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Calpain-14 and its association with eosinophilic esophagitis

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Abstract

Calpains are a family of intracellular, calcium-dependent cysteine proteases involved in a variety of regulatory processes, including cytoskeletal dynamics, cell-cycle progression, signal transduction, gene expression, and apoptosis. These enzymes have been implicated in a number of disease processes, notably for this review involving eosinophilic tissue inflammation, such as eosinophilic esophagitis (EoE), a chronic inflammatory disorder triggered by allergic hypersensitivity to food and associated with genetic variants in calpain 14 *(CAPN14)*. Herein we review the genetic, structural, and biochemical properties of *CAPN14* and its gene product CAPN14, and its emerging role in patients with EoE. The *CAPN14* gene is localized at chromosome 2p23.1-p21 and is most homologous to *CAPN13* (36% sequence identity), which is located 365 kb downstream of *CAPN14*. Structurally, CAPN14 has classical calpain motifs, including a cysteine protease core. In comparison with other human calpains, *CAPN14* has a unique expression pattern, with the highest levels in the upper gastrointestinal tract, particularly in the squamous epithelium of the esophagus. The *CAPN14* gene is positioned in an epigenetic hotspot regulated by IL-13, a T_H2 cytokine with increased levels in patients with EoE that has been shown to be a mediator of the disease. CAPN14 induces disruptive effects on the esophageal epithelium by impairing epithelial barrier function in association with loss of desmoglein-1 expression and has a regulatory role in repairing epithelial changes induced by IL-13. Thus CAPN14 is a unique protease with distinct tissue-specific expression and function in patients with EoE and is a potential therapeutic target for EoE and related eosinophilic and allergic diseases.

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Keywords

Calpains; calpain-14; calpainopathy; IL-13; desmoglein-1; epithelial barrier; enzymes; food allergy; genetics; eosinophils; eosinophilic esophagitis; limb-girdle muscular dystrophy; myositis

Calpains are a class of intracellular, calcium-dependent cysteine (Cys) proteases.[1,2] Unlike other families of proteolytic enzymes (eg, proteasomes and lysosomes), calpains do not digest proteins to complete degradation; instead, they regulate intracellular cascades (eg, those found in proteasomes and lysosomes), especially intermediate signaling molecules, cytoskeletal dynamics,[3] cell-cycle progression, signal transduction, gene expression,[4] and apoptosis.[5] Under physiologic conditions, calpain activation is regulated by transient and localized calcium fluxes from extracellular or intracellular calcium stores.[6] In human subjects there are 16 calpain proteins that are classified into several groups according to their expression profile[7] or domain structure.[8] Among them, calpain-14 (CAPN14) is a comparatively new classical representative of calpains that has been relatively unexamined until our recent finding that it is etiologically associated with eosinophilic esophagitis (EoE).[9,10]

Calpain dysregulation has been implicated in patients with a number of genetic and acquired diseases.[1,11] For example, limb-girdle muscular dystrophy type 2A (LGMD2A) is an autosomal recessive disorder caused by homozygous and compound heterozygous mutations in the *CAPN3* gene,[12,13] resulting in loss of CAPN3 proteolytic activity.[14] Notably, idiopathic eosinophilic myositis[15] is a known pathophysiologic component of LGMD2A. Mechanistically, it is possible that CAPN3 acts as a sensor of sarcomeric integrity and function and is involved in its repair and maintenance.[16] Deficiency in CAPN3 also increases oxidative stress in the mitochondria because of accumulation of mitochondrial proteins involved in β-oxidation of fatty acids,[17] resulting in decreased nuclear localization of nuclear factor κB. Attenuated nuclear factor κB signaling leads to increased susceptibility to myocyte apoptosis,[18–21] production of eosinophil chemoattractants,[22] and eosinophil accumulation and activation,[13] as seen in patients with LGMD2A. The inflammatory component of LMGD2A is thought to be mediated by the eosinophil-derived secretory granule proteins, such as eosinophil cationic protein[23] and major basic protein.[24] Eosinophil cationic protein is involved in degrading myofibrils and membrane-associated cytoskeletal proteins, and major basic protein induces muscle fiber membrane damage through nonenzymatic interactions, leading to degeneration or necrosis. CAPN3 also has an important role in forming the dysferlin protein membrane repair complex,[25] and thus the lack of calpain activity might also adversely affect the repair process of muscle membrane lesions induced by eosinophils.[22] Collectively, CAPN3 acts as a gatekeeper, maintaining muscle cell integrity.

On the basis of genetic association and tissue-specific expression of CAPN14 in the esophagi of patients,[9] dysregulated expression of CAPN14 (either increased or decreased[26]) is linked with the development of EoE. This review is focused on characterizing CAPN14, including the gene structure, expression profile, protein structure, function, and role in EoE.

GENE STRUCTURE AND CONSERVATION

The *CAPN14* gene is 44,490 bp long, as defined by RefSeq annotation in the UCSC Genome Browser (Fig 1, *A*).[27] It contains 26 exons encoding a protein that is 684 amino acids long. *CAPN14* is located at 2p23.1 in juxtaposition to the polypeptide N-acetylgalactosaminyltransferase 14 *(GALNT14)* and encoding homology domain containing 3 *(EHD3)* genes.

