Microarray Analysis of Gene Expression in Human Donor Sclera

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Background

ABSTRACT

OBJECTIVES: To develop gene expression profiles of human sclera to allow for the identification of novel, uncharacterized genes, 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. Resulting cDNA was *in vitro* transcribed to produce biotin-labeled cRNA, fragmented, and mixed with hybridization controls before a 16-hour incubation/ hybridization step to 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 normalized to the same target intensity using all probe sets. Reverse- transcription polymerase chain reaction (RT-PCR) was performed to validate the microarray results.

RESULTS: Labelled, fragmented scleral cRNA hybridized to more than 58 % of the 12,626 probe sets represented on the microarray chip. There were 3751 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, versican, and dystroglycan have not previously been shown to be expressed in sclera.
RT-PCR results confirmed expression in all genes examined.

CONCLUSIONS: This study demonstrates the utility of gene microarray technology in identifying global patterns of scleral gene expression, and provides a comprehensive list of genes expressed in human sclera. Identification of genes expressed preferentially or exclusively in sclera contributes to our understanding of scleral biology, and potentially provides positional candidate genes for scleral disorders such as high myopia.

Methods

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 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 hours post mortem. Total RNA was extracted from pulverized samples using TRIZOL reagent (Invitrogen Inc., Carlsbad, CA).

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:isolamyl alcohol (25:24:1). Approximately 1 µg of cDNA was used as template in an *in vitro* transcription assay reaction (Enzo Biochem, Inc., New York, 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 ºC for 35 minutes in fragmentation buffer. Following removal of the prehybridization buffer, the 6 Affymetrix chips were filled with 200 µl of the hybridization mixture and incubated at 45 ºC at 60 RPM for 16 hours.

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. The chips were then scanned and the data analyzed using the Affymetrix Microarray Analysis Suite software as described below. The Affymetrix HG-U95A chip contains 12,626 probe sets.

Scanning, Data Collection, and Analysis

Following staining and washing, each chip was scanned twice at 570 nm with a con-focal scanner (Agilent, 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 identified expressed genes with present calls for all 6 samples were compared with the following databases: Genebank, On-Line Mendelian Inheritance in Man (OMIM), and PubMed -all accessed at http://ncbi.nlm.nih.gov.

Reverse Transcription – Polymerase Chain Reaction

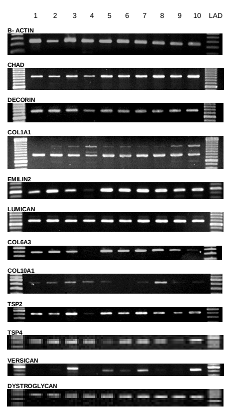
Total RNA was extracted from 4 pooled, human donor sclera using TRIZOL reagent. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed with oligo-dT oligonucleotide primer using standard methods to synthesize cDNA. One microgram of total RNA from sclera, other eye tissues such as optic nerve, retina and cornea, as well as commercially prepared poly-A RNA from various human organs were used as a template for first-strand cDNA synthesis. The 5' sense and 3' antisense PCR primer pairs designed for collagens 6A3 and 10A1, thrombospondins 2 and 4, versican, 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 Beta actin as a control were visualized on 2% agarose gels after electrophoresis and staining with ethidium bromide. **(Figure 1)**

