Decorin Accumulation Contributes to the Stromal Opacities Found in Congenital Stromal Corneal Dystrophy

Cecilie Bredrup,[1,2,3] *Espen Stang,*[4] *Ove Bruland,*[3] *Barbara P. Palka,*[5] *Robert D. Young,*[5] *Jan Haavik,*[6] *Per M. Knappskog,*[1,3] *and Eyvind Rødahl*[1,2]

Purpose. Congenital stromal corneal dystrophy (CSCD) is characterized by stromal opacities that morphologically are seen as interlamellar layers of amorphous substance with small filaments, the nature of which has hitherto been unknown. CSCD is associated with truncating mutations in the decorin gene (*DCN*). To understand the molecular basis for the corneal opacities we analyzed the expression of decorin in this disease, both at the morphologic and the molecular level.

Methods. Corneal specimens were examined after contrast enhancement with cuprolinic blue and by immunoelectron microscopy. Decorin protein from corneal tissue and keratocyte culture was studied by immunoblot analysis before and after O- and N-deglycosylation. The relative level of *DCN* mRNA expression was examined using Q-RT-PCR, and cDNA was sequenced. Recombinant wild-type and truncated decorin transiently expressed in HEK293 cells were analyzed by gel filtration and immunoblotting.

Results. The areas of interlamellar filaments were stained by cuprolinic blue. Immunoelectron microscopy using decorin antibodies revealed intense labeling of these areas. Both wild-type and truncated decorin protein was expressed in corneal tissue and keratocytes of affected persons. When decorin expressed in HEK293 cells was examined by gel filtration, the truncated decorin eluted as high molecular weight aggregates.

Conclusions. Accumulation of decorin was found in the interlamellar areas of amorphous substance. The truncated decorin is present in CSCD corneas, and there is evidence it may aggregate in vitro. Thus, decorin accumulation appears to contribute to the stromal opacities that are characteristic of CSCD. (*Invest Ophthalmol Vis Sci.* 2010;51:5578–5582) DOI:10.1167/iovs.09-4933

Congenital stromal corneal dystrophy (CSCD; Online Mendelian Inheritance in Man 610048) is a rare autosomal dominant disorder characterized by small opacities found throughout the stroma. These small flakes and spots are present shortly after birth and are thought to be slowly progressive. Most patients will need bilateral corneal transplantations as young adults. By transmission electron microscopy, the opacities can be visualized as layers of thin filaments embedded in an amorphous material that separates apparently normal stromal lamellae. The nature of these opacities has hitherto been unknown. Genetic analysis in two CSCD families has revealed frameshift mutations in the C-terminal part of the decorin gene in affected persons.[1,2] Both mutations (c.967delT and c.941delC) are predicted to introduce the same premature stop codon, causing the deletion of 33 amino acids in the C-terminal end of the decorin protein.[1,2]

Decorin is a small leucine-rich proteoglycan (SLRP) involved in several important biological processes, including collagen fibrillogenesis and matrix assembly.[3,4] Decorin-deficient mice have abnormal collagen morphology in skin and tendons but have clear corneas.[5] This indicates that decorin is not essential for corneal transparency. To improve our understanding of why heterozygous truncating mutations in the decorin gene cause corneal clouding, we analyzed decorin expression in CSCD corneas morphologically and at a molecular level.

Materials and Methods

Patients with CSCD and unaffected persons were invited to participate in the study. Informed written consent was obtained from all participants. The study was approved by the Regional Committee for Medical and Research Ethics, Western Norway (IRB 00001872) and adhered to the tenets of the Declaration of Helsinki.

Tissue Samples

Skin biopsy samples were obtained from the forearm skin of affected persons carrying the c.967delT mutation and from healthy family members. A CSCD corneal button was available after therapeutic corneal grafting. The clinical findings of the patient have previously been described (V-6 in Bredrup et al.[1]). An unaffected cornea was obtained from an unrelated person who had an eye removed because of malignant choroidal melanoma.