Phylogenetically, *CAPN14* is most closely related to *CAPN13* (Fig 1, *B*), with 36% DNA sequence identity based on Clustal Omega multiple sequence alignment.[28] Both CAPN13 and CAPN14 are derived from a single classical calpain present in the jawed vertebrate ancestor[7] and are located in the same chromosomal band (approximately 365 kb apart). *CAPN13* is considered to be the parent of all classical calpains: *CAPN1* to *CAPN3*, *CAPN8*, *CAPN9*, and CAPN*11* to *CAPN14*.[7]

In the course of evolution, a genomic duplication event is thought to have separated *CAPN13* and *CAPN14*, a theory supported by the duplicate copies of *CAPN14* present in some animal species, such as pigs and Tasmanian devils.[7] The *CAPN14* gene is conserved in vertebrate species spanning from lobe-finned fish to human subjects (see Fig E1 in this article's Online Repository at www.jacionline.org),[29] but human CAPN14 protein is the only orthologue that has been isolated and characterized thus far.[26] The amino acid alignment of the predicted CAPN14 protein (see Table E1 in this article's Online Repository at www.jacionline.org) in primates reveals 90% or greater identity to human CAPN14. In domesticated animals the CAPN14 orthologues share 76% to 88% identity with the human protein; homology of human CAPN14 to other mammals and birds, reptiles, and frogs is 61% to 77% and 44% to 61%, respectively. Surprisingly, there is no evidence of the *Capn14* gene in mice or rats, although its presence is suggested in other rodents (eg, guinea pigs and squirrels).

CAPN14 is expressed at the highest level in the esophagus and has been identified as a tissue identity marker.[9,30] Expression of CAPN13, the most closely related molecule to CAPN14, is highest in the stomach and small intestine[31]; it is scarcely detectable in the esophagus. It is tempting to speculate that esophagus-specific proteases, such as CAPN14, might have appeared as a means of protection of the integrity of esophageal tissue. Because esophageal epithelium is prone to damage because of food consumption, there are various defense mechanisms against esophageal damage. One of these mechanisms is keratinization of esophageal epithelium, which increases its mechanical stability[32]; for example, animals consuming a diet rich in grains and plants have a highly keratinized esophagus. Another protective mechanism includes higher expression of protective molecules, such as the cationic antimicrobial peptides cathelicidin and β-defensins 2 and 3,[33] which are characteristic of carnivorous animals that have a less keratinized esophagus. The human and primate esophagi[34] are not keratinized, and therefore it is plausible that esophagus-specific proteases, such as CAPN14, are a means of protecting the integrity of esophageal tissue (Fig 2). This hypothesis is supported by correlative evidence that *CAPN14* mRNA expression in pigs, a species with keratinized esophagus,[32] is virtually undetectable[7] and by evolution of cationic antimicrobial peptides,[35] particularly defensins,[36] that provide antifungal resistance

of human esophageal surface.[37] CAPN14's role in regulation of esophageal tissue barrier function can account for the fact that an imbalance in proteolytic function in the human esophagus caused by genetic[9] or environmental[38] factors can result in predisposition to tissue damage, particularly loss of esophageal tissue integrity.[26]

EXPRESSION AND REGULATION OF *CAPN14*

The first evidence of *CAPN14* expression in human tissue emerged in 2001 from radiation hybrid mapping.[39] In this initial study *CAPN14* mRNA was not detected by means of either RT-PCR or Northern dot blot analysis.[39] Later, moderate-to-high levels of *CAPN14* mRNA were detected in human conjunctival[40] and corneal epithelial cells,[41] as well as in the superior frontal gyrus of patients with schizophrenia.[42] Notably, expression of *CAPN14* mRNA and protein is orders of magnitude greater in the esophagus compared with any other tissue.[9,10,26,43] Other tissues with significant *CAPN14* expression, as determined by using a microarray, are (from higher to lower) pharyngeal mucosa, tonsil epithelium, tongue squamous cells, head and neck epithelial cells, and nasopharyngeal epithelial cells (see Fig E2 in this article's Online Repository at www.jacionline.org).[44] A unifying characteristic of each of the tissues with CAPN14 expression is a similar cellular architecture that forms a stratified squamous epithelium.

The *CAPN14* gene is dynamically upregulated by the 2 related proallergic T_H2 cytokines IL-4 and IL-13, which share a common subunit (IL-4 receptor α) in their receptor complexes.[45] IL-13 stimulation of EPC2-immortalized esophageal epithelial cells results in a 100-fold increase in the relative expression of *CAPN14*,[9] whereas stimulation of IL-4 in conjunctival epithelial cells leads to a 4- to 22-fold upregulation of *CAPN14*.[40] Notably, in esophageal epithelial cells the induction effect of IL-13 is specific to CAPN14 because other calpains are not induced by this interleukin. The kinetics of IL-13–induced CAPN14 expression are rapid and parallel the induction of CCL26 (eotaxin-3), a markedly induced IL-13–upregulated gene product (Fig 3).[26]