Results

TABLE 1. RT-PCR PRIMERS OF EXPRESSED GENES

Primer Name	Gene	Sequence	Product Size (bp)
COL6A3-F	COL6A3	AGGAGAAATTACTGCCGTACA	
COL6A3-R	COL6A3	CGGCTGAACTCGTGAATAGGT	390
COL10A1-F-2	COL10A1	CCCAACACCAAGACACAGTTC	
COL10A1-R-2	COL10A1	CCCTTTCTGTCCATTCATAC	462
VERS-F	Versican	GCGGCAACCAGCAGGTACAC	
VERS-R	Versican	CATCCGACAGCCACCCGTAAT	439
DAG1-F	Dystroglycan	CTGCCTGTGCTGCGGATGAAC	
DAG1-R	Dystroglycan	GGGCTTCTTATTGGCGATGTG	374
THBS2-F	Thrombospondin 2	AGGACGGGATTGGCGATG	
THBS2-R	Thrombospondin 2	GGTTGGCGTTGGAGATGTAGG	453
THBS4-F	Thrombospondin 4	CCAGGTGGATTCCGTTCACAA	
THBS4-R	Thrombospondin 4	CGGGTAGCAGGGATGGTATT	568
COL1A1-F	COL1A1	GAGGGAAGCCGCAAGAA	
COL1A1-R	COL1A1	GCGCGGATCTCGATCTC	455
CHAD-F	Chondroadherin	ATGCCGAACCTCGTGTC	
CHAD-R	Chondroadherin	CTCCACCACCCGTAGC	447
LUM-F	Lumican	TGCCCTGAAAGCTACC	
LUM-R	Lumican	CTCTTTCAGCCGATTGT	393
DCN-F	Decorin	AACCTTCACGCATTGAT	
DCN-R	Decorin	AATCCCAACTTAGCCA	440
EMILIN-2F	EMLIN-2	CATCGTGAACAAGAATGTGAG	
EMILIN2-R	EMLIN-2	CAAGTTGGGGACAAAGTCT	317
BGN-F	Biglycan	AACTACCTGCGCATCTC	
BGN-R	Biglycan	TGACGCAGCGGAAAG	436

FIGURE 1. RT-PCR PRODUCTS



1 - Sclera
2 - Cornea
3 - Optic Nerve
4 - Retina
5 - Lung
6 - Skeletal Muscle
7 - Heart
8 -Trachea
9 - Kidney

TABLE 2. EXTRACELLULAR MATRIX EXPRESSED GENES

GenBank #	Extracellular matrix Description	SYMBOL	Cyto Pos
NM_001135	aggrecan 1 (chondroitin sulfate proteoglycan 1, large aggregating proteoglycan, antigen identified by monoclonal antibody A0122)	AGC1	15q26.1
NM_001135	NM_001723, 230/240kDa	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, 72kDa gelatinase, 72kDa 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 180kDa (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

TABLE 3. CHROMOSOME 18p11.31 LOCUS EXPRESSED GENES

Genbank #	Name	Gene	Cyto Locus
NM_006796	AFG3 (ATPase family gene 3, yeast)-like 2	AFG3L2	18p11
NM_004338	chromosome 18 open reading frame 1	C18orf1	18p11.2
NM_001967	eukaryotic translation initiation factor 4A, isoform 2	EIF4A2	18p11.2
AF515797	erythrocyte membrane protein band 4.1-like 3	EPB41L3	18p11.32
inositol(myo)-1(or 4)-monophosphatase 2	inositol(myo)-1(or 4)-monophosphatase 2	IMPA2	18p11.2
NM_000529	melanocortin 2 receptor (adrenocorticotropic hormone)	MC2R	18p11.2
myosin, light polypeptide, regulatory, non-sarcomeric (20kD)	myosin, light polypeptide, regulatory, non-sarcomeric (20kD)	MLCB	18
NM_003803	myomesin 1 (skelemin) (185kD)	MYOM1	18p11.31-p11.32
NM_021074	NADH dehydrogenase (ubiquinone) flavoprotein 2 (24kD)	NDUFV2	18p11.31-p11.2
NM_002828	protein tyrosine phosphatase, non-receptor type 2	PTPN2	18p11.3-p11.2
NM_002845	protein tyrosine phosphatase, receptor type, M	PTPRM	18p11.2
NM_006868	RAB31, member RAS oncogene family	RAB31	18p11.3
NM_006788	ralA binding protein 1	RALBP1	18p11.3
X89750	TGFB-induced factor (TALE family homeobox)	TGIF	18p11.3
NM_001071	thymidylate synthetase	TYMS	18p11.32
NM_005151	ubiquitin specific protease 14 (tRNA-guanine transglycosylase)	USP14	18p11.32
NM_005433	v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1	YES1	18p11.31-p11.21

