | <i>WDR76</i>         | WD repeat domain 76  | -2.3   | 0.0029464  |
| 77  | NM_004456      | <i>EZH2</i>          | Enhancer of zeste homolog 2 (Drosophila)   | -2.3   | 0.000033   |

(continues)

TABLE 5 (continued). Downregulated Homozygous GCD II-Related Genes

| No. | Gene Accession  | Gene Symbol | Gene Description   | Change | P         |
|-----|-----------------|-------------|--|--------|-----------|
| 78  | NM_198433       | AURKA       | Aurora kinase A  | -2.3   | 0.0009593 |
| 79  | NM_001878       | CRABP2      | Cellular retinoic acid binding protein 2   | -2.3   | 0.0003869 |
| 80  | NM_014865       | NCAPD2      | Non-SMC condensin 1 complex, subunit D2  | -2.3   | 0.0000146 |
| 81  | NM_006479       | RAD51AP1    | RAD51 associated protein 1   | -2.3   | 0.0004349 |
| 82  | NM_003541       | HIST1H4K    | Histone cluster 1, H4k   | -2.3   | 0.0001022 |
| 83  | NM_005480       | TROAP       | Trophinin associated protein (tastin)  | -2.3   | 0.0007361 |
| 84  | NM_130398       | EXO1        | Exonuclease 1  | -2.3   | 0.0007009 |
| 85  | NM_003530       | HIST1H3D    | Histone cluster 1, H3d   | -2.3   | 0.0000989 |
| 86  | NM_012484       | HMMR        | Hyaluronan-mediated motility receptor (RHAMM)  | -2.3   | 0.0010368 |
| 87  | BX641032        | WEE1        | WEE1 homolog (S. pombe)  | -2.3   | 0.0020117 |
| 88  | NM_005491       | CXorf6      | Chromosome X open reading frame 6  | -2.3   | 0.000069  |
| 89  | NM_004900       | APOBEC3B    | Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B                        | -2.3   | 0.002503  |
| 90  | NM_020890       | KIAA1524    | KIAA1524   | -2.4   | 0.0035147 |
| 91  | NM_012177       | FBXO5       | F-box protein 5  | -2.4   | 0.0029497 |
| 92  | ENST00000377738 | KRTAP2-4    | Keratin associated protein 2-4 (KRTAP2-4), mRNA  | -2.4   | 0.0003921 |
| 93  | NM_013230       | CD24        | CD24 molecule  | -2.4   | 0.0032149 |
| 94  | NM_001813       | CENPE       | Centromere protein E, 312kDa   | -2.4   | 0.0019189 |
| 95  | NM_007174       | CIT         | Citron (rho-interacting, serine/threonine kinase 21)                                       | -2.4   | 0.0006063 |
| 96  | NM_002106       | H2AFZ       | H2A histone family, member Z   | -2.4   | 0.0010744 |
| 97  | NM_003524       | HIST1H2BH   | Histone cluster 1, H2bh  | -2.4   | 0.0001753 |
| 98  | NM_003784       | SERPINB7    | Serpin peptidase inhibitor, clade B (ovalbumin), member 7                                  | -2.4   | 0.0011738 |
| 99  | NM_017669       | ERCC6L      | Excision repair cross-complementing rodent repair deficiency, complementation group 6-like | -2.4   | 0.0037181 |
| 100 | ENST00000377738 | KRTAP2-4    | Keratin associated protein 2-4 (KRTAP2-4), mRNA  | -2.4   | 0.0006498 |
| 101 | ENST00000377738 | KRTAP2-4    | Keratin associated protein 2-4 (KRTAP2-4), mRNA  | -2.4   | 0.0006498 |
| 102 | NM_021052       | HIST1H2AE   | Histone cluster 1, H2ae  | -2.4   | 0.0004919 |
| 103 | NM_002263       | KIFC1       | Kinesin family member C1   | -2.4   | 0.002479  |
| 104 | NM_003258       | TK1         | Thymidine kinase 1, soluble  | -2.4   | 0.0006185 |
| 105 | NM_001048198    | SNHG3-RCC1  | Regulator of chromosome, condensation 1  | -2.4   | 0.0000584 |
| 106 | NM_013296       | GPSM2       | G-protein signaling modulator 2 (AGS3-like, C. elegans)                                    | -2.4   | 0.0006993 |
| 107 | NM_031217       | KIF18A      | Kinesin family member 18A  | -2.5   | 0.0001562 |
| 108 | NM_001017420    | ESCO2       | Establishment of cohesion 1 homolog 2 (S. cerevisiae)                                      | -2.5   | 0.0013571 |
| 109 | NM_014264       | PLK4        | Polo-like kinase 4 (Drosophila)  | -2.5   | 0.0031529 |
