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Microarray analysis of gene expression in human donor sclera

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Purpose: To develop gene expression profiles of human sclera to allow for the identification of novel, uncharacterized genes in this tissue-type, and to identify candidate genes for scleral disorders.
Methods: Total RNA was isolated from 6 donor sources of human sclerae, and reverse transcribed into cDNA using a T7-(dT) 24 primer. The resulting cDNA was in vitro transcribed to produce biotin-labeled cRNA, fragmented, and mixed with hybridization controls before a 16 h hybridization step with oligonucleotide probes on 6 Affymetrix U95A chips. The chips were scanned twice at 570 nM and the data collected using GeneChip software. Array analyses were carried out with Microarray Suite, version 5.0 (Affymetrix), using the expression analysis algorithm to run an absolute analysis after cell intensities were computed. All arrays were scaled to the same target intensity using all probe sets. Reverse-transcription polymerase chain reaction (RT-PCR) was performed to validate the microarray results.
Results: There were 3,751 genes with "present" calls assigned independently to all six human scleral samples. These genes could be clustered into 4 major categories; transcription (10%), metabolism (8.8%), cell growth and proliferation (5.4%), and extracellular matrix (2%). Many extracellular matrix proteins, such as collagens 6A3 and 10A1, thrombospondins 2 and 4, and dystroglycan have not previously been shown to be expressed in sclera. RT-PCR results confirmed scleral expression in 7 extracellular matrix genes examined.
Conclusions: This study demonstrated the utility of gene microarray technology in identifying global patterns of scleral gene expression, and provides an extended list of genes expressed in human sclera. Identification of genes expressed in sclera contributes to our understanding of scleral biology, and potentially provides positional candidate genes for scleral disorders such as high myopia.

The sclera, the tough outer wall of the eye, is a specialized connective tissue that provides the structural framework that defines the shape (such as the axial length) of the eye. It consists largely of collagenous lamellae in close association with proteoglycans and glycoproteins [1-5]. Changes in the extracellular matrix components of the sclera or in molecules required for the synthesis and degradation of the scleral matrix may lead to significant changes in scleral biomechanical properties, leading to changes in scleral shape, ocular size and therefore the refractive state of the eye [6-10].

The profiling of gene expression in specific tissues provides useful information to characterize gene function and tissue physiology [11-13]. This baseline knowledge should facilitate the identification of alterations from normal gene expression that play important roles in disease pathogenesis. Prior research on the human sclera has focused on the detection of individual proteins of interest using techniques such as immunohistochemistry and in situ hybridization. Due to the rapid progress of the Human Genome Project and the development of high-throughput techniques such as cDNA microarray analysis [14-16], messenger RNA (mRNA) expression can be determined on a global scale with parallel assessment of gene expression for hundreds or thousands of genes in a single experiment.

Microarray expression analysis has features that make it the most widely used method for profiling mRNA expression. DNA segments representing the collection of genes to be assayed are amplified by polymerase chain reaction (PCR) and mechanically spotted at high density on glass microscope slides or nylon membranes using robotic systems. Experimental mRNA is labeled as a complex mixture and exposed to the microarray. Labeled mRNA will bind to complementary sequences on the microarray and can be detected in a semi-quantitative manner using automated techniques. The microrarrays are queried in a co-hybridization assay using fluorescently labeled probes prepared from mRNA from the cellular phenotypes of interest. The kinetics of hybridization allows relative expression levels to be determined based on the ratio with which each probe hybridizes to an individual array element. Hybridization is assayed using a confocal laser scanner to measure fluorescence intensities, which allows the simultaneous determination of the relative expression levels of all the genes represented in the array [16-19].

Because of the success of microarray analysis use in other biological systems and other eye tissue types [16-23], we sought to apply this technology to study gene expression in human donor scleral tissue. This analysis provides baseline expression information regarding the genetic basis of normal

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scleral function, as well as for scleral disease processes such as pathologic myopia with excess axial elongation, microphthalmia, scleral ectasia, focal staphylomatous formation, and inflammation. Knowledge of genes expressed or not expressed in a particular scleral disorder could lead to novel and definitive treatment strategies, such as interventional or gene therapies. These strategies may be particularly relevant for the sclera because the tissue is relatively less complex, can be manipulated ex vivo, and can be readily assessed visually.

METHODS

Affymetrix prefabricated chips: Each probe set on an Affymetrix chip is represented by multiple features or probes consisting of synthesized oligonucleotides. Half of the features are exact match representations at different positions along the length of the expressed regions of the gene. The other half contains the same oligonucleotide probes, but with a single mismatch in the middle. The range of 22-40 cells on the HG-U95A chip includes some controls for which there is a smaller or larger number than the 32 used as the probe sets for experimental genes. On the Affymetrix HG-U95A chip, for any given experimental gene there are 16 positions at which hybridization can be assessed, and the comparison between the perfect match and mismatch probes provides a control for non-specific hybridization. The Affymetrix HG-U95A chip contains 12,626 probe sets with some redundancy for certain genes or splice variants.

Target preparation, hybridization, and washing: Total RNA was isolated from the sclera of 6 human donor eyes. The eyes were from both male and female donors, ages 35-68 years. The human eyes were obtained from the Lions Eye Bank of Minnesota under an approved Institutional Review Board protocol at the University of Minnesota. The procurement and use of human tissues was in compliance with the tenets of the Declaration of Helsinki. The donor eyes were obtained as either whole globes or posterior poles with the cornea removed. The eyes were treated by submersion in RNA*Later* solution (Ambion Inc., Austin, TX) within 2-6 h post mortem. The entire sclera, minus the lamina cribosa was used for extraction of total RNA. The scleral tissue was snap frozen, and stored in a -80 °C freezer until RNA extraction. Frozen scleral tissue was cryogenically ground into a powder form using a freezer mill (6750 SPEX, CertiPrep Inc., Metuchen, NJ). Total RNA was extracted from pulverized samples using TRIZOL reagent (Invitrogen Inc., Carlsbad, CA) [24]. A high salt (0.8 M sodium citrate and 1.2 M NaCl) precipitation step was added to increase yield and improve the quality of the total RNA. The quantity and quality of RNA was assessed by obtaining the ratio of absorbance values at 260 and 280 nm using a Beckman spectrophotometer, and by visualization of intact 28S and 18S ribosomal RNA bands on denaturing formaldehyde agarose gels after electrophoresis. Individual RNA samples were assessed with electrophoresis to document RNA integrity using the Agilent Caliper system (Agilent Technologies Inc., Palo Alto, CA) [25]. The yield of total RNA per scleral sample ranged from 89-201 μg/eye. There was no correlation of quality with donor age.

Target was prepared using 5-20 μg of total RNA for each donor sclera. First strand cDNA was synthesized using Superscript II Reverse Transcriptase (Invitrogen Corporation, Carlsbad, CA) and a T7-$(dT)_{24}$ primer to incorporate the T7 priming site into the cDNA. Following RNA degradation with RNase H and second strand cDNA synthesis with DNA polymerase I, the double-stranded cDNA was extracted with phenol:chlorform:isoamyl alcohol (25:24:1). Approximately 1 μg of cDNA was used as template in an in vitro transcription assay reaction (Enzo Life Sciences, Inc., Farmingdale, NY) that incorporates biotin into the resulting cRNA. The cRNA was fragmented to a size range of 35-200 bases prior to use in hybridization by incubation at 94 °C for 35 min in fragmentation buffer (40 mM Tris acetate, pH 8.1, 125 mM KOAc, 30 mM MgOAc). Fifteen μg of fragmented probe was mixed with hybridization controls, herring sperm DNA (final concentration 100 μg/ml), and acetylated BSA (final concentration 100 μg/ml) in hybridization buffer (100 mM MES, 1 M [Na^+], 20 mM EDTA, 0.01% Tween 20). The hybridization mixture was heated at 99 °C for five min, incubated at 45 °C for five min, and centrifuged at 13,000x g for 5 min. Test 3 chips were pre-hybridized with 80 μl of 1X hybridization buffer for 10 min at 45 °C and 60 RPM in the hybridization oven. Following removal of the pre-hybridization buffer, the 6 Affymetrix chips were filled with 200 μl of the hybridization mixture and incubated at 45 °C and 60 RPM for 16 h [26,27].

Hybridization mixture was removed and saved, and each chip was filled with 250 μl of non-stringent wash buffer (6X SSPE, 0.01% Tween 20). Further washing and staining of the chips was conducted on the fluidics station with non-stringent washing buffer, stringent washing buffer (100 mM MES, 0.1 M [Na^+], 0.01% Tween 20), and stain buffer (100 mM MES, 1 M [Na^+], 0.05% Tween 20) containing 10 μg /ml of streptavidin phycoerythrin (SAPE, Molecular Probes, Inc., Eugene, OR). The signal was amplified by an additional treatment with goat IgG (0.1 mg/ml), biotinylated antibody (3 μg/ml) and a second staining with SAPE. The chips were then scanned and the data analyzed using the Affymetrix Microarray Analysis Suite software as described below.

Scanning, data collection, and analysis: Following staining and washing, each chip was scanned twice at 570 nm with a confocal scanner (Agilent Technologies, Inc., Palo Alto, CA). The output fluorescence was collected using the Affymetrix Microarray Analysis Suite 5.0 software and the average for the two scans yielded an image file used for further data analysis.

The Microarray Analysis Suite software performs absolute analysis of the data from each chip, including background calculation, detection call (present, absent, or marginal) and signal, which serves as a measure of relative expression level for the various genes. Several metrics are used in these calculations, including the intensities of each probe pair (perfect match and mismatch) in a probe set (the 11-20 pairs representing each gene) and a decision matrix is formed from which the absolute call is made. The software can also perform comparative analysis between any two chips, normalizing signals, calculating more metrics and using them to make a difference

call (increased, decreased, marginally increased, marginally decreased, no change), to generate an average difference change between the same genes (i.e., probe sets) on the different chips, and to provide a fold change calculation about the magnitude of any expression difference measured.

