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Lacritin and Other New Proteins of the Lacrimal Functional Unit

Robert L. McKown^a, Ningning Wang^b, Ronald W. Raab^a, Roy Karnati^b, Yinghui Zhang^b, Patricia B. Williams^c, and Gordon W. Laurie^{b,*}

^a Department of Integrated Science and Technology, James Madison University, Harrisonburg VA, 22807 USA

^b Department of Cell Biology, University of Virginia, P.O. Box 800732 UVa Health System, Charlottesville VA, 22908-0732 USA

^c T. R. Lee Center for Ocular Pharmacology, Department of Physiological Sciences, Eastern Virginia Medical School, Norfolk VA, 23501 USA

Abstract

The lacrimal functional unit (LFU) is defined by the 2007 International Dry Eye WorkShop as ‘an integrated system comprising the lacrimal glands, ocular surface (cornea, conjunctiva and meibomian glands) and lids, and the sensory and motor nerves that connect them’. The LFU maintains a healthy ocular surface primarily through a properly functioning tear film that provides protection, lubrication, and an environment for corneal epithelial cell renewal. LFU cells express thousands of proteins. Over two hundred new LFU proteins have been discovered in the last decade. Lacritin is a new LFU-specific growth factor in human tears that flows through ducts to target corneal epithelial cells on the ocular surface. When applied topically in rabbits, lacritin appears to increase the volume of basal tear secretion. Lacritin is one of only a handful of tear proteins preliminarily reported to be downregulated in blepharitis and in two dry eye syndromes. Computational analysis predicts an ordered C-terminal domain that binds the corneal epithelial cell surface proteoglycan syndecan-1 (SDC1) and is required for lacritin’s low nanomolar mitogenic activity. The lacritin binding site on the N-terminus of SDC1 is exposed by heparanase. Heparanase is constitutively expressed by the corneal epithelium and appears to be a normal constituent of tears. Binding triggers rapid signaling to downstream NFAT and mTOR. A wealth of other new proteins, originally designated as hypothetical when first identified by genomic sequencing, are expressed by the human LFU including: ALS2CL, ARHGEF19, KIAA1109, PLXNA1, POLG, WIP1 and ZMIZ2. Their demonstrated or implied roles in human genetic disease or basic cellular functions are fuel for new investigation. Addressing topical areas in ocular surface physiology with new LFU proteins may reveal interesting new biological mechanisms and help get to the heart of ocular surface dysfunction.

Keywords

dry eye; cornea; lacrimal; meibomian; LACRT; LFU

*Corresponding author: Email address: E-mail: glaurie@virginia.edu (G.W. Laurie).

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1. Introduction

The lacrimal functional unit (LFU; Fig. 1) is 'an integrated system comprising the lacrimal glands, ocular surface (cornea, conjunctiva and meibomian glands) and lids, and the sensory and motor nerves that connect them' (2007 International Dry Eye WorkShop; reviewed by Rolando & Zierhut, 2001, and Stern et al., 2004). Corneal mechanonociceptors, polymodal nociceptors and cold receptors send afferent impulses to the CNS. Reflex efferent innervation stimulates secretion by main and accessory lacrimal glands. It also likely stimulates secretion by conjunctival goblet cells and the Meibomian gland (Stern et al., 2004). Stimulated or basal protein and lipid secretions flow onto the ocular surface to form the biophysically complex tear film. An estimated 15% of the tear film is expended per minute increasing to as much as 500% upon irritation (Jordan & Baum, 1980). Trans epithelial water transport also contributes to the tear film and replaces water loss from evaporation (Levin & Verkman, 2004). This constant state of sensory and secretory flux - likely involving thousands of soluble agonists, surface receptors and signaling mediators - is responsible for maintaining the health of the ocular surface epithelium.

Much of the cellular and molecular LFU machinery is well-known, but many gaps in the understanding of LFU proteins remain. Largely unaddressed are contributions of alternative splicing, post-translational modifications, upstream regulators and heterocomplex formation. Also, little is known about transient molar amounts of LFU proteins in tissues and how these doses affect other transient agonists and antagonists. At a proteomic level, the complexity of new proteins and their interactions with others is challenging. The human corneal BodyMap and NEIBank human keratocornea databases separately list over 2000 distinct mRNAs, and over 200 are listed in the human conjunctival BodyMap database, each coding for distinct LFU proteins. Similarly, the human tear proteome is more complex than previously appreciated with over 400 proteins apparently constituting the tear proteome (de Souza et al., 2006; Laurie et al., 2008). Preliminary studies suggest that only 4 – 5% may be downregulated in dry eye or dry eye-related syndromes, one of which is lacritin (Koo et al., 2005; Green-Church et al., 2007; Kitagawa et al., 2007).

This review of new proteins of the human LFU proteome begins with lacritin (Sanghi et al., 2001; Ma et al., 2008), a 12.3 kDa secreted glycoprotein in tears whose normal expression is largely restricted to the LFU. Lacritin is mitogenic for subconfluent human corneal epithelial cells (Wang et al., 2006), and promotes rat lacrimal acinar (Sanghi et al., 2001) and human corneal epithelial cell secretion. Although a tear protein, when applied topically in rabbits lacritin appears to increase the volume of basal tear secretion. Lacritin also appears to be protective against the inflammatory cytokines interferon- γ and TNF (Wang & Laurie, unpublished). Orthologs of the human LACRT gene are represented in more than thirteen different mammalian genomes and may be absent from lower vertebrates.

1.1 Lacritin structural features

Considering lacritin's widespread expression in the LFU and its presence in tears, it is surprising that lacritin was not discovered earlier. Lacritin's protein backbone has a predicted isoelectric point (pI) of 5, and eleven to twelve predicted O-glycosylation sites (Fig. 2; NetOGlyc 3.1 above threshold prediction) that support some FASTA homology with the mucin-like region of fibulin-2 and glycosaminoglycan binding region of neuroglycan C (Sanghi et al., 2001). Predicted O-glycosylation sites are concentrated in the N-terminal half of human lacritin (Fig. 2A) and orthologs (not shown). Unglycosylated recombinant human lacritin generated in *E. coli* is mitogenic (Sanghi et al., 2001; Wang et al., 2006). This activity is unaffected by deletion of up to 65 N-terminal amino acids (Wang, McKown, Laurie, unpublished). Expression of N-65 lacritin (lacking 65 amino acids from the N-terminus) in mammalian cells

would exclude all predicted O-glycosylation sites, and yet O-glycosylation of the N-terminal half appears to be conserved in all lacritins, although possibly at different levels.