Cuprolinic Blue Staining

CSCD and healthy corneal samples were plunge frozen and stored in liquid nitrogen. Thawed samples were fixed overnight in 2.5% glutaraldehyde in 25 mM sodium acetate buffer, pH 5.7, containing 0.1 M magnesium chloride and 0.05% cuprolinic blue (BioSciences, Warrington, PA). The samples were washed in 25 mM sodium acetate buffer, then transferred to aqueous 0.5% sodium tungstate for 15 minutes, followed by 50% ethanolic 0.5% sodium tungstate for 15 minutes. Specimens were dehydrated in an ascending ethanol series, followed by propylene oxide, and were infiltrated with Araldite resin. They were embedded in moulds with fresh resin and were cured for 24

From the Departments of [1]Clinical Medicine and [6]Biomedicine, University of Bergen, Bergen, Norway; the [2]Department of Ophthalmology and the [3]Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway; the [4]Department of Pathology, Oslo University Hospital, Rikshospitalet, Oslo, Norway; and the [5]Structural Biophysics Group, School of Optometry and Vision Sciences, Cardiff University, Cardiff, Wales, United Kingdom.

Supported by grants from the Western Norway Regional Health Authority.

Submitted for publication November 16, 2009; revised March 26 and April 16, 2010; accepted May 8, 2010.

Disclosure: **C. Bredrup**, None; **E. Stang**, None; **O. Bruland**, None; **B.P. Palka**, None; **R.D. Young**, None; **J. Haavik**, None; **P.M. Knappskog**, None; **E. Rødahl**, None

Corresponding author: Eyvind Rødahl, Department of Ophthalmology, Haukeland University Hospital, 5021 Bergen, Norway; eyvind.rodahl@helse-bergen.no.

hours at 60°C. Ultrathin sections were cut with a diamond knife, collected on uncoated copper grids, stained with 1% phosphotungstic acid and saturated uranyl acetate solutions, and examined in a transmission electron microscope (EM208; Philips, Eindhoven, The Netherlands).

Immunoelectron Microscopy

Corneal specimens were fixed using 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer for 2 hours and processed for cryosectioning and immunolabeling essentially as previously described.[6] Some sections were incubated with 0.4 U/mL chondroitinase ABC in 0.1 M Tris acetate, pH 6.8, for 1 hour at room temperature and washed in PBS before blocking and immunolabeling. Bound antibodies were visualized using protein A gold (G. Posthuma, Utrecht, The Netherlands). The sections were examined using an electron microscope (CM 120; Philips).[7]

Three decorin antibodies were used—goat anti–human decorin antibody AF143 (R&D Systems, Minneapolis, MN) and two rabbit antisera, LF-136 and LF-122, generously provided by Larry Fisher (Dept. of Health and Human Services, NIH, Bethesda, MD). The target for LF-136 is an N-terminal peptide,[8] and that for LF-122[9] and AF143 is recombinant human decorin. When labeling with the goat anti-decorin antibody, a rabbit anti-goat IgG antibody (Cappel; ICN Biochemicals Costa Mesa, CA) was used as secondary antibody before incubation with protein A gold.

Extraction of Decorin from Corneal Tissue

Untreated corneal samples that had been stored in liquid nitrogen were added to 200 μL lysis buffer (5 mM EDTA, 150 mM NaCl, 0.002 M phenylmethylsulfonyl fluoride, 50 mM Tris-HCl, pH 7.5) containing 1 μL protease inhibitor cocktail (P8340; Sigma-Aldrich, St. Louis, MO). The sample was homogenized (TissueLyser II; Qiagen, Hilden, Germany) for 200 seconds before the addition of 1 μL Nonidet P-40, 1 μL Tween 20, and 1 μL 20% sodium dodecyl sulphate (SDS) and was incubated at 37°C for 30 minutes. The sample was centrifuged at 16000g for 10 minutes, and the supernatant was collected.

Keratocyte and Fibroblast Cultures

Skin biopsy specimens and part of the corneal buttons were cut into small pieces. Keratocytes and fibroblasts were cultivated (Amniochrome II with Amniochrome II Supplement modified; Lonza, Verviers, Belgium) at 37°C with 5% CO_2. When fully confluent, the cells were transferred (Amniochrome II without Supplement; Lonza), and the medium was harvested after 6 hours. Cells were lysed using 50 mM Tris-HCl, pH 7.5, containing 200 mM NaCl, 5 mM EDTA, 1% Igepal, 1 μg/mL aprotinin, 1 mM phenylmethylsulfonyl fluoride, complete protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany), 0.5% Tween, and 0.1% SDS.

