

The New Human Tissue Kallikrein Gene Family: Structure, Function, and Association to Disease*

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ABSTRACT

The human tissue kallikrein gene family was, until recently, thought to consist of only three genes. Two of these human kallikreins, prostate-specific antigen and human glandular kallikrein 2, are currently used as valuable biomarkers of prostatic carcinoma. More recently, new kallikrein-like genes have been discovered. It is now clear that the human tissue kallikrein gene family contains at least 15 genes. All genes share important similarities, including mapping at the same chromosomal locus (19q13.4), significant homology at both the nucleotide and protein level, and similar genomic organization.

All genes encode for putative serine proteases and most of them are regulated by steroid hormones. Recent data suggest that at least a few of these kallikrein genes are connected to malignancy. In this review, we summarize the recently accumulated knowledge on the human tissue kallikrein gene family, including gene and protein structure, predicted enzymatic activities, tissue expression, hormonal regulation, and alternative splicing. We further describe the reported associations of the human kallikreins with various human diseases and identify future avenues for research. (*Endocrine Reviews* 22: 184–204, 2001)

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I. Introduction

KALLIKREINS are a group of serine proteases that are found in diverse tissues and biological fluids. The term “Kallikrein” was introduced in the 1930s by Werle and colleagues (1, 2) who found high levels of their original isolates in the pancreas (in Greek, the “Kallikreas”). The kallikrein enzymes are now divided into two major categories: plasma kallikrein and tissue kallikrein (3, 4). These two categories differ significantly in their molecular weight, substrate specificity, immunological characteristics, gene struc-

ture, and type of kinin released. Plasma kallikrein or Fletcher factor (official symbol KLKB1)¹ is encoded by a single gene, which is located on human chromosome 4q35 (5, 6). The gene is composed of 15 exons and encodes for an enzyme that releases the bioactive peptide bradykinin from a high molecular weight precursor molecule (high mol wt kininogen) produced by the liver. Plasma kallikrein is exclusively expressed by liver cells. The function of plasma kallikrein includes its participation in the process of blood clotting and fibrinolysis and, through the release of bradykinin, in the regulation of vascular tone and inflammatory reactions (7). Plasma kallikrein will not be discussed further in this review since the gene encoding for this enzyme has no similarities with the tissue kallikrein genes and clearly, is not a member of this multigene family. A historical perspective on the discovery of the kallikrein-kinin system and bradykinin has recently been published (8).

Tissue kallikreins are members of a large multigene family and demonstrate considerable similarities at the gene and protein level as well as in tertiary structure. In this review, we will describe recent developments, exclusively pertinent to the human family of enzymes.

The term “kallikrein” is usually used to describe an enzyme that acts upon a precursor molecule (kininogen) for release of a bioactive peptide (kinin) (7–10). Another term that is also frequently used to describe these enzymes is

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¹ KLK, kallikrein; KLK-L, kallikrein-like; EMSP1, enamel matrix serine proteinase 1; hGK-1, human glandular kallikrein-1; HSCTE, human stratum corneum tryptic enzyme; HSCCE, human stratum corneum chymotryptic enzyme; TADG-14, tumor-associated differentially expressed gene-14; TLSP, trypsin-like serine protease; NES1, normal epithelial cell-specific 1 gene; PRSS, protease serine; PRSSL, protease serine-like; HRE, hormone response element; ARE, androgen response element; CNS, central nervous system; HUGO, human genome organization; uPA, urokinase type plasminogen activator; TGF- β , transforming growth factor β ; PSA, prostate specific antigen.

"kininogenases." The term "kininase" is used to describe other enzymes that can inactivate kinins. Among the known human and animal tissue kallikreins, only one enzyme has the ability to release efficiently a bioactive kinin from a kininogen. In humans, this enzyme is known as pancreatic/renal kallikrein or, with the new nomenclature, as the KLK1 gene, encoding for human kallikrein 1 (hK1 protein) (9–12). This enzyme acts upon a liver-derived kininogen (low mol wt kininogen) to release lysyl-bradykinin (also known as kallidin), which is involved in the control of blood pressure, electrolyte balance, inflammation, and other diverse physiological processes. Tissue kallikrein (hK1) may further enzymatically digest other substrates, including growth factors, hormones, and cytokines, to mediate pleiotropic effects (7).