These and other properties in some non-human primates appear to differ. Predicted molecular weights, *pI* and O-glycosylation site values for secreted lacritin in confirmed or predicted orthologs include the primates: chimpanzee (12.6 kDa, 5.2, 13), orangutan (12.4 kDa, 5, 13), rhesus monkey (12.2 kDa, 5, 14) and mouse lemur (12.3 kDa, 4.9, 9); and non-primates: house cat (10.7 kDa, 5, 14), common shrew (12.9 kDa, 10.2, 4), northern tree shrew (11.9 kDa, 5.4, 20), lesser hedgehog (10.43 kDa, 6.6, 17), little brown bat (9.3 kDa, 5, 10), and nine-banded armadillo (10 kDa, 10.1, 10). Human lacritin's single predicted N-linked glycosylation site towards the C-terminus (Fig. 2A) is conserved in chimpanzee and orangutan, but not in the others. Estimated molecular weights and isoelectric points for several other orthologues were provided in Ma et al. (2008). Mouse and rat lacritin orthologs are currently not listed (Ensembl release version 49), as is the case for abundant human tear protein proline rich 4 (PRR4). We noted previously that human genes displaying preferential lacrimal gland expression in humans do not do so in mouse (Ozyildirim et al., 2005). Possibly these human and rat or mouse LFU differences (Ozyildirim et al., 2005) are habitat-related and linked to absence of a human Harderian gland. If so, our dependence on other animals for disease models and preclinical studies may require more informed interpretations, perhaps from parallel use of human LFU cell lines.

An InterPro IPR003982 Leukotriene B4 type 2 receptor domain extending into the signal peptide has been assigned to human (Ma et al., 2008) and chimp lacritin, but not to other orthologues. Other proteins with an IPR003982 domain vary widely in function and include a transcription factor, an NF κ B complex inhibitor, a cytokine, a receptor, a phosphatase inhibitor and a furin-dependent secreted protein. Deletion mutants appear to rule out a functional role for this domain (Wang, McKown, Laurie, unpublished).

PONDR (Predictor of Naturally Disordered Regions) analyses of human lacritin (Fig. 2A) and orthologues (Fig. 2B) suggest that the N- and C-terminal halves are respectively disordered and ordered. This is in keeping with more α -helical structure in the C-terminal half (Fig. 2A), as confirmed by circular dichroism using synthetic peptides (Wang et al., 2006) and recombinant lacritins (McKown, unpublished). The disordered domain may contribute to secreted lacritin's anomalously slow migration in SDS-PAGE (observe 18 kDa vs expected 12.3 kDa for non-glycosylated) as is the case for XPA (Iakoucheva et al., 2001). Also slowing migration may be its uneven distribution of charge residues (Garfin, 1990), ie. N-71 is highly acidic (*pI* 10.7) and α -helix-rich, whereas C-59 is basic (*pI* 3.8).

Progressive deletion analysis (Fig. 2C; Wang et al., 2006) localized lacritin's mitogenic and syndecan-1 binding activities to the α -helical region (NGSEFAQKLL) between C-terminal amino acids 99 and 109 of secreted lacritin. Helical wheel predicts that NGSEFAQKLL and flanking residues form a conserved amphipathic α -helix. The hydrophobic face of amphipathic α -helices is capable of mediating low nanomolar protein-protein interactions. An amphipathic α -helix in parathyroid hormone is responsible for ligating parathyroid hormone receptor 1 (Pioszak and Xue, 2008), a G-protein coupled receptor (GPCR). A similar mechanism mediates the interaction of exendin-4 with the GPCR glucagon-like peptide-1 receptor (Runge et al., 2008), and binding of small ankyrin 1 of the sarcoplasmic reticulum with obscurin (Borzok et al., 2007). At micromolar concentrations, some amphipathic α -helices insert in membranes. This is the approach by which RGS2 (regulator of G-protein signaling2) targets the plasma membrane to inhibit the M1 muscarinic receptor (Gu et al., 2007) and by which antimicrobial peptides are effective (Hawrani et al., 2008). Actual molar levels of lacritin in human tears are currently unknown, but one preliminary estimate (McKown, unpublished) puts it in the nanomolar range. In vitro dose response studies with human recombinant lacritin reveal a

biphasic low nanomolar dose optimum for signaling and mitogenesis. This is in keeping with a co-receptor or receptor-binding mechanism (see below 1.3 Lacritin cell targeting).

Dermcidin (DCD) was proposed to be homologous to lacritin (Porter et al., 2003), but is not listed as a homologue by Homologene. Dermcidin's structure is not known. Although the best alignment shown was between signal peptides (Porter et al., 2003), several other regions are similar (Fig. 2D; alignment without signal peptide), and the functional and structural parallels are intriguing. Dermcidin has been reported to be mitogenic (Porter et al., 2003). Like lacritin dermcidin displays PONDR-predicted N-terminal half disorder and C-terminal half order, as well as aberrant mobility in SDS PAGE. Both appear to be relatively unstable in solution, a condition possibly attributable to the disordered half (Majczak et al., 2007). Dermcidin is proteolytically processed to generate N- and C-terminal peptides respectively with neural cell survival and skin antimicrobial activities, a process that has neither been demonstrated nor found necessary for lacritin mitogenic and prosecretory activities. Interestingly, LACRT and DCD genes are closely adjacent on human chromosome 12q13 and have been reported to be co-amplified in some metastatic breast cancers (Porter et al., 2003).

1.2 Lacritin expression and regulation

Lacritin expression is mainly restricted to the LFU (Fig. 1) and in tissue sections is detected most strongly in lacrimal acinar cell secretory granules (Sanghi et al., 2001) from which it is released after carbachol stimulation (Nakajima et al., 2007). In the monkey LFU, quantitative PCR suggests the following decreasing levels of tissue expression: lacrimal gland>conjunctiva>meibomian gland>cornea (Nakajima et al., 2007). Recent mass spec studies have detected lacritin in human meibomian gland secretions (Tsai et al., 2005), in human tears (Zhou et al., 2006; Koo et al., 2005) and deposited on contact lenses (Green-Church & Nichols, 2008). Its eye-specific transcriptional regulation appears to exceed that of α -crystallin, rhodopsin and keratocan. Lacritin is also expressed in salivary gland (staining of some ductal cells [Sanghi et al., 2001], in saliva [Ramachandran et al., 2006]) and thyroid (Sanghi et al., 2001) glands, and in lung bronchoalveolar lavage (Human Proteinpedia: HuPA_00022). No information is available on mechanisms of transcriptional regulation. Lacritin mRNAs are the sixth most common behind lysozyme, proline rich 4, lipocalin-1, lactotransferrin and proline rich 1 in NEIBank's human lacrimal EST database (Ozyildirim et al., 2005) from mixed female (51 yrs) and male (75 yrs) normal lacrimal gland.