The identified expressed genes with present calls for all 6 samples were compared with the following NCBI databases: GenBank, On-Line Mendelian Inheritance in Man (OMIM), and PubMed.

Reverse transcription-polymerase chain reaction: Total RNA was extracted from 4 pooled, human donor sclera using TRIZOL reagent as described above. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed with oligo-dT oligonucleotide primer using standard methods to synthesize cDNA with SuperScript II (Invitrogen Corporation, Carlsbad, CA). Total RNA from sclera (1 μg) or eye tissues such as optic nerve, retina, and cornea, as well as commercially prepared poly-A RNA from various human organs (Clontech Inc., Pal Alto, CA) were used as a template for first-strand cDNA synthesis. PCR was performed using Platinum Taq polymerase using 2 μl of each cDNA sample in a final reaction volume of 50 μl. A final concentration of 2.5 μM was used for each PCR primer. The PCR cycling conditions included an initial denaturation for 120 s at 95 °C, followed by 34 cycles of denaturation for 15 s at 95 °C, annealing for 30 s at 54 °C, extension for 45 s at 68 °C, and a final extension for 4 min at 68 °C. The 5' sense and 3' antisense PCR primer pairs designed for collagens 6A3 and 10A1, thrombospondins 2 and 4, dystroglycan, biglycan, and decorin are listed in Table 1, along with their corresponding amplicon size. The RT-PCR products, along with the amplicon products of the housekeeping gene β-actin as a control were visualized on 2% agarose gels after electrophoresis and staining with ethidium bromide.

Table 1. Primer pair sequence of select expressed scleral genes for RT-PCR

Primer name	Gene	Sequence	Primer size	Product size
COL6A3-F	Collagen 6A3	AGGAGAAATTACTGCCGTACA	21	390
COL6A3-R	Collagen 6A3	CGGCTGAACTCGTGAATAGGT	21	390
COL10A1-F	Collagen 10A1	CCCAACACCAAGACACAGTTC	21	462
COL10A1-R	Collagen 10A1	CCCTTTCTGTCCATTCATAC	20	462
THBS2-F	Thrombospondin 2	AGGACGGGATTGGCGATG	18	453
THBS2-R	Thrombospondin 2	GGTTGGCGTTGGAGATGTAGG	21	453
THBS4-F	Thrombospondin 4	CCAGGTGGATTCCGTTCACAA	21	568
THBS4-R	Thrombospondin 4	CGGGTAGCAGGGATGGTATT	20	568
DSGN-F	Dystroglycan	CTGCCTGTGCTGCGGATGAAC	21	374
DSGN-R	Dystroglycan	GGGCTTCTTATTGGCGATGTG	21	374
LUM-R	Lumican	CTCTTTCAGCCGATTGT	17	393
DCN-F	Decorin	AACCTTCACGCATTGAT	17	440
DCN-R	Decorin	AATCCCAACTTAGCCA	16	440
BGN-F	Biglycan	AACTACCTGCGCATCTC	17	436
BGN-R	Biglycan	TGACGCAGCGGAAAG	15	436
DCN-F	Decorin	AACCTTCACGCATTGAT	17	440
DCN-R	Decorin	AATCCCAACTTAGCCA	16	440

The 5' sense and 3' antisense polymerase chain reaction primer pairs designed for collagens 6A3 and 10A1, thrombospondins 2 and 4, dystroglycan, biglycan, and decorin with their corresponding amplicon sizes. In the first column, F indicates the forward primer and R indicates the reverse primer.

RESULTS

There were 3,751 genes with "present" calls assigned independently to all six human scleral samples. Only the 3,751 genes confirmed by all 6 microarrays as expressed in sclera were used in subsequent analyses in this study. These genes could be clustered into 4 major categories; transcription (10%), metabolism (8.8%), cell growth and proliferation (5.4%), and extracellular matrix (2%). The 3,751 genes with confirmed scleral expression were analyzed using GenBank and LocusLink at NCBI. Of the 3,751 genes, 3,096 (82.5%) had assigned chromosomal loci. Six hundred fifty-five confirmed scleral genes (17.5%) had unassigned chromosomal loci.

The 3,751 genes detected with this microarray analysis form the basis of a scleral genetics sub-web site named ScleraNet at our research laboratory web site. The raw data for this study can also be found in Appendix 1. ScleraNet includes the 3,751 genes identified in the present study in addition to genes identified from the literature (sclera or scleral fibroblast cell lines), and non-overlapping genes identified from a human scleral cDNA library developed previously in our laboratory [28].

Table 2 displays all extracellular matrix genes identified by microarray analysis, along with their chromosomal locus and GenBank accession numbers. The primary structural proteins which sclera is comprised of are the collagens. As expected, a variety of collagen genes were expressed in all samples. These include COL1A2, COL2A1, COL3A1, COL4A1-4,6, COL5A1-3, COL6A1-3, COL7A1, COL8A1-2, COL9A1-3, COL10A1, COL11A1-2, COL12A1, COL13A1, COL14A1, COL15A1, COL18A1, and COL19A1. Many of these extracellular matrix proteins, such as collagens 6A3 and 10A1, have not been shown to be expressed in sclera previously. Interestingly, there was minimal overlap with collagens expressed in the cornea, where only 6 collagens (COL4A1, 11A1, 16A1, 5A2, 4A3, and 6A3) were detected by similar microarray analysis [20].

Even though the scleral samples were from adult human eyes presumptively no longer in a growth phase, evidence of continued remodeling is supported by the detection of expressed regulators of collagen metabolism. Secreted proteases are known to play a major role in remodeling the stromal extracellular matrix [29,30]. Multiple human metalloproteinase proteins (MMP) were detected (MMP 1-3, 7-17, 20, and 24). These are proteins originally identified in macrophages [31]. MMP 12, for example, has multiple extracellular matrix substrates and disrupts basement membranes. Manganese superoxide dismutase and leukotriene C4 synthase were detected, possibly indicating regulated scleral degradation by MMPs based on other scleral studies [32,33].

Secreted proteases are in turn regulated by various inhibitors. These have been shown to help control the degradation of the extracellular matrix [34,35]. Three tissue inhibitor metalloproteinases were detected (TIMPs 1, 2, and 3). The tissue inhibitors of metalloproteinases (TIMPs) block matrix metalloproteinase (MMP)-mediated increases in cell proliferation, migration, and invasion that are associated with ex-

Table 2. Extracellular matrix genes identified by microarray analysis

GenBank accession number	Extracellular matrix description	Symbol	Cytogenetic position
NM_001135	aggrecan 1 (chondroitin sulfate proteoglycan 1, large aggregating proteoglycan, antigen identified by monoclonal antibody A0122)	AGC1	15q26.1
NM_001135	NM_001723, 230/240 kDa	BPAG1	6p12-p11
NM_001728	basigin (OK blood group)	BSG	19p13.3
NM_000610	CD44 antigen (homing function and Indian blood group system)	CD44	11p13
NM_000493	collagen, type X, alpha 1(Schmid metaphyseal chondrodysplasia)	COL10A1	6q21-q22
NM_001854	collagen, type XI, alpha 1	COL11A1	1p21
NM_001855	collagen, type XV, alpha 1	COL15A1	9q21-q22
NM_000089	collagen, type I, alpha 2	COL1A2	7q22.1
NM_000090	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	COL3A1	2q31
NM_001846	collagen, type IV, alpha 2	COL4A2	13q34
NM_000393	collagen, type V, alpha 2	COL5A2	2q14-q32
NM_001848	collagen, type VI, alpha 1	COL6A1	21q22.3
NM_001849	collagen, type VI, alpha 2	COL6A2	21q22.3
NM_004369	collagen, type VI, alpha 3	COL6A3	2q37
NM_001850	collagen, type VIII, alpha 1	COL8A1	3q12-q13.1
NM_001853	collagen, type IX, alpha 3	COL9A3	20q13.3
NM_000095	cartilage oligomeric matrix protein (pseudoachondroplasia, epiphyseal dysplasia 1, multiple)	COMP	19p13.1
NM_004385	chondroitin sulfate proteoglycan 2 (versican)	CSPG2	5q14.3
NM_001901	connective tissue growth factor	CTGF	6q23.1
NM_004393	dystroglycan 1 (dystrophin-associated glycoprotein 1)	DAG1	3p21
NM_004425	extracellular matrix protein 1	ECM1	1q21
NM_001393	extracellular matrix protein 2, female organ and adipocyte specific	ECM2	9q22.3
NM_004105	EGF-containing fibulin-like extracellular matrix protein 1	EFEMP1	2p16
NM_016938	EGF-containing fibulin-like extracellular matrix protein 2	EFEMP2	11q13
NM_006487	fibulin 1	FBLN1	22q13.31
NM_001998	fibulin 2	FBLN2	3p25-p24
NM_006329	fibulin 5	FBLN5	14q32.1
NM_000138	fibrillin 1 (Marfan syndrome)	FBN1	15q21.1
NM_002023	fibromodulin	FMOD	1q32
NM_002026	fibronectin 1	FN1	2q34
NM_002081	glypican 1	GPC1	2q35-q37
NM_004484	glypican 3	GPC3	Xq26.1
NM_000426	laminin, alpha 2 (merosin, congenital muscular dystrophy)	LAMA2	6q22-q23
NM_000227	laminin, alpha 3	LAMA3	18q11.2
NM_002290	laminin, alpha 4	LAMA4	6q21
NM_005560	laminin, alpha 5	LAMA5	20q13.2-q13.3
NM_002291	laminin, beta 1	LAMB1	7q22
NM_002292	laminin, beta 2 (laminin S)	LAMB2	3p21
NM_002293	laminin, gamma 1 (formerly LAMB2)	LAMC1	1q31
NM_000428	latent transforming growth factor beta binding protein 2	LTBP2	14q24
NM_003573	latent transforming growth factor beta binding protein 4	LTBP4	19q13.1-q13.2
NM_002345	lumican	LUM	12q21.3-q22
NM_005926	microfibrillar-associated protein 1	MFAP1	15q15-q21
NM_002404	microfibrillar-associated protein 4	MFAP4	17p11.2
NM_012215	meningioma expressed antigen 5 (hyaluronidase)	MGEA5	10q24.1-q24.3
NM_000900	matrix Gla protein	MGP	12p13.1-p12.3
NM_004530	matrix metalloproteinase 2 (gelatinase A, 72 kDa gelatinase, 72 kDa type IV collagenase)	MMP2	16q13-q21
NM_002422	matrix metalloproteinase 3 (stromelysin 1, progelatinase)	MMP3	11q22.3
NM_002508	nidogen (enactin)	NID	1q43
NM_007361	nidogen 2 (osteonidogen)	NID2	14q21-q22
NM_000917	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I	P4HA1	10q21.3-q23.1
NM_004199	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide II	P4HA2	5q31
NM_000918	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), beta polypeptide (protein disulfide isomerase; thyroid hormone binding protein p55)	P4HB	17q25
NM_002593	procollagen C-endopeptidase enhancer	PCOLCE	7q22
NM_000302	procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase, Ehlers-Danlos syndrome type VI)	PLOD	1p36.3-p36.2
NM_002725	proline arginine-rich end leucine-rich repeat protein	PRELP	1q32
NM_004587	ribosome binding protein 1 homolog 180 kDa (dog)	RRBP1	20p12
NM_002639	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5	SERPINB5	18q21.3
NM_000112	solute carrier family 26 (sulfate transporter), member 2	SLC26A2	5q31-q34
NM_003102	superoxide dismutase 3, extracellular	SOD3	4p16.3-q21
NM_003118	secreted protein, acidic, cysteine-rich (osteonectin)	SPARC	5q31.3-q32
NM_000582	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	SPP1	4q21-q25
NM_003247	thrombospondin 2	THBS2	6q27
NM_003248	thrombospondin 4	THBS4	5q13
NM_003254	tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor)	TIMP1	Xp11.3-p11.23
NM_000362	tissue inhibitor of metalloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory)	TIMP3	22q12.3
NM_003278	tetranectin (plasminogen binding protein)	TNA	3p22-p21.3
NM_002160	tenascin C (hexabrachion)	TNC	9q33