| 110 | ENST00000302536 | KIAA1576    | KIAA1576 protein (KIAA1576), mRNA  | -2.5   | 0.0000329 |
| 111 | NM_031966       | CCNB1       | Cyclin B1  | -2.5   | 0.0000813 |
| 112 | NM_003877       | SOCS2       | Suppressor of cytokine signaling 2   | -2.5   | 0.0000898 |
| 113 | NM_005325       | HIST1H1A    | Histone cluster 1, H1a   | -2.5   | 0.0000106 |
| 114 | NM_138555       | KIF23       | Kinesin family member 23   | -2.5   | 0.0005602 |
| 115 | NM_001012507    | C6orf173    | Chromosome 6 open reading frame 173  | -2.5   | 0.0003908 |
| 116 | NM_002466       | MYBL2       | V-myb mycloblastosis viral oncogene homolog (avian)-like 2                                 | -2.5   | 0.0000271 |
| 117 | NM_013372       | GREM1       | Gremlin 1, cysteine knot superfamily, homolog (Xenopus laevis)                             | -2.5   | 0.000007  |
| 118 | NM_003529       | HIST1H3A    | Histone cluster 1, H3a   | -2.5   | 0.0012216 |
| 119 | NM_001034       | RRM2        | Ribonucleotide reductase M2 polypeptide  | -2.5   | 0.0034171 |
| 120 | NM_000599       | IGFBP5      | Insulin-like growth factor binding protein 5   | -2.6   | 0.0039748 |
| 121 | NM_001033049    | ADARB1      | Adenosine deaminase, RNA-specific, B1 (RED1 homolog rat)                                   | -2.6   | 0.0000097 |
| 122 | NM_000561       | GSTM1       | Glutathione S-transferase M1   | -2.6   | 0.0003801 |
| 123 | NM_005517       | HMGN2       | High-mobility group nucleosomal binding domain 2   | -2.6   | 0.0001929 |
| 124 | NM_175065       | HIST2H2AB   | Histone cluster 2, H2ab  | -2.6   | 0.0001294 |
| 125 | NM_006682       | FGI2        | Fibrinogen-like 2  | -2.6   | 0.0004615 |
| 126 | NM_001012410    | SGOL1       | Shugoshin-like 1 (S. pombe)  | -2.6   | 0.0072724 |
| 127 | NM_003544       | HIST1H4B    | Histone cluster 1, H4b   | -2.6   | 0.0001657 |
| 128 | NM_153262       | SYT14       | Synaptotagmin XIV  | -2.6   | 0.0000915 |
| 129 | NM_002263       | KIFC1       | Kinesin family member C1   | -2.7   | 0.0013435 |
| 130 | NM_182705       | FAM101B     | Family with sequence similarity 101, member B  | -2.7   | 0.0000477 |
| 131 | NM_006558       | KHDRBS3     | KH domain containing, RNA binding, signal transduction associated 3                        | -2.7   | 0.0000787 |
| 132 | NM_001943       | DSG2        | Desmoglein 2   | -2.7   | 0.0000628 |
| 133 | NM_015975       | TAF9B       | TAF9B RNA polymerase II, TATA box binding protein (TBP)-associated factor, 31kDa           | -2.7   | 0.0001503 |
| 134 | NM_016591       | GCNT4       | Glucosaminyl (N-acetyl) transferase 4, core 2 (beta-1,6-N-acetylglucosaminyltransferase)   | -2.7   | 0.0002032 |
| 135 | NM_014875       | KIF14       | Kinesin family member 14   | -2.7   | 0.0026412 |
| 136 | BX641032        | WEE1        | WEE1 homolog (S. pombe)  | -2.7   | 0.0018471 |
| 137 | NM_016588       | NRN1        | Neuritin 1   | -2.7   | 0.0000023 |
| 138 | NM_003546       | HIST1H4L    | Histone cluster 1, H4l   | -2.7   | 0.0012995 |
| 139 | NM_006845       | KIF2C       | Kinesin family member 2C   | -2.7   | 0.0020828 |
| 140 | NR_002612       | DLEU2       | Deleted in lymphocytic leukemia, 2   | -2.7   | 0.0017015 |
| 141 | NM_031299       | CDCA3       | Cell division cycle associated 3   | -2.7   | 0.0002869 |
| 142 | NM_018849       | ABCB4       | ATP-binding cassette, sub-family B (MDR/TAP), member 4                                     | -2.8   | 0.000121  |
| 143 | NM_001067       | TOP2A       | Topoisomerase (DNA) II alpha 170kDa  | -2.8   | 0.0004533 |
| 144 | NM_020242       | KIF15       | Kinesin family member 15   | -2.8   | 0.0002493 |

(continues)