Aceview documents lacritin-b, lacritin-c (Ma et al., 2008) and lacritin-d splice variants largely from NEIBank lacrimal transcripts (Ozyildirim et al., 2005). The most common form is lacritin-a. Currently 80 accessions are reported for lacritin-a versus one accession each for lacritin-b and -d; and eight accessions for -c. Lacritin-a is the representative RefSeq transcript. The LACRT gene is composed of five exons. Lacritin-b mRNA encodes a predicted secreted glycoprotein (MW 11.1 kDa, pI 5.3, 12 predicted O-linked sites) deficient in the sequence SIVEKSILTE from an abbreviated exon 4. This was not detected in an early RT-PCR that in retrospect was capable of resolving lacritin-b (but not lacritin-c or -d; Sanghi et al., 2001). Amplification was performed from human lacrimal and submandibular RNA each pooled from three females (66, 69, 71 yrs) and one male (38 yr). Further variant-specific PCR and antibody-based assays are warranted. Lacritin-c mRNA encodes a predicted secreted glycoprotein (MW 10.7 kDa, pI 4.6, 13 predicted O-linked sites) lacking sequence from exons 4 and 5 and instead displaying a novel C-terminus from intron 3. The lacritin-d prediction is not as strong. Its putative mRNA lacks exon 1 and part of exon 2, and codes for a non-secreted, possibly nuclear protein (MW 8.7, pI 7.3) with a potential PEST sequence for rapid turnover. The incidence of lacritin splice variants in tears of normal versus dry eye populations has not been studied but could be fruitful for both lacritin-b and -c are predicted to be inactive.

1.3 Lacritin cell targeting

Screening of seventeen different cell types suggests that lacritin may target an unusually narrow selection of epithelia, and not lymphoblastic, fibroblastic or glioma cells (Wang et al., 2006). Lacritin appears to promote constitutive tear secretion by cultured rat lacrimal acinar cells (Sanghi et al., 2001), and both secretion (Wang & Laurie, unpublished) and mitogenesis of human corneal epithelial cells (Wang et al., 2006). The lacrimal acinar cell response implies an autocrine or paracrine stimulatory mechanism since lacritin is itself a stimulated secretory product of acinar cells.

A mechanism of targeting has been partially elucidated by recombinant protein engineering and receptor screening strategies (Fig. 2B, 3A; Wang et al., 2006; Ma et al., 2006). The lacritin C-terminal amphipathic α -helix targets the cell surface heparan sulfate proteoglycan syndecan-1 (SDC1), but not syndecan-2 or -4. Syndecan-1 is a common cell surface heparan sulfate proteoglycan that surrounds all human corneal and conjunctival epithelia cells (Fig. 1; Heimann et al., 2001). Deletion analysis narrowed the lacritin binding site to the first 51 N-terminal amino acids of SDC1. Binding requires heparanase (HPSE) cleavage of the three heparan sulfate chains attached in this region (Fig. 3; Ma et al., 2006). This previously undescribed mechanism may at first glance seem counterintuitive to the health of the ocular surface since corneal stromal release of heparanase by infiltrating lymphocytes is a component of the inflammatory response (reviewed by Nasser, 2008). However, heparanase is constitutively expressed throughout the normal corneal epithelium (Fig. 1; Berk et al., 2004), and may be an important element in normal epithelial physiology. It also appears to be a normal constituent of human tears (Ma and Laurie, unpublished). In skin, heparanase is involved in the migration of stem cell progeny (Zcharia et al., 2005), and antisense knockdown of heparanase in *Xenopus* embryos promotes cell death, a phenotype that can be rescued by co-transfection of a heparanase construct (Bertolesi et al., 2008). Salivary branching morphogenesis is dependent on heparanase cleavage of heparan sulfate to release FGF10 (Patel et al., 2007). Secretion of heparanase that has been processed from its latent 65 kDa to active 58 kDa heterodimeric form can be stimulated by UTP (Shafat et al., 2006). UTP is in late clinical trials for the treatment of dry eye (Tauber et al., 2004) via a mechanism thought to involve the production of mucins (Murakami et al., 2003). These observations suggest a potential linkage between lacritin, UTP and heparanase in ocular surface physiology.

1.4 Lacritin cell signaling

Lacritin binding of syndecan-1 sets up a cascade of intracellular prosecretory and promitogenic signaling that has been partially worked out using pharmacological inhibitors and siRNA transfections (Fig. 4; Wang et al., 2006). Interestingly, proximal signaling is inhibited by pertussis toxin. Pertussis toxin blocks the ADP-ribosylation of the G proteins $G\alpha_i$ and $G\alpha_o$. This suggests that lacritin signaling is generated via a G-protein coupled receptor (GPCR) or G-protein-dependent ion channel that develops affinity for lacritin when lacritin is immobilized on syndecan-1. Lacritin signaling proceeds from $G\alpha_i$ and $G\alpha_o$ to the rapid dephosphorylation of protein kinase C α (PKC α) by an unidentified phosphatase. This targets PKC α to the perinuclear Golgi region where it complexes and activates phospholipase D1 (PLD1) and phospholipase C γ 2 (PLC γ 2). Activation is not detected after knockdown of PKC α by siRNA.

PLD1 is a key mediator of cell secretion and proliferation by catalyzing the generation of phosphatidic acid to promote secretory granule release from the Golgi apparatus (Chen et al., 1997). Phosphatidic acid is important in regulated insulin secretion from pancreatic β -cells (Hughes et al., 2004). It also activates mTOR. mTOR is a serine/threonine kinase regulating multiple cellular processes including protein translation, cell proliferation and survival (Bhaskar & Hay, 2007). Cells depleted of mTOR (or PKC α by siRNA) are unresponsive to lacritin in mitogenic assays (Wang et al., 2006).

Activation of PLC γ 2 triggers a separate chain of events. Activated PLC γ 2 catalyzes the hydrolysis of phosphatidylinositol biphosphate to generate inositol triphosphate (IP $_3$) and diacylglycerol (DAG), likely in the perinuclear Golgi region. IP $_3$ then binds IP $_3$ receptors on the endoplasmic reticulum to drive Ca $^{2+}$ mobilization from endoplasmic reticulum into the cytoplasm and then Ca $^{2+}$ entry into the cell via the Ca $^{2+}$ sensor STIM1. Prolonged Ca $^{2+}$ entry activates the cyclosporin A-sensitive serine/threonine phosphatase calcineurin to in turn dephosphorylate the cytoplasmic form of transcription factor nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 - also known as NFATC1. Despite its name, NFATs are widely expressed in epithelia and other cell types (Horsley et al., 2008). Dephosphorylated NFATC1 translocates into the nucleus where it functions as a transcription factor in an obligatory cooperative manner with several other transcription factors to regulate growth and secretion (Heit et al., 2006). Cells depleted of NFATC1 by siRNA are unresponsive to lacritin in mitogenic assays (Wang et al., 2006). Thus lacritin signaling involves G α_i and G α_o /PKC α /PLC γ 2/Ca $^{2+}$ /calcineurin/NFATC1 and G α_i and G α_o /PKC α /PLD1/mTOR.

