Structural and Biochemical Analyses of Choroidal Thickness in Human Donor Eyes

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PURPOSE. The choroid plays a vital role in the health of the outer retina. While measurements of choroid using optical coherence tomography show altered thickness in aging and macular disease, detailed histopathologic and proteomic analyses are lacking. In this study we sought to evaluate biochemical differences in human donor eyes between very thin and thick choroids.

METHODS. One hundred forty-one eyes from 104 donors (mean age $\pm$ standard deviation, 81.5 $\pm$ 12.2) were studied. Macular sections were collected, and the distance between Bruch's membrane and the inner surface of the sclera was measured in control, early/dry age-related macular degeneration (AMD), neovascular AMD, and geographic atrophy eyes. Proteins from the RPE-choroid of eyes with thick and thin choroids were analyzed using two-dimensional electrophoresis and/or mass spectrometry. Two proteins with altered abundance were confirmed using Western blot analysis.

RESULTS. Donor eyes showed a normal distribution of thicknesses. Eyes with geographic atrophy had significantly thinner choroids than age-matched controls or early AMD eyes. Proteomic analysis showed higher levels of the serine protease SERPINA3 in thick choroids and increased levels of tissue inhibitor of metalloproteinases-3 (TIMP3) in thin choroids.

CONCLUSIONS. Consistent with clinical imaging observations, geographic atrophy was associated with choroidal thinning. Biochemical data suggest an alteration in the balance between proteases and protease inhibitors in eyes that lie at the extremes of choroidal thickness. An improved understanding of the basic mechanisms associated with choroidal thinning may guide the development of new therapies for AMD.

Keywords: choroid, age-related macular degeneration, protease

The choroid is the essential vascular bed that supplies the retinal pigment epithelium and the outer retina.^{1–4} The choroid and choriocapillaris play a critical role in diseases affecting the eye, including uveitic diseases,^{5–10} infections,^{8,11–13} and both primary^{14–16} and metastatic^{17–19} ocular tumors. More recently, there is accumulating evidence that variations in choroidal thickness may be associated with certain eye diseases. For example, in vivo spectral-domain optical coherence tomography (OCT) of the choroid consistently demonstrates a decrease in thickness with advancing age and myopia,^{20–24} both risk factors for central vision-disabling choroidal neovascularization (CNV). Several OCT studies of eyes with early age-related macular degeneration (AMD) without CNV show a thinning of choroid compared to that in age-matched controls.^{25,26} Eyes with AMD complicated by CNV show decreased subfoveal choroidal thickness after intravitreal anti-vascular endothelial growth factor (anti-VEGF) therapy.²⁷ Choroidal thickness changes after anti-VEGF therapy have even been observed in eyes being treated for diseases of the retinal vasculature.²⁸ On the other hand, eyes with central serous chorioretinopathy and idiopathic polypoidal choroidopathy have a significantly thicker choroid on OCT^{29–33} that can persist despite anti-VEGF therapy. While OCT has proven to be a valid measure for choroidal thickness with highly reproduc-

ible results^{34–37} and the occurrence of choroidal thinning has been described in clinical studies, the molecular basis of this phenomenon is not well understood. In the current study we sought to evaluate the most prominent molecular differences in human donor eyes with very thick and very thin choroids, and identified an imbalance between proteases and protease inhibitors in eyes with the thickest and thinnest choroids.

METHODS

Human Donor Eyes

Donor eyes were obtained from the Iowa Lions Eye Bank within 8 hours of death, following informed consent of the donors' next of kin. All research adhered to the tenets of the Declaration of Helsinki; Institutional Review Board approval is not required at the University of Iowa for donors post mortem. Eyes were either fixed in toto in 4% paraformaldehyde in phosphate-buffered saline (PBS) or dissected such that macular, temporal, inferior, and nasal punches of neural retina and RPE-choroid were flash frozen in liquid nitrogen for biochemical studies. The average age of eyes used in this study was 81.5 and standard deviation was 12.2, with a range from 21 to 100 years

FIGURE 1. Distribution of choroidal thicknesses in human donor maculae. Examples of thick (A) and thin (B) choroids in sections labeled with anti-CD45 antibody (green fluorescence) and *Ulex europaeus* agglutinin-I lectin (red fluorescence). The yellow lines indicate the measured distances used for donor eyes (i.e., the distance between Bruch's membrane and the inner surface of the sclera). Note the cross section of a short posterior ciliary artery in the sclera in (A) (arrow). A histogram showing the distribution of thicknesses of 141 donor eyes is illustrated in (C). Scale bar: 100 μm.

of age. All experiments were performed in accordance with the Declaration of Helsinki.

