

Supplementary Online Content

Schmidt TA, Sullivan DA, Knop E, et al. Transcription, translation, and function of lubricin, a boundary lubricant, at the ocular surface. *JAMA Ophthalmol*. Published online April 18, 2013. doi:10.1001/jamaophthalmol.2013.2385.

eTable 1. Identification of lubricin amplicon sequences from human corneal, conjunctival and liver RNA samples.

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This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1. Identification of lubricin amplicon sequences from human corneal, conjunctival and liver RNA samples

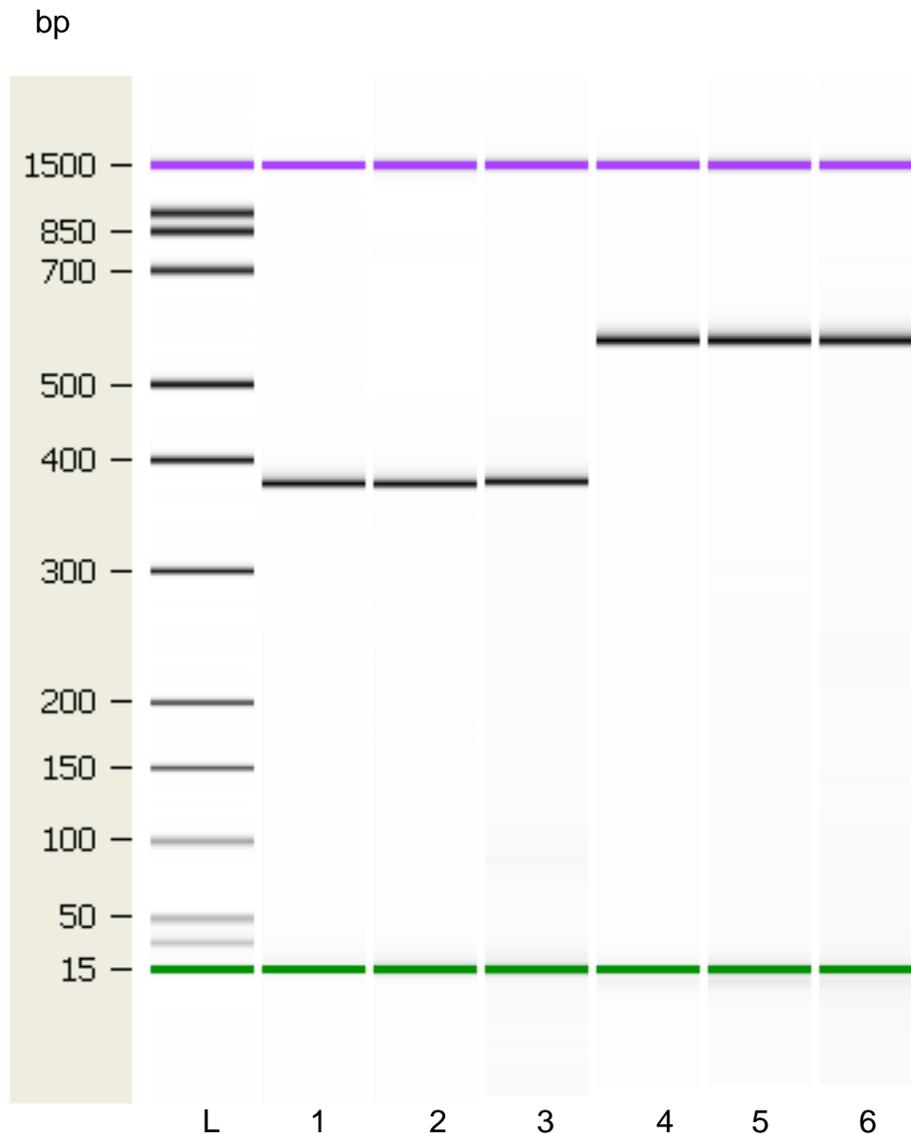
Sample	Sequencing Direction	Aligned Base Pairs to Human lubricin (PRG4)	Total Base Pairs from Amplicon	BLASTn Search Identity
Liver Standard				
A	Forward	495	500	Human PRG4
A	Reverse	488	491	Human PRG4
B	Forward	496	499	Human PRG4
B	Reverse	498	500	Human PRG4
Corneal Epithelial Cells (1)				
A	Forward	497	499	Human PRG4
A	Reverse	490	492	Human PRG4
B	Forward	500	504	Human PRG4
B	Reverse	498	501	Human PRG4
Cornea Epithelial Cells (2)				
A	Forward	498	499	Human PRG4
A	Reverse	474	489	Human PRG4
B	Forward	496	498	Human PRG4
B	Reverse	490	491	Human PRG4
Immortalized Conjunctival Epithelial Cells				
A	Forward	496	499	Human PRG4
A	Reverse	490	492	Human PRG4
B	Forward	495	499	Human PRG4
B	Reverse	491	491	Human PRG4