The two diverging downstream pathways may interact (Wang et al., 2006), but are far from being completely understood. Deciphering cell signaling is at the heart of new drug design and understanding how drugs work. Although cyclosporin A suppresses T cell function to inhibit the inflammatory component of dry eye, a potential overlooked adverse effect is blockage of calcineurin/NFAT signaling in ocular surface epithelium. Many epithelial cells are absolutely dependent on calcineurin/NFAT signaling, ie. pancreatic β -cells (proliferation, differentiation and insulin production; Heit et al., 2006; Gurda et al., 2008) and lung epithelium (expression of aqaporin-1 and -5 and lysozyme; Dave et al., 2006).

1.5 Lacritin function: preliminary preclinical testing

In a small preclinical study, recombinant human lacritin was topically applied to rabbit eyes and basal tear flow measured (Samudre et al., submitted). A tear volume increase of 40% over vehicle control emerged, despite variability inherent in the Schirmer strip basal tear volume assay and problems with recombinant protein stability. Tear composition was normal with no significant changes noted in tear sodium, potassium, pH or protein concentration. Efficacy was apparent at the first time point (60 min) and was most significant at the last time point (240 min). Topical application of lacritin was also well tolerated by ocular tissues as indicated by negative Rose Bengal and lissamine green staining. Additional testing in normal and dry eye rodent models, and in non-human primates is needed to confirm these preliminary results. How might lacritin be prosecretory on the eye? The mechanism is unknown, but new data suggests that lacritin is a secretagogue for the ocular surface mucin MUC16 in corneal epithelial cell culture (Wang & Laurie, unpublished). MUC16 and other ocular mucins have short O-linked oligosaccharide chains, at least in human, rabbit and dog. Mucins together may form a continuous hydrophilic barrier on the ocular surface. Shedding of MUC16 leaves MUC1, MUC4 and other elements of the glycocalyx to help shield the cell surface. However, experimental knockdown of MUC16 (Blalock et al., 2007), dysregulation of MUC16 glycation (Sumiyoshi et al., 2008), or shedding of MUC16 (Blalock et al., 2008) appears to partially expose the corneal epithelial surface to bacteria and disrupt the normal tear film.

1.6 Lacritin and disease

Although it is still not clear what proteins make up the human tear proteome, over 400 have been reported. Preliminary studies involving small trials suggest that only 4 – 5% of these may be dysregulated in ocular surface disease, of which lacritin is the only one apparently capable of promoting tear production. Most have utilized 2-D SDS PAGE to compare quantities of proteins from normal tears versus tears from dry eye-related conditions. An estimate is generated from densitometry of silver-stained protein dots or by fluorescent measurement of cye3/cye5-stained protein dots, and protein identity of extracted dots is determined by mass

spectrometric protein sequencing. In tears from patients with blepharitis, lacritin was downregulated 'about 50%' (Koo et al., 2005). Blepharitis is a common inflammation of the eyelid, associated with evaporative dry eye. 27 normals and 19 patients participated. A smaller study (Green-Church et al., 2007) comparing tears from 10 normals and 10 contact lens-related dry eye patients reported that 'lacritin showed a ~7-fold downregulation'. The same group recently reported that lacritin is one of six tear proteins non-specifically absorbed to four different contact lenses (Green-Church & Nichols, 2008). LC-MS/MS suggests that lacritin may also be downregulated in Sjögren's syndrome tears (Kitagawa et al., 2007). This follows an earlier SELDI study by the same group indicating differences in peak heights of unidentified proteins from normal versus Sjögren's syndrome tears (Tomosugi et al., 2005). Large trials and quantitative capture assays are needed to transform lacritin tear data into molar levels that have physiological implications. Attention should also be paid to cell targeting (ie. levels of tear heparanase, disease-associated shedding of syndecan-1) and even cell signaling components.

2.1 Other new LFU proteins - originally hypothetical proteins

Over two hundred new human LFU proteins have been discovered over the past decade, most from unbiased sequencing of thousands of human lacrimal gland cDNAs for NEIBank's human lacrimal gland EST database (Ozyildirim et al., 2005). Here we define new proteins as those that were originally designated as hypothetical indicating a protein predicted from informatic analysis of the human genome. Of these, only the cytoskeletal protein myosin heavy chain 14 (MYH14) overlaps with the de Souza et al. (2006) tear proteome. Dermokine (DMKN) is extracellular but not apparently in tears. The remainder appear to be intracellular. We previously noted that more hypotheticals per total number of sequenced transcripts are concentrated in human lacrimal gland than in any other human organ (Ozyildirim et al., 2005). Although this term is slowly disappearing from GenBank with gradual protein characterization, we have the original human lacrimal gland EST data. Table 1 documents all past and current LFU hypothetical proteins integrated from NCBI Protein, BodyMap and NEIBank databases. Their current RefSeq protein designation, and known or predicted functions are indicated. Many are highly conserved (Ensembl). Some are disease genes or associated with disease genes (OMIM). Others may be important in LFU cell biology and androgen or estrogen responsiveness. We briefly highlight several in the following sections.

2.2 Other new LFU proteins: Risk factor locus for autoimmune disease

KIAA1109 is a large (556 kDa) integral membrane protein with at least twelve splice variants and a predicted nuclear location. Although highly conserved, no functional or structural information is available. The KIAA1109 gene is proximal to ADAD1 (adenosine deaminase domain containing 1), IL2 (interleukin 2) and IL21 (interleukin 21) genes. All three reside on a 480 kb section of human chromosome 4q27 in linkage disequilibrium for celiac disease, rheumatoid arthritis and type 1 diabetes - suggesting that this locus may be a general risk factor for autoimmune disease (Zhernakova et al., 2007). Secondary Sjögren's syndrome, an autoimmune disease of the LFU, is associated with rheumatoid arthritis. Although IL2 and IL21 are the suspected disease genes, the KIAA1109 SNP (single nucleotide polymorphism) rs13119723 is more significantly linked to celiac disease than any other SNP outside of the HLA region. Further meta-analysis identified a strong association at SNP rs6822844 which is 24 kb 5' of IL21 (van Heel et al., 2007).