PROTEIN STRUCTURE AND FUNCTION

The human calpain family consists of 16 members defined by their structure[2,8] and the calcium concentration[46] required for exhibiting activity: micromolar for μ-calpains (eg, calpain-1) and millimolar for m-calpains (eg, calpain-2). The structure of classical calpains, such as CAPN1 and CAPN2, consists of the N-terminal anchor helix region, the Cys protease core (PC) domain, the C2-like (C2L) domain, and the penta-EF-hand domain (PEF). In turn, the PC domain is divided into 2 subdomains. Subdomain 1 (PC1) contains the catalytic Cys residue, and subdomain 2 (PC2) contains the other 2 members of the catalytic triad: histidine (His) and asparagine (Asn). There are 3 other proteins associated with calpains: regulatory calpain small subunit (CAPNS) 1 and CAPNS2 and calpastatin (CAST). CAPNS1 contains a glycine-rich domain and a PEF domain, and its role is to stabilize the large catalytic subunit[47] by heterodimerizing through the PEF domains, although this has only been shown for CAPN1 and CAPN2.[48,49] The structure of CAPNS2 is similar to that of CAPNS1, but its physiologic role is not yet clear.[8] CAST is the only known specific endogenous inhibitor for classical calpains,[1] except for CAPN3, for which it

is a substrate.[50] Classical calpains do not necessarily interact with the small regulatory subunit,[51] but they do possess all of the domains present in CAPN1 and CAPN2. Nonclassical calpains exclude either the C2L and/or PEF domains from their catalytic subunit.[8] On the basis of sequence analysis using the National Center for Biotechnology Information (NCBI) Conserved Domain Database (CDD),[52] CAPN14 belongs to the family of classical calpains.

We have generated a 3-dimensional model of CAPN14 (Fig 4, *A*) by using the Phyre2 server[53] based on the structure of human CAPN2 (m-calpain) as a template. Phyre2 identifies templates for modeling from the Protein Databank,[54] even with remote homology based on similarity to the predicted protein secondary structure. The proposed CAPN14 structure includes an N-terminal anchor (29 amino acids in length), PC domain (Cys protease core, 316 amino acids in length), C2L domain (158 amino acids in length), and PEF domain (181 amino acids in length). In turn, the PC domain is divided into 2 subdomains: PC1 (176 amino acids in length) and PC2 (140 amino acids in length). The secondary structure profile of CAPN14 consists of 31% α-helices, 20% β-strands, and 49% C-unstructured coils, as computed by using Polyview-2D from the 3-dimensional model (see Fig E3, *A*, in this article's Online Repository at www.jacionline.org).[55]

The model reveals the location of the predicted catalytic site of CAPN14, Cys101 in PC1 and His254/Asn278 in PC2, and putative calcium-binding sites (Fig 4, *B*) mapped from multiple sequence alignment with other annotated human calpain proteins (see Fig E3, *B*). The presumed mechanism of substrate cleavage (Fig 4, *C*) is the attack of the ionized Cys101 thiol, which has a low acid dissociation constant, pK_a, at the carbon atom of the carbonyl group attached to the peptide bond, which undergoes cleavage in the course of enzyme-driven hydrolysis (the scissile bond; red in Fig 4, *C*). This process is assisted by protonation of the carbonyl oxygen by the His254 residue, the second member of the catalytic triad, leading to formation of the covalent tetrahedral intermediate, decay of which results in cleavage of the scissile C-N bond to release fragment 1. The other fragment, still bound to the Cys101 residue of the enzyme as a thioester, is released by means of hydrolysis through a similar tetrahedral intermediate (implied but not shown in Fig 4, *C*). By analogy with the papain system,[56] Asn278, the third residue of the catalytic triad, presumably orients the proximate His254 member by forming a hydrogen bond between the carbonyl oxygen of Asn278 and the hydrogen atom attached at the N-3 atom of the His254 imidazole ring. Multiple sequence alignment with other annotated human calpains,[49,57–59] as well as the NCBI-CDD annotation, suggests that CAPN14 binds 4 calcium ions, 2 in the catalytic domain and 2 in the PEF domain (Fig 4, *B*).

CAPN14 proteolytic activity has been demonstrated by using a classical Cys protease detection assay[26] based on cleavage of the peptide substrate Suc-Lys-Lys-Val-Tyr bearing either a fluorogenic group (eg, 7-amino-4-methylcoumarin), the release of which caused by hydrolysis results in fluorescence, or a substrate for luciferase (eg, aminoluciferin), the release of which results in luminescence. CAPN14 shows significantly slower 7-amino-4-methylcoumarin substrate cleavage compared with CAPN1. There were also kinetic differences because CAPN14 shows continued reaction progress for at least 2 hours, whereas the activity of CAPN1 reaches a plateau after 20 minutes (see Fig E4, *A*, in this

article's Online Repository at www.jacionline.org). CAPN14 is inhibited by various classical calpain inhibitors,[26] such as E-64 (competitive, irreversible Cys residue binding agent), acetyl-CAST (a derivative of the endogenous competitive, reversible, tight binding protein [CAST]), PD-151746 (noncompetitive, reversible, presumably allosteric site binding[60] agent), and MDL-28170 (competitive, reversible Cys residue binding moiety). These findings suggest that CAPN14 has a similar catalytic unit to that of the most studied representatives of the calpain family, CAPN1 and CAPN2. Compared with CAPN1, the inhibitors demonstrate less efficient inactivation of CAPN14 (see Fig E4, *B–D*). Collectively, CAPN14 cleaves overlapping substrates with CAPN1 and is inhibited by Cys protease inhibitors, but it has a unique activity and inhibition profile.

ROLE IN EOE

EoE is a food antigen–induced chronic inflammatory disease of the esophagus, with clinical symptoms that include dysphagia, vomiting, and severe chest pain. Dysregulation of CAPN14 expression and activity in the esophagus provides a potential explanation for the tissue specificity of EoE.[9,10,26,43,61–63] The initial genome-wide association studies identified genetic association between the *CAPN14* gene locus and EoE (Fig 5),[9,10] with the leading G/A single nucleotide polymorphism (SNP) rs76562819 ($P = 3.3 \times 10^{-10}$) located close to the *CAPN14* transcription start site (Fig 6, *A*). The SNP is localized within the promoter region, specifically in an epigenetic hotspot characterized by increased histone 3 acetylation at lysine 27 after IL-13 treatment.[9] Electrophoretic mobility shift assays have revealed that DNA around rs76562819 binds nuclear factors in a genotype-dependent manner, with the risk allele preferentially binding to an unidentified nuclear protein that is present in IL-13–stimulated esophageal epithelial cells. The data available to date suggest that this SNP and other variants in linkage disequilibrium are statistically associated with genotype-dependent *CAPN14* expression (Fig 6, *A*). Importantly, no other diseases or phenotypes have been associated with variants at the *CAPN14* locus to date.