Two different samples (A and B) of each preparation were sequenced in forward and reverse directions. The human cornea samples were epithelial cells from the corneoscleral rims of 24 (1) and 51 (2) year old female donors. The BLASTn program usually cuts off the last 3 to 4 base pairs, because the end region typically has high ambiguity. The gene accession number for human lubricin (PRG4) for these analyses was NM_005807.2. The lubricin (PRG4) mRNA sequence has recently been updated to NM_005807.3.

eTable 2. Identification of lubricin amplicon sequences from human and mouse tissues

	Number of Aligned Base Pairs to Lubricin (PRG4)	
	Sample 1	Sample 2
Human		
Uterus	487/494	476/483
Cervix	483/493	476/479
Bladder	485/491	478/482
Prostate	333/355	483/488
Mouse		
Lacrimal gland (M)	338/343	332/335
Lacrimal gland (F)	339/343	329/334
Submandibular gland (M)	332/335	328/336
Submandibular gland (F)	338/343	338/343
Bladder (M)	332/335	332/335
Bladder (F)	332/334	333/336
Vagina/cervix	332/335	341/345
Seminal vesicles	340/344	332/335

Human samples were obtained from Ambion, and murine tissues were collected from 129/SvEv mice (n = 3 males and 3 females [except for bladder, which came from 2 females]). For the human samples two aliquots (1 and 2) of the same preparation were sequenced in forward and reverse directions. The mouse samples (1 and 2) originated from different animals and were sequenced in the forward reverse direction. Data were evaluated by BLASTn searches of GenBank databases. The numerator in the ratios equals the number of aligned base pairs to lubricin (PRG4), whereas the denominator equals the total number of amplicon base pairs. The ratios for each human sample were identical for alignments to lubricin (PRG4) transcript variants A (NM_005807.3), B (NM_001127708.1), C (NM_001127709.1) and D (NM_001127710.1). Similarly, the ratios for each mouse sample were identical for alignments to lubricin (PRG4) transcripts 1 (NM_021400.3) and 2 (NM_001110146.1).