It should be emphasized that the generic term "tissue kallikrein" is not restricted to the description of enzymes that release bioactive peptides from precursor molecules. The term is used to describe a group of enzymes with highly conserved gene and protein structure, which also share considerable sequence homology and colocalize in the same chromosomal locus as the KLK1 gene. In this review, the term "kallikrein" will be used to describe a family of 15 genes that have a number of striking similarities, as outlined in point format in Table 1 (13). The use of the term "kallikrein" does not necessarily imply that any of these family members (with the exception of KLK1) have kininogenase activity. In fact, for human family members that have been functionally tested, it was found that they possess very low (hK2) (14, 15) or no kininogenase activity [prostate-specific antigen (PSA)] (14). These enzymes are grouped together with KLK1, based on the similarities outlined in Table 1.

II. The Human and Rodent Families of Kallikrein Genes

The tissue kallikrein literature can be roughly separated into various periods as follows. Early in the 1920s and 30s, researchers discovered the basic components of the kallikrein-kinin system and identified the molecular structure of bradykinin and kallidin (lysyl-bradykinin) in the 1960s (8). The molecular biology of the tissue kallikrein gene family was worked out in detail in both the human and rodents in the 1980s (16–19). It was then concluded that the mouse and rat gene families were composed of many genes, clustered in the same chromosomal locus. In particular, the mouse tissue kallikrein gene family is localized on chromosome 7 and consists of 24 genes, of which at least 14 encode for active

proteins (the remaining being pseudogenes) (16, 20–22). The area on chromosome 7 encompassing the mouse kallikreins is homologous to an area on human chromosome 19q13.4 that harbors the human kallikrein gene family. The rat tissue kallikrein gene family is composed of approximately 20 homologous genes of which at least 10 are expressed (18, 23–30).

Most of the rodent tissue kallikreins are expressed in the salivary glands, but a few, including the prostate, pituitary gland, and endometrium, have more diverse tissue expression (7, 9, 31–33). It is not the purpose of this review to describe in detail the rodent or other animal tissue kallikrein gene families. Excellent reviews on this subject already exist (9, 16, 17, 21, 22).

The human tissue kallikrein gene family was also discovered in the 1980s and it was then concluded that the entire family is composed of only three genes, namely KLK1, encoding for pancreatic/renal kallikrein (hK1 protein), the KLK2 gene, encoding for human glandular kallikrein 2 (hK2), and the KLK3 gene, encoding for PSA (hK3) (34–38). The major interest in human kallikreins lies in the very restricted tissue expression of hK2 and hK3 in the prostate, which qualifies them as candidate biomarkers for prostatic diseases (39–43). hK3 (PSA), in particular, has gained prominence in recent years as the most valuable tumor marker ever discovered and is currently used widely for the diagnosis, monitoring, and population screening for prostate cancer (44–51). The introduction of this test has had a major impact on prostate cancer diagnosis and monitoring and this field is still evolving (52, 53). More recently, PSA applications have extended beyond the prostate, including breast and other cancers (54–57). Over the last few years, human glandular kallikrein 2 is emerging as an additional prostatic and breast cancer biomarker, and it is now clear that it can supplement PSA testing for improved identification and differential diagnosis of prostate cancer (43, 58–66). It is thus logical to exploit the possible applications of other members of this gene family for cancer and other disease diagnosis and monitoring.

In the last 3 yr, we have witnessed the emergence of new knowledge related to the human kallikrein gene family (13). Independent researchers have cloned a number of new serine protease genes that show significant homologies with the classical human kallikreins; in addition, when these new protease genes were mapped, they were found to colocalize in the known human kallikrein gene locus on chromosome 19q13.3–q13.4 (67–90). The recent detailed molecular description of the human kallikrein gene locus (67, 68) enabled us to construct a physical map containing 15 genes that share

TABLE 1. Similarities between members of the new human kallikrein gene family

1. All genes localize to the same chromosomal region (19q13.4).
2. All genes encode for putative serine proteases with a conserved catalytic triad (histidine, aspartic acid, and serine in the appropriate positions).
3. All genes have five coding exons (some members contain one or more 5'-untranslated exons).
4. Coding exon sizes are similar or identical.
5. Intron phases are fully conserved among all 15 human members and among members of the rodent kallikrein gene families.^a
6. All genes have significant sequence homologies at the DNA and amino acid levels (40–80%).
7. Many of these genes are regulated by steroid hormones.