2.3 Other new LFU proteins: Human genetic diseases

ALS2CL (ALS2 C-terminal like) is a conserved 108 kDa endosomal protein with eight MORN (membrane occupation and recognition nexus), one PH (pleckstrin homology) and one VPS9 (domain present in yeast vacuolar sorting protein 9 and other proteins) domains. Aceview

predicts eighteen or more splice variants. Its C-terminal Rab5-GDP/GTP exchange (GEF) activity region (Hadano et al., 2004) is homologous with the larger Golgi protein ALS2 (amyotrophic lateral sclerosis 2) that also shares MORN and VPS9 domains. ALS2 is the disease gene for juvenile recessive amyotrophic lateral sclerosis. ALS2 is also associated with primary lateral sclerosis and hereditary spastic paraplegia (Chandran et al., 2007). All are neurological degenerative disorders of motor neurons. ALS2CL homodimers bind ALS2 oligomers on membranes to reduce ALS2-dependent enlargement of endosomes (Suzuki-Utsunomiya et al., 2007). Yang et al (1999) noted changes in the distribution of lacrimal acinar cell Rab5 in endosomal compartments with carbachol stimulation as part of a larger theme of aberrant endosomal sorting in development of LFU autoimmune disease. APBB2 (amyloid beta precursor protein-binding, family B, member 2) is a conserved 81 kDa nuclear or cytoplasmic protein with WW (two conserved tryptophan residues) and two PTP (phosphotyrosine binding) domains. WW domains bind proline and phosphoserine/phosphothreonine motifs. Aceview predicts seventeen splice variants.

APBB2 binds APP (amyloid beta precursor protein), whose mutated gene is associated with autosomal dominant early onset Alzheimer disease in individuals developing disease prior to 75 years old (Li et al., 2005).

CREB3L2 (cAMP responsive element binding protein 3-like 2) is a conserved 57.4 kDa integral membrane protein with a predicted nuclear location and single BRLZ (basic region leucine zipper), transmembrane and CC (coiled coil) domains. Aceview predicts nine splice variants. Translocation of CREB3L2 and FUS chromosome bands form a chimera associated with human fibrosarcomas (Mertens et al., 2005).

MYH14 (myosin, heavy chain 14) is a 229 kDa cytoskeletal protein with myosin, IQ (calmodulin-binding) and CC domains. Aceview predicts six splice variants. MYH14 gene point mutations in some individuals have been associated with progressive hearing loss (Donaudy et al., 2004). From NOD mouse studies and developmental expression of the transcription factor Hox11 (Tlx1) in pancreas, salivary gland, tongue and cochlea, Lonyai et al. (2008) recently proposed a Hox11-associated developmental link for the hearing loss observed in some Sjögren's syndrome patients.

POLG (polymerase [DNA directed], gamma) is a conserved 139.6 kDa mitochondrial DNA polymerase. POLG contains one CC motif. Aceview predicts ten splice variants. Human missense mutations and deletions in the POLG gene are associated with several human genetic diseases including progressive external ophthalmoplegia or ophthalmoparesis (extraocular muscle weakness or paralysis; Van Goethem et al., 2002) and Alper's syndrome (cerebral degenerative disease in children).

SPG11 (spastic paraplegia 11 [autosomal recessive]) is evolutionarily conserved and is predicted to be a large secreted protein of approximately 275 kDa without signal peptide. SPG11 contains one CC motif. Aceview predicts eighteen splice variants. Point mutations in the SPG11 gene are associated with neurodegenerative thinning of the corpus callosum leading to spastic paraplegia in children (Stevanin et al., 2007).

TMEM43 (transmembrane protein 43) is a conserved 44.9 kDa integral membrane protein likely of the endoplasmic reticulum and Golgi apparatus. TMEM43 contains four predicted transmembrane domains. Aceview predicts six splice variants. Missense mutation of the TMEM43 gene causes arrhythmogenic right ventricular dysplasia (Merner et al., 2008).

ZFYVE27 (zinc finger, FYVE domain containing 27) is a conserved 46.4 kDa integral membrane protein with three transmembrane and one C-terminal FYVE (protein present in Fab1, YOTB, Vac1, and EEA1) domains. Aceview predicts 28 splice variants. Point mutation

in the ZFYVE27 gene is associated with spastic paraplegia (Mannan et al., 2006). ZFYVE27 is an ERK phosphorylated regulator of Rab11 membrane trafficking (Shirane and Nakayama, 2006). Active Rab11 is an endosomal GTPase. Its major roles are in sorting within the endosomal pathway and in regulation of transcytosis. Changes in its activity in transcytosis might result in abnormalities in the flow of proteins into tears. Rab11 also appears to play a role in docking multivesicular bodies with autophagosomes (Fader et al., 2008). Autophagosomes and autophagy are mechanistically linked to cell survival or death mechanisms and innate defense.

2.4 Other new LFU proteins: Androgen or estrogen signaling

WIPI1 (WD repeat domain, phosphoinositide interacting 1) is a conserved 48.7 kDa cytoplasmic protein with two WD40 repeats suggesting a scaffolding function in protein-protein interactions. Aceview predicts eleven splice variants. In pull-down assays, WIPI1 binds androgen, estrogen and retinoic acid receptors. The LFU's androgen-dependence has focused much attention on the contribution of androgen insufficiency to dry eye (reviewed by Sullivan, 2004). Retinoic acid is an LFU androgen antagonist (Ubels et al., 2002). Use of estrogen in postmenopausal hormone replacement therapy places women at increased risk of dry eye syndrome (Schaumberg et al., 2001). Thus WIPI1 potentially integrates three key elements of LFU physiology. WIPI1 appears to accumulate in trans-Golgi and endosomal membranes, and on LC3-positive autophagic membranes in a PI3K dependent manner (Proikas-Cezanne et al., 2007). Autophagy is a complicated cell survival mechanism that can be also utilized as a cell death pathway.

ZMIZ2 is a conserved 96.5 kDa cytoplasmic or nuclear protein with one NLS (nuclear localization) domain that colocalizes with and binds the androgen receptor. Binding enhances androgen receptor transcription (Huang et al., 2005). Aceview predicts twelve splice variants.

2.5 Other new LFU proteins: Cell biological features

ARHGEF19 (Rho guanine nucleotide exchange factor [GEF] 19) is an 89.2 kDa conserved nuclear or cytoplasmic Rho activating GEF with RhoGEF, PH and SH3 (Src homology 3) domains. Aceview predicts 9 splice variants.

PLXNA1 (plexin A1) is a conserved 208.6 kDa plasma membrane protein with one Sema (semaphorin), two PSI (domain found in plexins, semaphorins and integrins) and three IPT (Ig-like, plexins, transcription factors) domains. Aceview predicts seven splice variants. PLXNA1 is a cell surface signaling receptor for its ligands Sema3A and Sema6D. Sema6D targeting of dendritic cells induces IL-12 production; and PLXNA1^{-/-} dendritic cells poorly stimulate allogeneic and antigen-specific T-cells versus wild type cells. PLXNA1 thus appears to play a key role in T-cell stimulation (Takegahara et al., 2006). Although PLXNA1 has not apparently been examined in Sjögren's syndrome, the disease is associated with invading dendritic cells and increased IL-12 (Manoussakis et al., 2007). PLXNA1 also is a negative regulator of epithelial and endothelial migration and axonal migration (Bachelder et al., 2003).