RNA Analyses

Total RNA was purified from the corneal specimen and HEK293 cells (TissueLyzer and RNeasy kits; Qiagen). The quality of the purified RNA was analyzed (Experion system; Bio-Rad, Hercules, CA), and cDNA synthesis was performed (TaqMan Reverse Transcription kit; ABI, Foster City, CA). *DCN* RNA expression was determined using quantitative reverse transcriptase-PCR (Q-RT-PCR) using ABI primers and probes (Assay on Demand; HS00266491) and was analyzed (7900 instrument; ABI). Expression of β-actin was used as an endogenous normalization control.

PCR amplification of *DCN* from cDNA of cultured corneal cells was performed with forward primer (5′-GAGGGAGCTTCACTTGGACAACA-3′) and reverse primer (5′-GAATGGCAGAGCGCACGTAG-3′), and the PCR product was sequenced.

Construction of Plasmids

Both wild-type and mutant *DCN* from a heterozygous sample were cloned by PCR amplification of cDNA using forward primer (5′-cgcggatccGCAAATTCCCGGATTAAA-3′) and reverse primer (5′-ctagtctagaTGCATAATAAGTCATGTGGGTAA-3′), with sequences incorporating restriction sites facilitating cloning added at the 5′-end (lower caps). PCR products were digested and ligated into the pCDNA3.1(+) vector (Invitrogen, Carlsbad, CA) using the *Bam*HI and *Xba*I restriction enzymes (NEB; Ipswich, MA). All plasmids were verified by DNA sequencing.

Transient Transfection of Cells

Human embryonic kidney cells (HEK293) were grown in Dulbecco's modified Eagle medium (DMEM; Gibco, Auckland, New Zealand) supplemented with 10% fetal calf serum. Six million cells were plated onto 10-cm dishes the day before transfection to obtain 90% to 95% confluence at transfection. The transfection mixture contained 60 μL reagent (Lipofectamine 2000; Invitrogen), 24 μg expression vector (wild-type and mutated decorin), or empty vector as a negative control. Transfection was performed according to the manufacturer's recommendations. A GFP expression vector (pSiren RetroQ, 2.5 μg; Clontech Laboratories, Mountain View, CA) was added as an internal control of transfection efficacy. The medium was changed daily. At 48 hours after transfection, the cells were transferred to serum-free DMEM for 6 hours, and the medium was then harvested.

N- and O-Deglycosylation Using Chondroitinase ABC and N-Glycosidase F

Homogenized corneal tissue or cell culture medium (50 μL) with 1 μL protease inhibitor cocktail P8340 and 10 μL chondroitinase ABC (Sigma-Aldrich; 0.01 U/μL in 50 mM Tris-HCl buffer, pH 8.0, containing 60 mM sodium acetate and 0.02% bovine serum albumin [BSA]) was incubated at 37°C for 6 hours. After acetone precipitation, the pellet was dissolved in either 20 μL sample buffer (Invitrogen) for immunoblotting or in 25 μL of 20 mM sodium phosphate buffer, pH 7.2, containing 1% SDS for further N-deglycosidase F digestion.

For N-deglycosylation alone, a sample with acetone-precipitated cell culture medium (50 μL) dissolved in 25 μL of 20 mM sodium phosphate buffer, pH 7.2, containing 1% SDS was prepared. Both this and the sample that had previously undergone O-deglycosylation were denaturated by heating to 100°C for 2 minutes. The reaction mixtures were then adjusted to contain 10 mM EDTA, 0.5% Nonidet P-40, 0.1% SDS, and 1% 2-mercaptoetanol in 20 mM sodium phosphate buffer, pH 7.2, in a total volume of 150 μL. One unit of N-glycosidase F (Roche Diagnostics GmbH) was added, and the samples were incubated for 1 hour at 37°C. One additional unit of N-glycosidase F was then added, and the incubations continued for another hour.

Western Blot Analysis

Proteins were separated with a high-resolution gel system (12% NuPAGE Novex Bis-Tris Gel; Invitrogen) according to the manufacturer's instructions and were electrophoretically transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA). After blocking with 5% nonfat dry milk (Bio-Rad), 1% glycine, and 1% BSA in PBS-T buffer (PBS containing 0.05% Tween 20), the membranes were incubated overnight at 4°C with goat anti–human decorin antibody (R&D Systems) at a dilution of 0.05 μg/mL. Membranes were washed with PBS containing 0.05% Tween 20 and incubated with horseradish peroxidase–conjugated anti–goat IgG (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 0.2 μg/mL for 2 hours at room temperature. Proteins were visualized (Super Signal West Pico system; Pierce, Rockford, IL), and protein standard (MagicMark; Invitrogen) was used as a molecular weight marker.