TABLE 4. . CHROMOSOME 17q21-22 LOCUS EXPRESSED GENES

GenBank #	Description	Gene	Cyto Locus
NM_001096	ATP citrate lyase	ACLY	17q12-q21
NM_003488	A kinase (PRKA) anchor protein 1	AKAP1	17q21-q23
NM_006380	amyloid beta precursor protein (cytoplasmic tail)-binding protein 2	APPBP2	17q21-q23
NM_003766	"beclin 1 (coiled-coil, myosin-like BCL2-interacting protein)"	BECN1	17q21
NM_006807	chromobox homolog 1 (Drosophila HP1 beta)	CBX1	17q
NM_001256	cell division cycle 27	CDC27	17q12-17q23.2
NM_004375	"COX11 (yeast) homolog, cytochrome c oxidase assembly protein"	COX11	17q22
NM_006688	C1q-related factor	CRF	17q21
NM_004396	"DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (RNA helicase, 68kD)"	DDX5	17q21
NM_001991	enhancer of zeste (Drosophila) homolog 1	EZH1	17q21.1-q21.3
NM_021078	"GCN5 (general control of amino-acid synthesis, yeast, homolog)-like 2"	GCN5L2	17q21
NM_006460	HMBA-inducible	HIS1	17q21.32
NM_006460	HMBA-inducible	HIS1	17q21.32
NM_002126	hepatic leukemia factor	HLF	17q22
NM_005533	interferon-induced protein 35	IFI35	17q21
NM_001552	insulin-like growth factor-binding protein 4	IGFBP4	17q12-q21.1
NM_002230	junction plakoglobin	JUP	17q21
XM_290758	KIAA0553 protein	KIAA0553	17q21.31
NM_153490	keratin 13	KRT13	17q21-q23
AF202321	keratin 19	KRT19	17q21
NM_006148	LIM and SH3 protein 1	LASP1	17q11-q21.3
NM_016001	CGI-48 protein	LOC51096	17q21.33
NM_005899	"membrane component, chromosome 17, surface marker 2 (ovarian carcinoma antigen CA125)"	M17S2	17q21.1
NM_007359	MLN51 protein	MLN51	17q11-q21.3
NM_004687	myotubularin related protein 4	MTMR4	17q22-q23
NM_025233	nucleotide binding protein	NBP	17q12-q21
NM_025233	nucleotide binding protein	NBP	17q12-q21
NM_005831	nuclear domain 10 protein	NDP52	17q21.32
NM_000269	"non-metastatic cells 1, protein (NM23A) expressed in"	NME1	17q21.3
NM_002512	"non-metastatic cells 2, protein (NM23B) expressed in"	NME2	17q21.3
NM_002512	"non-metastatic cells 2, protein (NM23B) expressed in"	NME2	17q21.3
NM_006178	N-ethylmaleimide-sensitive factor	NSF	17q21
NM_002634	prohibitin	PHB	17q21
NM_004574	peanut (Drosophila)-like 2	PNUTL2	17q22-q23
AF039571	peripheral benzodiazepine receptor-associated protein 1	PRAX-1	17q22-q23
NM_002824	parathymosin	PTMS	17q12-q22
NM_004583	"RAB5C, member RAS oncogene family"	RAB5C	17q21.2
NM_000978	ribosomal protein L23	RPL23	17q
NM_000988	ribosomal protein L27	RPL27	17q21.1-q21.2
AF519531	"small inducible cytokine A2 (monocyte chemotactic protein 1, homologous to mouse Sig-je)"	SCYA2	17q11.2-q21.1
NM_006924	"splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)"	SFRS1	17q21.3-q22
NM_003079	"SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1"	SMARCE1	17q21.1
NM_022739	E3 ubiquitin ligase SMURF2	SMURF2	17q22-q23
NM_003563	speckle-type POZ protein	SPOP	17q21.32
NM_139276	signal transducer and activator of transcription 3 (acute-phase response factor)	STAT3	17q21
NM_170608	transcription factor-like 4	TCFL4	17q21.1
NM_021137	"tumor necrosis factor, alpha-induced protein 1 (endothelial)"	TNFAIP1	17q22-q23
NM_005749	"transducer of ERBB2, 1"	TOB1	17q21
NM_005121	"thyroid hormone receptor-associated protein, 240 kDa subunit"	TRAP240	17q22-q23
NM_015294	tripartite motif-containing 37	TRIM37	17q22-q23
NM_004773	thyroid hormone receptor interactor 3	TRIP3	17q21.1
NM_001070	"tubulin, gamma 1"	TUBG1	17q21
NM_014233	"upstream binding transcription factor, RNA polymerase I"	UBTF	17q21.3
NM_006373	membrane protein of cholinergic synaptic vesicles	VATI	17q21