3.0 Conclusions

Exploring lacritin and the over two hundred new LFU proteins that have been discovered over the last decade together offer a unique opportunity to understand human ocular surface physiology at a level of complexity previously unimagined. Some will expand the frontiers of biology to benefit all fields. This back to basics molecular approach gets to the heart of ocular surface dysfunction.

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Abbreviations

LACRT

lacritin

SDC1

syndecan-1

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Figure 1.

Schematic depiction of key cellular components of the lacrimal functional unit (LFU), and expression of prosecretory mitogen lacritin, cell surface proteoglycan syndecan-1 (to which lacritin binds) and heparanase (necessary for lacritin binding of syndecan-1). The LFU consists of lacrimal gland acinar cells, cornea epithelial cells, the goblet cell-enriched conjunctiva, the lipid and protein releasing meibomian gland from the lid margin, and (not shown) sensory and motor nerves. Corneal afferent fibers are sensory for mechanical, chemical and thermal reduction stimuli from mechanonociceptor, polymodal nociceptor and cold receptors. Impulses pass to the CNS. Efferents (mainly parasympathetic) help coordinate ocular surface wetting. They are motor to main and accessory lacrimal gland secretion. They are also thought to promote goblet cell and meibomian gland secretion (Stern et al., 2004).

Figure 2.

Structural and functional features of lacritin. (A) Comparison of lacritin PONDR (Predictor Of Naturally Disordered Regions; red line) analysis to a linear model of lacritin (purple) with boxes indicating PSIPRED-predicted alpha helices. Lacritin's N-terminal and C-terminal halves are respectively predicted to be disordered and ordered. Disordered implies absence of fixed tertiary structure. The ordered half contains more predicted α -helices. Vertical lines are sites of predicted glycosylation. Most are N-terminal. An IPR003982 Leukotriene B4 type 2 receptor (LTB4R2) domain is predicted by InterPro. The cytokine TNFSF8 also contains an LTB4R2 domain. Shown is human lacritin without signal peptide. (B) Similar PONDR analysis of six lacritin orthologues, all without signal peptide. (* lacritin ortholog length is shorter;

tracing was scaled to match the others). (C) The C-terminal α -helix in the ordered half contains mitogenic and syndecan-1 binding domain(s), as determined by deletion analysis (Wang et al., 2006). (D) Near-Optimal Global Alignment (Smoot et al., 2004) of human lacritin and dermcidin. Level of green indicates robustness of alignment. Red and pink underline respectively indicates identity and similarity.

Figure 3.

Off/on switch for lacritin function. (A) Partial deletion analysis reveals that lacritin binds an N-terminal region in the core protein of syndecan-1 after heparanase (hep-ase) treatment. (B) Heparanase cleaves heparan sulfate chains to expose the binding site.

TABLE 1

Other new (formerly hypothetical) proteins of the LFU

Gene Symbol	Protein
	<u>(i) Apoptosis/Autophagy</u>
FAM82C	family with sequence similarity 82, member C ^{3,6}
RNF144B	ring finger 144B ^{1,6}
WIP1I	WD repeat dom., phosphoinositide interact. 1 ^{1,6}
ZC3H12A	Zn finger CCCH-type containing 12A ^{1,6}
	<u>(ii) ATPase/Transferase/Other Enzyme</u>
ANKRD36B	ankyrin repeat domain 36B ^{1,7}
ATP13A3	ATPase type 13A3 ^{1,6}
C14orf172	chromosome 14 open reading frame 172 ^{1,6}
C18orf25	chromosome 18 open reading frame 25 ^{1,6}
CARS2	cysteinyI-tRNA synth. 2, mitochond. (putative) ^{3,10}
CCDC46	coiled-coil domain containing 46 ^{1,6}
CCDC57	coiled-coil domain containing 57 ^{1,6}
CHD1L	chromodom. helicase DNA bp 1-like ^{1,9}
COX18	COX18 cytochr. c oxidase assem. hom. ^{1,10}
DALRD3	DALR anticodon binding domain containing 3 ^{1,6}
EME2	essential meiotic endonuclease 1 homolog 2 ^{3,6}
FLJ20628	hypothetical protein FLJ20628 ^{1,6}
HECTD3	HECT domain containing 3 ^{1,6}
LOC100134-860	hypothetical protein LOC100134860 ^{3,9}
MANSC1	MANSC domain containing 1 ^{1,6}
N4BP2L2	NEDD4 binding protein 2-like 2 ^{1,6}
PCMTD1	prot.-L-isoasp. O-methyltransf. dom. contain. 1 ^{3,6}
POLG	polymerase (DNA directed), gamma ^{3,8,9}
TARS2	threonyI-tRNA synthetase 2, mitochond. (put.) ^{3,6}
UPRT	uracil phosphoribosyltransf. (FUR1) homol. ^{3,6}
ZDHHC21	zinc finger, DHHC-type containing 21 ^{3,6}
	<u>(iii) Calcium</u>
C9orf140	chromosome 9 open reading frame 140 ^{2,6}
	<u>(iv) Cell Adhesion/Motility/Structure</u>
CEP192	centrosomal protein 192kDa ^{1,6}
CGNL1	cingulin-like 1 ^{1,6}
FNBP1L	formin binding protein 1-like ^{5,7}
MPZL3	myelin protein zero-like 3 ^{3,7}
MYH14	myosin, heavy chain 14 ^{5,6}
	<u>(v) Cell Growth</u>
C2orf29	chromosome 2 open reading frame 29 ^{2,6}
LYAR	Ly1 antibody reactive homolog (mouse) ^{3,10}
	<u>(vi) Endosomal/Lysosomal</u>
ALS2CL	ALS2 C-terminal like ^{1,6}
CHMP7	CHMP family, member 7 ^{3,6}