TABLE 5 (continued). Downregulated Homozygous GCD II-Related Genes

| No. | Gene Accession  | Gene Symbol      | Gene Description   | Change | P         |
|-----|-----------------|------------------|--|--------|-----------|
| 145 | NM_004439       | <i>EPHA5</i>     | EPH receptor A5  | -2.8   | 0.0000033 |
| 146 | NM_003981       | <i>PRC1</i>      | Protein regulator of cytokinesis 1                                   | -2.8   | 0.0054775 |
| 147 | NM_002398       | <i>MEIS1</i>     | Meis homeobox 1  | -2.8   | 0.0003329 |
| 148 | NM_173084       | <i>TRIM59</i>    | Tripartite motif-containing 59                                       | -2.8   | 0.001123  |
| 149 | NM_003533       | <i>HIST1H31</i>  | Histone cluster 1, H3i   | -2.8   | 0.0000119 |
| 150 | NM_145061       | <i>C13orf3</i>   | Chromosome 13 open reading frame 3                                   | -2.8   | 0.0003047 |
| 151 | NM_001786       | <i>CDC2</i>      | Cell division cycle 2, G1 to S and G2 to M                           | -2.8   | 0.0035238 |
| 152 | NM_006101       | <i>NDC80</i>     | NDC80 homolog, kinetochore complex component (S. cerevisiae)         | -2.9   | 0.0012085 |
| 153 | NM_012112       | <i>TPX2</i>      | TPX2, microtubule-associated, homolog (Xenopus laevis)               | -2.9   | 0.000403  |
| 154 | NM_016359       | <i>NUSAP1</i>    | Nucleolar and spindle associated protein 1                           | -2.9   | 0.0011078 |
| 155 | NM_003540       | <i>HIST1H4F</i>  | Histone cluster 1, H4f   | -2.9   | 0.0010387 |
| 156 | NM_015341       | <i>NCAPH</i>     | Non-SMC condensin 1 complex, subunit H                               | -2.9   | 0.0003153 |
| 157 | NM_012310       | <i>KIF4A</i>     | Kinesin family member 4A   | -2.9   | 0.0001634 |
| 158 | NM_001211       | <i>BUB1B</i>     | BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)    | -2.9   | 0.0000898 |
| 159 | NM_003522       | <i>HIST1H2BF</i> | Histone cluster 1, H2bf  | -2.9   | 0.0011487 |
| 160 | NM_152515       | <i>CKAP2L</i>    | Cytoskeleton associated protein 2-like                               | -3.0   | 0.0008625 |
| 161 | NM_152562       | <i>CDCA2</i>     | Cell division cycle associated 2                                     | -3.0   | 0.0001478 |
| 162 | NM_178229       | <i>IQGAP3</i>    | IQ motif containing GTPase activating protein 3                      | -3.0   | 0.0006081 |
| 163 | NM_001884       | <i>HAPLN1</i>    | Hyaluronan and proteoglycan link protein 1                           | -3.0   | 0.0003171 |
| 164 | NR_001544       | <i>CYorf14</i>   | Chromosome Y open reading frame 14                                   | -3.0   | 0.0001937 |
| 165 | NM_022346       | <i>NCAPG</i>     | Non-SMC condensin 1 complex, subunit G                               | -3.0   | 0.0016196 |
| 166 | NM_002867       | <i>RAB3B</i>     | RAB3B, member RAS oncogene family                                    | -3.0   | 0.0000771 |
| 167 | NM_001080480    | <i>MBOAT1</i>    | Membrane bound O-acyltransferase domain containing 1                 | -3.1   | 0.0000156 |
| 168 | NM_006350       | <i>FST</i>       | Follistatin  | -3.1   | 0.0008267 |
| 169 | ENST00000389239 | <i>FAM64A</i>    | Family with sequence similarity 64, member A (FAM64A), mRNA          | -3.1   | 0.0003557 |
| 170 | NM_020675       | <i>SPC25</i>     | SPC25, NDC80 kinetochore complex component, homolog (S. cerevisiae)  | -3.1   | 0.0024131 |
| 171 | NM_170589       | <i>CASC5</i>     | Cancer susceptibility candidate 5                                    | -3.1   | 0.0006033 |
| 172 | NM_002497       | <i>NEK2</i>      | NIMA (never in mitosis gene a)-related kinase 2                      | -3.1   | 0.0035344 |
| 173 | NM_004701       | <i>CCNB2</i>     | Cyclin B2  | -3.1   | 0.0006401 |
| 174 | NM_003318       | <i>TTK</i>       | TTK protein kinase   | -3.1   | 0.0011001 |
| 175 | NM_002129       | <i>HMGB2</i>     | High-mobility group box 2  | -3.1   | 0.0003609 |
| 176 | NM_018131       | <i>CEP55</i>     | Centrosomal protein 55kDa  | -3.1   | 0.0053938 |