Gel Filtration of Decorin

Medium from transfected HEK293 cells was concentrated approximately 50 times with a centrifugal filter (Amicon Ultra, 10K NMWL; Millipore, Billerica, MA) and diluted in 10 mM HEPES buffer, pH 7.4,

containing 150 mM NaCl, 3.4 mM EDTA, and 0.005% (vol/vol) surfactant P20.

Samples (500 μL) were run on a column (Superdex 200 HR 10/30; Amersham Biosciences, Piscataway, NJ) connected to a chromatography system (BioLogic HR; Bio-Rad) with absorbance detection at 280 nm and a flow rate of 0.2 mL/min at 4°C. The column was equilibrated in 10 mM HEPES buffer, pH 7.4, containing 150 mM NaCl, 3.4 mM EDTA, and 0.005% (vol/vol) surfactant P20. The column was calibrated using the following proteins as standards: catalase (MWt. 250 kDa), BSA (MWt, 66 kDa), and lysozyme (MWt, 14.3 kDa). Fractions (500 μL) were collected and subjected to immunoblot analysis using affinity-purified antibodies against recombinant human decorin.

Results

Cuprolinic Blue Staining

The healthy corneal sample had a normal ultrastructure. Some fracture lines, interpreted as freeze-thaw artifacts, were found (Fig. 1A). In contrast, the CSCD cornea was severely disorganized. Areas of amorphous substance with small filaments disrupted layers of apparently normal collagen fibrils. These areas were often located in the immediate vicinity of keratocytes. In some places, the lamellar structure of collagen fibrils appeared to disintegrate (Fig. 1B). The abnormal filaments seen in the CSCD cornea stained with cuprolinic blue, indicating the presence of sulfated glycosaminoglycans (GAGs; Fig. 1D). Cuprolinic blue staining was also found along the collagen fibrils in CSCD and in healthy corneas (Fig. 1).

Immunoelectron Microscopy

When examining normal and CSCD corneas by immunoelectron microscopy, decorin immunolabeling was located along collagen fibrils. This was particularly prominent when using the R&D System goat anti-decorin antibody (Fig. 2). A similar pattern was seen with antibody LF-136, whereas LF-122 did not label the collagen fibrils (data not shown). In addition, strong decorin immunolabeling was found in the abnormal areas of CSCD cornea, particularly in the areas with abnormal filaments. This finding was similar for all three decorin antibodies. Decorin immunolabeling of keratocytes was not above background. Chondroitinase ABC treatment strongly increased decorin immunolabeling (Fig. 2), particularly for antibody LF-122 (data not shown).

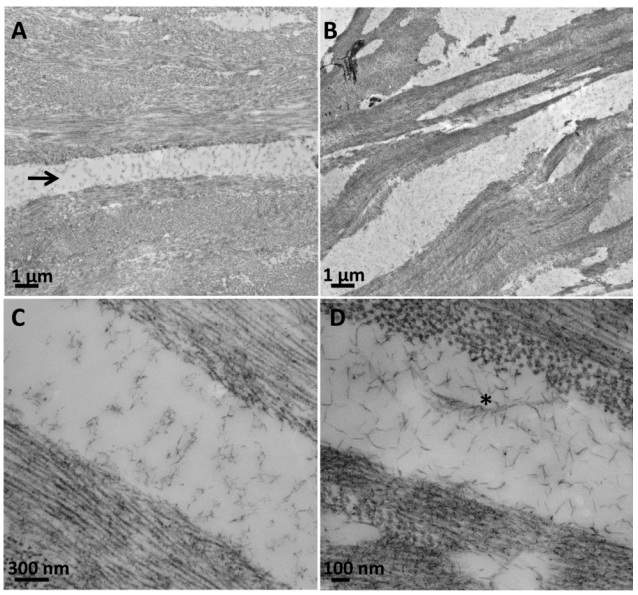

Figure 1. Electron micrographs of corneal stroma after cuprolinic blue staining. (**A**) Overview of healthy cornea showing normal stromal architecture with some freeze-thaw breaks (*arrow*). (**B**) CSCD cornea with areas of small filaments separating what appear to be normal lamellae. (**C**) Detail of a freeze-thaw break in healthy cornea showing minimal staining with cuprolinic blue. (**D**) Detail of an area with abnormal filaments showing strong staining with cuprolinic blue. *Asterisk*: in some places the filaments seem to form longer structures.