Quantification of Macular Thickness

Supertemporal wedges that included the macula or 8-mm punches, collected from the macula and centered on the fovea centralis, were cryoprotected in sucrose solution and embedded for cryostat sectioning, as described previously.³⁸ Cryostat sections were collected and stained with hematoxylin/eosin and photographed using a microscope (BX41; Olympus, Center Valley, PA) and a digital camera, calibrated using a stage micrometer. The distance between Bruch's membrane and the inner surface of the sclera, assessed on hematoxylin/eosin-stained sections, was determined in 141 eyes from 104 donors (Figs. 1A, 1B). Scatter plots were generated in control eyes plotting age against thickness, and statistical significance was determined by linear regression analysis using an online analysis tool (http://www.wessa.net/rwasp_linear_regression.wasp/ [in the public domain]). Ultrastructural analysis was also performed in temporal, juxtamacular choroid in four eyes with thick and four eyes with thin choroids (mean thickness 293

and 53.1 μm, respectively). Transmission electron microscopy was performed as described previously.³⁹

Identification of Proteins With Altered Abundance in Thin Choroids

A subset of eyes studied for morphology was also suitable for biochemical analyses. For initial experiments to identify major differences between thick and thin choroids, we prepared two pools of macular RPE-choroid protein; results were replicated in different eyes for confirmation (see below). Donors with thin ($n = 2$ eyes from two separate donors) and thick ($n = 2$ eyes from two separate donors) choroids were selected. The measured values for the thin set were 74.8 and 94.1 μm and for the thick group were 198.3 and 210.1 μm, respectively. Samples were homogenized using disposable tissue grinders (Kontes, Kimble Chase, Vineland, NJ) in an ice-cold solution consisting of 1% Triton X-100 in PBS with protease inhibitors (Complete kit; Roche, Indianapolis, IN), and two pools were generated, consisting of 100 μg total protein each.

Proteins were separated using two-dimensional (2D) gel electrophoresis (Kendrick Laboratories, Madison, WI). The conditions for the separation were 2% ampholyte in the first dimension and 10% acrylamide in the second (SDS-PAGE) dimension. Gels from the thin and thick pools were stained with mass spectrometry-compatible silver stain⁴⁰ and were aligned using UnwarpJ (a plug-in for ImageJ available at <http://rsb.info.nih.gov/ij/index.html> [in the public domain]). Spots were selected that differed between the two pools, and mass spectrometry of tryptic peptides was performed using liquid chromatography–tandem mass spectrometry (LC-MS/MS) on a LTQ XL linear ion trap mass spectrometer (Thermo Scientific, Waltham, MA) at the University of Iowa Proteomics Core. Tryptic peptides were matched to known proteins in the Swiss Prot database using a commercial software package (Scaffold 3; <http://www.proteomesoftware.com> [in the public domain]).

In parallel, a shotgun proteomic experiment was conducted in which two pools were generated from two thin and two thick choroids, respectively, using the same protein samples as above. Protein profiling was performed using a commercial vendor (Bioproximity LLC, Chantilly, VA). Tryptic peptides were generated and separated using LC-MS/MS (Easy nano LC II HPLC system; Thermo Scientific). Normalization and quantification was performed using the normalized spectral abundance factor (NSAF) method.⁴¹

Proteins whose abundances varied by more than 1.5-fold between thick and thin choroids were evaluated using the DAVID functional annotation website (david.abcc.ncifcrf.gov/ [in the public domain]).^{42,43}

Validation of Altered Expression of SERPINs and TIMP3

For assessment of levels of alpha-1-antichymotrypsin/serine protease SERPINA3 identified by 2D gel electrophoresis, additional thin and thick choroids were evaluated by Western blot analysis. Protein was extracted from juxtamacular, superior 6-mm punches of RPE-choroid, and blots were probed using antibodies directed against alpha-1-antichymotrypsin (0.7 µg/mL; Abcam, Cambridge MA) as described previously⁴⁴ except that Alexa 488-conjugated secondary antibodies (Invitrogen, Carlsbad, CA) were used for detection. Blots were stained with Ponceau-S (ST-180; Boston BioProducts, Ashland, MA) to verify that differences in protein abundance were not due to grossly different amounts of protein in each well.

