

Module 2: The Biology of Human Kallikreins (KLKs)

The human kallikrein (KLK) gene family consists of 15 highly homologous serine protease genes clustered in tandem on chromosome 19q13.33-q13.41, spanning approximately 280 kb.

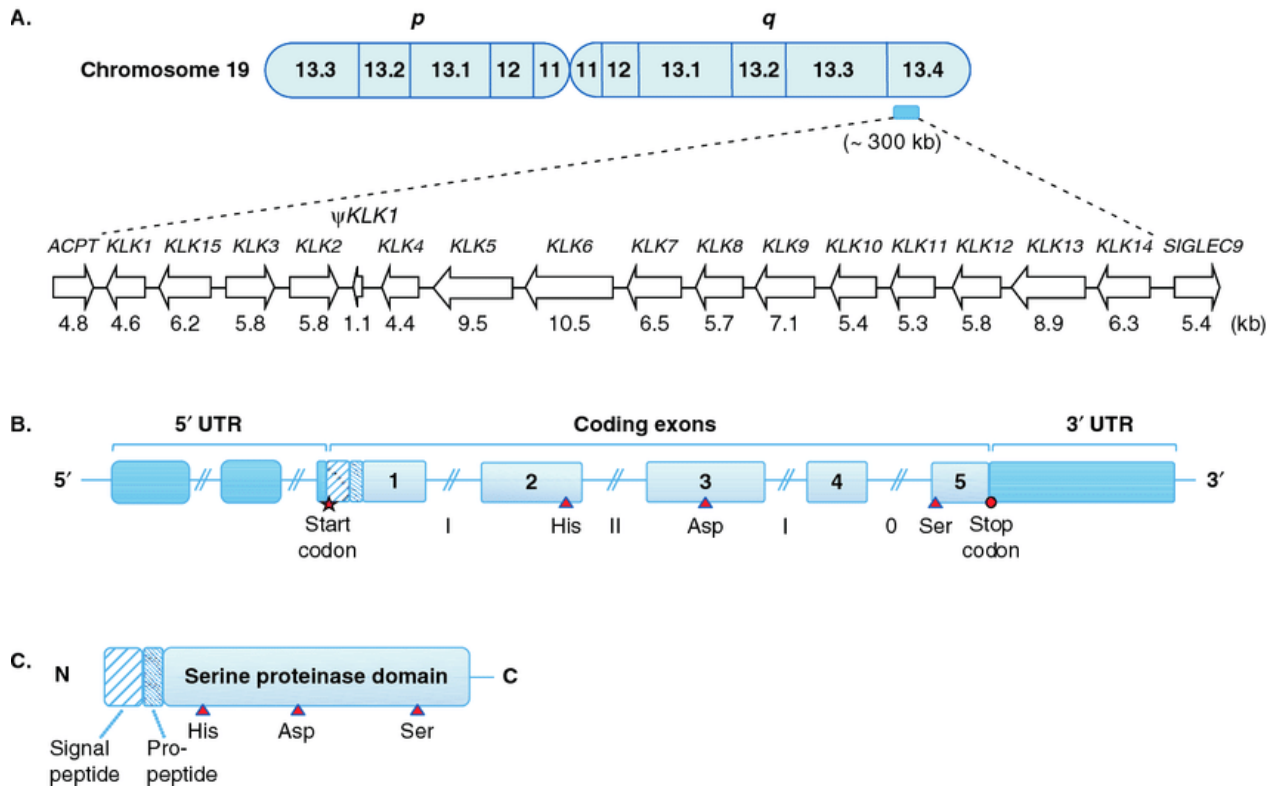


Figure 1. Gene locus, organisation pattern and protein characteristics of the human tissue kallikrein family (https://www.researchgate.net/figure/Gene-locus-organisation-pattern-and-protein-characteristics-of-the-human-tissue_fig1_232052911)