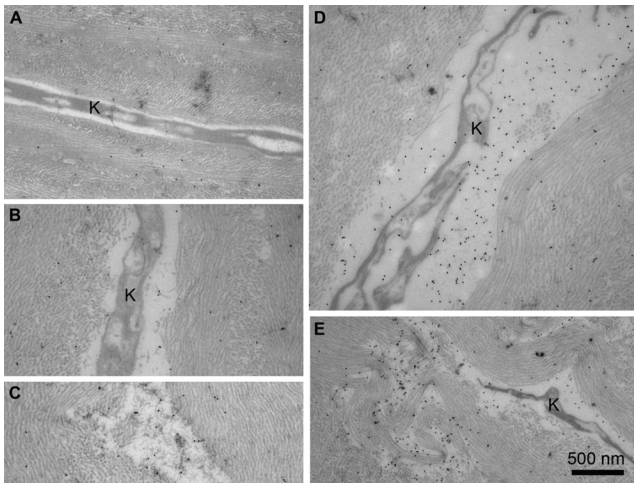

Figure 2. Electron micrographs of corneal stroma after labeling with an anti–decorin antibody. (**A**) Section of healthy cornea treated with chondroitinase ABC before labeling. Labeling is diffusely distributed along collagen fibrils. (**B**–**E**) Sections of CSCD cornea without (**B**, **C**) or with (**D**, **E**) chondroitinase ABC treatment before labeling. Labeling is localized to small filaments in the amorphous area near keratocytes (K; **B**, **D**), and in areas with abnormal filaments (**C**, **E**).

Decorin Expression in Patient Samples

Primary keratocyte cultures from a person heterozygous for the c.967delT mutation (hetDCN) and from an unaffected person (normDCN) were established. No major differences regarding the morphology of the cells could be observed. Both cell types had to be fully confluent for high levels of decorin to be secreted into the medium. Sequencing of cDNA from cultured hetDCN corneal cells revealed that both wild-type transcripts and transcripts with the deletion were present (data not shown).

Supernatant from homogenized cornea and medium from cultured keratocytes were examined by immunoblot analysis before and after treatment with chondroitinase ABC and N-glycosidase F. In both hetDCN and normDCN samples, decorin was detected as a diffuse band with molecular weights of approximately 80 kDa and 90 kDa in the medium from keratocyte culture and homogenized cornea, respectively. The wild-type decorin expressed in hetDCN samples, particularly from corneal tissue, appeared to migrate slightly faster than that expressed in the normDCN samples. No difference in glycosylation was observed, as assessed by chondroitinase ABC or N-glycosidase F treatment between hetDCN and normDCN samples (Fig. 3).

After treatment with chondroitinase ABC and N-glycosidase F, a single band of approximately 41 kDa, as determined by molecular weight markers (MagicMark; Invitrogen) was observed in the normDCN samples, whereas two clearly distinguishable bands (41 and 37 kDa) were seen in the hetDCN samples (Fig. 3). The same pattern was seen in cultured fibroblasts (data not shown). Decorin was not detected by immunoblot analysis of normDCN and hetDCN keratocyte lysates (data not shown).