Gene Symbol	Protein
KIAA0528	KIAA0528 ^{2,6}
NECAP2	NECAP endocytosis associated 2 ^{3,8,9}
STX17	syntaxin 17 ^{1,6}
TTC13	tetratricopeptide repeat domain 13 ^{1,6}
UFM1	ubiquitin-fold modifier 1 ^{2,6}
VPS37A	vacuolar protein sorting 37 homolog A ^{3,6}
ZFYVE27	zinc finger, FYVE domain containing 27 ^{1,6}
	<u>(vii) Extracellular</u>
DMKN	dermokine ^{3,6}
FAM20B	family with sequence similarity 20, member B ^{2,6}
	<u>(viii) Immune</u>
KIAA1109	KIAA1109 ^{1,6}
	<u>(ix) Lipid/Cholesterol</u>
IAH1	soamyl acetate-hydrolyz. esterase 1 homol. ^{3,6}
LASS4	LAG1 homolog, ceramide synthase 4 ^{3,6}
MTMR10	myotubularin related protein 10 ^{1,6}
PLCXD1	PI-spec. phospholipase C, X dom. contain. 1 ^{1,6}
	<u>(x) Other/Unknown</u>
ARGLU1	arginine and glutamate rich 1 ^{1,6}
C1orf58	chromosome 1 open reading frame 58 ^{1,6}
C1orf80	chromosome 1 open reading frame 80 ^{1,6}
C1orf109	chromosome 1 open reading frame 109 ^{2,6}
C1orf163	chromosome 1 open reading frame 163 ^{2,6}
C4orf18	chromosome 4 open reading frame 18 ^{2,6}
C4orf28	chromosome 4 open reading frame 28 ^{2,6}
C5orf24	chromosome 5 open reading frame 24 ^{2,6}
C5orf28	chromosome 5 open reading frame 28 ^{2,6}
C5orf30	chromosome 5 open reading frame 30 ^{2,10}
C6orf70	chromosome 6 open reading frame 70 ^{1,6}
C6orf106	chromosome 6 open reading frame 106 ^{1,6}
C7orf47	chromosome 7 open reading frame 47 ^{2,10}
C8orf42	chromosome 8 open reading frame 42 ^{1,6}
C9orf41	chromosome 9 open reading frame 41 ^{2,6}
C10orf104	chromosome 10 open reading frame 104 ^{1,6}
C11orf48	chromosome 11 open reading frame 48 ^{1,10}
C11orf56	chromosome 11 open reading frame 56 ^{1,6}
C12orf30	chromosome 12 open reading frame 30 ^{1,6}
C12orf35	chromosome 12 open reading frame 35 ^{1,6}
C12orf44	chromosome 12 open reading frame 44 ^{1,6}
C14orf159	chromosome 14 open reading frame 159 ^{1,8}
C16orf58	chromosome 16 open reading frame 58 ^{2,6}
C17orf45	chromosome 17 open reading frame 45 ^{2,6}

Gene Symbol	Protein
C17orf56	chromosome 17 open reading frame 56 ^{2,10}
C19orf42	chromosome 19 open reading frame 42 ^{1,10}
CAMSAP1	calmodulin regulated spectrin-assoc. prot. 1 ^{3,8,9}
CCDC49	coiled-coil domain containing 49 ^{3,6}
CCDC94	coiled-coil domain containing 94 ^{3,6}
CCDC109A	coiled-coil domain containing 109A ^{3,6}
CCDC117	coiled-coil domain containing 117 ^{3,6}
CCNYL1	cyclin Y-like 1 ^{3,6}
CXorf39	chromosome X open reading frame 39 ^{2,6}
FAM46A	family with sequence similarity 46, member A ^{1,6}
FAM46C	family with sequence similarity 46, member C ^{1,6}
FAM65A	family with sequence similarity 65, member A ^{1,6}
FAM76B	family with sequence similarity 76, member B ^{1,6}
FAM83E	family with sequence similarity 83, member E ^{1,6}
FAM96A	family with sequence similarity 96, member A ^{1,10}
FAM107B	family with sequence similarity 107, member B ^{2,6}
FAM114A1	family with sequence similarity 114, mem. A1 ^{2,6}
FAM128B	family with sequence simil. 128, member B ^{1,10}
FAM133B	family with sequence simil. 133, member B ^{1,10}
FAM116A	family with sequence simil. 116, member A ^{2,10}
FCHO2	FCH domain only 2 ^{3,6}
FLJ14154	hypothetical protein FLJ14154 ^{1,10}
FLJ20254	FLJ20254 ^{1,6}
FLJ23584	hypothetical FLJ23584 ^{4,9}
FLJ31818	hypothetical protein FLJ31818 ^{1,6}
JAGN1	jagunal homolog 1 (Drosophila) ^{3,6}
KIAA0515	KIAA0515 ^{1,6}
KIAA0913	KIAA0913 ^{1,9}
KIAA1545	KIAA1545 protein ^{4,10}
KRCC1	lysine-rich coiled-coil 1 ^{3,10}
LMF2	lipase maturation factor 2 ^{3,6}
LOC26010	viral DNA polymerase-transactivated protein 6 ^{1,8}
LOC129293	hypothetical protein LOC129293 ^{2,10}
LOC201229	hypothetical protein LOC201229 ^{1,10}
LOC283951	hypothetical protein LOC283951 ^{2,10}
LOC441150	similar to RIKEN cDNA 2310039H08 ^{3,10}
MGC3207	hypothetical protein MGC3207 ^{1,10}
MGC70857	similar to RIKEN cDNA C030006K11 gene ^{2,10}
NHLRC2	NHL repeat containing 2 ^{3,6}
NOL10	nucleolar protein 10 ^{1,6}
NBPF15	neuroblastoma breakpoint family, member 15 ^{2,10}
NPC-A-5	NPC-A-5 ^{1,10}

Gene Symbol	Protein
ODAM	odontogenic, ameloblast associated ^{1,6}
PRR14	proline rich 14 ^{3,10}
PRRC1	proline-rich coiled-coil 1 ^{3,6}
RWDD4A	RWD domain containing 4A ^{1,6}
SERTAD4	SERTA domain containing 4 ^{3,6}
SETD5	SET domain containing 5 ^{3,6}
SPG11	spastic paraplegia 11 (autosomal recessive) ^{1,6}
SPTY2D1	SPT2, Suppressor of Ty, domain containing 1 ^{1,6}
STAG3L4	stromal antigen 3-like 4 ^{1,10}
SYCP2L	synaptonemal complex protein 2-like ^{3,6}
TMCO4	transmembrane and coiled-coil domains 4 ^{3,6}
TMEM25	transmembrane protein 25 ^{3,6}
TMEM32	transmembrane protein 32 ^{3,6}
TMEM43	transmembrane protein 43 ^{3,6}
TMEM44	transmembrane protein 44 ^{1,6}
TMEM62	transmembrane protein 62 ^{1,6}
TMEM66	transmembrane protein 66 ^{3,6}
TMEM85	transmembrane protein 85 ^{3,6}
TMEM100	transmembrane protein 100 ^{1,10}
TMEM109	transmembrane protein 109 ^{3,6}
TMEM125	transmembrane protein 125 ^{3,6}
TMEM127	transmembrane protein 127 ^{3,6}
TMEM134	transmembrane protein 134 ^{2,10}
TMEM168	transmembrane protein 168 ^{1,6}
TMEM184A	transmembrane protein 184A ^{1,6}
TTC17	tetratricopeptide repeat domain 17 ^{3,6}
TTC19	tetratricopeptide repeat domain 19 ^{5,6}
TTC21B	tetratricopeptide repeat domain 21B ^{1,6}
TTC31	tetratricopeptide repeat domain 31 ^{3,6}
UHRF1BP1	UHRF1 (ICBP90) binding protein 1 ^{1,6}
WDR52	WD repeat domain 52 ^{3,6}
WDR60	WD repeat domain 60 ^{1,6}
	<u>(xi) Protein Production/Binding/Complex</u>
C4orf34	chromosome 4 open reading frame 34 ^{2,6}
CCDC130	coiled-coil domain containing 130 ^{3,6}
EIF4B	eukaryotic translation initiation factor 4B ^{1,6}
FAM96A	FAM96A ^{1,6}
FAM136A	family with sequence similarity 136, member A ^{2,6}
NOC4L	nucleolar complex associated 4 homolog ^{3,6}
ORMDL1	ORM1-like 1 ^{3,6}
PHF15	PHD finger protein 15 ^{3,6}
	<u>(xii) Proteinase/Hydrolase/Lysase</u>