Extracellular matrix genes identified by microarray analysis with present calls in all 6 samples including GenBank Accession number, description of the expressed extracellular matrix gene, gene symbol, and published cytogenetic position.

TABLE 3. TRANSCRIPTION FACTOR GENES IDENTIFIED BY MICROARRAY ANALYSIS

GenBank accession number	Transcription factor description	Gene symbol	Cytogenetic position
NM_012138	apoptosis antagonizing transcription factor	AATF	17q11.2-q12
NM_001129	AE binding protein 1	AEBP1	7p13
NM_001621	aryl hydrocarbon receptor	AHR	7p15
NM_006885	AT-binding transcription factor 1	ATBF1	16q22.3-q23.1
NM_001675	activating transcription factor 4 (tax-responsive enhancer element B67)	ATF4	22q13.1
NM_000489	alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae)	ATRX	Xq13.1-q21.1
NM_001186	BTB and CNC homology 1, basic leucine zipper transcription factor 1	BACH1	21q22.11
NM_023005	bromodomain adjacent to zinc finger domain, 1B	BAZ1B	7q11.23
NM_013449	bromodomain adjacent to zinc finger domain, 2A	BAZ2A	12q24.3-qter
NM_001706	B-cell CLL/lymphoma 6 (zinc finger protein 51)	BCL6	3q27
NM_003670	basic helix-loop-helix domain containing, class B, 2	BHLHB2	3p26
NM_006624	adenovirus 5 E1A binding protein	BS69	10p14
NM_001206	basic transcription element binding protein 1	BTEB1	9q13
NM_014739	Bcl-2-associated transcription factor	BTF	6q22-q23
NM_001207	basic transcription factor 3	BTF3	5q13.1
NM_006763	BTG family, member 2	BTG2	1q32
NM_003203	chromosome 2 open reading frame 3	C2orf3	2p11.2-p11.1
NM_016604	chromosome 5 open reading frame 7	C5orf7	5q31
NM_005760	CCAAT-box-binding transcription factor	CBF2	2p23.1
NM_004349	core-binding factor, runt domain, alpha subunit 2; translocated to, 1; cyclin D-related	CBFA2T1	8q22
NM_022845	core-binding factor, beta subunit	CBFB	16q22.1
NM_005194	CCAAT/enhancer binding protein (C/EBP), beta	CEBPB	20q13.1
NM_005195	CCAAT/enhancer binding protein (C/EBP), delta	CEBPD	8p11.2-p11.1
NM_001806	CCAAT/enhancer binding protein (C/EBP), gamma	CEBPG	19q13.11
NM_005197	checkpoint suppressor 1	CHES1	14q24.3-q31
NM_004882	CBF1 interacting corepressor	CIR	2q31.1
NM_006079	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	CITED2	6q23.3
NM_004898	clock homolog (mouse)	CLOCK	4q12
NM_006837	COP9 constitutive photomorphogenic homolog subunit 5 (Arabidopsis)	COPS5	8q12.3
NM_004379	cAMP responsive element binding protein 1	CREB1	2q34
NM_006368	cAMP responsive element binding protein 3 (luman)	CREB3	9pter-p22.1
NM_004380	CREB binding protein (Rubinstein-Taybi syndrome)	CREBBP	16p13.3
NM_001310	cAMP responsive element binding protein-like 2	CREBL2	12p13
NM_003851	cellular repressor of E1A-stimulated genes	CREG	1q24
NM_001881	cAMP responsive element modulator	CREM	10p12.1-p11.1
NM_004270	cofactor required for Sp1 transcriptional activation, subunit 9, 33 kDa	CRSP9	5q33.3
NM_003651	cold shock domain protein A	CSDA	12p13.1
NM_006565	CCCTC-binding factor (zinc finger protein)	CTCF	16q21-q22.3
NM_001904	catenin (cadherin-associated protein), beta 1, 88 kDa	CTNNB1	3p21
NM_001913	cut-like 1, CCAAT displacement protein (Drosophila)	CUTL1	7q22
NM_022105	death associated transcription factor 1	DATF1	20q13.33
NM_021008	deformed epidermal autoregulatory factor 1 (Drosophila)	DEAF1	11p15.5
NM_032998	death effector domain containing	DEDD	1q23.1
NM_003472	DEK oncogene (DNA binding)	DEK	6p23
NM_021145	cyclin D binding myb-like transcription factor 1	DMTF1	7q21
NM_001938	down-regulator of transcription 1, TBP-binding (negative cofactor 2)	DR1	1p22.1
NM_004147	developmentally regulated GTP binding protein 1	DRG1	22q12.2
NM_004414	Down syndrome critical region gene 1	DSCR1	21q22.12
NM_004089	delta sleep inducing peptide, immunoreactor	DSIPI	Xq22.3
NM_172373	E74-like factor 1 (ets domain transcription factor)	ELF1	13q13
NM_006874	E74-like factor 2 (ets domain transcription factor)	ELF2	4q28
NM_001421	E74-like factor 4 (ets domain transcription factor)	ELF4	Xq26
NM_001428	enolase 1, (alpha)	ENO1	1p36.3-p36.2
NM_006209	ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	ENPP2	8q24.1

TABLE 3. CONTINUED.

GenBank accession number	Transcription factor description	Gene symbol	Cytogenetic position
NM_000122	excision repair cross-complementing rodent repair deficiency, complementation group 3 (xeroderma pigmentosum group B complementing)	ERCC3	2q21
NM_006494	Ets2 repressor factor	ERF	19q13
NM_004907	immediate early protein	ETR101	19p13.12
NM_005239	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	ETS2	21q22.2
NM_004454	ets variant gene 5 (ets-related molecule)	ETV5	3q28
NM_004459	fetal Alzheimer antigen	FALZ	17q24
NM_018416	FOXJ2 forkhead factor	FHX	12p13.31
NM_005252	v-fos FBJ murine osteosarcoma viral oncogene homolog	FOS	14q24.3
NM_006732	FBJ murine osteosarcoma viral oncogene homolog B	FOSB	19q13.32
NM_005253	FOS-like antigen 2	FOSL2	2p23-p22
NM_001452	forkhead box F2	FOXF2	6p25.3
NM_021953	forkhead box M1	FOXM1	12p13
NM_002015	forkhead box O1A (rhabdomyosarcoma)	FOXO1A	13q14.1
NM_001455	forkhead box O3A	FOXO3A	6q21
NM_003902	far upstream element (FUSE) binding protein 1	FUBP1	1p31.1
XM_033327	far upstream element (FUSE) binding protein 3	FUBP3	9q34.2
NM_003910	maternal G10 transcript	G10	7q22.1
NM_003644	growth arrest-specific 7	GAS7	17p
NM_002051	GATA binding protein 3	GATA3	10p15
NM_004492	general transcription factor IIA, 2, 12 kDa	GTF2A2	15q21.3
NM_001514	general transcription factor IIB	GTF2B	1p22-p21
NM_002095	general transcription factor IIE, polypeptide 2, beta 34 kDa	GTF2E2	8p21-p12
NM_002096	general transcription factor IIF, polypeptide 1, 74 kDa	GTF2F1	19p13.3
NM_005316	general transcription factor IIH, polypeptide 1, 62 kDa	GTF2H1	11p15.1-p14
NM_032999	general transcription factor II, i	GTF2I	7q11.23
NM_002097	general transcription factor IIIA	GTF3A	13q12.3-q13.1
NM_001520	general transcription factor IIIC, polypeptide 1, alpha 220 kDa	GTF3C1	16p12
NM_001521	general transcription factor IIIC, polypeptide 2, beta 110 kDa	GTF3C2	2p23.3
NM_007067	histone acetyltransferase	HBOA	17q21.32
NM_005334	host cell factor C1 (VP16-accessory protein)	HCFC1	Xq28
NM_005535	hematopoietic cell-specific Lyn substrate 1	HCLS1	3q13
NM_004964	histone deacetylase 1	HDAC1	1p34
HDAC2	histone deacetylase 2	HDAC2	6q21
NM_001530	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	HIF1A	14q21-q24
NM_002126	hepatic leukemia factor	HLF	17q22
NM_002129	high-mobility group box 2	HMGB2	4q31
NM_004965	high-mobility group nucleosome binding domain 1	HMGN1	21q22.2
AF264785	hairy homolog (Drosophila)	HRY	3q28-q29
NM_001537	heat shock factor binding protein 1	HSBP1	16q23.3
NM_004506	heat shock transcription factor 2	HSF2	6q22.32
NM_001538	heat shock transcription factor 4	HSF4	16q21
NM_014500	HIV TAT specific factor 1	HTATSF1	Xq26.1-q27.2
NM_005533	interferon-induced protein 35	IFI35	17q21
NM_004514	interleukin enhancer binding factor 1	ILF1	17q25
NM_004515	interleukin enhancer binding factor 2, 45 kDa	ILF2	1q21.3
NM_004516	interleukin enhancer binding factor 3, 90 kDa	ILF3	19p13.2
NM_006084	interferon-stimulated transcription factor 3, gamma 48 kDa	ISGF3G	14q11.2
NM_004973	jumonji homolog (mouse)	JMJ	6p24-p23
AY217548	v-jun sarcoma virus 17 oncogene homolog (avian)	JUN	1p32-p31
NM_002229	jun B proto-oncogene	JUNB	19p13.2
NM_005354	jun D proto-oncogene	JUND	19p13.2
NM_015874	H-2K binding factor-2	KBF2	9
XM_043272	KIAA0346 protein	KIAA0346	17p13.1
NM_014947	KIAA1041 protein	KIAA1041	1pter-q31.3
XM_171233	KIAA1111 protein	KIAA1111	Xp11.22
NM_006769	LIM domain only 4	LMO4	1p22.3
NM_004735	leucine rich repeat (in FLII) interacting protein 1	LRRFIP1	2q37.3
NM_007358	likely ortholog of mouse metal response element binding transcription factor 2	M96	1p22.1
NM_005902	MAD, mothers against decapentaplegic homolog 3 (Drosophila)	MADH3	15q21-q22
NM_005359	MAD, mothers against decapentaplegic homolog 4 (Drosophila)	MADH4	18q21.1
NM_005360	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)	MAF	16q22-q23