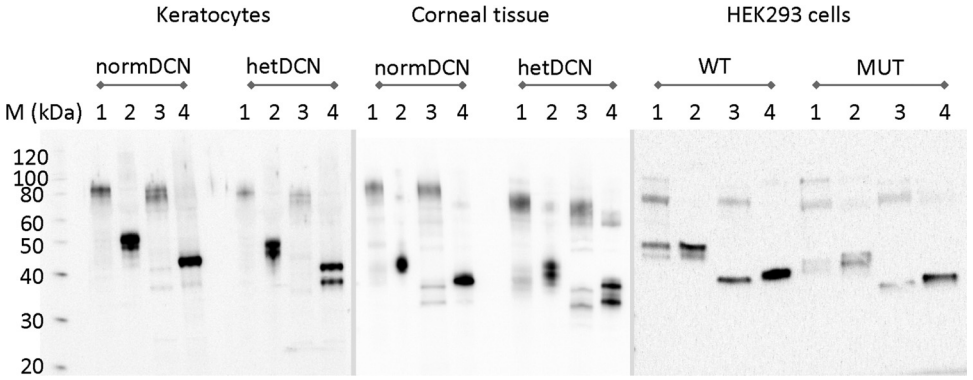

FIGURE 3. Western blot analysis of decorin. Shown are untreated samples (1), samples treated with chondroitinase ABC (2), N-glycosidase F (3), and samples treated with both chondroitinase ABC and N-glycosidase F (4) from keratocytes, corneal tissue, and HEK293 cells. Keratocytes and corneal tissue were obtained from either a healthy cornea (normDCN) or a CSCD cornea heterozygous for the c.967delT mutation (hetDCN). The HEK293 cells had been transfected with either WT or MUT *DCN* cDNA. Separate gels were used for samples from keratocytes, corneal tissue, and HEK 293 cells. Different migration between gels is attributed to variation in electrophoresis time. M, molecular weight marker.

Transient Transfection of Cells

HEK293 cells were transfected with plasmids containing wild-type decorin (WT) or *DCN* cDNA with the c.967delT mutation (MUT). Low endogenous expression of decorin was found in HEK293 cells by Q-RT-PCR, with a cycle threshold of approximately 35. Decorin could not be identified by immunoblot analysis of cells transfected with GFP expression vector alone (data not shown). On immunoblot analysis, decorin was detected as four bands (80, 65, 48, and 45 kDa; Fig. 3). After complete N- and O-deglycosylation, a single band of 39 kDa and 35 kDa in the WT and MUT samples, respectively, was left. All bands in the MUT sample had slightly lower molecular weight corresponding to the expression of a truncated protein (Fig. 3).

Gel Filtration

Medium from WT- and MUT-transfected HEK293 cells were subjected to size exclusion chromatography using a high-resolution gel filtration column (Superdex 200 HR 10/30; Amersham Biosciences; Freiburg, Germany). The fractionation range for globular proteins on this column is between 10 and 600 kDa. The UV absorption profile was similar for WT and MUT media. Fractions were subjected to immunoblot analysis; in the WT medium, decorin was detected across 12 fractions in a broad molecular weight range. Medium from MUT-transfected cells showed a different pattern. Here decorin was found in only five fractions starting in the void volume of the column, corresponding to the presence of decorin as aggregates (Fig. 4).

Immunoblot analyses of early WT fractions showed decorin as a diffuse band with an estimated molecular weight around 80 kDa. The molecular weight was gradually reduced in later fractions, suggesting varying degrees of glycosylation of decorin. In all MUT fractions, decorin was detected as a major band with an estimated molecular weight of 40 kDa. Higher molecular weight bands were also observed (Fig. 4).