^a Intron phase refers to the location of the intron within the codon: intron phase I, the intron occurs after the first nucleotide of the codon; II, the intron occurs after the second nucleotide; 0, the intron occurs between codons.

significant structural similarities (Table 1). Some of these genes appear to be related to breast, ovarian, and other human cancers, and a few of them appear to encode for functional tumor suppressor genes. In view of these very recent developments, we will describe, in this review, the knowledge that has accumulated on these genes, with special emphasis on the structure of the genes and proteins, their tissue expression and hormonal regulation, and their connection to various human diseases. Where possible, functional aspects of these enzymes will also be described. We hope that the summary of these new findings on the human kallikrein gene family will facilitate further research toward better understanding their physiological function, their pathophysiology and connection to human diseases, and their possible applications in the diagnosis and monitoring of various malignancies and their future suitability as therapeutic targets.

III. Nomenclature

Until 2–3 yr ago, only three human kallikrein genes were recognized: the pancreatic/renal kallikrein (KLK1), the human glandular kallikrein 2 (KLK2), and PSA (KLK3). Rittenhouse and co-workers (43, 49) have recently published the revised nomenclature for these three genes. New developments led to the identification of 15 different genes exhibiting significant homologies and other similarities, as described in Table 1 (13). Since many of these genes were cloned independently by different investigators, various empirical names were initially used for their description.

The Human Genome Organization (HUGO) has recently proposed guidelines for human gene nomenclature. Initially, some members of the new kallikrein gene family were classified by HUGO along with other serine proteases under the prefix "PRSS", standing for "protease serine." It is now clear that this designation does not serve well the needs of the future since members of this multigene family are classified together with other serine proteases that map in different locations of the genome.

The construction of the first detailed map of the human kallikrein gene locus (13, 67, 68) allows for a more rational assignment of official gene symbols. Since the rodent and other animal species kallikrein multigene families were known before 1992, an international working party had reached agreement in 1992 on uniform nomenclature of the animal kallikreins and the three human kallikreins known at that time (91). Based on this paradigm and the guidelines of HUGO (for details please visit the Website: <http://www.gene.ucl.ac.uk/nomenclature/>), an international group of scientists working in the field agreed to adopt a nomenclature for the newer human kallikreins, consistent with that already defined for KLK1–3, as shown in Table 2 (92). In the same table, we also include previous symbols based on the PRSS system as well as names originally proposed by the discoverers of these genes (93–100). Gene numbering starts from centromere to telomere on chromosome 19q13.4 with the exception of the three classical kallikreins for which the existing nomenclature was retained and one newly discovered gene, which maps between KLK1 and KLK2 genes (69). It is possible that, in the future, new members of this gene family may be identified, either centromeric to KLK1 or telomeric to KLK14 (see below). If new kallikrein genes are identified in this locus, they will be sequentially numbered, starting with KLK16.

IV. The Human Kallikrein Gene Locus

A. Locus organization

The availability of linear genomic sequences around chromosome 19q13.3–q13.4 from the human genome project (the sequences were generated by the Lawrence Livermore National Laboratory) allowed the precise localization of the 15 members of the new human kallikrein gene family with high accuracy (± 1 nucleotide) (68) (Fig. 1). The three classical kallikreins, KLK1, KLK3, and KLK2, cluster together within a 60-kb region, as previously described by Riegman *et al.* (36,

TABLE 2. Proposed new nomenclature for human kallikreins

New gene symbol ^{a,b}	Previous gene symbol(s)	New protein symbol	Other protein names/symbols	GenBank accession no.	Reference
KLK1	KLK1	hK1	Pancreatic/renal kallikrein, hPRK	M25629, M33105	34, 93
KLK2	KLK2	hK2	Human glandular kallikrein 1, hGK-1	M18157	94
KLK3	KLK3	hK3	Prostate-specific antigen, PSA	X14810, M24543, M27274	95–97
KLK4	PRSS17, KLK-L1, KLK4	hK4	Protease, KLK-L1 protein, EMSP1	AF113141, AF135023, AF148532	70–72, 79
KLK5	KLK-L2	hK5	KLK-L2 protein; HSCTE	AF135028, AF168768	80, 81
KLK6	PRSS9	hK6	Zyme, protease M, neurosin	AF013988, AF149289, U62801, D78203	73, 74, 82, 83
KLK7	PRSS6	hK7	HSCCE	L33404, AF166330	84, 85
KLK8	PRSS19	hK8	Neurosin; ovasin; TADG-14	AB009849, AF095743, AB010780, AF055982	86, 98
KLK9	KLK-L3	hK9	KLK-L3 protein	AF135026	67
KLK10	PRSSL1, NES1	hK10	NES1 protein	AF055481, NM_002776	76, 99, 87
KLK11	PRSS20	hK11	TLSP/hippostasin	AB012917, AF164623	88, 89, 100
KLK12	KLK-L5	hK12	KLK-L5 protein	AF135025	77
KLK13	KLK-L4	hK13	KLK-L4 protein	AF135024	78
KLK14	KLK-L6	hK14	KLK-L6 protein	AF161221	90
KLK15		hK15		AF242195	69

^a The order of the genes on chromosome 19q13.4 is shown in Fig. 1.