Several lines of evidence suggest that CAPN14 plays important regulatory roles in the esophageal epithelium, as demonstrated by studies that overexpress or silence *CAPN14* gene expression. In patients with active EoE, there is a 2- to 6-fold increase in *CAPN14* mRNA levels, and the level of CAPN14 correlates with disease activity. Although there is a positive correlation between CAPN14 and EoE disease activity, the 2p23 risk haplotype of CAPN14 is associated with a 30% reduction in *CAPN14* gene expression.[9] Microarray expression analysis of the calpain family in esophageal biopsy specimens[9] revealed that in addition to upregulation of *CAPN14*, there is also a significant increase in *CAPN3* levels, whereas expression levels of *CAPN7*, *CAPN5*, *CAPNS2*, and the intracellular calpain inhibitor *CAST* are decreased (Fig 6, *B*). However, the expression level of CAPN13 is unaffected.

Although these data are currently limited to analysis of mRNA levels, it is tempting to speculate that the calpain family might be coordinately regulated in patients with EoE. For example, in patients with therapy-resistant EoE (EoE resistant), upregulation of *CAPN14* remains high, whereas the expression of *CAPN3* is increased only slightly and expression of *CAST* is normal. Expression analysis of the biopsy specimens of patients with EoE in remission shows normal expression levels of all calpain family members, suggesting a

dynamic regulation of the calpain pathway. Consistently, CAPN14 protein is upregulated in active EoE cases (Fig 6, *C*).[26]

EoE is driven in part by increased levels of IL-13 in the esophagus,[43,64] which result in a characteristic gene expression profile, the EoE transcriptome.[43] IL-13 stimulation of differentiated esophageal epithelial cells leads to disruption of epithelial cell architecture and impaired barrier function (see Fig E5, *A*, in this article's Online Repository at www.jacionline.org), which has been attributed to at least partial reduction of desmoglein-1 (DSG1) expression.[65] DSG1 is a transmembrane glycoprotein that forms a major component of the desmosome (cell-cell junctions in epithelial cells that help resist shearing forces and are found in high concentrations in cells subject to mechanical stress). Data have implicated a strong connection between CAPN14 and IL-13–induced responses in esophageal epithelial cells, with CAPN14 potentially playing an intermediary role. For example, in the air-liquid interface system, *CAPN14* mRNA expression is increased 100-fold after IL-13 exposure.[9] Overexpression of CAPN14 is sufficient to decrease DSG1 expression,[26] disrupt epithelial cellular architecture (see Fig E5, *A*), decrease transepithelial resistance (see Fig E5, *B*), and increase fluorescein isothiocyanate–dextran flux through the epithelial layer compared with control cells (see Fig E5, *C*). As a control, none of these changes are induced by CAPN1, thus demonstrating specificity of these effects by CAPN14.[26]

Importantly, IL-13–induced loss of DSG1 expression is dependent on *CAPN14* expression, as revealed by gene-silencing experiments.[26] It is notable that a severe atopy syndrome (which includes EoE) is induced by homozygous mutations of the *DSG1* gene,[66] and there is a substantial decrease in the expression of DSG1 protein in the esophagi of patients with EoE.[65] Furthermore, loss of DSG1 expression *in vitro* is sufficient to impair barrier function and promote an esophageal epithelial transcript signature that includes increased gene expression of the proinflammatory extracellular matrix molecule periostin and thymic stromal lymphopoietin.[65] Collectively, these observations implicate the involvement of CAPN14 in impaired barrier function partially mediated by loss of DSG1 (Fig 7) and raise the hypothesis that CAPN14 is an intermediary in eliciting IL-13–induced responses in esophageal epithelial cells. However, CAPN14 involvement in IL-13–induced responses is not simply linear because silencing of the *CAPN14* gene leads to defects in repair of IL-13–induced dilated intercellular spaces and IL-13–induced disruption of basal cell organization (see Fig E5, *D*).[26] Interestingly, in patients with EoE, although *CAPN14* is overexpressed compared with that in control subjects, the disease risk allele is associated with modest reductions (30%) in *CAPN14* mRNA levels compared with the nondisease risk allele.[9]

Collectively, upregulation of CAPN14 is linked to impairment of the epithelial barrier, whereas its downregulation leads to failure to repair IL-13–induced epithelial changes (see Fig E6 in this article's Online Repository at www.jacionline.org), which is consistent with a gatekeeper role for CAPN14 similar to CAPN3's role in muscle. Similarly, patients with active EoE have increased CAPN14 expression in their esophageal epithelia; however, those with the EoE risk genetic haplotype have half of the induction seen in subjects with the nonrisk EoE haplotype. Taken together, we propose that CAPN14 has a regulatory role in IL-13–induced epithelial cell responses (Fig 7), which makes selective modulation of CAPN14 activity a potentially promising target for the treatment of EoE.[67] It is quite clear

that a cohesive picture has not yet emerged, and causality between CAPN14 and EoE might be plausible but remains to be established.