TABLE 5. CHROMOSOME 12q21-23 LOCUS EXPRESSED GENES

GenBank #	Description	Gene	Cyto Locus
NM_015270	adenylate cyclase 6	ADCY6	12q12-q13
NM_000690	aldehyde dehydrogenase 2 family (mitochondrial)	ALDH2	12q24.2
NM_001659	ADP-ribosylation factor 3	ARF3	12q13
NM_001177	ADP-ribosylation factor-like 1	ARL1	12q23.3
NM_005719	actin related protein 2/3 complex, subunit 3 (21 kD)	ARPC3	12q24
NM_170665	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	ATP2A2	12q23-q24.1
NM_001682	ATPase, Ca++ transporting, plasma membrane 1	ATP2B1	12q21-q23
NM_006356	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d	ATP5H	12q13
NM_001731	B-cell translocation gene 1, anti-proliferative	BTG1	12q22
NM_006817	chromosome 12 open reading frame 8	C12orf8	12q24.13
NM_006431	chaperonin containing TCP1, subunit 2 (beta)	CCT2	12q15
AY095373	CD36 antigen (collagen type I receptor, thrombospondin receptor)-like 1	CD36L1	12q24.31
NM_001798	cyclin-dependent kinase 2	CDK2	12q13
NM_004642	CDK2-associated protein 1	CDK2AP1	12q24.31
NM_006825	cytoskeleton-associated protein 4	CKAP4	12q24.11
NM_014515	CCR4-NOT transcription complex, subunit 2	CNOT2	12q15
NM_004373	cytochrome c oxidase subunit VIa polypeptide 1	COX6A1	12q24.2
NM_003805	CASP2 and RIPK1 domain containing adaptor with death domain	CRADD	12q21.33-q23.1
NM_004075	cryptochrome 1 (photolyase-like)	CRY1	12q23-q24.1
NM_001321	cysteine and glycine-rich protein 2	CSRP2	12q21.1
NM_006400	dynactin 2 (p50)	DCTN2	12q13.2-q13.3
NM_001345	diacylglycerol kinase, alpha (80kD)	DGKA	12q13.3
NM_015416	DKFZP586A011 protein	DKFZP586A011	12q13.13
NM_014033	DKFZP586A0522 protein	DKFZP586A0522	12q13.12
AL050100	DKFZP586D0919 protein	DKFZP586D0919	12q13.2
NM_001946	dual specificity phosphatase 6	DUSP6	12q22-q23
NM_003583	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	DYRK2	12q14.3
NM_001417	eukaryotic translation initiation factor 4B	EIF4B	12q13.11-12q14.3
NM_001982	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 3	ERBB3	12q13
NM_033624	F-box only protein 21	FBXO21	12q24.23
NM_023012	hypothetical protein FLJ11021 similar to splicing factor, arginine/serine-rich 4	FLJ11021	12q24.31
NM_006700	FLN29 gene product	FLN29	12q
XM_045792	GCN1 (general control of amino-acid synthesis 1, yeast)-like 1	GCN1L1	12q24.2
NM_001487	GCN5 (general control of amino-acid synthesis, yeast, homolog)-like 1	GCN5L1	12q13-q14
NM_005895	golgi autoantigen, golgin subfamily a, 3	GOLGA3	12q24.33
NM_002136	heterogeneous nuclear ribonucleoprotein A1	HNRPA1	12q13.1
NM_000618	insulin-like growth factor 1 (somatomedin C)	IGF1	12q22-q23
NM_002178	insulin-like growth factor binding protein 6	IGFBP6	12q13
NM_002206	integrin, alpha 7	ITGA7	12q13
NM_006082	tubulin, alpha, ubiquitous	K-ALPHA-1	12q12-12q14.3
BC021901	KIAA0118 protein	KIAA0118	12q15
D63879	KIAA0156 gene product	KIAA0156	12q24.11
NM_014840	KIAA0537 gene product	KIAA0537	12q24.11
AB011118	KIAA0546 protein	KIAA0546	12q15
AB014514	KIAA0614 protein	KIAA0614	12q24.13
NM_014871	KIAA0710 gene product	KIAA0710	12q13.2-q13.3
AB018290	KIAA0747 protein	KIAA0747	12q13.13
AB023155	KIAA0938 protein	KIAA0938	12q21.2
XM_034056	KIAA1025 protein	KIAA1025	12q24.22
AB028998	KIAA1075 protein	KIAA1075	12q13.13
NM_020898	KIAA1536 protein	KIAA1536	12q13.13
NM_002272	keratin 4	KRT4	12q12-q13
D50666	keratin 5 (epidermolysis bullosa simplex, Dowling-Meara/Kobner/Weber-Cockayne types)	KRT5	12q12-q13
NM_002273	keratin 8	KRT8	12q13
NM_005475	lymphocyte adaptor protein	LNK	12q24
NM_002332	low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor)	LRP1	12q13-q14
NM_000895	leukotriene A4 hydrolase	LTA4H	12q22