^b For full gene names, see abbreviation footnote.

37). and Richards *et al.* (35). Another newly discovered gene, KLK15, maps between KLK1 and KLK2 (69). The remaining kallikrein genes are aligned within this locus, as shown in Fig. 1, without intervention by other genes. The direction of transcription is from telomere to centromere with the exception of KLK3 and KLK2. The genomic lengths of all these genes are relatively small, ranging from 4–10 kb. It is unlikely that this locus harbors more kallikrein-like genes either centromeric from KLK1 or telomeric from KLK14. The next neighboring gene to KLK1 is testicular acid phosphatase (ACPT; GenBank Accession no. AF321918), which is not related to kallikreins. The next neighboring gene from KLK14 is Siglec 9 (101). Siglecs belong to the immunoglobulin superfamily and encode for transmembrane receptors that have the ability to bind sialic acid (102, 103). These genes have no structural or functional relationship to the human kallikreins.

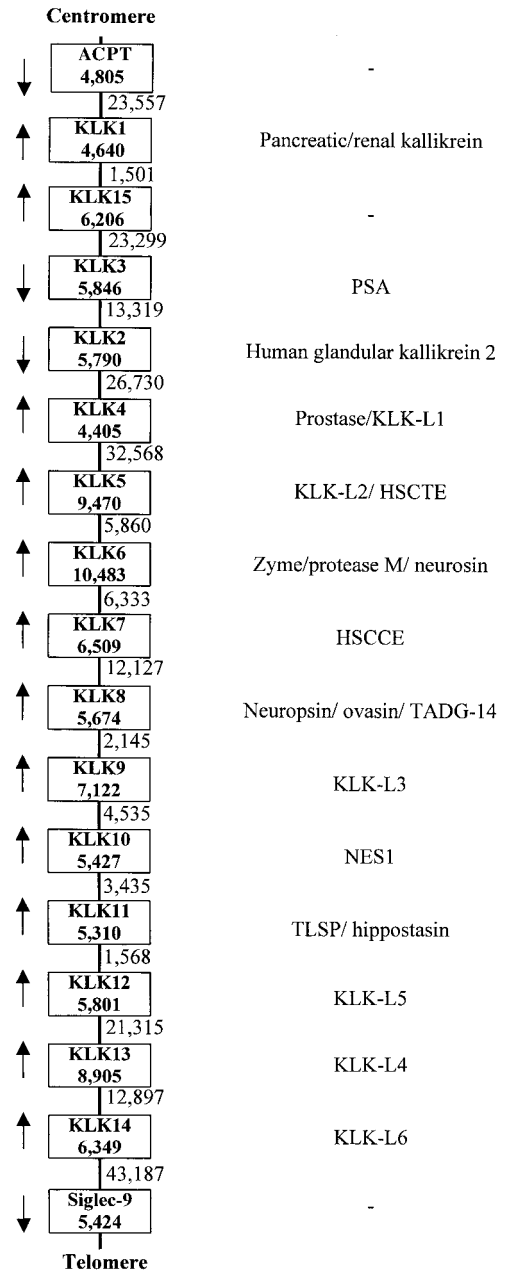
B. Gene organization

All members of the new human kallikrein multigene family encode for serine proteases. All genes consist of five coding exons, as shown in Fig. 2. The organization of all genes is very similar, with the first coding exon having a short 5'-untranslated region, the second exon harboring the amino acid histidine of the catalytic triad toward the end of the exon, the third exon harboring the aspartic acid of the catalytic triad around the middle, and the fifth exon harboring the serine of the catalytic triad, at the beginning of the exon. Beyond the stop codon, there is a 3'-untranslated region of variable length.

While it is certain that the classical kallikreins do not have 5'-untranslated exons, most other members of this multigene family have one or two 5'-untranslated exons, as shown in Fig. 2. It is possible that some other members of this gene family also harbor 5'-untranslated exons, which have not as yet been identified. In addition, the 3'-untranslated region of many of these genes is sometimes variable, giving rise to variants with different mRNA lengths, but encoding for the same protein (variant kallikrein transcripts are described under a separate heading). It is thus possible that the actual lengths of these genes, as shown in Figs. 1 and 2, may change slightly in the future.

Although the intron lengths of these genes vary considerably, the exon lengths are quite comparable or identical. Additionally, the intron phases between coding exons of all these genes (and those of the rodent kallikreins) are completely conserved among all members, with phases I–II–I–O. The intron phases are defined in Fig. 2.