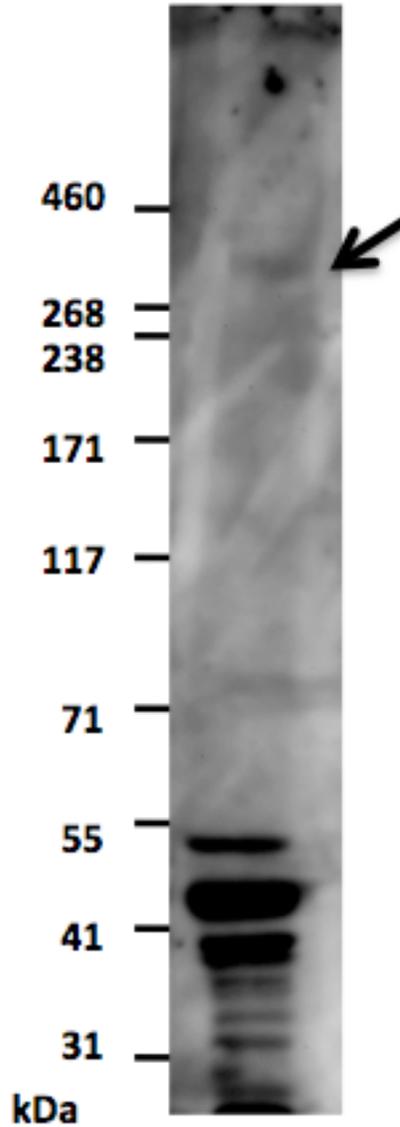


eFigure 1. Identification of lubricin mRNA in human and mouse tissues by RT-PCR

RNA lane: 1) female C57BL/6 mouse conjunctiva, 2) female C57BL/6 mouse cornea, 3) male and female BALB/c mouse meibomian gland positive control, 4) 56 year old male synovial membrane, 5) 56 year old male articular cartilage, 6) human prostate standard. The anticipated amplicon size of the mouse and human lubricin transcripts were 367 and 526 bp, and all bands fell within the 10% error of the Bioanalyzer when using a 1,000 bp sizing kit (internal controls = 15 and 1,500 bp). Analogous bands were also found in RNAs prepared from 3 additional C57BL/6 female conjunctivae, 2 more C57BL/6 female corneas, 2 more C57BL/6 male corneas, and a 49 year old female's articular cartilage and synovial membrane (data not shown). Abbreviations: bp = base pair; L = ladder.