TABLE 3. CONTINUED.

GenBank accession number	Transcription factor description	Gene symbol	Cytogenetic position
NM_012323	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	MAFF	22q13.1
NM_002383	MYC-associated zinc finger protein (purine-binding transcription factor)	MAZ	16p11.2
NM_015846	methyl-CpG binding domain protein 1	MBD1	18q21
NM_003791	membrane-bound transcription factor protease, site 1	MBTPS1	16q24
NM_005466	mediator of RNA polymerase II transcription, subunit 6 homolog (yeast)	MED6	14q24.1
NM_005587	MADS box transcription enhancer factor 2, polypeptide A (myocyte enhancer factor 2A)	MEF2A	15q26
NM_005924	mesenchyme homeo box 2 (growth arrest-specific homeo box)	MEOX2	7p22.1-p21.3
NM_032638	hypothetical protein MGC2306	MGC2306	3q21.3
NM_033290	midline 1 (Opitz/BBB syndrome)	MID1	Xp22
NM_000248	microphthalmia-associated transcription factor	MITF	3p14.1-p12.3
NM_005935	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 2	MLLT2	4q21
NM_020310	MAX binding protein	MNT	17p13.3
NM_012330	monocytic leukemia zinc finger protein-related factor	MORF	10q22.2
NM_005098	musculin (activated B-cell factor-1)	MSC	8q21
NM_078629	male-specific lethal 3-like 1 (Drosophila)	MSL3L1	Xp22.3
NM_002448	msh homeo box homolog 1 (Drosophila)	MSX1	4p16.3-p16.1
NM_005955	metal-regulatory transcription factor 1	MTF1	1p33
NM_002382	MAX interacting protein 1	MXI1	10q24-q25
NM_002467	v-myc myelocytomatosis viral oncogene homolog (avian)	MYC	8q24.12-q24.13
NM_173165	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3	NFATC3	16q22.2
NM_003204	nuclear factor (erythroid-derived 2)-like 1	NFE2L1	17q21.3
NM_006164	nuclear factor (erythroid-derived 2)-like 2	NFE2L2	2q31
NM_005596	nuclear factor I/B	NFIB	9p24.1
NM_005384	nuclear factor, interleukin 3 regulated	NFIL3	9q22
NM_003998	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)	NFKB1	4q24
NM_020529	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	NFKBIA	14q13
NM_006165	nuclear factor related to kappa B binding protein	NFRKB	11q24-q25
NM_006166	nuclear transcription factor Y, beta	NFYB	12q22-q23
NM_014223	nuclear transcription factor Y, gamma	NFYC	1p32
NM_002518	neuronal PAS domain protein 2	NPAS2	2q11.2
NM_003297	nuclear receptor subfamily 2, group C, member 1	NR2C1	12q21.32-q21.33
NM_021005	nuclear receptor subfamily 2, group F, member 2	NR2F2	15q26
NM_000176	nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	NR3C1	5q31
NM_000901	nuclear receptor subfamily 3, group C, member 2	NR3C2	4q31.1
NM_002135	nuclear receptor subfamily 4, group A, member 1	NR4A1	12q13
XM_009699	nuclear receptor interacting protein 1	NRIP1	21q11.2
NM_006469	NS1-binding protein	NS1-BP	1q25.1-q31.1
NM_004559	nuclease sensitive element binding protein 1	NSEP1	1p34
NM_015069	OLF-1/EBF associated zinc finger gene	OAZ	16q12
NM_001604	paired box gene 6 (aniridia, keratitis)	PAX6	11p13
NM_003466	paired box gene 8	PAX8	2q12-q14
NM_006195	pre-B-cell leukemia transcription factor 3	PBX3	9q33-q34
NM_006713	activated RNA polymerase II transcription cofactor 4	PC4	5p13.3
L41559	6-pyruvoyl-tetrahydropterin synthase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1)	PCBD	10q22
NM_002622	prefoldin 1	PFDN1	5q31
NM_002636	PHD finger protein 1	PHF1	6p21.3
NM_005392	PHD finger protein 2	PHF2	9q22.31
NM_015153	PHD finger protein 3	PHF3	
AF010312	LPS-induced TNF-alpha factor	PIG7	16p13.3-p12
NM_002650	phosphatidylinositol 4-kinase, catalytic, alpha polypeptide	PIK4CA	22q11.21
NM_002653	paired-like homeodomain transcription factor 1	PITX1	5q31
NM_153427	paired-like homeodomain transcription factor 2	PITX2	4q25-q27

TABLE 3. CONTINUED.

GenBank accession number	Transcription factor description	Gene symbol	Cytogenetic position
NM_002656	pleiomorphic adenoma gene-like 1	PLAGL1	6q24-q25
NM_006618	putative DNA/chromatin binding motif	PLU-1	1q32.1
NM_007221	polyamine-modulated factor 1	PMF1	1q12
NM_033238	promyelocytic leukemia	PML	15q22
NM_017443	polymerase (DNA directed), epsilon 3 (p17 subunit)	POLE3	9q33
NM_006233	polymerase (RNA) II (DNA directed) polypeptide I, 14.5 kDa	POLR2I	19q12
NM_006237	POU domain, class 4, transcription factor 1	POU4F1	13q21.1-q22
NM_002714	protein phosphatase 1, regulatory subunit 10	PPP1R10	6p21.3
NM_005710	polyglutamine binding protein 1	PQBP1	Xp11.23
NM_002804	proteasome (prosome, macropain) 26S subunit, ATPase, 3	PSMC3	11p12-p13
NM_015167	phosphatidylserine receptor	PTDSR	17q25
NM_005859	purine-rich element binding protein A	PURA	5q31
NM_000965	retinoic acid receptor, beta	RARB	3p24
NM_000321	retinoblastoma 1 (including osteosarcoma)	RB1	13q14.2
NM_005056	retinoblastoma binding protein 2	RBBP2	12p11
NM_002908	v-rel reticuloendotheliosis viral oncogene homolog (avian)	REL	2p13-p12
NM_021975	v-rel reticuloendotheliosis viral oncogene homolog A, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3, p65 (avian)	RELA	11q13
NM_002938	ring finger protein 4	RNF4	4p16.3
NM_002957	retinoid X receptor, alpha	RXRA	9q34.3
NM_021976	retinoid X receptor, beta	RXRB	6p21.3
NM_012234	RING1 and YY1 binding protein	RYBP	3p13
NM_006089	sex comb on midleg-like 2 (Drosophila)	SCML2	Xp22
NM_004630	splicing factor 1	SF1	11q13
NM_000451	short stature homeobox	SHOX	Xpter-p22.32
NM_006930	S-phase kinase-associated protein 1A (p19A)	SKP1A	5q31
NM_014267	skeletal muscle abundant protein	SMAP	5q31
NM_003071	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 3	SMARCA3	3q25.1-q26.1
NM_003072	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	SMARCA4	19p13.13
NM_003601	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5	SMARCA5	4q31.1-q31.2
NM_003073	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1	SMARCB1	22q11.23
NM_003074	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 1	SMARCC1	3p23-p21
NM_003075	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2	SMARCC2	12q13-q14
NM_003077	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 2	SMARCD2	17q23-q24
NM_004187	Smcx homolog, X chromosome (mouse)	SMCX	Xp11.22-p11.21
NM_006049	small nuclear RNA activating complex, polypeptide 5, 19 kDa	SNAPC5	15q22.2
NM_006941	SRY (sex determining region Y)-box 10	SOX10	22q13.1
NM_006942	SRY (sex determining region Y)-box 15	SOX15	17p13
NM_003107	SRY (sex determining region Y)-box 4	SOX4	6p22.3
NM_000346	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	SOX9	17q24.3-q25.1
XM_092672	Sp3 transcription factor	SP3	2q31
NM_004176	sterol regulatory element binding transcription factor 1	SREBF1	17p11.2
NM_003131	serum response factor (c-fos serum response element-binding transcription factor)	SRF	6p21.1
NM_007315	signal transducer and activator of transcription 1, 91 kDa	STAT1	2q32.2
NM_005419	signal transducer and activator of transcription 1, 91 kDa	STAT2	2q32.3
NM_139276	signal transducer and activator of transcription 3 (acute-phase response factor)	STAT3	17q21
NM_003152	signal transducer and activator of transcription 5A	STAT5A	17q11.2
NM_003153	signal transducer and activator of transcription 6, interleukin-4 induced	STAT6	12q13
NM_003168	suppressor of Ty 4 homolog 1 (S. cerevisiae)	SUPT4H1	17q21-q23

TABLE 3. CONTINUED.