| 177 | NM_003513       | <i>HIST1H2AB</i> | Histone cluster 1, H2ab  | -3.1   | 0.000027  |
| 178 | NM_001032283    | <i>TMPO</i>      | Thymopoietin   | -3.2   | 0.0003481 |
| 179 | NM_001255       | <i>CDC20</i>     | Cell division cycle 20 homolog (S. cerevisiae)                       | -3.2   | 0.0000258 |
| 180 | NM_001711       | <i>BGN</i>       | Biglycan   | -3.2   | 0.0000375 |
| 181 | NM_001071       | <i>TYMS</i>      | Thymidylate synthetase   | -3.2   | 0.0032506 |
| 182 | NM_004934       | <i>CDH18</i>     | Cadherin 18, type 2  | -3.2   | 0.0000862 |
| 183 | NM_018136       | <i>ASPM</i>      | Asp (abnormal spindle) homolog, microcephaly associated (Drosophila) | -3.2   | 0.0001314 |
| 184 | NM_020859       | <i>SHROOM3</i>   | Shroom family member 3   | -3.2   | 0.0002947 |
| 185 | NM_006147       | <i>IRF6</i>      | Interferon regulatory factor 6                                       | -3.2   | 0.0002558 |
| 186 | NM_018685       | <i>ANLN</i>      | Anillin, actin binding protein                                       | -3.2   | 0.0004383 |
| 187 | NM_005321       | <i>HIST1H1E</i>  | Histone cluster 1, H1e   | -3.3   | 0.0000589 |
| 188 | NM_017779       | <i>DEPDC1</i>    | DEP domain containing 1  | -3.3   | 0.0005434 |
| 189 | NM_002783       | <i>PSG7</i>      | Pregnancy specific beta-1-glycoprotein 7                             | -3.3   | 0.0002987 |
| 190 | NM_005556       | <i>KRT7</i>      | Keratin 7  | -3.4   | 0.0000318 |
| 191 | NM_005322       | <i>HIST1H1B</i>  | Histone cluster 1, H1b   | -3.4   | 0.000073  |
| 192 | NM_014736       | <i>KIAA0101</i>  | KIAA0101   | -3.4   | 0.0073048 |
| 193 | NM_202002       | <i>FOXM1</i>     | Forkhead box M1  | -3.4   | 0.0009265 |
| 194 | NM_005030       | <i>PLK1</i>      | Polo-like kinase 1 (Drosophila)                                      | -3.4   | 0.0000316 |
| 195 | NM_006461       | <i>SPAG5</i>     | Sperm associated antigen 5   | -3.4   | 0.0002157 |
| 196 | NM_016343       | <i>CENPF</i>     | Centromere protein F, 350/400ka (mitosis)                            | -3.5   | 0.0053205 |
| 197 | NM_000609       | <i>CXCL12</i>    | Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)    | -3.5   | 0.0000196 |
| 198 | NM_018689       | <i>KIAA1199</i>  | KIAA1199   | -3.6   | 0.0000461 |
| 199 | NM_145697       | <i>NUF2</i>      | NUF2, NDC80 kinetochore complex component, homolog (S. cerevisiae)   | -3.6   | 0.0008586 |
| 200 | NM_005573       | <i>LMNB1</i>     | Lamin B1   | -3.7   | 0.0000014 |
| 201 | NM_203401       | <i>STMN1</i>     | Stathmin 1/oncoprotein 18  | -3.8   | 0.0000397 |
| 202 | NM_006013       | <i>RPL10</i>     | Ribosomal protein L10  | -3.8   | 0.0000282 |
| 203 | NM_014750       | <i>DLG7</i>      | Discs, large homolog 7 (Drosophila)                                  | -3.9   | 0.0004194 |
| 204 | NM_003638       | <i>ITGA8</i>     | Integrin, alpha 8  | -4.0   | 0.0005969 |
| 205 | NM_003537       | <i>HIST1H3B</i>  | Histone cluster 1, H3b   | -4.0   | 0.0033207 |
| 206 | NM_014932       | <i>NLGN1</i>     | Neurologin 1   | -4.2   | 0.0048918 |
| 207 | NR_003106       | <i>PWCR1</i>     | Prader-Willi syndrome chromosome region 1                            | -4.2   | 0.0041387 |
| 208 | NM_002276       | <i>KRT19</i>     | Keratin 19   | -4.2   | 0.0002805 |
| 209 | NM_005733       | <i>KIF20A</i>    | Kinesin family member 20A  | -4.3   | 0.005152  |
| 210 | NM_001290       | <i>LDB2</i>      | LIM domain binding 2   | -4.4   | 0.0000202 |
| 211 | NM_001080428    | <i>ODZ2</i>      | Odz, odd Oz/ten-m homolog 2 (Drosophila)                             | -4.5   | 0.0001199 |
| 212 | NM_003521       | <i>HIST1H2BM</i> | Histone cluster 1, H2bm  | -4.5   | 0.0000106 |
| 213 | NM_018284       | <i>GBP3</i>      | Guanylate binding protein 3  | -4.6   | 0.0002522 |
| 214 | NM_013381       | <i>TRHDE</i>     | Thyrotropin-releasing hormone degrading enzyme                       | -4.7   | 0.0001288 |