SUMMARY

CAPN14 is a classical calpain protease with a gene that is encoded in chromosome 2p23 and has a unique relative selective expression in the esophagus. It is most closely related to CAPN13 and is divergent from the rest of the calpain family. CAPN14 cleaves the same short dye-labeled peptide detector, as do CAPN1 and CAPN2, and is inhibited by the same Cys protease inhibitors (Table I), yet the relative potency of these inhibitors is unique, indicating that the development of CAPN14-specific modulators is feasible. It is now appreciated that *CAPN14* is an IL-13–induced gene in epithelial cells. Overexpression of CAPN14 in esophageal epithelial cells results in a substantially impaired barrier function, as well as loss of DSG1 expression; the latter is presumed to cause the loss of barrier integrity, at least in part. At the same time, decreased CAPN14 expression increases IL-13–mediated epithelial changes, indicating that CAPN14 might be involved in multiple aspects of IL-13–induced epithelial cell responses, which is consistent with the regulatory function of calpain family members. We propose that calpainopathy is not just a term that should be associated with CAPN3 deficiency (a cause for LGMD2A, eosinophilic myositis, or both) but should be extended to EoE because the disease is mediated by dysregulated expression, function, or both of CAPN14. The molecular steps that link tissue-specific eosinophilic disorders, such as myositis and esophagitis, with genetic variants in *CAPN3* and *CAPN14*, respectively, are an important area of research investigation because selective modulation of calpain activity might be a valuable therapeutic target for eosinophilic and allergic disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

Asn	Asparagine
CAPN	Calpain
CAPNS	Calpain small subunit
CAST	Calpastatin
CDD	Conserved Domain Database
C2L	C2-like

Cys	Cysteine
DSG1	Desmoglein-1
EoE	Eosinophilic esophagitis
His	Histidine
LGMD2A	Limb-girdle muscular dystrophy type 2A
NCBI	National Center for Biotechnology Information
PC	Protease core
PEF	Penta-EF-hand
SNP	Single nucleotide polymorphism

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What do we know?

- CAPN14 is a Cys protease that belongs to the family of classical calpains.
- CAPN14 is inhibited by classical calpain inhibitors, although with lower efficiency than CAPN1.
- CAPN14 is expressed most highly in human esophagus.
- CAPN14 is genetically linked to EoE.
- CAPN14 is transcriptionally and epigenetically upregulated by the T_H2 cytokine IL-13 in esophageal epithelium.
- CAPN14 is implicated in downregulation of DSG1, a protein that is important for epithelial barrier function.
- CAPN14 is involved in repairing IL-13–induced epithelial changes.

We propose that EoE is a calpainopathy.

What is still unknown?

- What are CAPN14's real physiologic substrates?
- Identity of the binding partners (eg, small regulatory subunits and CAST)
- Identity of the conserved covalent bonds subjected to proteolysis (eg, scissile bonds)
- Detailed enzymatic properties (ie, conformational differences between the inactive and active forms of the enzyme, location of Ca^{2+}-binding sites, calcium concentration required for the enzyme's activation, and kinetic parameters for substrate cleavage)
- Selective modulators of activity (ie, activators, stabilizers, and inhibitors)
- Molecular steps that link EoE to dysregulated CAPN14 function

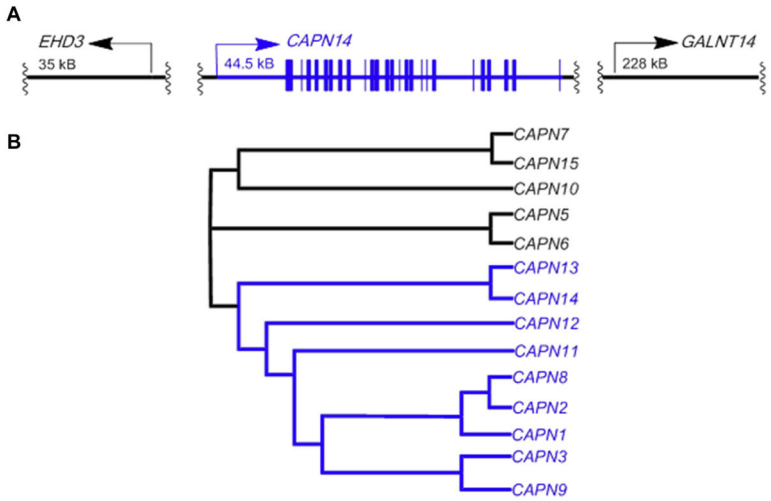

FIG 1.

A, The structure of the *CAPN14* gene locus. Human *CAPN14* (blue) is located at chromosome 2p23, according to RefSeq human genome alignment GRCh38/hg38, in juxtaposition to the polypeptide N-acetylgalactosaminyltransferase 14 *(GALNT14)* and encoding homology domain containing 3 *(EHD3)* genes. It has 26 exons *(blue vertical lines)*. **B,** Phylogenetic tree of human calpains based on multiple sequence alignment by using Clustal Omega. Classical calpains are shown in blue. *CAPN14* has the closest distance to *CAPN13*.