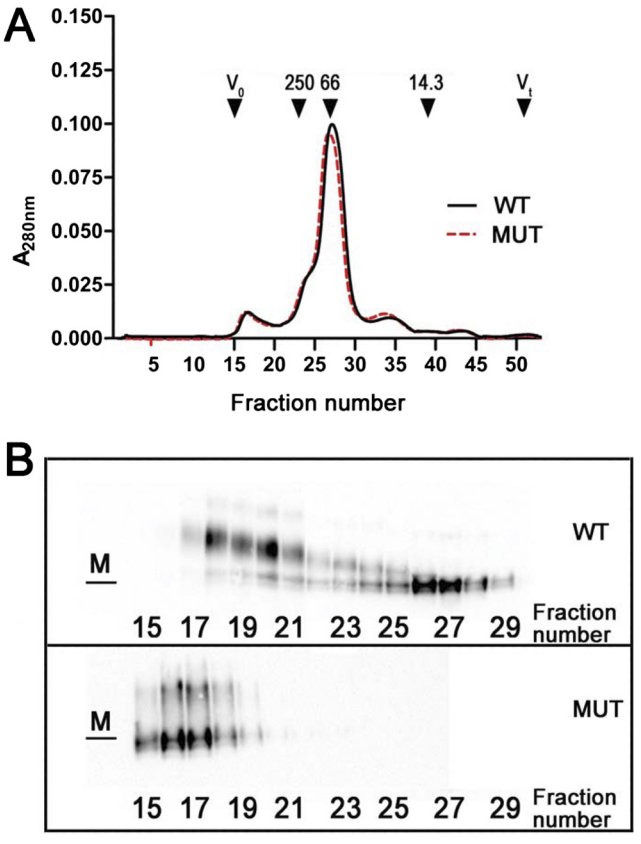

FIGURE 4. Size exclusion chromatography of medium from transfected HEK293 cells. (**A**) UV absorption of WT and MUT media. The fractionation range for globular proteins on this column is between 10 and 600 kDa. (**B**) Immunoblot analysis of WT and MUT fractions. In the WT medium, decorin is present in a broad molecular weight range, whereas in the MUT medium, decorin is found in a few early fractions, starting in the void volume of the column. *Downward arrows*: position of protein standards; molecular weights are shown above. Catalase (MWt, 250 kDa), BSA (MWt, 66 kDa), lysozyme (MWt, 14.3 kDa). Vo, void volume; V_t, total volume; M, molecular weight marker corresponding to 40 kDa.

DISCUSSION

CSCD is characterized by stromal layers of abnormal filaments, randomly arranged and loosely embedded in an amorphous substance.[1,10–12] In the present study a CSCD cornea, exhibiting these typical findings, was available for cuprolinic blue staining and immunoelectron microscopy. The abnormal filaments were found to stain with cuprolinic blue, indicating the

presence of sulfated GAGs. Immunoelectron microscopy revealed staining with decorin antibodies in these areas and where disorganized lamellae were found. Staining was stronger after treatment with chondroitinase ABC, most likely because the GAG chain of decorin disturbs antibody recognition.[13] Deposition of various substances in the keratocytes or extracellular matrix has been found in several other stromal dystrophies. Examples include the transforming growth factor-β–induced gene (*TGFBI*) corneal dystrophies, lattice corneal dystrophy type II, Schnyder corneal dystrophy, and macular corneal dystrophy.[14,15]

The two decorin mutations found associated with CSCD, c.967delT and c.941delC, are predicted to produce a truncated protein because of the deletion of 33 C-terminal amino acids. Analyses of mRNA in a person carrying the c.967delT mutation showed that both the wild-type and the mutated allele are transcribed. By Western blot analysis, the presence of both WT and truncated decorin protein could be observed in corneal tissue and in keratocyte and fibroblast cell cultures. Decorin contains a single chondroitin/dermatan sulfate GAG chain attached to a serine residue near the N terminus[16] and three N-linked oligosaccharides.[17] These would theoretically not be affected by the C-terminal decorin truncation found in CSCD.

Decorin is a class I SLRP with a core of 12 leucine-rich-repeats (LRR) flanked by cysteine-rich capping domains important for the stability of the protein.[18] The LRR domain forms a curved solenoid fold, with the second-to-last LRR (the ear repeat) spanning away from the main solenoid and coming back at the last LRR. The truncation is likely to disrupt the disulfide bond between Cys283 and Cys316 in the mature protein (corresponding to amino acids 313–346 in the unprocessed gene product) that connects the last LRR to the top of the ear repeat. It is thought that this disulfide bond is important for folding the ear repeat back to complete the solenoid and thus to maintain the structure of the protein.[17,18] The decorin mutations found associated with CSCD are, therefore, likely to result in an altered protein structure with other properties than WT.

When concentrated medium from WT HEK293 cells was subjected to size exclusion chromatography, decorin eluted over a broad molecular weight range. In contrast, when medium from MUT HEK293 cells was examined, decorin eluted in the void volume of the column. The finding of decorin from the MUT sample as high molecular weight complexes suggested that the truncated decorin was more likely than the WT decorin to aggregate in vitro. Decorin is known to form dimers[19] and probably also larger protein complexes, and it interacts with a number of other proteins. The precise nature of the high molecular weight complexes observed in the MUT sample is unknown. Varying degrees of glycosylation were observed in the WT sample, a feature of HEK293 cells previously described by others.[20] The same pattern was not observed in the MUT sample, possibly because the presence of truncated decorin in aggregates prevented the separation of proteins with varying degrees of glycosylation.

In conclusion, we provide evidence that decorin accumulates in the amorphous areas with small filaments that cause the stromal opacities in CSCD. Truncated decorin is expressed in patients with CSCD and is more likely than WT to form high-molecular weight complexes. These altered molecular features could be important for the accumulation of decorin in CSCD.

Acknowledgments

The authors thank Helge Boman for valuable advice and continuous support throughout this work, Andrew Quantock and Ingeborg Winge for professional assistance, and Jorunn Bringsli for technical assistance.