GenBank accession number	Transcription factor description	Gene symbol	Cytogenetic position
NM_003169	suppressor of Ty 5 homolog (S. cerevisiae)	SUPT5H	19q13
NM_003170	suppressor of Ty 6 homolog (S. cerevisiae)	SUPT6H	17q11.2
NM_004264	SRB7 suppressor of RNA polymerase B homolog (yeast)	SURB7	12p11.23
NM_006354	transcriptional adaptor 3-like	TADA3L	3p25.3
NM_006284	TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 30 kDa	TAF10	11p15.3
NM_005644	TAF12 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 20 kDa	TAF12	1p35.2
NM_139215	TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 68 kDa	TAF15	17q11.1-q11.2
NM_005679	TATA box binding protein (TBP)-associated factor, RNA polymerase I, C, 110 kDa	TAF1C	16q24
NM_003184	TAF2 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 150 kDa	TAF2	8q24.12
NM_005642	TAF7 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 55 kDa	TAF7	5q31
NM_003187	TAF9 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 32 kDa	TAF9	5q11.2-q13.1
NM_007375	TAR DNA binding protein	TARDBP	1p36.21
NM_005149	T-box 19	TBX19	1q23-q24
NM_006756	transcription elongation factor A (SII), 1	TCEA1	3p22-p21.3
NM_004780	transcription elongation factor A (SII)-like 1	TCEAL1	Xq22.1
NM_005648	transcription elongation factor B (SIII), polypeptide 1 (15 kDa, elongin C)	TCEB1	8q13.3
NM_006706	transcription elongation regulator 1 (CA150)	TCERG1	5q31
NM_003205	transcription factor 12 (HTF4, helix-loop-helix transcription factors 4)	TCF12	15q21
NM_003200	transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)	TCF3	19p13.3
NM_030751	transcription factor 8 (represses interleukin 2 expression)	TCF8	10p11.2
NM_005997	transcription factor-like 1	TCFL1	1q21
NM_170608	transcription factor-like 4	TCFL4	17q21.1
NM_006602	transcription factor-like 5 (basic helix-loop-helix)	TCFL5	20q13.3-qter
NM_020755	likely ortholog of mouse tumor differentially expressed 1, like	TDE1L	6q22.32
NM_003214	TEA domain family member 3	TEAD3	6p21.2
NM_003220	transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)	TFAP2A	6p24
NM_003223	transcription factor AP-4 (activating enhancer binding protein 4)	TFAP4	16p13
NM_007111	transcription factor Dp-1	TFDP1	13q34
NM_006521	transcription factor binding to IGHM enhancer 3	TFE3	Xp11.22
NM_170695	TGFB-induced factor (TALE family homeobox)	TGIF	18p11.3
NM_003250	thyroid hormone receptor, alpha (erythroblastic leukemia viral (v-erb-a) oncogene homolog, avian)	THRA	17q11.2
NM_003252	TIA1 cytotoxic granule-associated RNA binding protein-like 1	TIAL1	10q
NM_005655	TGFB inducible early growth response	TIEG	8q22.2
NM_001068	topoisomerase (DNA) II beta 180 kDa	TOP2B	3p24
TP63	tumor protein p63	TP63	3q27-q29
NM_006470	tripartite motif-containing 16	TRIM16	17p11.2
NM_006074	tripartite motif-containing 22	TRIM22	11p15
NM_012101	tripartite motif-containing 29	TRIM29	11q22-q23
NM_015906	tripartite motif-containing 33	TRIM33	1p13.1
NM_006022	transforming growth factor beta-stimulated protein TSC-22	TSC22	13q14
NM_005726	Ts translation elongation factor, mitochondrial	TSFM	12q13-q14
NM_018433	zinc finger protein	TSGA	2p11.2
BC036704	twist homolog (acrocephalosyndactyly 3; Saethre-Chotzen syndrome; Drosophila)	TWIST	7p21.2
NM_016936	ubinuclein 1	UBN1	16p13.3
NM_014233	upstream binding transcription factor, RNA polymerase I	UBTF	17q21.3
NM_000376	vitamin D (1,25-dihydroxyvitamin D3) receptor	VDR	12q12-q14
NM_005080	X-box binding protein 1	XBP1	22q12.1
NM_005748	YY1 associated factor 2	YAF2	12q12
NM_003403	YY1 transcription factor	YY1	14q
NM_004926	zinc finger protein 36, C3H type-like 1	ZFP36L1	14q22-q24
NM_006887	zinc finger protein 36, C3H type-like 2	ZFP36L2	2p22.3-p21

TABLE 3. CONTINUED.

GenBank accession number	Transcription factor description	Gene symbol	Cytogenetic position
NM_003434	zinc finger protein 133 (clone pHZ-13)	ZNF133	20p11.23-20p11.22
NM_007144	zinc finger protein 144 (Mel-18)	ZNF144	17q21.1
NM_006006	zinc finger protein 145 (Kruppel-like, expressed in promyelocytic leukemia)	ZNF145	11q23.1
NM_021964	zinc finger protein 148 (pHZ-52)	ZNF148	3q21
NM_007146	zinc finger protein 161	ZNF161	17q23.2
NM_007147	zinc finger protein 175	ZNF175	19q13.4
NM_003452	zinc finger protein 189	ZNF189	9q22-q31
NM_006299	zinc finger protein 193	ZNF193	6p21.3
NM_003457	zinc finger protein 207	ZNF207	17q11.2
NM_006526	zinc finger protein 217	ZNF217	20q13.2
NM_006352	zinc finger protein 238	ZNF238	1q44-qter
NM_014323	zinc finger protein 278	ZNF278	22q12.2
NM_024493	zinc finger protein 306	ZNF306	6p21.33
NM_133476	zinc finger protein 384	ZNF384	
NM_003422	zinc finger protein 42 (myeloid-specific retinoic acid-responsive)	ZNF42	19q13.2-q13.4
NM_003418	zinc finger protein 9 (a cellular retroviral nucleic acid binding protein)	ZNF9	3q21

A list of transcription factors expressed in human sclera that showed present calls in all 6 samples including GenBank Accession number, description of the expressed transcription factor gene, gene symbol, and published cytogenetic position.

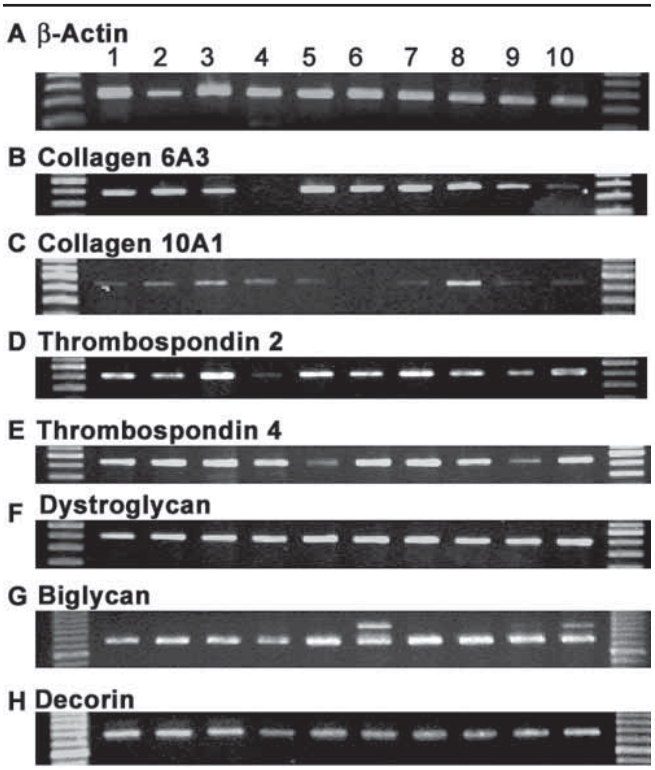

Figure 1. Confirmed scleral expression of genes tested by reverse transcription-polymerase chain reaction. The expressed genes tested were β-actin (control, **A**), collagens 6A3 (**B**) and 10A1 (**C**), thrombospondins 2 (**D**) and 4 (**E**), dystroglycan (**F**), biglycan (**G**), and decorin (**H**). The PCR amplicons were derived from cDNA from reverse-transcribed RNA of human ocular tissues and are commercially available as RNA from various human tissue types (column 1 is sclera, column 2 is cornea, column 3 is optic nerve, column 4 is retina, column 5 is lung, column 6 is skeletal muscle, column 7 is heart, column 8 is trachea, column 9 is kidney, and column 10 is brain).