Replication Study

For confirmation of upregulated tissue inhibitor of metalloproteinases-3 (TIMP3) levels, protein samples of RPE-choroid from five additional eyes with thin choroids (mean thickness 61.1 µm) and five with thick choroids (mean thickness 244.1 µm) were evaluated and probed with anti-TIMP3 antibody as described previously.⁴⁴ Protein loading was assessed using Ponceau-S as above.

RESULTS

Variability of Choroidal Thickness in Human Donor Eyes

In human choroids, with or without disease, we observed a range of macular thicknesses from ~31 to ~385 µm (Fig. 1C). The mean thickness was 150.0 µm, and the standard deviation was 71.1 µm. These measurements were somewhat lower than those observed clinically in perfused eyes using OCT (e.g., see Ref. 22).

Effects of Aging and AMD

Maculas from eyes without AMD were evaluated as a function of age. Hematoxylin-eosin-stained sections from 22 normal eyes, ages 57 to 93, were measured as described above. Eyes under 50 years of age were excluded for this comparison since they could not be designated as AMD or non-AMD. We observed decreasing thickness with increasing age (coefficient of determination = 0.19) that was statistically significant (*t*-statistic −2.7, *P* < 0.05).

In addition to these 22 control eyes, we also studied eyes with early/dry AMD (*n* = 21), neovascular AMD (*n* = 6), and geographic atrophy (*n* = 12), which were selected from our research eye bank collection as described previously.⁴⁵ There was no significant difference in choroidal thickness between age-matched donors and early AMD or eyes with CNV. In contrast, eyes with geographic atrophy showed significantly thinner choroids than any of the other categories (*P* < 0.01 for control versus geographic atrophy, *P* < 0.05 for dry AMD versus geographic atrophy, Fig. 2).

Ultrastructural Evaluation

Transmission electron microscopy was performed on a subset of eyes (thick and thin, four each). The most salient difference between the stroma of thick and thin choroids was the dense and abundant fibrillar collagen in the latter. While thick choroids showed a considerable network of bundled collagen, loosely packed and separated by fibroblast processes and other cellular elements, as well as ground substance, thin choroids showed a much higher density of collagen and elastin (Fig. 3).

Biochemical Detection of Differentially Expressed Proteins in Thick and Thin Choroids

Pools of thick and thin choroid proteins were prepared and separated using 2D gel electrophoresis. The overall pattern of protein spots was remarkably similar between thin and thick choroids (Fig. 4). Positive matches from differential spots included SERPINA3/alpha-1-antichymotrypsin (α-1-ACT) (spot series A), vimentin (spot B), and SERPINA1/alpha-1-antitrypsin (α-1-AT) (spot C).

Proteins with attenuated expression in thin choroids were validated by Western blot, using the original pools (Supplementary Fig. S1) on an additional five thin and five thick RPE-choroid punches (Fig. 5). The decreased abundance of α-1-antichymotrypsin/SERPINA3 was confirmed in both pools and in additional, unpooled, individual samples.

In a shotgun proteomics experiment, the protein with the largest increase in thick choroid samples, based on fold change, was another member of the serine protease inhibitor family (SERPINB1), an inhibitor of neutrophil elastase (Table 1). Thick choroid samples also showed higher levels of retinal proteins, which we assume is due to retinal contamination of one or both of the thick samples during dissection. In addition, thick choroid contained more C-reactive protein and macrophage-associated CD163. Proteins with at least 1.5-fold higher abundance in thick choroids were assessed using the DAVID analysis platform and were found to be most enriched in translational elongation/ribosome categories (Benjamini *P* values < 10^{−12}). Plasma proteins albumin and immunoglobulin heavy and light chains were similar between thick and thin pools (log2 ratio between 0 and 0.8), indicating that differences in plasma proteins are not a major source of difference between thick and thin pools in this experiment.