(continues)

TABLE 5 (continued). Downregulated Homozygous GCD II-Related Genes

| No. | Gene Accession | Gene Symbol | Gene Description   | Change | P         |
|-----|----------------|-------------|--|--------|-----------|
| 215 | NM_001040152   | PEG10       | Paternally expressed 10  | -4.7   | 0.0000323 |
| 216 | NM_005159      | ACTC1       | Actin, alpha, cardiac muscle 1   | -4.7   | 0.0000545 |
| 217 | NM_003542      | HIST1H4C    | Histone cluster 1, H4c   | -5.2   | 0.0002514 |
| 218 | NM_022350      | LRAP        | Leukocyte-derived arginine aminopeptidase                              | -5.2   | 0.0000817 |
| 219 | NM_002421      | MMP1        | Matrix metalloproteinase 1 (interstitial collagenase)                  | -5.5   | 0.0047864 |
| 220 | NM_002781      | PSG5        | Pregnancy specific beta-1-glycoprotein 5                               | -5.6   | 0.0000083 |
| 221 | NM_005711      | EDIL3       | EGF-like repeats and discoidin 1-like domains 3                        | -6.5   | 0.0000014 |
| 222 | NM_003411      | ZFY         | Zinc finger protein, Y-linked  | -6.5   | 0.0000338 |
| 223 | NM_005434      | MALL        | Mal, T-cell differentiation protein-like                               | -7.5   | 0.0000132 |
| 224 | NM_004653      | JARID1D     | Jumonji, AT rich interactive domain 1D                                 | -9.0   | 0.0000055 |
| 225 | NM_001005852   | CYorf15A    | Chromosome Y open reading frame 15A                                    | -9.7   | 0.0000309 |
| 226 | NM_004932      | CDH6        | Cadherin 6, type 2, K-cadherin (fetal kidney)                          | -10.2  | 0.0000005 |
| 227 | NM_032576      | CYorf15B    | Chromosome Y open reading frame 15B                                    | -11.1  | 0.0000012 |
| 228 | NM_007125      | UTY         | Ubiquitously transcribed tetratricopeptide repeat gene, Y-linked       | -11.2  | 0.0000027 |
| 229 | NM_014893      | NLGN4Y      | Neuroigin 4, Y-linked  | -11.8  | 0.0001143 |
| 230 | NM_021013      | KRT34       | Keratin 34   | -12.1  | 0.0000017 |
| 231 | NM_004654      | USP9Y       | Ubiquitin specific peptidase 9, Y-linked (fat facets-like, Drosophila) | -16.3  | 0.0000009 |
| 232 | NM_005045      | RELN        | Reelin   | -17.4  | 0.0001692 |
| 233 | NM_004681      | EIF1AY      | Eukaryotic translation initiation factor 1A, Y-linked                  | -19.5  | 0.0014353 |
| 234 | NM_001008      | RPS4Y1      | Ribosomal protein S4, Y-linked 1                                       | -27.2  | 0.0000016 |
| 235 | NM_001999      | FBN2        | Fibrillin 2 (congenital contractural arachnodactyly)                   | -47.8  | 0.0000903 |
| 236 | NM_004660      | DDX3Y       | DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked                     | -59.9  | 0.0002855 |