Table 4. Genes expressed in human donor sclera that map to chromosome loci with defined intervals

Accession number	Name	Gene	Cytogenetic position
NM_006788	ralA binding protein 1	RALBP1	18p11.3
NM_021074	NADH dehydrogenase (ubiquinone) flavoprotein 2 (24kD)	NDUFV2	18p11.31-p11.2
NM_003803	myomesin 1 (skelemin, 185 kD)	MYOM1	18p11.31-p11.32
NM_005433	v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1	YES1	18p11.31-p11.21
NM_174886	TGFB-induced factor (TALE family homeobox)	TGIF	18p11.3
NM_013292	myosin regulatory light chain	MLC-B	18p11.31
NM_004583	Cluster Incl. U18420:Human ras-related small GTP binding protein Rab5 (rab5) mRNA, complete cds /cds=(135,785) /gb=U18420 /gi=642531 /ug=Hs.479 /len=1579	RAB5C	17q21.2
NM_001096	Cluster Incl. X64330:H.sapiens mRNA for ATP-citrate lyase /cds=(84,3401) /gb=X64330 /gi=28934 /ug=Hs.174140 /len=4297	ACLY	17q12-q21
NM_006807	Cluster Incl. U35451:Homo sapiens heterochromatin protein p25 mRNA, complete cds /cds=(216,773) /gb=U35451 /gi=1177844 /ug=Hs.77254 /len=2148	CBX1	17q
NM_003488	Cluster Incl. X97335:H.sapiens mRNA for kinase A anchor protein /cds=(124,2835) /gb=X97335 /gi=1507823 /ug=Hs.78921 /len=3758	AKAP1	17q21-q23
NM_002634	Cluster Incl. S85655:prohibitin [human, mRNA, 1043 nt] /cds=(50,868) /gb=S85655 /gi=246482 /ug=Hs.75323 /len=1024	PHB	17q21
NM_006380	Cluster Incl. D86981:Human mRNA for KIAA0228 gene, partial cds /cds=(0,2045) /gb=D86981 /gi=1504035 /ug=Hs.84084 /len=6465	APPBP2	17q21-q23
NM_014233	Cluster Incl. X53390:Human mRNA for upstream binding factor (hUBF) /cds=(147,2441) /gb=X53390 /gi=509240 /ug=Hs.89781 /len=3097	UBTF	17q21.3
NM_002512	Cluster Incl. X58965:H.sapiens RNA for nm23-H2 gene /cds=(72,530) /gb=X58965 /gi=35069 /ug=Hs.227823 /len=670	NME2	17q21.3
NM_022739	Cluster Incl. AA630312:ac08f05.s1 Homo sapiens cDNA, 3 end /clone=IMAGE-855873 /clone_end=3 /gb=AA630312 /gi=2552923 /ug=Hs.21806 /len=685	SMURF2	17q22-q23
NM_025233	Cluster Incl. U18919:Human chromosome 17q12-21 mRNA, clone pOV-2, partial cds /cds=(0,886) /gb=U18919 /gi=894177 /ug=Hs.74130 /len=1009	NBP	17q12-q21
NM_000988	Cluster Incl. AA044823:zk72a10.s1 Homo sapiens cDNA, 3 end /clone=IMAGE-488346 /clone_end=3 /gb=AA044823 /gi=1523026 /ug=Hs.111611 /len=649	RPL27	17q21.1-q21.2
NM_005749	Cluster Incl. D38305:Human mRNA for Tob, complete cds /cds=(43,1080) /gb=D38305 /gi=1580723 /ug=Hs.178137 /len=1206	TOB1	17q21
M23410	HUMPLAKO Human plakoglobin (PLAK) mRNA; complete cds.	JUP	17q21
AF519531	Human interferon gamma treatment inducible mRNA	SCYA2	17q11.2-q21.1
XM_290758	Cluster Incl. AB011125:Homo sapiens mRNA for KIAA0553 protein, partial cds /cds=(0,3289) /gb=AB011125 /gi=3043629 /ug=Hs.105749 /len=5574	KIAA0553	17q21.31
NM_004396	Cluster Incl. X52104:Human mRNA for p68 protein /cds=(175,2019) /gb=X52104 /gi=35219 /ug=Hs.76053 /len=2330	DDX5	17q21
NM_153490	Cluster Incl. X14640:Human mRNA for keratin 13 /cds=(42,1418) /gb=X14640 /gi=34032 /ug=Hs.74070 /len=1691	KRT13	17q21-q23
NM_005899	Cluster Incl. D30756:Human mRNA for KIAA0049 gene, complete cds /cds=(140,3040) /gb=D30756 /gi=488500 /ug=Hs.233745 /len=4654	M17S2	17q21.1
NM_006688	Cluster Incl. AF095154:Homo sapiens C1q-related factor mRNA, complete cds /cds=(13,789) /gb=AF095154 /gi=3747096 /ug=Hs.134012 /len=1284	CRF	17q21
NM_006460	Cluster Incl. AB021179:Homo sapiens mRNA for HEXIM1 protein, complete cds /cds=(689,1768) /gb=AB021179 /gi=4062855 /ug=Hs.15299 /len=3597	HIS1	17q21.32
NM_021137	Cluster Incl. M80783:Human B12 protein mRNA, complete cds /cds=(153,1103) /gb=M80783 /gi=179303 /ug=Hs.76090 /len=3512	TNFAIP1	17q22-q23
NM_004773	Cluster Incl. L40410:Homo sapiens thyroid receptor interactor (TRIP3) mRNA, 3 end of cds /cds=(0,458) /gb=L40410 /gi=703109 /ug=Hs.2210 /len=867	TRIP3	17q21.1
NM_025233	Cluster Incl. U18919:Human chromosome 17q12-21 mRNA, clone pOV-2, partial cds /cds=(0,886) /gb=U18919 /gi=894177 /ug=Hs.74130 /len=1009	NBP	17q12-q21
NM_001070	Cluster Incl. M61764:Human gamma-tubulin mRNA, complete cds /cds=(24,1379) /gb=M61764 /gi=183702 /ug=Hs.21635 /len=1568	TUBG1	17q21
AF073312	Cluster Incl. AF035811:Homo sapiens protein H5 (H5) mRNA, complete cds /cds=(129,1565) /gb=AF035811 /gi=2665833 /ug=Hs.155524 /len=1737	PNUTL2	17q22-q23
NM_170608.	Cluster Incl. AW005997:wz91c01.x1 Homo sapiens cDNA, 3 end /clone=IMAGE-2566176 /clone_end=3 /gb=AW005997 /gi=5854775 /ug=Hs.78185 /len=702	TCFL4	17q21.1
NM_015294	Cluster Incl. AB020705:Homo sapiens mRNA for KIAA0898 protein, partial cds /cds=(0,2939) /gb=AB020705 /gi=4240284 /ug=Hs.8164 /len=4111	TRIM37	17q22-q23
NM_006148	Cluster Incl. X82456:H.sapiens MLN50 mRNA /cds=(75,860) /gb=X82456 /gi=2407912 /ug=Hs.75080 /len=3846	LASP1	17q11-q21.3
NM_005121	Cluster Incl. AB011165:Homo sapiens mRNA for KIAA0593 protein, partial cds /cds=(0,2844) /gb=AB011165 /gi=3043709 /ug=Hs.11861 /len=4924	TRAP240	17q22-q23
NM_004375	Cluster Incl. U79270:Human clone 23707 mRNA, partial cds /cds=(0,460) /gb=U79270 /gi=1710235 /ug=Hs.239420 /len=1255	COX11	17q22
NM_002512	H.sapiens RNA for nm23-H2 gene.	NME2	17q21.3
NM_000269	Homo sapiens NM23-H1 mRNA	NME1	17q21.3
NM_001552	Human insulin-like growth factor binding protein 4 (IGFBP4) mRNA, complete cds	IGFBP4	17q12-q21.1
NM_002824	Cluster Incl. M24398:Human parathymosin mRNA, complete cds /cds=(300,608) /gb=M24398 /gi=339698 /ug=Hs.171814 /len=1109	PTMS	17q12-q22
NM_006373	Cluster Incl. U18009:Human chromosome 17q21 mRNA clone LF113 /cds=(0,939) /gb=U18009 /gi=602277 /ug=Hs.157236 /len=2381	VAT1	17q21
NM_003766	Cluster Incl. U17999:HSU17999 Homo sapiens cDNA /clone=B49B32B27 /gb=U17999 /gi=602263 /ug=Hs.12272 /len=1758	BECN1	17q21
NM_005831	Cluster Incl. U22897:Homo sapiens nuclear domain 10 protein (ndp52) mRNA, complete cds /cds=(54,1394) /gb=U22897 /gi=984286 /ug=Hs.154230 /len=2387	NDP52	17q21.32
NM_001991	Cluster Incl. AB002386:Human mRNA for KIAA0388 gene, complete cds /cds=(100,2343) /gb=AB002386 /gi=2224716 /ug=Hs.194669 /len=4606	EZH1	17q21.1-q21.3
NM_002126	Cluster Incl. M95585:Human hepatic leukemia factor (HLF) mRNA, complete cds /cds=(322,1209) /gb=M95585 /gi=184223 /ug=Hs.101047 /len=3865	HLF	17q22

TABLE 4. CONTINUED.