In the same analysis, TIMP3 was found to be more abundant in thin choroids (Table 2), in contrast to other protease inhibitors. Bioinformatic analysis of proteins that were more

FIGURE 2. Effects of age and AMD on choroidal thickness. (A) Scatter plot of age (x-axis) and choroidal thickness (y-axis) of non-AMD controls used in the study. Among samples included in this study, age showed a statistically significant negative relationship to thickness (slope $P < 0.05$), although the correlation was weak ($r^2 = 0.19$). (B) Donor maculae were categorized as controls (CTL), early/dry AMD (ARM), neovascular AMD (CNV), or geographic atrophy (GA). Eyes with geographic atrophy showed significant choroidal thinning compared to the other categories ($P < 0.05$). *Diamonds* indicate individuals, and *filled squares* indicate averages for each category.

abundant in thin choroids (≥ 1.5 -fold enriched) using the DAVID tool showed enrichment of proteins associated with oxidation-reduction and fatty acid metabolism (Benjamini corrected P values = 2.2×10^{-4} and $P = 7.2 \times 10^{-5}$, respectively).

Validation of TIMP3 levels was performed using an additional five thin and five thick samples, which confirmed an overall increase of TIMP3 protein in eyes with thin choroids (Fig. 6).

DISCUSSION

The choroid provides oxygen and other nutrients to the highly metabolic outer retina as well as to the RPE. With the widespread application of enhanced depth imaging (EDI)-

OCT, clinicians and scientists can now routinely visualize the choroid, qualitatively and quantitatively. One outcome of these studies in living patients is that the macular choroid has been found to vary in thickness by anatomic location, age, and, to a lesser degree, pharmacologic exposures. While there is not universal agreement on the importance of choroidal thickness in eye disease, choroidal atrophy is a recognized component of some forms of AMD where it is accompanied by subretinal drusenoid deposits.^{46,47} In this study we sought to characterize the anatomical and molecular bases of choroidal thickness in the maculas of human eye donors.

We found that donor eyes displayed a normal distribution of thickness (Fig. 1). The measurements made in the current study were overall somewhat thinner than those observed using OCT. This may be due to the nonlinearity of the OCT, which assesses the reflectivity of different interfaces, or it may

FIGURE 3. Examples of ultrastructure of thick (A, B) and thin (C, D) choroidal stroma. Collagen fibrils (f) in thick choroids were present in bundles that were loosely packed, with considerable intervening ground substance and fibrocyte processes (fp). Thin choroids showed much higher density of fibrillar collagen, without appreciable ground substance (electron-lucent material in [A] and [B]). Note the large coarse collagen fibrils (cf) in (C). ec, endothelial cell; m, melanocytes; v, vascular lumen. Scale bar: 2 μ m.

simply reflect the lack of choroidal perfusion in a deceased individual. The latter possibility is supported by findings that drugs that increase blood flow, such as sildenafil citrate, induce choroidal swelling that is measurable by EDI-OCT.⁴⁸

Ramrattan and colleagues⁴⁹ previously characterized changes in several morphometric parameters including choroidal thickness in aging in donor eyes. One interesting finding in their study was that choroidal thickness appears to decline

FIGURE 4. Comparison of thick and thin choroids by 2D gel electrophoresis. Pools of thick (left) and thin (right) RPE-choroids were evaluated. Three differences that were also observed with mass spectrometry are indicated by arrows (A, alpha-1-antichymotrypsin; B, vimentin; C, alpha-1-antitrypsin).

FIGURE 5. Western blot showing elevated alpha-1 antichymotrypsin/SERPINA3 in thick choroids. *Left:* Ponceau S protein stain; *right:* same blot probed with antibodies directed against SERPINA3/alpha-1 antichymotrypsin.

with age, with most of the trend over the first five decades of life. Our findings reveal a statistically significant but numerically modest age-related decline; however, it should be noted that only three eyes under age 50 were included in the current study. It is also noteworthy that our thickness measurements made using frozen sections were in good agreement with those obtained by Ramrattan et al.⁴⁹ using paraffin sections.

To identify factors associated with the extremes of choroidal thickness, we performed an experiment to evaluate differences in proteins between the thickest and thinnest samples. We found that eyes with thin choroids have reduced levels of vimentin, a cytoskeletal protein expressed by choroidal fibroblasts,⁵⁰ which may be consistent with loss of cellularity in thin choroids. Thin choroids also showed reduced levels of serine protease inhibitors (SERPINs) compared to those with thick choroids. In contrast, the metalloprotease inhibitor TIMP3 was more abundant in thin choroids than in thick choroids. These findings were validated by Western blot experiments using unpooled samples ($n = 5$ human eyes/category).

