

Involvement of TGF- β Receptor- and Integrin-Mediated Signaling Pathways in the Pathogenesis of Granular Corneal Dystrophy II

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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- β 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- β 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.

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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- β -induced gene (*TGFBI*) on chromosome 5, region q31.^{1,2} *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.¹ TGFBIp is a component of extracellular matrix (ECM) that mediates cell adhesion and migration by interacting with integrins.³⁻⁵ More recently, it has been shown that loss of TGFBIp induces cell proliferation and spontaneous tumor development in *TGFBI*-knockout mice.⁶

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.⁷

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.^{8,9} 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,¹⁰ and ablation of the corneal stroma by laser in situ keratomileusis (LASIK) in GCD II patients accelerates TGFBIp deposition.^{11,12} Overexpression of mutant *TGFBI* induces apoptotic cell death in human corneal epithelial cells,¹³ and age-dependent retinal degeneration has been observed in transgenic mice that express a mutant form of the human *TGFBI*.¹⁴ More recently, we have reported that PCFs are most susceptible to oxidative stress.¹⁵ 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.

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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 RT-PCR Western blot Cell-adhesion assay
2	M/10	25.3	F/20	35.3	M/10	16.6	Microarray RT-PCR Cell-adhesion assay
3	M/45		F/49		F/27		Microarray 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.¹⁵ 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 (α -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 β -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.¹⁵ The following antibodies were used: anti-integrin α_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-β1</i>	NM_000660	5'-GGGACTATCCACCTGCAAGA-3'	5'-CCTCCTTGGCGTAGTAGTCG-3'	239	60
<i>TGF-β2</i>	NM_001135599	5'-CCGAGGTGATTTCCATCTA-3'	5'-CTCCATTGCTGAGACGTCAA-3'	287	58
<i>TGF-β3</i>	NM_003239	5'-GAGTCAGAGCCCAGCAAAAC-3'	5'-AGAAGGAGGGAGGAAAACCA-3'	246	58
<i>TGFBI</i>	NM_000358	5'-GTGTGTGCTGTGCAGAAGGT-3'	5'-TTGAGAGTGGTAGGGGTGCT-3'	172	58
<i>β-Actin</i>	NM_001101	5'-GGACTTCGAGCAAGAGATGG-3'	5'-AGCACTGTGTTGGCGTACAG-3'	234	58

mecula, CA); anti-TGF- β receptors I, II, and III (1:200, 1:200, and 1:100, respectively; Santa Cruz Biotechnology, Santa Cruz, CA); and anti-fibronectin (1 μ 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 β -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- β 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, $P < 0.0017777$), *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 α_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- β 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 β 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- β Signaling Pathway

The TGF- β signaling pathway has been implicated as a regulator in numerous cellular and physiological processes including ECM homeostasis.¹⁶ The expression of TGF- β in corneal fibroblast cultures also suggests that it plays a role in regulating ECM metabolism through the TGF- β signaling pathway.¹⁷⁻¹⁹ TGF- β initiates signaling through a complex made up of TGF- β receptor (T β R) I and T β RII²⁰ and signals to the nucleus through the Smads protein.²¹ Our results show that T β 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, WFDCL1, 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, PCDH3, CCL2, TNFAIP6, LGALS3BP, ROBO1, CNTNAP2, ITGB3, COL8A1, SRPX, ADAM23, RASSF2, HMCN1, TNC, LAMC2, CASK, THBS3, PCDH13, 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, TLL7, 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 CFBs

GO ID	GO Category	Genes		n
		Up	Down	
GO:0051318	G ₁ 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

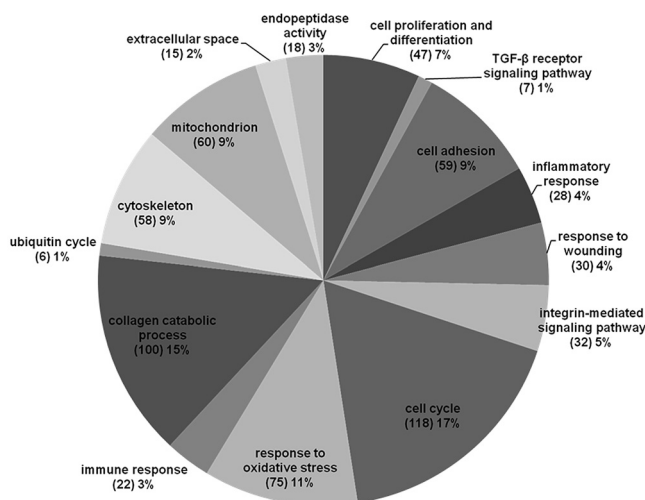


FIGURE 1. Gene ontological classification of differentially regulated genes in homozygous GCD II PCFs. Genes that were differentially expressed at a minimum of twofold were included in the analysis. Numbers in parentheses represent the number of genes.

compared with wild-type. Moreover, we identified different gene expression levels of the T β R complex assembly gene (*FMOD*; 3.2-fold increased, $P < 0.000113$) in homozygous GCD II PCFs compared with wild-type PCFs (Table 4). This suggests that TGF β I β expression may occur more rapidly in GCD II than in wild-type PCFs under various physiological conditions and could result in accelerated TGF β I β deposition and ultimate GCD II disease. Also, because overexpression of misfolded or mutant proteins could induce protein aggregation or deposition, increased T β 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- β after LASIK surgery.^{11,12,22} However, the specific mechanism(s) regarding the increase in T β R expression in GCD II corneal fibroblasts remains unknown.