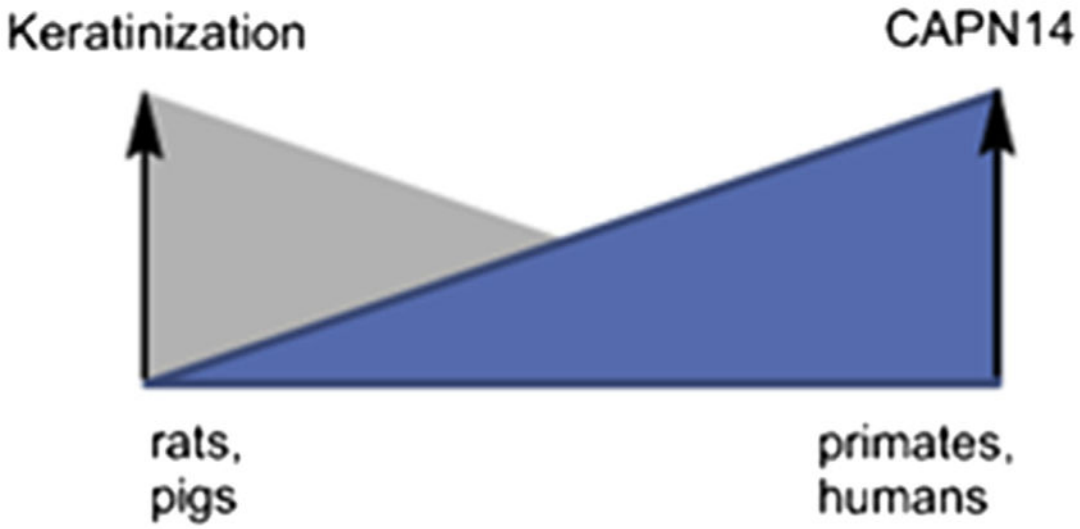

FIG 2.

Hypothetical connection between the 2 defense mechanisms against esophageal damage: keratinization of esophagus versus esophageal expression of protecting molecules (CAPN14).

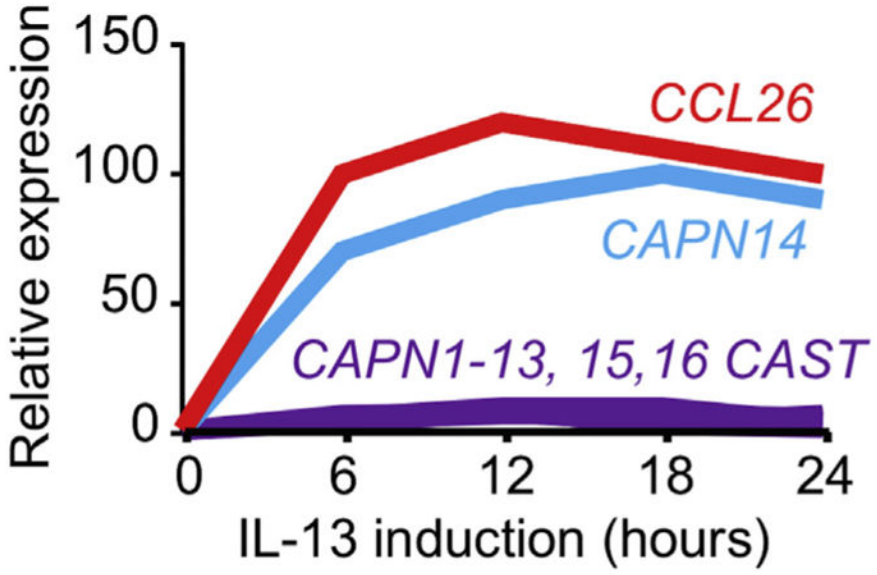

FIG 3.

Expression of mRNA of calpain family members relative to *GAPDH* in EPC2 cells exposed to IL-13 for the indicated times. Eotaxin-3 (*CCL26*) is a positive control for IL-13 stimulation.