Although TATA boxes have been identified within the proximal promoter of the classical kallikrein genes (Table 3), no such elements were found for most of the other kallikreins. This may be due to the absence of these elements or to the fact that the proximal promoter of some of these genes has not been accurately defined due to the presence of as yet unidentified 5'-untranslated exons. This issue merits further investigation. Classical (AATAAA) or variant polyadenylation signals have been identified 10–20 bases away from the poly A tail of all kallikrein mRNAs (Table 3). With only one exception, all splice-junction sites are fully conserved among the human kallikrein genes (Table 3).



Chromosomal locus 19q13.3-q13.4

FIG. 1. An approximate 300-kb region of contiguous genomic sequence around chromosome 19q13.4. The direction of transcription of each gene is illustrated by arrows. Boxes represent genes and contain the gene names and their genomic length, in base pairs. Other commonly used names for these genes are also mentioned. Distances between genes in base pairs are shown between boxes. The Siglec and ACPT (testicular acid phosphatase) genes do not belong to the tissue kallikrein gene family. Figure is not drawn to scale. For full gene names, see Table 2 and abbreviation footnote.

V. Protein Homologies and Predicted Enzymatic Activity

The 15 members of the new human kallikrein gene family have been aligned to identify similarities (Fig. 3). Maximum homology between all these proteins is found around the catalytic amino acids histidine (with the conserved region