The SERPINs alpha-1-antitrypsin and alpha-1-antichymotrypsin inhibit a range of serine proteases and elastases involved in tissue remodeling, including neutrophil elastases.^{51,52} A reduction in the activity of SERPINs, observed in the thin choroid group, might be expected to allow increased

proteolysis by enzymes such as cathepsin G, which has been linked to degradation of proteoglycans present in ground substance of connective tissues.⁵³ Increased degradation of extracellular ground substance components may lead to collapse of the choroid and choroidal thinning. Thus, the general finding that SERPINs were less abundant in thin choroids seems mechanistically logical and straightforward.

In contrast, the relative abundance of TIMP3 in thin choroids seems counterintuitive, since TIMP3 inhibits several metalloproteases and, at least simplistically, would be expected to prevent matrix breakdown. However, mutations in the *TIMP3* gene are responsible for Sorsby fundus dystrophy, an autosomal dominant maculopathy characterized by extracellular matrix imbalance in Bruch's membrane^{54,55} and a dramatic thickening of the latter structure. Also, mice lacking TIMP3 develop an abnormal, dilated choroidal vasculature,⁵⁶ and TIMP3 itself interferes with VEGF-VEGF receptor interactions.⁵⁷ It is therefore plausible that an increased level of TIMP3 might inhibit normal physiological vascular repair and turnover in the choroid, resulting in eventual choroidal thinning. Consistent with this notion, induction of TIMP3 overexpression in tumors was recently shown to be associated with a high density of endothelial cells, but without feeder vessels or pericytes, and with reduced VE-cadherin expression.⁵⁸ Thus, at least in the abnormal tumor microenviron-

TABLE 1. Proteins More Abundant in Thick RPE-Choroid

ENSEMBL Protein Name	NSAF Thin	NSAF Thick	Base 2 Log Ratio	Gene Name	Description
ENSP00000370115	2.83	71.75	-4.67	<i>SERPINA1</i>	Serpin peptidase inhibitor, clade B (ovalbumin), member 1
ENSP00000255030	4.78	117.22	-4.62	<i>CRP</i>	C-reactive protein, pentraxin-related
ENSP00000278833	3.05	66.79	-4.45	<i>ROM1</i>	Retinal outer segment membrane protein 1
ENSP00000354513	1.99	43.58	-4.45	<i>CALD1</i>	Caldesmon 1
ENSP00000230381	3.10	48.79	-3.98	<i>PRPH2</i>	Peripherin 2 (retinal degeneration, slow)
ENSP00000255266	1.25	19.63	-3.98	<i>PDE6A</i>	Phosphodiesterase 6A, cGMP-specific, rod, alpha
ENSP00000352071	1.85	26.77	-3.85	<i>CD163</i>	CD163 molecule
ENSP00000255945	3.26	42.76	-3.72	<i>GIMAP4</i>	GTPase, IMAF family member 4
ENSP00000307705	4.89	64.23	-3.72	<i>HIST1H1E</i>	Histone cluster 1, H1e
ENSP00000319690	3.50	45.97	-3.72	<i>HNRNPC</i>	Heterogeneous nuclear ribonucleoprotein C (C1/C2)
ENSP00000369741	23.54	298.85	-3.67	<i>RPS6</i>	Ribosomal protein S6
ENSP00000274364	0.68	7.74	-3.51	<i>IQGAP2</i>	IQ motif containing GTPase activating protein 2
ENSP00000345156	4.98	56.70	-3.51	<i>RPL14</i>	Ribosomal protein L14
ENSP00000239761	0.74	7.73	-3.39	<i>MRC1</i>	Mannose receptor, C type 1
ENSP00000264605	1.79	18.76	-3.39	<i>MLPH</i>	Melanophilin
ENSP00000354791	0.84	8.81	-3.39	<i>DCTN1</i>	Dynactin 1
ENSP00000334876	1.90	18.32	-3.27	<i>GRK1</i>	G protein-coupled receptor kinase 1
ENSP00000354739	6.49	62.52	-3.27	<i>RPL12</i>	Ribosomal protein L12
ENSP00000359345	7.21	66.31	-3.20	<i>RPL5</i>	Ribosomal protein L5
ENSP00000371393	1.75	15.32	-3.13	<i>PGM2</i>	Phosphoglucomutase 2