Gene Symbol	Protein
AMZ2	archaelysin family metallopeptidase 2 ^{1,6}
CMBL	carboxymethylenebutenolidase homolog ^{1,6}
ECHDC2	enoyl Coenzyme A hydratase dom. contain. 2 ^{1,6}
FAM111A	family with sequence similarity 111, member A ^{1,6}
FAM113B	family with sequence similarity 113, member B ^{2,6}
FLJ35220	hypothetical protein FLJ35220 ^{2,10}
KIAA1434	hypothetical protein KIAA1434 ^{1,10}
NUDT16	nudix-type motif 16 ^{3,6}
SCRN2	secernin 2 ^{3,6}
SERHL	serine hydrolase-like ^{1,6}
SPATA20	spermatogenesis associated 20 ^{3,6}
UFSP2	UFM1-specific peptidase 2 ^{3,10}
	<u>(xiii) Receptor/Channel/Transport</u>
GPRC5B	G prot.-coupled recept., fam. C, grp 5, mem. B ^{5,6}
KCTD12	K channel tetramerisation dom. containing 12 ^{1,6}
PLXNA1	plexin A1 ^{1,6}
SHKBP1	SH3KBP1 binding protein 1 ^{3,6}
SLC25A46	solute carrier family 25, member 46 ^{2,6}
TMEM104	transmembrane protein 104 ^{1,6}
	<u>(xiv) RNA Processing</u>
CCDC131	coiled-coil domain containing 131 ^{1,6}
CNOT6L	CCR4-NOT transcripti. complex, subunit 6-like ^{1,6}
CWF19L1	CWF19-like 1, cell cycle control ^{1,6}
INTS3	integrator complex subunit 3 ^{1,6}
INTS10	integrator complex subunit 10 ^{1,6}
MAGOHB	mago-nashi homolog B ^{3,6}
NOL12	nucleolar protein 12 ^{1,6}
NPIP	nuclear pore complex interacting protein ^{1,6}
RBM35B	RNA binding motif protein 35B ^{1,6}
SFRS2	splicing factor, arginine/serine-rich 2 ^{3,6}
ZMAT5	zinc finger, matrin type 5 ^{1,6}
	<u>(xv) Signal Transduction</u>
ARHGEF19	Rho guanine nucleotide exchange factor 19 ^{3,6}
C10orf81	chromosome 10 open reading frame 81 ^{2,6}
C19orf50	chromosome 19 open reading frame 50 ^{2,6}
DEF8	differentially expressed in FDCP 8 homolog ^{1,6}
FLJ10357	hypothetical protein FLJ10357 ^{1,6}
KIAA1975	KIAA1975 protein similar to MRIP2 ^{1,10}
LOC440456	sim. plecks. homol. dom. fam. M memb. 1 ^{4,6}
NOMO1	NODAL modulator 1 ^{5,6}
SH2D4A	SH2 domain containing 4A ^{3,6}
SPATA13	spermatogenesis associated 13 ^{3,6}

Gene Symbol	Protein
TBC1D17	TBC1 domain family, member 17 ^{3,6} (xvi) Transcription/Nucleic Acid Binding
AFTPH	aftiphilin ^{1,5}
ANKZF1	ankyrin repeat and Zn finger dom. containing 1 ^{1,6}
APBB2	amyloid beta prec. prot.-bind., fam. B, mem. 2) ^{5,6}
ATPBD1B	ATP binding domain 1 family, member B ^{1,9}
CREB3L2	cAMP responsive element bind. prot. 3-like 2 ^{1,6}
BSDC1	BSD domain containing 1 ^{3,6}
BTBD14B	BTB (POZ) domain containing 14B ^{1,6}
C9orf86	chromosome 9 open reading frame 86 ^{1,6}
C9orf152	chromosome 9 open reading frame 152 ^{1,6}
CCDC75	coiled-coil domain containing 75 ^{1,6}
CXXC5	CXXC finger 5 ^{3,6}
MED25	mediator complex subunit 25 ^{1,6}
MGC16385	hypothetical protein MGC16385 ^{1,6}
NSMCE4A	non-SMC element 4 homolog A ^{3,6}
RBPJ	recombin. signal bp for Ig kappa J region ^{1,9}
STOX1	storkhead box 1 ^{3,6}
TCEAL4	transcription elongation factor A (SII)-like 4 ^{5,6}
ZCCHC6	zinc finger, CCHC domain containing 6 ^{1,6}
ZGPAT	zinc finger, CCCH-type with G patch domain ^{1,6}
ZMAT2	zinc finger, matrin type 2 ^{3,6}
ZMIZ2	zinc finger, MIZ-type containing 2 ^{1,6}
ZNF280D	zinc finger protein 280D ^{1,6}
ZNF517	zinc finger protein 517 ^{1,6}
ZNF557	zinc finger protein 557 ^{1,6}
ZNF618	zinc finger protein 618 ^{1,6}
ZNF664	zinc finger protein 664 ^{3,6}
ZNF700	zinc finger protein 700 ^{3,6}
ZNF821	zinc finger protein 821 ^{3,6}

Validated¹, predicted², provisional³, model⁴ or reviewed⁵ RefSeq proteins from: (1) accession number search of all human lacrimal⁶ EST's (Ozyildirim et al., 2005) originally linked to proteins bioinformatically designated as hypothetical, and (2) keyword 'eye and hypothetical and human' search of the NCBI Protein database. The latter hits were then restricted to all sources associated with the human lacrimal functional unit: 'cytoplasmic corneal stroma'⁷, 'eye' (then traced to lacrimal gland⁶ by accession number) and 'eye anterior segment'⁸. Hits ('hypothetical') from keratoconus⁹ and BodyMap¹⁰ cornea were included. Most derive from the human lacrimal gland⁶.