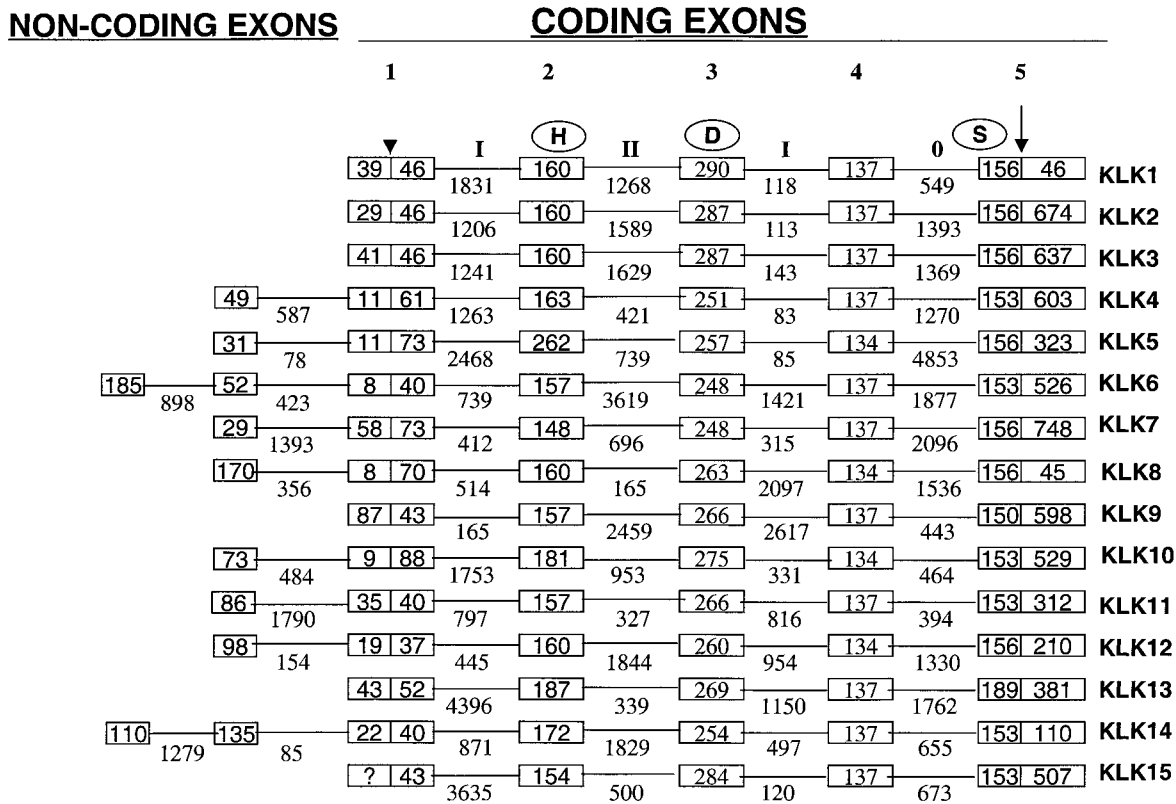


FIG. 2. Schematic diagram showing the comparison of the coding regions of the 15 kallikrein genes. Exons are shown by *solid bars* and introns by the *connecting lines*. *Letters above boxes* indicate relative positions of the catalytic triad that was found to be conserved in all genes; H, histidine; D, aspartic acid; and S, serine. *Roman numbers* indicate intron phases. The intron phase refers to the location of the intron within the codon; I denotes that the intron occurs after the first nucleotide of the codon; II, the intron occurs after the second nucleotide; 0, the intron occurs between codons. The intron phases are conserved in all genes. *Numbers inside boxes* indicate exon lengths and *numbers outside boxes* indicate intron lengths (in base pairs). The *arrowhead* represents the position of the start codon and the *arrow* indicates the position of the stop codon. *Question mark* denotes that region length is unknown. Figure is not drawn to scale.

TABLE 3. TATA, polyadenylation signals, and splice/junctions for the KLK genes^a

Gene	TATA box	Polyadenylation signal ^b	Splice junctions
KLK1	TTTAAA (-21 bp) ^c	AGTAAA (-15 bp)	Fully conserved
KLK2	TTTATA (-35 bp)	AATAAA (-19 bp)	Fully conserved
KLK3	TTTATA (-22 bp)	AATAAA (-16 bp)	Fully conserved
KLK4	TTATAA (-30 bp)	AATAAA (-15 bp)	Fully conserved
KLK5	Not found	AATAAA (-8 to -11 bp)	Fully conserved
KLK6	Not found	AATAAA (-14 bp)	Fully conserved
KLK7	Not found	AATAAA (-16 bp)	Fully conserved
KLK8	Not found	AATAAA (-15 to -18 bp)	Fully conserved
KLK9	Not found	AGTAAA (-14 bp)	Fully conserved
KLK10	TTAAAA (-35 bp)	ACTAAA (-17 bp)	gc for gt at beginning of intron 4 ^d
KLK11	Not found	AATAAA (-17 bp)	Fully conserved
KLK12	Not found	AATAAA (-16 bp)	Fully conserved
KLK13	Not found	TATAAA (-16 bp)	Fully conserved
KLK14	Not found	Putative AATAAA	Fully conserved
KLK15	Not found ^e	ATTAAA (-17 bp)	Fully conserved

^a The information was derived from the GenBank entries shown in Table 2.

^b Position of polyadenylation signal in base pairs before the poly-A tail.

^c Denotes position of TATA box considering first nucleotide of start codon as 1.

^d See Ref. 87.

^e TATA box may be present in some genes for which the 5'-proximal promoter sequences have not as yet been accurately defined.

WVLTAAHC), aspartic acid (DLMLL), and serine (GD~~S~~G-GPL). In general, the amino acid identity between the various members of this family ranges from about 40–80%. The number and position of cysteine residues are highly con-

served among the 15 human kallikreins and among other serine proteases. All members of this family possess between 10–12 cysteine residues, which are expected to form disulfide bridges. A number of other invariant amino acids (~25–30),

especially those around the active site of serine proteases, have been described (104). In the case of the human family of genes, there are 39 amino acids that are completely conserved among all 15 kallikreins (Fig. 3). Numerous other conservative amino acid substitutions are shown in Fig. 3. A phylogenetic tree of all human kallikreins and a few other serine proteases is shown in Fig. 4.

All proteins encoded by these genes are initially synthesized as preproenzymes that are then proteolytically processed to yield proenzymes by removal of the signal peptide, followed by activation (also by proteolysis) to the mature, enzymatically active forms. In Table 4, we present the reported signal and activation peptides as well as the length of the mature proteins that are encoded by these genes. It is important to mention that most of these cleavage sites have been predicted by computer programs and have been verified experimentally for only a few members.

The data of Table 4 suggest that most of the pro-forms of these enzymes are activated by cleavage at the carboxy-terminal end of either arginine (R) or lysine (K) residues (the preferred trypsin cleavage site). Since most of the human kallikrein enzymes have trypsin-like activity, they may potentially act as activating enzymes for either themselves (autoactivation) or other pro-forms of kallikreins. Kallikreins may participate in cascade pathways similar to those demonstrated for the digestive enzymes, coagulation, and apoptosis. These possibilities merit further investigation.

Protein sequence examination (Fig. 3) reveals that the three classical kallikreins possess an amino acid sequence of approximately 9–11 amino acids (the kallikrein loop) preceding the aspartic acid residue of serine proteases, which is not present in its entirety in any of the other 12 enzymes. This short sequence is thought to confer specificity for kininogenase activity but, as already mentioned, only hK1 is a potent kininogenase. KLK15 has a unique 8-amino acid sequence at positions 148–155, not found in any other kallikrein protein. Similarly, KLK13 possesses a unique amino-terminal and a unique carboxy-terminal end.