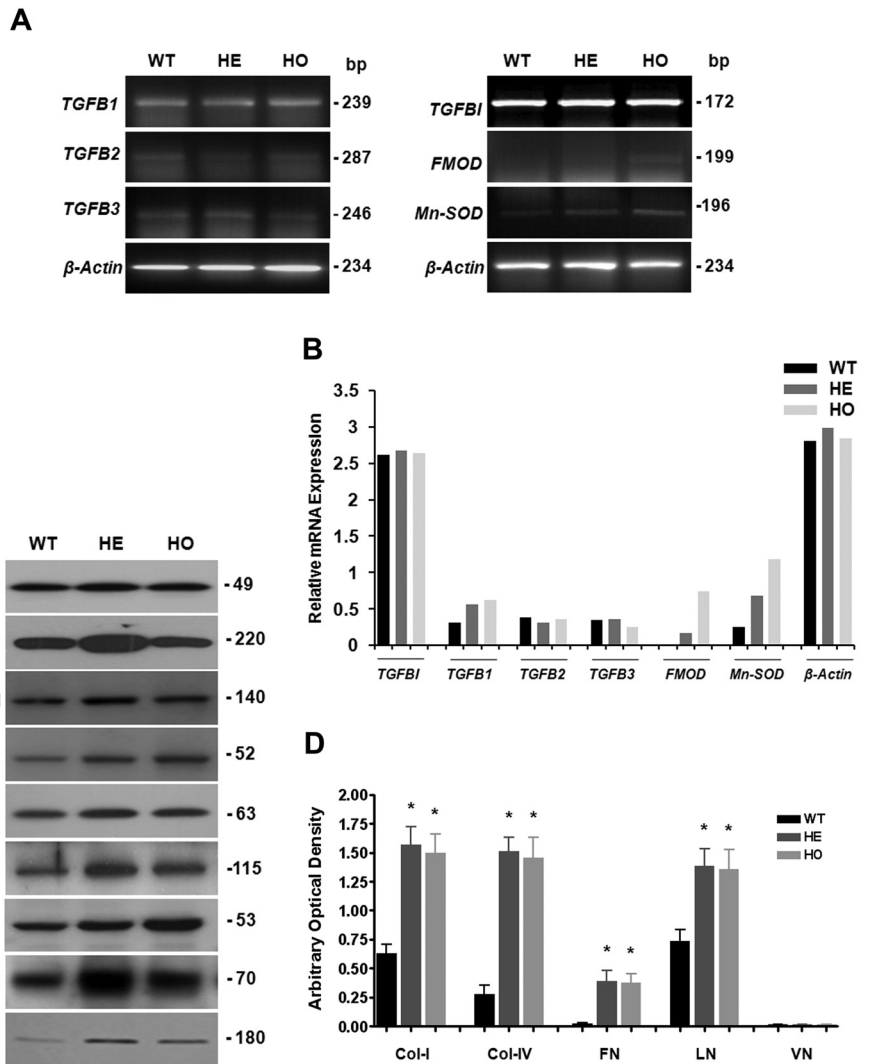
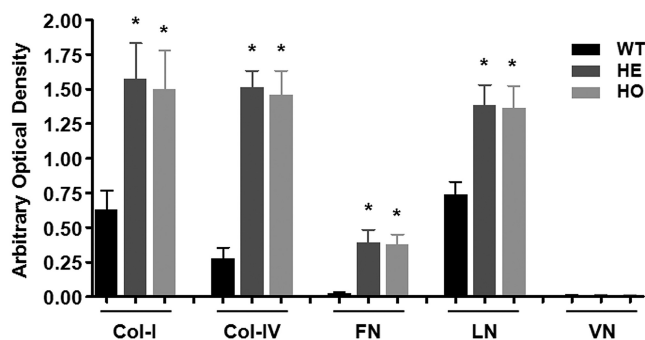


FIGURE 2. Relative quantification of 10 representative genes identified in the microarray. (A) RT-PCR was performed, and amplification products were subjected to agarose gel electrophoresis and were stained with ethidium bromide. (B) Each group was represented by two different cases whose RNA was pooled (sample pairs 1 and 2). Relative mRNA expression levels were normalized against  $\beta$ -actin. (C) Western blot analysis was performed using heterozygous GCD II, homozygous GCD II, and wild-type PCFs. (D) Each group was represented by two different cases whose protein was pooled (sample pairs 2 and 3). Relative protein expression levels were normalized to  $\beta$ -actin. Antibodies used are indicated to the left of each blot. WT, wild-type; HE, heterozygous GCD II; HO, homozygous GCD II. \* $P < 0.05$ .



**FIGURE 3.** Cell adhesion profiles of heterozygous GCD II, homozygous GCD II, and wild-type PCFs. Each column represents the mean value of four microspots; error bar, SD. PCF attachment experiments were performed with different ECM proteins. The experiment was repeated three times. The results are expressed as the mean  $\pm$  SD ( $n = 3$ ). BSA, bovine serum albumin; Col-I, type I collagen; Col-IV, type IV collagen; FN, fibronectin; LN, laminin; TN, tenascin; VN, vitronectin; WT, wild-type; HE, heterozygous GCD II; HO, homozygous GCD II. \* $P < 0.05$ .