Accession number	Name	Gene	Cytogenetic position
NM_001256	Cluster Incl. AA166687:zq41h05.s1 Homo sapiens cDNA, 3 end /clone=IMAGE-632313 /clone_end=3 /gb=AA166687 /gi=1745142 /ug=Hs.172405 /len=703	CDC27	17q12-17q23.2
NM_139276	Cluster Incl. L29277:Homo sapiens DNA-binding protein (APRF) mRNA, complete cds /cds=(220,2532) /gb=L29277 /gi=475788 /ug=Hs.142258 /len=2787	STAT3	17q21
NM_004687	Cluster Incl. AB014547:Homo sapiens mRNA for KIAA0647 protein, partial cds /cds=(0,3051) /gb=AB014547 /gi=3327107 /ug=Hs.141727 /len=5183	MTMR4	17q22-q23
NM_003563	Cluster Incl. AJ000644:Homo sapiens mRNA for SPOP /cds=(157,1281) /gb=AJ000644 /gi=2695707 /ug=Hs.129951 /len=1642	SPOP	17q21.32
NM_002276	Cluster Incl. Y00503:Human mRNA for keratin 19 /cds=(32,1234) /gb=Y00503 /gi=34038 /ug=Hs.182265 /len=1360	KRT19	17q21
NM_006924	Cluster Incl. M72709:Human alternative splicing factor mRNA, complete cds /cds=UNKNOWN /gb=M72709 /gi=179073 /ug=Hs.73737 /len=1717	SFRS1	17q21.3-q22
AB014512	Cluster Incl. AB014512:Homo sapiens mRNA for KIAA0612 protein, partial cds /cds=(0,5212) /gb=AB014512 /gi=3327037 /ug=Hs.112499 /len=6458	PRAX-1	17q22-q23
NM_003079	Cluster Incl. AF035262:Homo sapiens BAF57 (BAF57) gene, complete cds /cds=(0,1235) /gb=AF035262 /gi=2914752 /ug=Hs.235497 /len=1236	SMARCE1	17q21.1
NM_007359	Cluster Incl. X80199:H.sapiens MLN51 mRNA /cds=(233,1837) /gb=X80199 /gi=2385366 /ug=Hs.83422 /len=4253	MLN51	17q11-q21.3
LOC51096	Cluster Incl. AI140114:qa95c06.x1 Homo sapiens cDNA, 3 end /clone=IMAGE-1694506 /clone_end=3 /gb=AI140114 /gi=3647571 /ug=Hs.6153 /len=741	LOC51096	17q21.33
NM_021078	Cluster Incl. AF029777:Homo sapiens histone acetyltransferase (GCN5) mRNA, partial cds /cds=(24,2537) /gb=AF029777 /gi=3220163 /ug=Hs.101067 /len=3074	GCN5L2	17q21
NM_005533	Human interferon-induced leucine zipper protein (IFP35) mRNA, partial cds	IFI35	17q21
NM_006178	Cluster Incl. U03985:Human N-ethylmaleimide-sensitive factor mRNA, partial cds /cds=(0,2255) /gb=U03985 /gi=467976 /ug=Hs.108802 /len=2263	NSF	17q21
NM_005895	Cluster Incl. D63997:Homo sapiens mRNA for GCP170, complete cds /cds=(269,4861) /gb=D63997 /gi=2662348 /ug=Hs.4953 /len=6640	GOLGA3	12q24.33
NM_002813	Cluster Incl. AI347155:tc04c11.x1 Homo sapiens cDNA, 3 end /clone=IMAGE-2062868 /clone_end=3 /gb=AI347155 /gi=4084361 /ug=Hs.5648 /len=750	PSMD9	12q24.31-q24.32
NM_004642	Cluster Incl. AF006484:Homo sapiens putative oral tumor suppressor protein (doc-1) mRNA, complete cds /cds=(522,869) /gb=AF006484 /gi=2738496 /ug=Hs.3436 /len=1608	CDK2AP1	12q24.31
Z22555	Cluster Incl. Z22555:H.sapiens encoding CLA-1 mRNA /cds=(69,1598) /gb=Z22555 /gi=397606 /ug=Hs.180616 /len=2552	CD36L1	12q24.31
NM_023012	Cluster Incl. W26521:32g11 Homo sapiens cDNA /gb=W26521 /gi=1307382 /ug=Hs.81648 /len=783	FLJ11021	12q24.31
NM_006815	Cluster Incl. X92098:H.sapiens mRNA for transmembrane protein rnp24 /cds=(27,632) /gb=X92098 /gi=1212964 /ug=Hs.75914 /len=780	RNP24	12q24.31

TABLE 4. CONTINUED.

Accession number	Name	Gene	Cytogenetic position
NM_003565	Cluster Incl. AF045458:Homo sapiens serine/threonine kinase ULK1 (ULK1) mRNA, complete cds /cds=(268,3420) /gb=AF045458 /gi=3435113 /ug=Hs.47061 /len=5211	ULK1	12q24.3
XM_055013	Human ubiquitin mRNA, complete cds	UBC	12q24.3
NM_002956	Cluster Incl. X64838:H.sapiens mRNA for restin /cds=(132,4415) /gb=X64838 /gi=35998 /ug=Hs.31638 /len=5857	RSN	12q24.3
NM_003299	HSTRA1 Human tra1 mRNA for human homologue of murine tumor rejection antigen gp96	TRA1	12q24.2-q24.3
NM_033624	Cluster Incl. AB020682:Homo sapiens mRNA for KIAA0875 protein, partial cds /cds=(0,1866) /gb=AB020682 /gi=4240238 /ug=Hs.184227 /len=4168	FBXO21	12q24.23
NM_002567	Cluster Incl. X75252:H.sapiens phosphatidylethanolamine binding protein mRNA /cds=(110,673) /gb=X75252 /gi=406289 /ug=Hs.80423 /len=1444	PBP	12q24.23
XM_034056	Cluster Incl. AB028948:Homo sapiens mRNA for KIAA1025 protein, partial cds /cds=(0,3441) /gb=AB028948 /gi=5689386 /ug=Hs.4084 /len=6131	KIAA1025	12q24.22
XM_045792	Cluster Incl. D86973:Human mRNA for KIAA0219 gene, partial cds /cds=(0,7239) /gb=D86973 /gi=1504019 /ug=Hs.75354 /len=7819	GCN1L1	12q24.2
NM_000690	Cluster Incl. X05409:Human RNA for mitochondrial aldehyde dehydrogenase I ALDH I (EC 1.2.1.3) /cds=(36,1586) /gb=X05409 /gi=28605 /ug=Hs.195432 /len=1989	ALDH2	12q24.2
NM_001002	Cluster Incl. M17885:Human acidic ribosomal phosphoprotein P0 mRNA, complete cds /cds=(77,1030) /gb=M17885 /gi=190231 /ug=Hs.73742 /len=1097	RPLP0	12q24.2
NM_004373	Cluster Incl. AI540925:PEC1.2_15_A02.r Homo sapiens cDNA, 5 end /clone_end=5 /gb=AI540925 /gi=4458298 /ug=Hs.180714 /len=777	COX6A1	12q24.2
NM_002710	Cluster Incl. X74008:H.sapiens mRNA for protein phosphatase 1 gamma /cds=(154,1125) /gb=X74008 /gi=402777 /ug=Hs.79081 /len=2263	PPP1CC	12q24.1-q24.2
AB014514	Cluster Incl. AB014514:Homo sapiens mRNA for KIAA0614 protein, partial cds /cds=(0,4893) /gb=AB014514 /gi=3327041 /ug=Hs.7314 /len=7084	KIAA0614	12q24.13
NM_006817	Cluster Incl. X94910:Homo sapiens mRNA for ERp28 protein /cds=(11,796) /gb=X94910 /gi=3413292 /ug=Hs.75841 /len=892	C12orf8	12q24.13
NM_004592	Cluster Incl. U08377:Human homolog of Drosophila splicing regulator suppressor-of-white-apricot mRNA, complete cds /cds=(120,2975) /gb=U08377 /gi=508230 /ug=Hs.84229 /len=3225	SFRS8	12q24.12
NM_006825	Cluster Incl. X69910:H.sapiens p63 mRNA for transmembrane protein /cds=(84,1889) /gb=X69910 /gi=297407 /ug=Hs.74368 /len=2898	CKAP4	12q24.11
AF387506	Cluster Incl. AB020880:Homo sapiens mRNA for squamous cell carcinoma antigen SART-3, complete cds /cds=(19,2910) /gb=AB020880 /gi=4996281 /ug=Hs.116875 /len=3788	KIAA0156	12q24.11
NM_007062	Cluster Incl. L07758:Human IEF SSP 9502 mRNA, complete cds /cds=(87,1592) /gb=L07758 /gi=177764 /ug=Hs.172589 /len=1853	PWP1	12q24.11
AB011109	Cluster Incl. AB011109:Homo sapiens mRNA for KIAA0537 protein, complete cds /cds=(1380,3365) /gb=AB011109 /gi=3043597 /ug=Hs.200598 /len=6828	KIAA0537	12q24.11

TABLE 4. CONTINUED.