TABLE 2. Proteins More Abundant in Thin RPE-Choroid

ENSEMBL Protein Name	NSAF Thin	NSAF Thick	Base 2 Log Ratio	Gene Name	Description
ENSP00000266085	416.25	4.44	6.55	<i>TIMP3</i>	TIMP metalloproteinase inhibitor 3
ENSP00000343577	105.16	3.41	4.95	<i>TPSAB1</i>	Tryptase alpha/beta 1
ENSP00000332256	43.93	1.83	4.58	<i>ALDH1A3</i>	Aldehyde dehydrogenase 1 family, member A3
ENSP00000311489	8.96	0.39	4.51	<i>SPTBN2</i>	Spectrin, beta, non-erythrocytic 2
ENSP00000313420	3.11	0.23	3.78	<i>PRKDC</i>	Protein kinase, DNA-activated, catalytic polypeptide
ENSP00000222573	13.93	1.22	3.51	<i>ITGB8</i>	Integrin, beta 8
ENSP00000231887	14.81	1.30	3.51	<i>EHHADH</i>	Enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase
ENSP00000273784	52.39	5.10	3.36	<i>AHSG</i>	Alpha-2-HS-glycoprotein
ENSP00000347665	6.35	0.62	3.36	<i>COL18A1</i>	Collagen, type XVIII, alpha 1
ENSP00000265631	12.69	1.39	3.19	<i>SLC25A13</i>	Solute carrier family 25 (aspartate/glutamate carrier), member 13
ENSP00000268251	17.14	1.88	3.19	<i>ABAT</i>	4-Aminobutyrate aminotransferase
ENSP00000348762	11.71	1.28	3.19	<i>LSS</i>	Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)
ENSP00000296046	17.98	2.25	3.00	<i>CPA3</i>	Carboxypeptidase A3 (mast cell)
ENSP00000321988	25.42	3.18	3.00	<i>SULT1A1</i>	Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1
ENSP00000369927	46.42	5.81	3.00	<i>AKR1C3</i>	Aldo-keto reductase family 1, member C3
ENSP00000221283	10.84	1.58	2.78	<i>STXBP2</i>	Syntaxin binding protein 2
ENSP00000225275	8.63	1.26	2.78	<i>MPO</i>	Myeloperoxidase
ENSP00000262211	12.96	1.89	2.78	<i>SGK3</i>	Serum/glucocorticoid regulated kinase family, member 3
ENSP00000363639	75.61	11.03	2.78	<i>TXN</i>	Thioredoxin
ENSP00000367281	12.90	1.88	2.78	<i>BEST1</i>	Bestrophin 1

ment, TIMP3 may suppress endothelial cell differentiation. A similar pathway in choroid could affect its thickness. It will be of interest to determine whether the choroidal endothelial cells show altered differentiation in cases of choroidal thinning.

In a large study combining RT-PCR and morphometry, Bailey and colleagues⁵⁹ evaluated the relationship between TIMP3 expression and Bruch's membrane thickness, and found that TIMP3 RNA was more abundant in eyes with a thin Bruch's membrane. Our findings on TIMP3 protein and choroidal thickness are consistent with these findings. Although Bruch's membrane thickness was contained within the measurements in the current study, it represented less than 5% of the measured choroidal thickness, and differences at the extremes of choroidal thickness were therefore not due to differences at the level of Bruch's membrane.

In summary, we have identified altered abundance of different classes of protease inhibitors in thick and thin human choroids. In view of the altered choroidal thickness observed in macular diseases, especially central serous chorioretinop-

athy and geographic atrophy, an understanding of the basic mechanisms associated with choroidal thinning will provide better insight into pathogenesis of these diseases and guide the development of novel therapies.

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FIGURE 6. Western blot showing abundance of TIMP3 in thin (left lanes) compared to thick (right lanes) choroids. Each lane contains 15 μ g total protein from a separate RPE-choroid punch and from a different donor. Blots were probed with anti-TIMP3 antibody. As a group, TIMP3 abundance was notably higher in thin choroids.

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