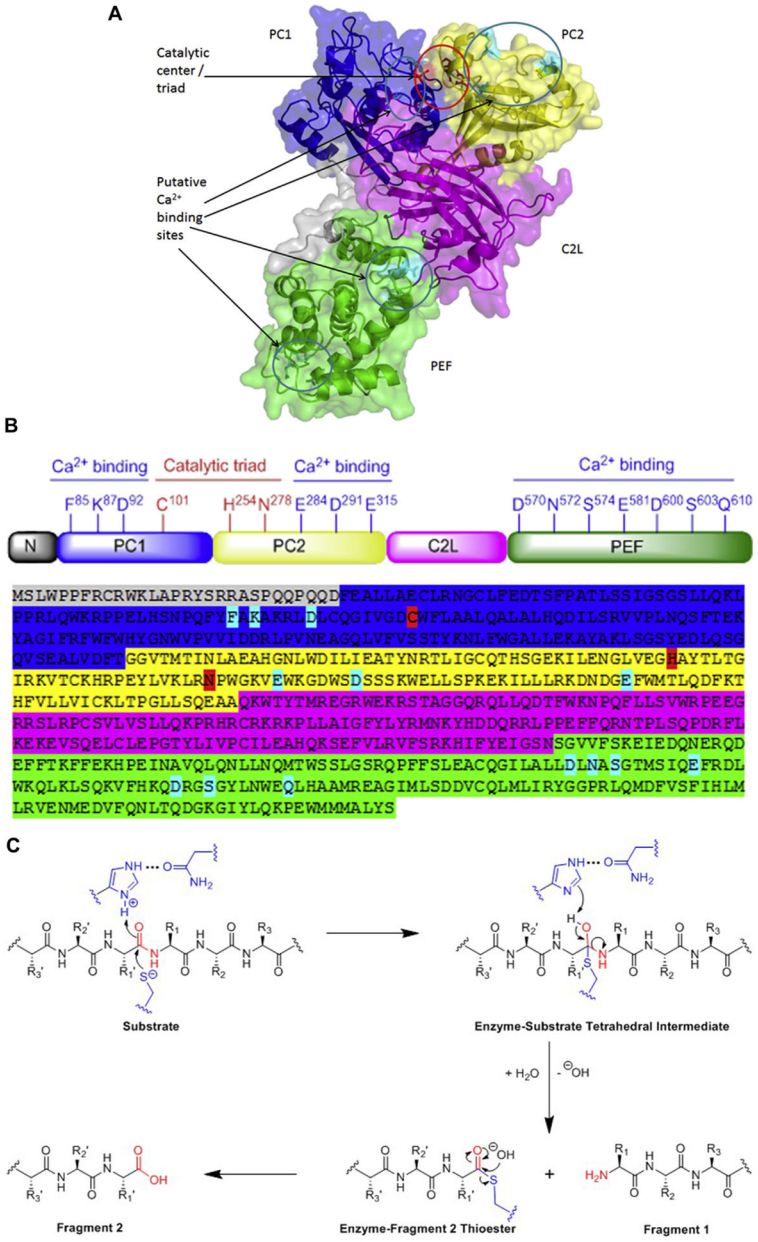

FIG 4.

A, A 3-dimensional model of human CAPN14 built by using Phyre2 based on a human CAPN2 (m-calpain; Protein Databank ID: 1KFX) template. Major domains are labeled and colored differently: gray is the N-terminal anchor, blue is the PC1 domain, yellow is the PC2 domain, purple is the C2L domain, and green is the PEF domain. The residues constituting the putative catalytic triad and the Ca^{2+}-binding sites are rendered with side chains and circled in red and cyan, respectively. Cartoon representation of secondary structures includes springs (α-helices), arrowed ribbons (β-strands), and irregular coils (unstructured loops). **B,** Schematic primary structure of human CAPN14. The amino acid sequences are highlighted in colors corresponding to the major domains, except for the catalytic triad (red) and calcium

ion-binding residues (cyan). **C,** Proposed mechanism of CAPN14 function. The thiol of ionized Cys101 attacks the scissile bond carbonyl carbon while protonated N_{D1} of the imidazole of His254 transfers the proton to the carbonyl oxygen of the substrate to form the covalent enzyme substrate tetrahedral intermediate. Decay of the tetrahedral intermediate results in cleavage of the scissile C-N bond in the substrate, releasing fragment 1, and subsequent hydrolysis of the remaining enzyme-protein thioester releases fragment 2.

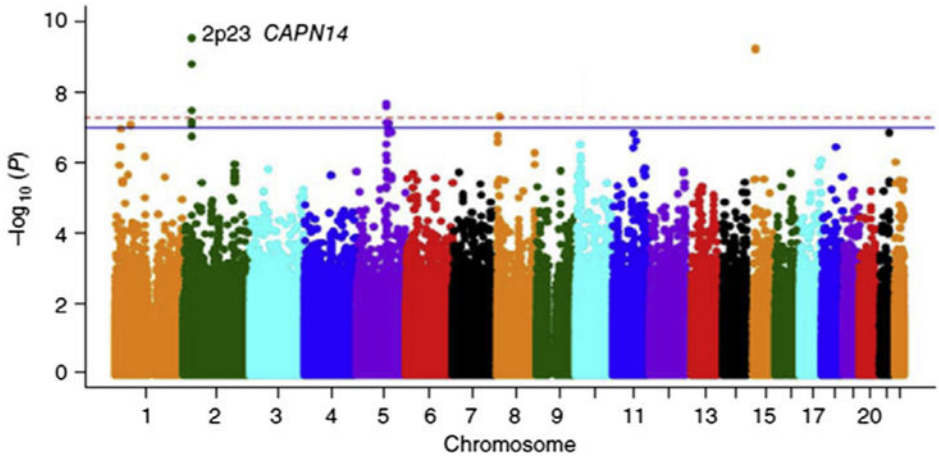

FIG 5.

Manhattan plot of *P* values obtained from the genome-wide association analysis. Data are from 736 patients with EoE and 9246 control subjects over 1,468,075 genetic variants, with minor allele frequencies of greater than 1% in the patients with EoE. The $-\log_{10}$ of the probability is shown as a function of genomic position on the autosomes. Indicated are genome-wide significance (*pink dashed line;* $P \leq 5 \times 10^{-8}$) and suggestive significance (*solid blue line;* $P \leq 1 \times 10^{-7}$). The figure is modified from Kottyan et al[9] with permission from *Nature Genetics*.