NM_002345	lumican	LUM	12q21.3-q22
NM_006301	mitogen-activated protein kinase kinase kinase 12	MAP3K12	12q13
NM_024056	hypothetical protein MGC5576	MGC5576	12q13.1
NM_002465	myosin-binding protein C, slow-type	MYBPC1	12q23.3
NM_005594	nascent-polypeptide-associated complex alpha polypeptide	NACA	12q23-q24.1
NM_139207	nucleosome assembly protein 1-like 1	NAP1L1	12q15
NM_006159	nel (chicken)-like 2	NELL2	12q13.11-q13.12
NM_006166	nuclear transcription factor Y, beta	NFYB	12q22-q23
NM_014301	nitrogen fixation cluster-like	NIFU	12q24.1
NM_003297	nuclear receptor subfamily 2, group C, member 1	NR2C1	12q23.1
NM_002135	nuclear receptor subfamily 4, group A, member 1	NR4A1	12q13
NM_005730	conserved gene amplified in osteosarcoma	OS4	12q13-q15
NM_006812	amplified in osteosarcoma	OS-9	12q13
XM_208234	unactive progesterone receptor, 23 kD	P23	12q13.13
NM_006191	proliferation-associated 2G4, 38kD	PA2G4	12q13
NM_002567	prostatic binding protein	PBP	12q24.23
NM_005016	poly(rC)-binding protein 2	PCBP2	12q13.12-q13.13
NM_000289	phosphofructokinase, muscle	PFKM	12q13.3
NM_002710	protein phosphatase 1, catalytic subunit, gamma isoform	PPP1CC	12q24.1-q24.2
NM_002480	protein phosphatase 1, regulatory (inhibitor) subunit 12A	PPP1R12A	12q15-q21
NM_002733	protein kinase, AMP-activated, gamma 1 non-catalytic subunit	PRKAG1	12q12-q14
NM_006262	peripherin	PRPH	12q12-q13
NM_002806	proteasome (prosome, macropain) 26S subunit, ATPase, 6	PSMC6	12q15
NM_002813	proteasome (prosome, macropain) 26S subunit, non-ATPase, 9	PSMD9	12q24.31-q24.32
NM_002822	protein tyrosine kinase 9	PTK9	12q12
NM_007062	nuclear phosphoprotein similar to S. cerevisiae PWP1	PWP1	12q24.11
NM_002870	RAB13, member RAS oncogene family	RAB13	12q13
NM_002868	RAB5B, member RAS oncogene family	RAB5B	12q13
NM_015646	RAP1B, member of RAS oncogene family	RAP1B	12q14
NM_006815	coated vesicle membrane protein	RNP24	12q24.31

Discussion

Because of the success of microarray analysis use in other biological systems and other eye tissue types, we sought to apply this technology to study gene expression in human donor scleral tissue. This analysis may prove useful for supplementing information regarding the genetic basis of normal 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 particular relevant for the sclera because the tissue is relatively less complex, can be manipulated *ex vivo*, and can be readily assessed visually.

Labeled, fragmented scleral cRNA hybridized to more than 58 % of the 12,626 probe sets represented on the microarray chip. There were 3751 genes with "present" calls assigned independently to all six human scleral samples. Only the 3751 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 3751 genes with confirmed scleral expression were analyzed using Genbank and LocusLink. Of the 3751 genes, 3096 (82.5%) had assigned chromosomal loci. Six hundred fifty five confirmed scleral genes (17.5 %) had unassigned chromosomal loci.

The 3751 genes detected with this microarray analysis form the basis of a scleral genetics web site named ScleraNet. ScleraNet includes the 3751 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. **Table 2** displays extracellular matrix genes expressed. **Tables 3-5** display expressed genes for three mapped loci for high myopia, chromosomes 18p11.31, 17q21-22, and 12q21-23, respectively.

Conclusion

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 has 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 protein 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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