Serine proteases can be divided into two main evolutionary families, the trypsin-like serine proteases and the subtilisin-like pro-protein convertases, which presumably evolved through convergent evolution (105). The trypsin-like serine proteases are believed to have evolved from a single ancestral gene that duplicated in the course of evolution to give rise to other genes that have gradually mutated and evolved to related proteases and protease subfamilies with new functions. The various serine proteases can be markedly different in relation to their substrate specificity (106, 107). The differences are due to very subtle variations in the substrate binding pocket. Trypsin-like serine proteases have an aspartic acid in their binding pocket, which can form strong electrostatic bonds with arginine or lysine residues, which are usually present at the carboxyl-terminal part of the cleavage site. The important amino acid of the binding pocket, responsible for substrate specificity, is usually found six amino acids before the catalytic serine residue. From the 15 proteins aligned in Fig. 3, 11 have aspartic acid in this position and are expected to have trypsin-like activity. The four remaining enzymes, namely hK3 (has serine), hK7 (has asparagine), hK9 (has glycine), and hK15 (has glutamic acid),

are expected to have chymotrypsin-like or other specific enzymatic activity (see also Table 4). The cleavage specificity of these enzymes needs to be established experimentally, with the exception of hK3, which has already been characterized (50).

VI. Hormonal Regulation of Kallikrein Genes

KLK1 expression has been studied in animals, and it was concluded, by using gene-specific probes, that this enzyme is not directly regulated by androgens either in the salivary glands or the kidney (31, 108–111). Similarly, no regulation of the KLK1 gene by thyroid hormones has been demonstrated (109–111). Results of KLK1 regulation by mineralocorticoids are inconclusive (112, 113). Other data support the transcriptional up-regulation of KLK1 by estrogens (114, 115) and by dopamine in rat pituitary (116). The demonstration that KLK1 expression in human endometrium is higher during the middle of the menstrual cycle is also suggestive of KLK1 up-regulation by estrogens in this tissue (117).

Murray *et al.* (19) have reported the presence of various motifs that are reminiscent of consensus estrogen-, progesterin-, glucocorticoid-, or cAMP-response elements in the 5'-flanking sequence of the human KLK1 gene (19). However, these putative elements have not been functionally tested. Consequently, no conclusion can be drawn regarding direct regulation of KLK1 transcription by steroid or other hormones.

The regulation of the PSA (KLK3) gene by steroid hormones has been extensively studied. Initially, two androgen-response elements were identified in the proximal PSA promoter, at positions –170 [ARE1] and –394 (ARE2), respectively (118–120). These AREs have been functionally tested and found to be active in LNCaP prostate cancer cells. More recently, Schuur *et al.* have identified various regions of 5'-sequences of the PSA gene around –6 to –4 kb and demonstrated presence of a putative androgen-response element at position –4,136 (ARE3), which markedly affects PSA transcription upon induction by androgens (121). It was also demonstrated that this area harbors an enhancer that is contained within a 440-bp fragment (121, 122). The upstream enhancer, containing the putative ARE3, has a dramatic effect on PSA transcription, in comparison to the two AREs in the proximal promoter (122). The hormonal regulation of the PSA gene is not tissue specific since PSA has also been found to be regulated by steroid hormones *in vitro* and *in vivo* in breast tissues and breast carcinoma cell lines (123–125). Despite this, a number of investigators have used the PSA promoter and enhancer region to deliver and express therapeutic vectors to prostate tissue, in experimental gene therapy protocols (126–132).

A number of investigations have clearly demonstrated hormonal regulation of the PSA gene primarily by androgens in the prostatic carcinoma cell line LNCaP (133) and by androgens and progestins in the breast carcinoma cell lines BT-474, T-47D, and MFM223 (123, 125, 134).

The 5'-promoter sequences of the KLK2 gene have been studied by Murtha *et al.* (135) who have identified functional androgen response elements in the promoter of this gene.