Differential Expression of Proteolytic Enzymes Involved in ECM Metabolism

T β 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.^{23,24} 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	0.000084
50	NM_018937	<i>PCDH3</i>	Protocadherin beta 3	4.4	0.0000115
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>FRIL2</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	0.0015163
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>HISTH4H</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>	Angiopoietin 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

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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
299	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.²⁵ 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.²⁶ 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,^{27,28} amyloid β precursor protein in familial Alzheimer's disease,^{29,30} and BRI2 in familial British dementia.^{31,32} 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	0.0041059
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	0.0065522
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	SERPINF7	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

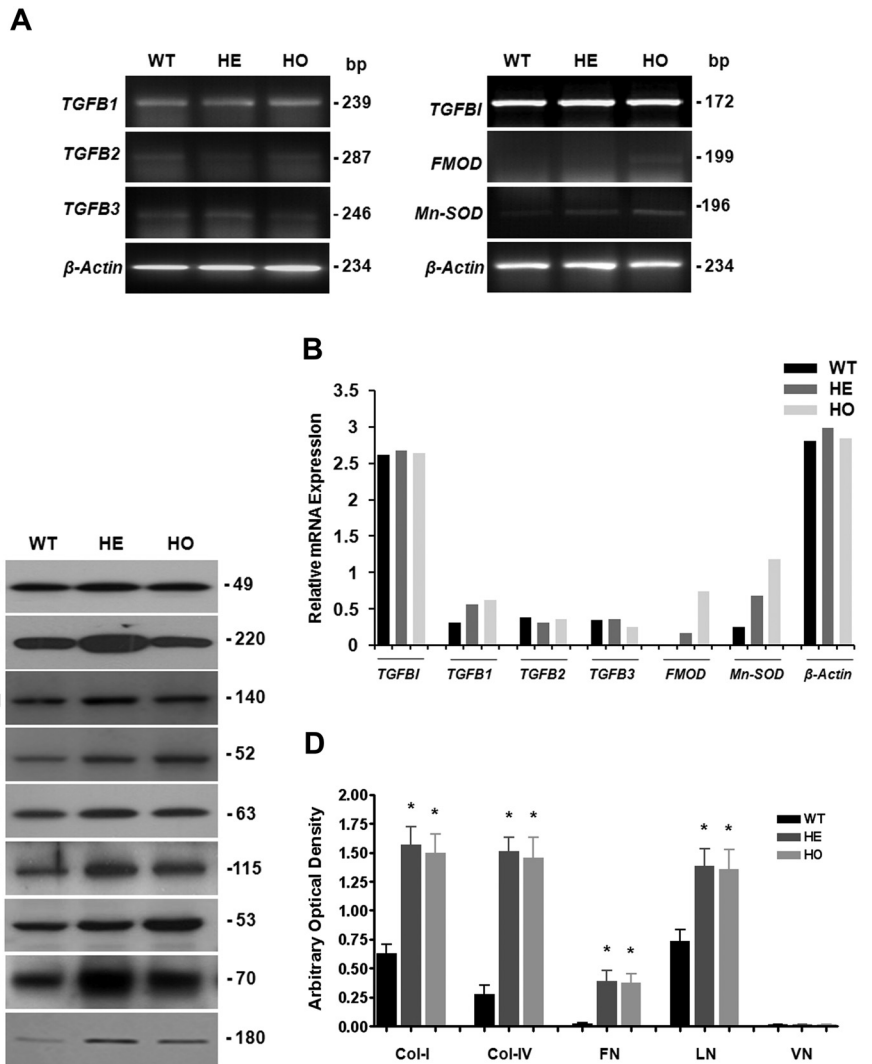


FIGURE 2. Relative quantification of 10 representative genes identified in the microarray. (A) RT-PCR was performed, and amplification products were subjected to agarose gel electrophoresis and were stained with ethidium bromide. (B) Each group was represented by two different cases whose RNA was pooled (sample pairs 1 and 2). Relative mRNA expression levels were normalized against β-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 β-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.

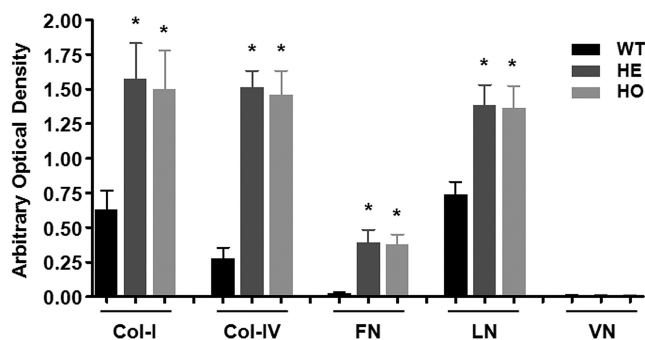


FIGURE 3. Cell adhesion profiles of heterozygous GCD II, homozygous GCD II, and wild-type PCFs. Each column represents the mean value of four microspots; error bar, SD. PCF attachment experiments were performed with different ECM proteins. The experiment was repeated three times. The results are expressed 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.³³ 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.¹⁵ Second, although TGFBIp can bind to collagens I, II, and III³⁴ and to fibronectin,³⁵ mutations in the *TGFBI* that commonly occur in certain corneal dystrophies do not apparently affect its binding to type I collagen, fibronectin, and laminin.³⁶ 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.³⁷ This possibility is supported by the fact that increased integrin α_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- β 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.

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