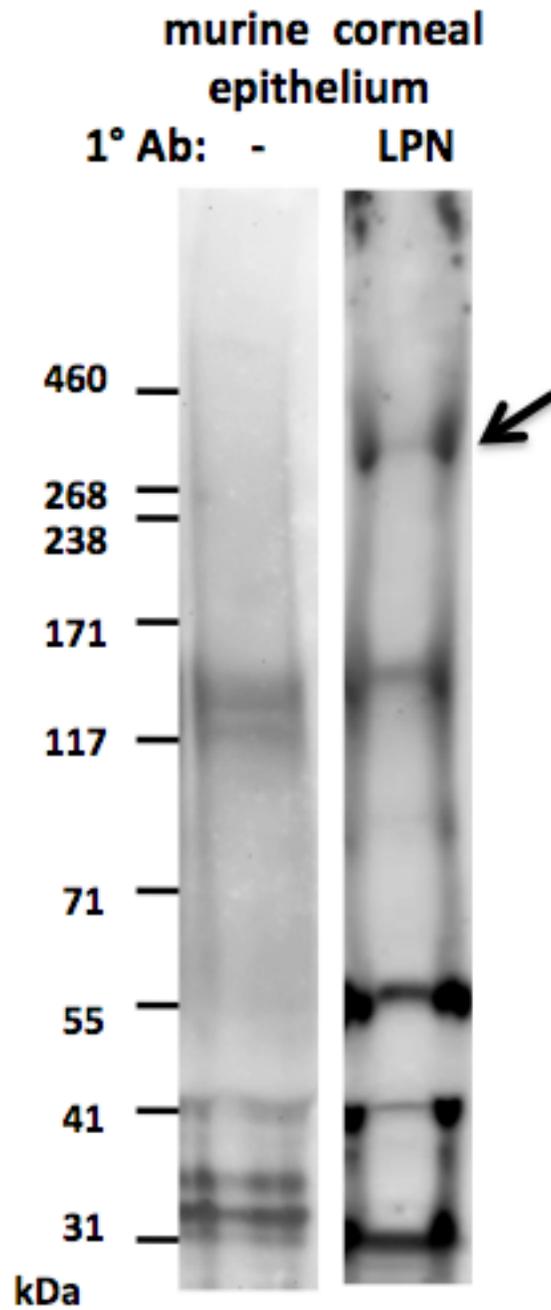
human cornea extract

1° Ab: LPN



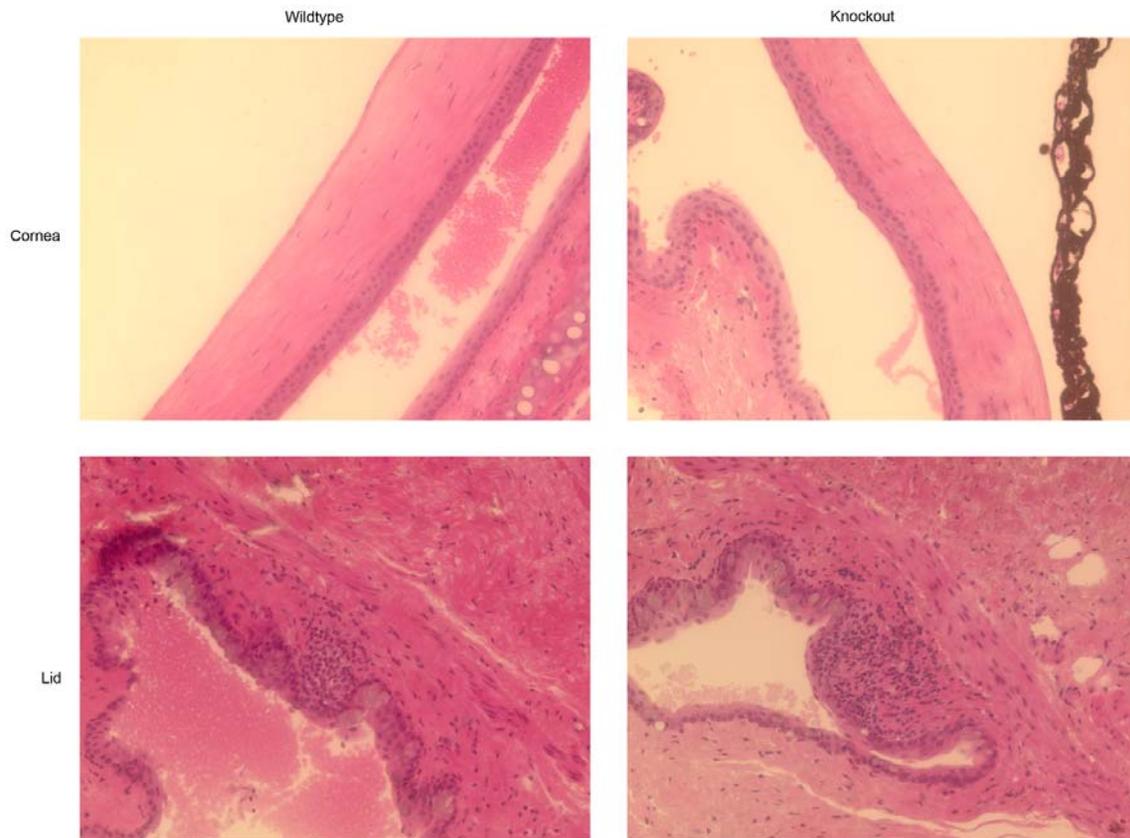
eFigure 2. Identification of lubricin protein in an extract of human corneal epithelium by Western blot

Numbers refer to molecular weight markers. The arrow points toward a protein band reactive with the “LPN” anti-lubricin antibody. The faint lubricin band may reflect the fact that these corneas were stored in Optisol, which, as noted in the Results, makes it difficult to detect this protein.



eFigure 3. Identification of lubricin protein in mouse corneal epithelium by Western blot

Numbers refer to molecular weight markers. The blot is shown with ("LPN") and without ("-") exposure to the "LPN" anti-lubricin antibody.



eFigure 4. Effect of lubricin insufficiency on the appearance of the murine cornea and lid

Corneal and lid tissues were obtained from male WT and KO mice and processed for histological evaluation. Photographs were taken at 20X magnification.