Current perspectives

Calpain-14 and its association with eosinophilic esophagitis

Vladislav A. Litosh, PhD,[a] Mark Rochman, PhD,[a] Jeffrey K. Rymer, BSci,[a,b] Aleksey Porollo, PhD,[c] Leah C. Kottyan, PhD,[c] and Marc E. Rothenberg, MD, PhD[a,d] *Cincinnati, Ohio*

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. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *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.

From [a]the Division of Allergy and Immunology and [c]the Center for Autoimmune Genomics and Etiology, Cincinnati Children's Hospital Medical Center, and [b]the Department of Molecular Genetics, Biochemistry, and Microbiology and [d]the Department of Pediatrics, University of Cincinnati College of Medicine.

Supported in part by National Institutes of Health (NIH) U19 AI070235, NIH R01 AI124355, NIH R01 DK107502, NIH P30 AR070549, R37 A1045898, the Campaign Urging Research for Eosinophilic Disease (CURED) Foundation, the Buckeye Foundation, the American Partnership for Eosinophilic Disorders (APFED; e10078), and the Sunshine Charitable Foundation and its supporters, Denise A. Bunning and David G. Bunning.

Disclosure of potential conflict of interest: L. C. Kottyan's and M. E. Rothenberg's institutions have received a grant from the National Institutes of Health. M. E. Rothenberg is a consultant for Immune Pharmaceuticals, NKT Therapeutics, Celgene, and Genetech and has an equity interest in the first two and receives royalties from Teva Pharmaceuticals for reslizumab. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication July 4, 2016; revised August 16, 2016; accepted for publication September 9, 2016.

Corresponding author: Marc E. Rothenberg, MD, PhD, Division of Allergy and Immunology, MLC 7028, 3333 Burnet Ave, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229. E-mail: marc.rothenberg@cchmc.org.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2016.09.027

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

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