with both  $\alpha v \beta 3$  and  $\alpha v \beta 5$  integrin receptors.<sup>33</sup> In the present investigation, we showed that heterozygous and homozygous GCD II PCFs tightly attached to collagen-I, collagen-IV, fibronectin, and laminin, compared with wild-type cells. These results support those in previous studies that show that TGFBIp plays a functional role in cell-ECM adhesion in the corneal stroma. However, our data suggest that tight attachment of heterozygous and homozygous GCD II PCFs to some ECM molecules is not due to a direct interaction between TGFBIp and the ECM. There are at least two possible explanations for this observation. First, TGFBIp expression was not significantly different in the heterozygous GCD II, homozygous GCD II, and wild-type PCFs.<sup>15</sup> Second, although TGFBIp can bind to collagens I, II, and III<sup>34</sup> and to fibronectin,<sup>35</sup> mutations in the *TGFBI* that commonly occur in certain corneal dystrophies do not apparently affect its binding to type I collagen, fibronectin, and laminin.<sup>36</sup> It is possible that signaling through integrins is involved in the attachment of GCD II PCFs to the ECM, especially since TGFBIp directly interacts with integrins.<sup>37</sup> This possibility is supported by the fact that increased integrin  $\alpha_2$  expression was detected in heterozygous and homozygous GCD II PCFs. Therefore, we suggest that increased cell adhesion does not result from structural changes, as a result of mutation, in the RGD motif and FAS domain of TGFBIp, but rather because of downstream signaling events that occur as a result of interactions between integrins and mutant TGFBIp.

In conclusion, our data suggest that the altered receptor-mediated signaling pathway of TGF- $\beta$  and integrins play a key role in GCD II pathophysiology. This study also identified other novel factors involved in this process that could aid in the design of future experiments to further investigate the development of this disease.

### Acknowledgments

The authors thank Mi-Na Park (Seoul National University, Seoul, Korea) for valuable discussions and Jung Wang Rim (DNALink, Inc., Seoul, Korea) for technical assistance.

### References

- Skonier J, Neubauer M, Madisen L, Bennett K, Plowman GD, Purchio AF. cDNA cloning and sequence analysis of beta ig-h3, a novel gene induced in a human adenocarcinoma cell line after treatment with transforming growth factor-beta. *DNA Cell Biol.* 1992;11:511-522.
- Klintworth GK. Advances in the molecular genetics of corneal dystrophies. *Am J Ophthalmol.* 1999;128:747-754.
- LeBaron RG, Bezverkov KI, Zimmer MP, Pavelec R, Skonier J, Purchio AF. Beta IG-H3, a novel secretory protein inducible by transforming growth factor-beta, is present in normal skin and promotes the adhesion and spreading of dermal fibroblasts in vitro. *J Invest Dermatol.* 1995;104:844-849.
- Billings PC, Herrick DJ, Kucich U, et al. Extracellular matrix and nuclear localization of beta ig-h3 in human bladder smooth muscle and fibroblast cells. *J Cell Biochem.* 2000;79:261-273.
- Kim JE, Jeong HW, Nam JO, et al. Identification of motifs in the fasciclin domains of the transforming growth factor-beta-induced matrix protein betaig-h3 that interact with the alphavbeta5 integrin. *J Biol Chem.* 2002;277:46159-46165.
- Zhang Y, Wen G, Shao G, et al. TGFBI deficiency predisposes mice to spontaneous tumor development. *Cancer Res.* 2009;69:37-44.
- Moon JW, Kim SW, Kim TI, Cristol SM, Chung ES, Kim EK. Homozygous granular corneal dystrophy type II (Avellino corneal dystrophy): natural history and progression after treatment. *Cornea.* 2007;26:1095-1100.
- Cintron C, Schneider H, Kublin C. Corneal scar formation. *Exp Eye Res.* 1973;17:251-259.
- Cintron C, Kublin CL. Regeneration of corneal tissue. *Dev Biol.* 1977;61:346-357.
- Takacs L, Csutak A, Balazs E, Berta A. Immunohistochemical detection of betaIG-H3 in scarring human corneas. *Graefes Arch Clin Exp Ophthalmol.* 1999;37:529-534.
- Wan XH, Lee HC, Stulting RD, et al. Exacerbation of Avellino corneal dystrophy after laser in situ keratomileusis. *Cornea.* 2002;21:223-226.
- Jun RM, Tchah H, Kim TI, et al. Avellino corneal dystrophy after LASIK. *Ophthalmology.* 2004;111:463-468.
- Morand S, Buchillier V, Maurer F, et al. Induction of apoptosis in human corneal and HeLa cells by mutated BIGH3. *Invest Ophthalmol Vis Sci.* 2003;44:2973-2979.
- Bustamante M, Tasinato A, Maurer F, et al. Overexpression of a mutant form of TGFBI/BIGH3 induces retinal degeneration in transgenic mice. *Mol Vis.* 2008;14:1129-1137.
- Choi SI, Kim TI, Kim KS, et al. Decreased catalase expression and increased susceptibility to oxidative stress in primary cultured corneal fibroblasts from patients with granular corneal dystrophy type II. *Am J Pathol.* 2009;175:1-14.
- O'Kane S, Ferguson MW. Transforming growth factor beta s and wound healing. *Int J Biochem Cell Biol.* 1997;29:63-78.
- Brown CT, Applebaum E, Banwatt R, Trinkaus-Randall V. Synthesis of stromal glycosaminoglycans in response to injury. *J Cell Biochem.* 1995;59:57-68.
- Hayashi K, Frangieh G, Wolf G, Kenyon KR. Expression of transforming growth factor-beta in wound healing of vitamin A-deficient rat corneas. *Invest Ophthalmol Vis Sci.* 1989;30:239-247.
- Wilson SE, He YG, Lloyd SA. EGF, EGF receptor, basic FGF, TGF beta-1, and IL-1 alpha mRNA in human corneal epithelial cells and stromal fibroblasts. *Invest Ophthalmol Vis Sci.* 1992;33:1756-1765.
- Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor beta in human disease. *N Engl J Med.* 2000;342:1350-1358.
- Monteleone G, Kumberova A, Croft NM, McKenzie C, Steer HW, MacDonald TT. Blocking Smad7 restores TGF-beta1 signaling in chronic inflammatory bowel disease. *J Clin Invest.* 2001;108:601-609.
- Roh MI, Grossniklaus HE, Chung SH, Kang SJ, Kim WC, Kim EK. Avellino corneal dystrophy exacerbated after LASIK: scanning electron microscopic findings. *Cornea.* 2006;25:306-311.
- Varga J, Jimenez SA. Stimulation of normal human fibroblast collagen production and processing by transforming growth factor-beta. *Biochem Biophys Res Commun.* 1986;138:974-980.
- Overall CM, Wrana JL, Sodek J. Independent regulation of collagenase, 72-kDa progelatinase, and metalloendoproteinase inhibitor expression in human fibroblasts by transforming growth factor-beta. *J Biol Chem.* 1989;264:1860-1869.
- Akhtar S, Meek KM, Ridgway AE, Bonshek RE, Bron AJ. Deposits and proteoglycan changes in primary and recurrent granular dystrophy of the cornea. *Arch Ophthalmol.* 1999;117:310-321.