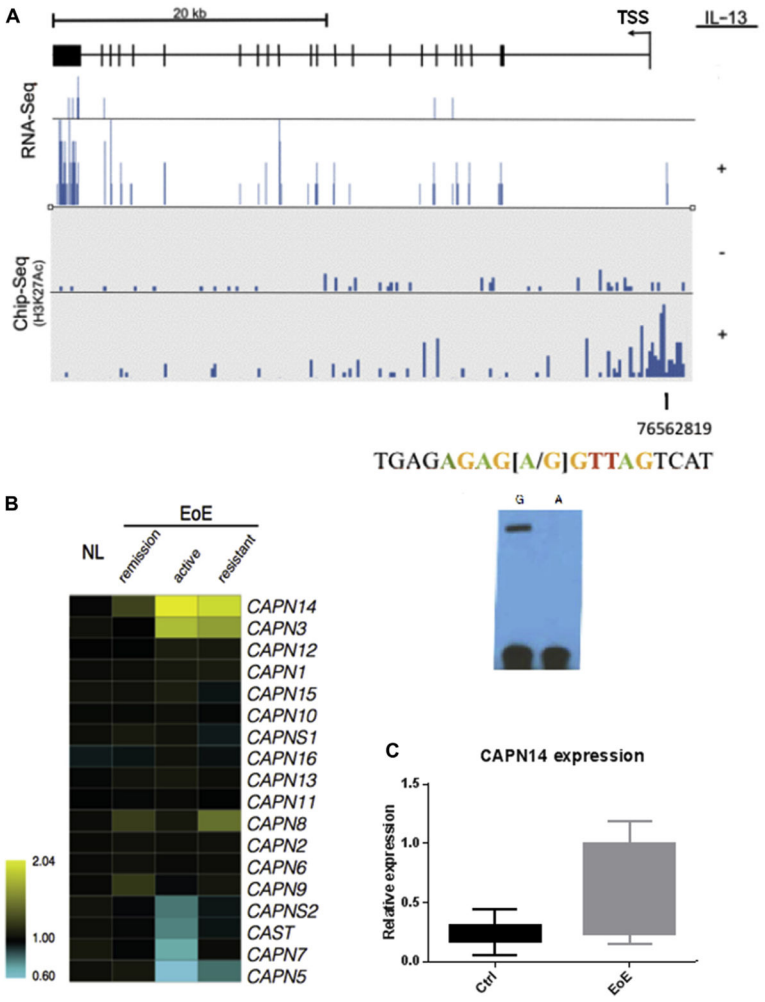

FIG 6.

Transcriptional and epigenetic analysis of *CAPN14* in esophageal epithelial cells. **A,** The most significantly associated SNP rs76562819 is located in the IL-13–stimulated acetylation region of histone 3 acetylated at lysine 27 *(H3K27ac)* over the transcription start site of *CAPN14*. Shown is an electrophoretic mobility shift assay of nuclear lysates from an esophageal epithelial cell line using oligonucleotides with the risk (G) and nonrisk (A) allele of rs76562819. **B,** Expression of *CAPN* genes in esophageal biopsy specimens. The heat map shows the relative expression of *CAPN* and *CAST* genes in the biopsy specimens taken from healthy control subjects (*NL*; 14 patients), patients with therapy-responsive EoE (*remission*; 18 patients), patients with active EoE (*active*; 18 patients), and patients with therapy-resistant EoE (*resistant*; 19 patients). Yellow and blue represent increased and decreased expression, respectively. **C,** Quantification of CAPN14 protein in control and active EoE biopsy specimens taken from 8 patients. *Error bars* indicate SEMs. The figure is modified from Kottyan et al[9] with permission from *Nature Genetics*.

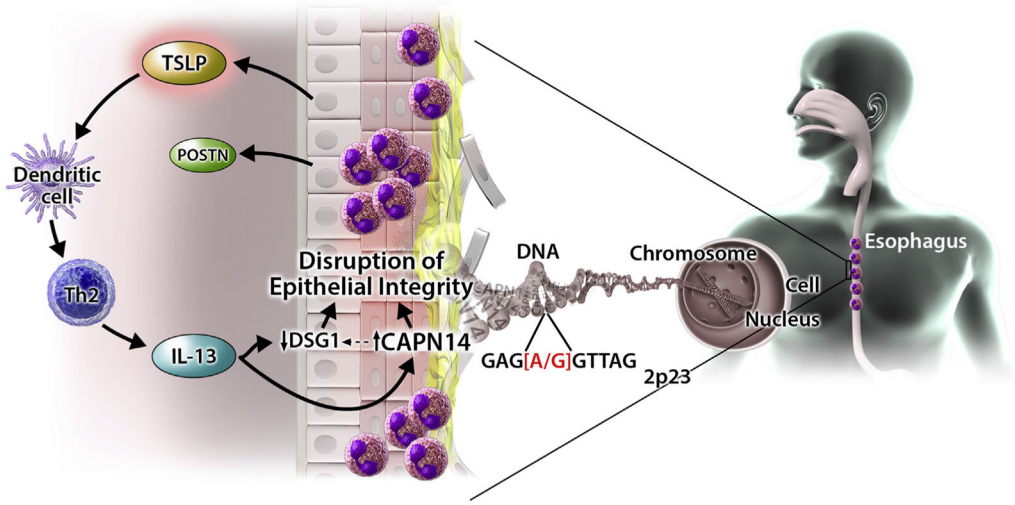

FIG 7.

Proposed role of CAPN14 activity in patients with EoE. IL-13 upregulates CAPN14, the increased expression of which leads to loss of DSG1, which results in impaired barrier function and violated homeostasis, ultimately leading to disrupted integrity of esophageal epithelium. CAPN14 exerts an effector and regulatory role in IL-13–induced responses.

TABLE I

Summary of CAPN14 properties

Genomic position	2p23.1
Gene length	44,490 bp
Exons	26
Protein length	684 amino acids
Protein type	Classical calpain
No. of putative Ca^{2+}-binding sites	4
No. of EF hands	5
Tissue specificity (from highest to lowest)	Esophagus
	Pharyngeal mucosa
	Tonsils epithelium
	Tongue squamous cells
	Head and neck epithelium
	Nasopharyngeal epithelium
Detectors	SucLLVY-detector (detector is 7-amino-4-methylcoumarin or aminoluciferin)
Inhibitors	E-64
	PD151746
	MDL-28170
	CAST
Disease involvement	EoE