1. Genomic organization

Genes are arranged in a head-to-tail orientation with most transcribed centromere-to-telomere (KLK3, KLK2 exceptions), exhibiting conserved 5-exon structure: exon 1 (signal peptide), exons 2-4 (catalytic domain with His-Asp-Ser triad), exon 5 (3' UTR/propeptide). Introns follow phase 1/2 patterns, enabling alternative splicing that generates multiple isoforms with tissue-specific expression (<https://www.sciencedirect.com/science/article/abs/pii/S0006291X0093448X?via%3DiHub>)

2. Protease activity: From zymogen to active catalyst

Kallikreins (KLKs) are secreted as inactive pre-pro-enzymes that require sequential processing to generate catalytically active serine proteases.

(<https://www.pnas.org/doi/10.1073/pnas.2014810118>)

Activation cascade

- **Pre-pro-KLK synthesis:** N-terminal signal peptide (15-19 aa) directs ER translocation and is cleaved co-translationally, yielding pro-KLK (zymogen) with an inhibitory pro-peptide (4-11 aa).
- **Zymogen secretion:** Pro-KLK exits via the classical secretory pathway into extracellular space, protecting against premature activation.
- **Proteolytic activation:** Pro-peptide removal exposes the N-terminal Ile/Ile-Val of the mature protease domain. KLK activation occurs via:
 - Autoactivation (e.g., KLK6 at acidic pH).
 - Cascade activation (e.g., matriptase activates pro-KLK11, which activates pro-KLK3/PSA).
 - Cross-activation by other proteases (e.g., plasmin, furin).

Catalytic properties

Active KLKs feature the conserved Ser195-His57-Asp102 triad with S1 pocket specificity:

- **Trypsin-like (KLK2,3,4,5,6,11,14):** Cleave after Arg/Lys (P1 basic).
- **Chymotrypsin-like (KLK7,9):** Prefer Phe/Tyr/Leu/Tyr (P1 hydrophobic).
- **Elastase-like (KLK13):** Cleave after Ala/Val.

This zymogen-to-catalyst transition enables tightly regulated extracellular proteolysis in cascades governing ECM remodeling, signaling, and cancer invasion.

3. Hormonal regulation and signaling

Human kallikreins (KLKs) exhibit tight transcriptional regulation by steroid hormones through response elements in their clustered 19q13 locus promoters.

- **Androgens (testosterone/DHT):** Strongly induce prostate-specific KLK2 and KLK3 (PSA) via AREs; weaker effects on KLK4/11.
- **Estrogens (E2):** Upregulate KLK5/6/10/13/14 in breast/ovarian tissues through EREs/half-sites.
- **Progestins (progesterone):** Activate KLK1/3/4/14 via PREs, relevant in endometrial/breast cancers.
- **Glucocorticoids (cortisol):** Repress most KLKs via GREs; KLK9 shows positive regulation.

Signaling Pathways

Hormone-bound nuclear receptors (AR/ER/PR/GR) dimerize, recruit coactivators (SRC-1/p300), and bind conserved HREs (-200 to -5kb upstream), driving chromatin remodeling and Pol II recruitment. Cross-talk with growth factors (EGF → MAPK → AR phosphorylation) amplifies expression in hormone-dependent tumors.

Cancer Relevance

Dysregulated hormonal control underlies elevated serum KLK3 (PSA) for prostate screening and KLK10/11 as ovarian/prostate progression markers, positioning HRE polymorphisms as risk modifiers.

4. Pathophysiology: Tissue remodeling and disease

Human kallikreins (KLKs) orchestrate extracellular matrix remodeling and cell signaling, with dysregulated cascades driving pathological progression from inflammation to malignancy.

Physiological roles

KLKs maintain tissue homeostasis through regulated proteolysis:

- **Skin barrier dynamics:** KLK5 initiates the desquamation cascade (KLK5→7→14), cleaving corneodesmosomes to shed dead cells while preserving barrier integrity.
- **Reproductive physiology:** KLK3 (PSA) and KLK4 liquefy seminal coagulum via fibronectin/fibrinogen degradation, enabling sperm motility.