26. Korvatska E, Henry H, Mashima Y, et al. Amyloid and non-amyloid forms of 5q31-linked corneal dystrophy resulting from kerato-epithelin mutations at Arg-124 are associated with abnormal turnover of the protein. *J Biol Chem*. 2000;275:11465-11469.
27. Huff ME, Page LJ, Balch WE, Kelly JW. Gelsolin domain 2 Ca<sup>2+</sup> affinity determines susceptibility to furin proteolysis and familial amyloidosis of finnish type. *J Mol Biol*. 2003;334:119-127.
28. Page LJ, Suk JY, Huff ME, et al. Metalloendoprotease cleavage triggers gelsolin amyloidogenesis. *EMBO J*. 2005;24:4124-4132.
29. Thinakaran G, Koo EH. Amyloid precursor protein trafficking, processing, and function. *J Biol Chem*. 2008;283:29615-29619.
30. Selkoe DJ. Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature*. 1999;399:A23-31.
31. Vidal R, Frangione B, Rostagno A, et al. A stop-codon mutation in the BRI gene associated with familial British dementia. *Nature*. 1999;399:776-781.
32. Choi SI, Vidal R, Frangione B, Levy E. Axonal transport of British and Danish amyloid peptides via secretory vesicles. *FASEB J*. 2004;18:373-375.
33. Shelton L, Summers-Rada J. Inhibition of human scleral fibroblast cell attachment to collagen type I by TGF $\beta$ 1p. *Invest Ophthalmol Vis Sci*. 2009;50(8):3542-3552.
34. Hashimoto K, Noshiro M, Ohno S, et al. Characterization of a cartilage-derived 66-kDa protein (RGD-CAP/beta ig-h3) that binds to collagen. *Biochim Biophys Acta*. 1997;1355:303-314.
35. Billings PC, Whitbeck JC, Adams CS, et al. The transforming growth factor-beta-inducible matrix protein (beta)ig-h3 interacts with fibronectin. *J Biol Chem*. 2002;277:28003-28009.
36. Kim JE, Park RW, Choi JY, et al. Molecular properties of wild-type and mutant beta1G-H3 proteins. *Invest Ophthalmol Vis Sci*. 2002;43:656-661.
37. Nam JO, Kim JE, Jeong HW, et al. Identification of the alphavbeta3 integrin-interacting motif of betaig-h3 and its anti-angiogenic effect. *J Biol Chem*. 2003;278:25902-25909.