References

1. Bredrup C, Knappskog PM, Majewski J, Rødahl E, Boman H. Congenital stromal dystrophy of the cornea caused by a mutation in the decorin gene. *Invest Ophthalmol Vis Sci.* 2005;46:420–426.
2. Rødahl E, Van Ginderdeuren R, Knappskog PM, Bredrup C, Boman H. A second decorin frame shift mutation in a family with congenital stromal corneal dystrophy. *Am J Ophthalmol.* 2006;142:520–521.
3. Goldoni S, Seidler DG, Heath J, et al. An antimetastatic role for decorin in breast cancer. *Am J Pathol.* 2008;173:844–855.
4. Goldoni S, Iozzo RV. Tumor microenvironment: modulation by decorin and related molecules harboring leucine-rich tandem motifs. *Int J Cancer.* 2008;123:2473–2479.
5. Danielson KG, Baribault H, Holmes DF, Graham H, Kadler KE, Iozzo RV. Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. *J Cell Biol.* 1997;136:729–743.
6. Griffiths G, McDowall A, Back R, Dubochet J. On the preparation of cryosections for immunocytochemistry. *J Ultrastruct Res.* 1984;89:65–78.
7. Stang E, Blystad FD, Kazazic M, et al. Cbl-dependent ubiquitination is required for progression of EGF receptors into clathrin-coated pits. *Mol Biol Cell.* 2004;15:3591–3604.
8. Fisher LW, Termine JD, Young MF. Deduced protein sequence of bone small proteoglycan I (biglycan) shows homology with proteoglycan II (decorin) and several nonconnective tissue proteins in a variety of species. *J Biol Chem.* 1989;264:4571-4576.
9. Fisher LW, Stubbs JT 3rd, Young MF. Antisera and cDNA probes to human and certain animal model bone matrix noncollagenous proteins. *Acta Orthop Scand Suppl.* 1995;266:61–65.
10. Witschel H, Fine BS, Grutzner P, McTigue JW. Congenital hereditary stromal dystrophy of the cornea. *Arch Ophthalmol.* 1978;96:1043–1051.
11. Van Ginderdeuren R, De Vos R, Casteels I, Foets B. Report of a new family with dominant congenital heredity stromal dystrophy of the cornea. *Cornea.* 2002;21:118–120.
12. Pouliquen Y, Lacombe E, Schreinzer C, Giraud JP, Savoldelli M. [Familial congenital dystrophy of the corneal stroma: Turpin's syndrome (author's transl)]. *J Fr Ophtalmol.* 1979;2:115–125.
13. Bianco P, Fisher LW, Young MF, Termine JD, Robey PG. Expression and localization of the two small proteoglycans biglycan and decorin in developing human skeletal and non-skeletal tissues. *J Histochem Cytochem.* 1990;38:1549–1563.
14. Weiss JS, Møller HU, Lisch W, et al. The IC3D classification of the corneal dystrophies. *Cornea.* 2008;27(suppl 2):S1–S83.
15. Vincent AL, Patel DV, McGhee CN. Inherited corneal disease: the evolving molecular, genetic and imaging revolution. *Clin Exp Ophthalmol.* 2005;33:303–316.
16. Chopra RK, Pearson CH, Pringle GA, Fackre DS, Scott PG. Dermatan sulphate is located on serine-4 of bovine skin proteodermatan sulphate: demonstration that most molecules possess only one glycosaminoglycan chain and comparison of amino acid sequences around glycosylation sites in different proteoglycans. *Biochem J.* 1985;232:277–279.
17. Scott PG, McEwan PA, Dodd CM, Bergmann EM, Bishop PN, Bella J. Crystal structure of the dimeric protein core of decorin, the archetypal small leucine-rich repeat proteoglycan. *Proc Natl Acad Sci U S A.* 2004;101:15633–15638.
18. McEwan PA, Scott PG, Bishop PN, Bella J. Structural correlations in the family of small leucine-rich repeat proteins and proteoglycans. *J Struct Biol.* 2006;155:294–305.
19. Scott PG, Grossmann JG, Dodd CM, Sheehan JK, Bishop PN. Light and X-ray scattering show decorin to be a dimer in solution. *J Biol Chem.* 2003;278:18353–18359.
20. Graham FL, Smiley J, Russell WC, Nairn R. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J Gen Virol.* 1977;36:59–74.