(<https://doi.org/10.1155/2015/946572>)

Inflammatory dysregulation

KLK overexpression disrupts epithelial barriers:

- **Netherton syndrome:** KLK5/7/14 hyperactivation causes corneodesmosome over-degradation → barrier loss → SPINK5 mutations amplify vicious cycle.
- **Atopic dermatitis:** KLK7 cleaves desmocollin-1, exacerbating Th2-driven inflammation and PAR2 signaling.

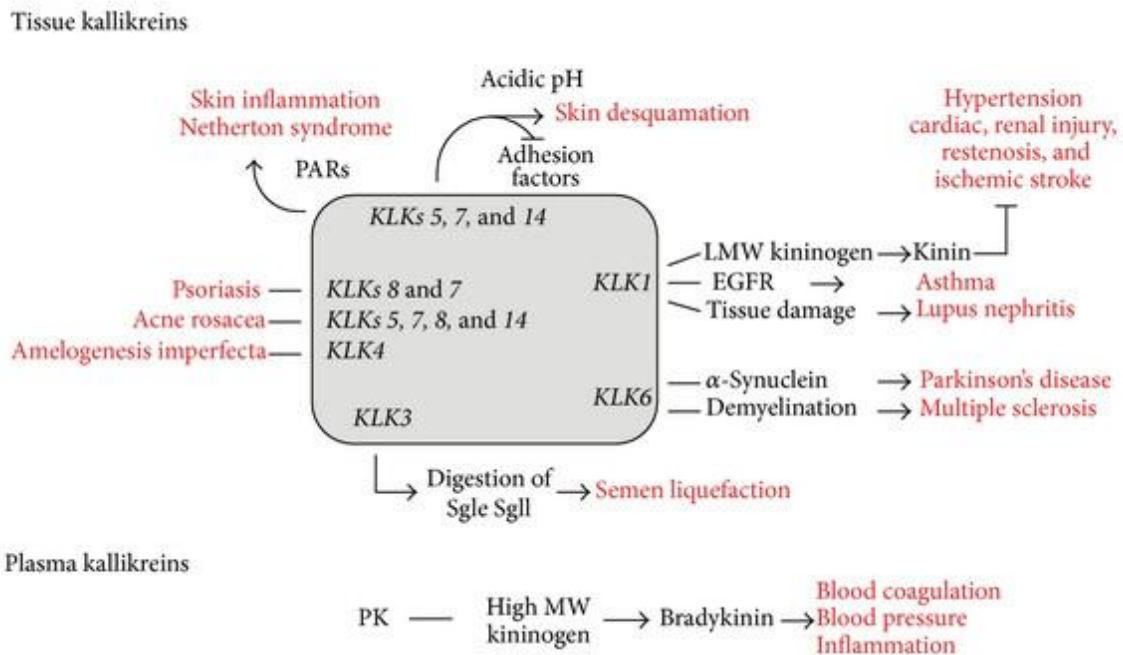


Figure 2. Schematic representation of KLK functions related to physiological and pathological conditions (<https://onlinelibrary.wiley.com/doi/10.1155/2015/946572>)

Dose-dependent tumorigenicity

ACDC Lab signature finding: KLK6 exhibits biphasic function in MDA-MB-231 breast cancer cells:

- Low expression (<10 ng/ml): Minimal proliferation/colony formation.
- High expression (>100 ng/ml): 4-fold ↑ soft agar colonies, 3-fold ↑ xenograft tumor volume via ECM degradation (collagen IV) and uPAR activation.

(Pampalakis G, Zingkou E, Sidiropoulos KG, Diamandis EP, Zoumpourlis V, Yousef GM, Sotiropoulou G. Biochemical pathways mediated by KLK6 protease in breast cancer. *Mol Oncol.* 2019 Nov;13(11):2329-2343. doi: 10.1002/1878-0261.12493. Epub 2019 Sep 30. PMID: 30980596; PMCID: PMC6822253.)