Accession number	Name	Gene	Cytogenetic position
NM_014301	Cluster Incl. U47101:Human NifU-like protein (hNifU) mRNA, partial cds /cds=(0,366) /gb=U47101 /gi=1685101 /ug=Hs.9908 /len=819	NIFU	12q24.1
NM_003211	Cluster Incl. U51166:Human G/T mismatch-specific thymine DNA glycosylase mRNA, complete cds /cds=(399,1631) /gb=U51166 /gi=1378106 /ug=Hs.173824 /len=3410	TDG	12q24.1
NM_005719	Cluster Incl. AI525393:PT1.1_07_A11.r Homo sapiens cDNA, 5 end /clone_end=5 /gb=AI525393 /gi=4439528 /ug=Hs.6895 /len=811	ARPC3	12q24
NM_005475	Cluster Incl. AF055581:Homo sapiens adaptor protein Lnk mRNA, complete cds /cds=(357,2084) /gb=AF055581 /gi=3845720 /ug=Hs.13131 /len=5403	LNK	12q24
NM_002973	Cluster Incl. Y08262:H.sapiens mRNA for SCA2 protein /cds=(0,2746) /gb=Y08262 /gi=1770389 /ug=Hs.76253 /len=4163	SCA2	12q24
NM_003362	Cluster Incl. Y09008:H.sapiens mRNA for uracil-DNA glycosylase /cds=(70,1011) /gb=Y09008 /gi=1850820 /ug=Hs.78853 /len=2036	UNG	12q23-q24.1
AY186578	Cluster Incl. M23114:Homo sapiens calcium-ATPase (HK1) mRNA, complete cds /cds=(163,3291) /gb=M23114 /gi=184100 /ug=Hs.1526 /len=4134	ATP2A2	12q23-q24.1
NM_003330	Cluster Incl. X91247:H.sapiens mRNA for thioredoxin reductase /cds=(439,1932) /gb=X91247 /gi=1237037 /ug=Hs.13046 /len=3826	TXNRD1	12q23-q24.1
NM_004075	Cluster Incl. D83702:Homo sapiens mRNA for photolyase, complete cds /cds=(586,2346) /gb=D83702 /gi=1304106 /ug=Hs.151573 /len=2981	CRY1	12q23-q24.1
NM_005594	Cluster Incl. AF054187:Homo sapiens alpha NAC mRNA, complete cds /cds=(309,956) /gb=AF054187 /gi=4092059 /ug=Hs.146763 /len=1059	NACA	12q23-q24.1
NM_001177	Cluster Incl. L28997:Homo sapiens ARL1 mRNA, complete cds /cds=(144,689) /gb=L28997 /gi=607027 /ug=Hs.77102 /len=1008	ARL1	12q23.3
NM_002465	Cluster Incl. X73114:H.sapiens mRNA for slow MyBP-C /cds=(81,3452) /gb=X73114 /gi=402618 /ug=Hs.169849 /len=3764	MYBPC1	12q23.3
NM_003297	Human steroid receptor (TR2-11) mRNA, complete cds	NR2C1	12q23.1
NM_005888	Cluster Incl. X60036:H.sapiens mRNA for mitochondrial phosphate carrier protein /cds=(48,1133) /gb=X60036 /gi=38261 /ug=Hs.78713 /len=1314	SLC25A3	12q23
AB013382	Cluster Incl. AB013382:Homo sapiens mRNA for DUSP6, complete cds /cds=(351,1496) /gb=AB013382 /gi=3869139 /ug=Hs.180383 /len=2390	DUSP6	12q22-q23
NM_000618	Human IGF-I mRNA for insulin-like growth factor I	IGF1	12q22-q23
NM_006166	Cluster Incl. AA621555:af53a04.s1 Homo sapiens cDNA, 3 end /clone=IMAGE-1035342 /clone_end=3 /gb=AA621555 /gi=2525494 /ug=Hs.84928 /len=791	NFYB	12q22-q23
NM_001682	Cluster Incl. J04027:Human plasma membrane Ca2+ pumping ATPase mRNA, complete cds /cds=(181,3843) /gb=J04027 /gi=950413 /ug=Hs.78546 /len=4398	ATP2B1	12q21-q23
NM_002345	Cluster Incl. U21128:Human lumican mRNA, complete cds /cds=(84,1100) /gb=U21128 /gi=699576 /ug=Hs.79914 /len=1717	LUM	12q21.3-q22
NM_003805	Human death domain containing protein CRADD mRNA, complete cds	CRADD	12q21.33-q23.1
NM_002847	Cluster Incl. U81561:Human protein tyrosine phosphatase receptor pi (PTPRP) mRNA, complete cds /cds=(42,3038) /gb=U81561 /gi=2351575 /ug=Hs.74624 /len=4699	PTPRN2	7q36

TABLE 4. CONTINUED.

Accession number	Name	Gene	Cytogenetic position
NM_002889	Cluster Incl. U77594:Human tazarotene-induced gene 2 (TIG2) mRNA, complete cds /cds=(96,587) /gb=U77594 /gi=1848263 /ug=Hs.37682 /len=708	RARRES2	7q36.1
NM_013316	Cluster Incl. U71267:Human potential transcriptional repressor NOT4Hp (NOT4H) mRNA, complete cds /cds=(281,2209) /gb=U71267 /gi=4097897 /ug=Hs.20423 /len=3297	CNOT4	7q22-qter
AF493921	Homo sapiens mRNA for ras-related GTP-binding protein; complete cds	RHEB2	7q36
NM_003592	Cluster Incl. AF062536:Homo sapiens cullin 1 mRNA, complete cds /cds=(124,2454) /gb=AF062536 /gi=3139076 /ug=Hs.14541 /len=2583	CUL1	7q36.1
NM_003040	Cluster Incl. U62531:Human AE2 anion exchanger (SLC4A2) mRNA, complete cds /cds=(174,3899) /gb=U62531 /gi=1809029 /ug=Hs.79410 /len=4054	SLC4A2	7q35-q36
D13635	Cluster Incl. D13635:Human mRNA for KIAA0010 gene, complete cds /cds=(303,3554) /gb=D13635 /gi=285982 /ug=Hs.155287 /len=5160	KIAA0010	7q36.3

A description of genes expressed in human donor sclera that map to chromosome loci with defined intervals (chromosomes 18p11.31, 17q21-22, 12q23-24, and 7q36) associated with non-syndromic high-grade myopia, associated accession number, gene symbol, and cytogenetic position.

tracellular matrix (ECM) turnover. TGFβ regulates collagen synthesis and deposition, and is activated by thrombospondin-1 [36,37]. Thrombospondins 1-3 were detected in the microarray analysis. Bone morphogenic protein (BMP-7) has been shown to regulate specific collagen deposition. Table 3 shows a list of transcription factors expressed in human sclera.

Collagen matrix organization is regulated by accessory proteins. Proteoglycans regulate collagen fibril spacing. Chondroitin 6-sulfotransferase-like protein was detected. Expression of this enzyme may be critical for maturation of the keratan sulfate proteoglycans, which are the major proteoglycans of the cornea and sclera. Several proteoglycans were identified, including glypicans 1 and 3-6, versican, dystroglycan 1, microfibril-associated glycoprotein-2, proline arginine-rich end leucine-rich repeat protein, lumican, aggrecan, and decorin. Another accessory protein identified was matrilin-3, which forms collagen dependent and independent fibrils, fibrillin, fibulin, various laminin subunits, fibronectin 1, fibromodulin, elastin, various cartilage matrix proteins, tenascin, and elastin microfibril interface located protein.

RT-PCR results confirmed expression in 7 of 7 genes examined (Figure 1). Genes tested were collagens 6A3 and 10A1, thrombospondins 2 and 4, and the proteoglycans dystroglycan, biglycan, and decorin. Biglycan and decorin are proteoglycans previously shown to be structural components of sclera [38].

DISCUSSION

These results have led to the identification of new scleral proteins that may be important in the maintenance of biochemical and biomechanical properties of the sclera. The list of genes reported here should not be considered comprehensive of all genes expressed in sclera, however. Limitations include those of the finite number of representative gene probe sets on the commercial chip itself, incomplete representation of all expressed genes in the mRNA, and differences in the developmental expression of various transcripts. Ideally, every gene that appears to be expressed should be confirmed with RT-PCR, immunostaining, and in situ hybridization. The present study chiefly serves to identify novel targets for future investigations. This is especially relevant for the extracellular matrix gene expression profile identified herein, which corresponds to other gene families under investigation that are known to be of major mechanistic interest.

Pathologic myopia can severely affect visual function and, at present, no effective pharmacologic agents are available to limit the development of this condition. It is clear, however, that in animal models of myopia the processes of biomechanical changes to produce axial elongation of the eye are regulated by the coordinated expression of genes encoding growth factors, cytokines, extracellular matrix molecules, metalloproteinases, specific glycoproteins, and glycosyltransferases. Investigators in past studies on gene expression during experimentally induced myopia have used traditional approaches, including quantitative reverse transcription-polymerase chain reaction (RT-PCR), in situ hybridization, RNase protection assays, and western blot and immunohistochemistry analyses. The disadvantages of these approaches have been that only a few genes are detected per assay. A more global approach is needed to elucidate the many factors that may play a role in scleral physiology. In the present study, gene array technology was used to examine gene expression in human scleral donor tissue. We identified many genes for the first time to be expressed in human sclera. These genes are novel factors for further study.

Progress is underway to identify the hereditary basis of high myopia at the molecular genetic level. Mutations in extracellular matrix genes have been associated with several syndromic genetic disorders that include myopia as a consistent clinical feature. Collagen 2A1 and 11A1 mutations have been identified for Stickler syndromes type 1 and 2, respectively. Mutations in lysyl-protocollagen hydroxylase have been shown to be responsible for type VI Ehlers-Danlos Syndrome. Collagen 18A1 mutations have been identified in Knobloch syndrome, and fibrillin defects have been shown to be responsible for Marfan syndrome [39-43]. Each of these genes was detected in this microarray study of human sclera and supports the notion of increased scleral elasticity and ocular axial elongation due to faulty structural protein function when its gene is mutated, leading to the high myopia observed in these syndromes. Therefore, knowledge of gene expression of the membranous scleral wall is critical to our understanding of the mechanisms that regulate ocular size and shape, as well as to our understanding of the etiology of abnormal eye expansion and myopia.

Five chromosomal loci have been identified for nonsyndromic high-grade myopia (MYP1 at Xq28, MYP2 at 18p11.31, MYP3 at 12q23-24, and loci at 17q21-22 and 7q36) suggesting significant genetic heterogeneity [44-49]. Despite these recent successes in mapping myopia loci and implicating specific extracellular matrix proteins with myopia development, no gene mutations for any loci have been identified to date, although transforming growth factor beta-induced has been implicated [50]. The search for these disease-causing genes could be facilitated by knowledge of which expressed scleral genes reside at specific chromosomal loci. Table 4 displays a list of genes expressed in human donor sclera that map to chromosome loci with defined intervals (chromosomes 7q36, 18p11.31, 12q23-24, and 17q21-22) associated with nonsyndromic high-grade myopia.

This study describes a reverse molecular genetic approach to identify genes involved in scleral composition. Microarray analyses of mRNA from 6 human donor scleral samples have identified several known genes, as well as previously uncharacterized novel genes expressed in this specialized connective tissue. Any of the genes identified in this cDNA library may serve as candidates for high myopia or other disorders of scleral growth and development, and may help to explain scleral involvement in a variety of heritable disorders.

These efforts are to our knowledge a first attempt using microarray analysis to obtain a broad picture of the composition of human scleral tissue. The results were used to create a preliminary, online database of genes expressed in normal human donor sclera.

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Appendix 1. Raw Affymetrix hybridization data

The raw data from each Affymetrix hybridization experiment is included as a supplement. The file contains data corresponding to the Affymetrix probes including Affymetrix probe number, raw averaged signal observed when scanned, detection call (absent, present, or marginal based on 150 signal units determining present calls), and detection p value.

To access this data, in the online version of this article, click or select the words "Raw Affymetrix data". This will initiate the download of a compressed (zip) archive. This file should be uncompressed with an appropriate program (the particular program will depend on your operating system). Once extracted, you will have a folder (or directory) containing a raw data file. The file is tab delimited text. Most spreadsheet programs will import files in this format.

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