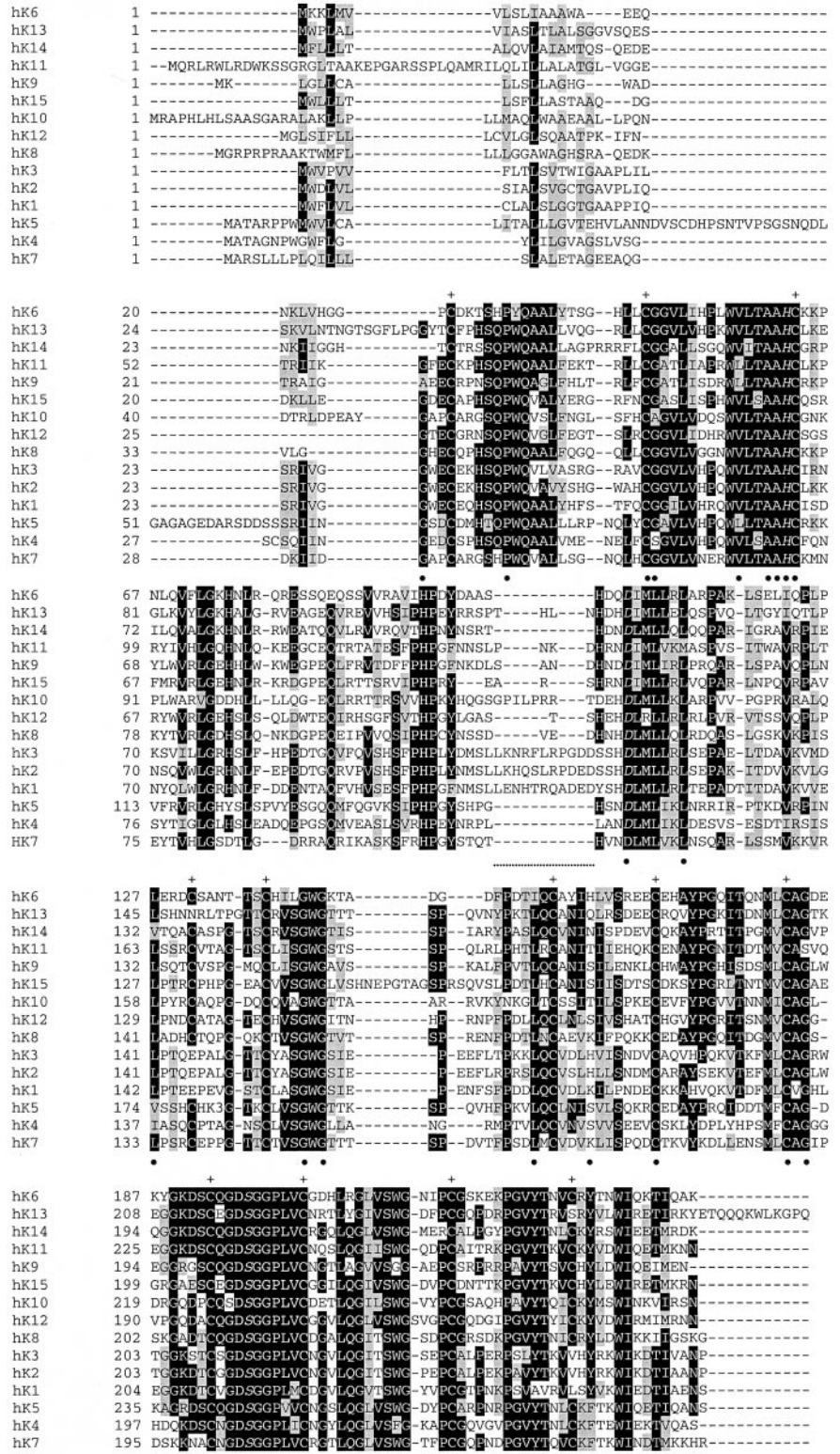


FIG. 3. Alignment of the deduced amino acid sequence of the 15 kallikrein proteins. Dashes represent gaps to bring the sequences to better alignment. The amino acids of the catalytic triad (H, D, S) are shown in *italics*. Identical amino acids are highlighted in *black* and similar residues in *gray*. The 29 invariant serine protease residues are marked by (●) on the *bottom*, and the cysteine residues by (+) on *top* of each block. The dotted area represents the kallikrein loop sequence. The asterisk (*) denotes the position of the amino acid of the binding pocket that is crucial for substrate specificity (for trypsin-like enzymes the amino acid is D). For more details, see text.

The same group has subsequently shown that KLK2 is up-regulated by androgens and progestins in the breast carcinoma cell line T47-D (136) while Riegman *et al.* (137) showed up-regulation by androgens. More recently, it has been demonstrated that, similarly to PSA, a 5'-enhancer region exists

about 3-5 kb upstream from the transcription site of the KLK2 gene (138). The enhancer region contains an androgen response element that was shown to be functionally active. Consistent with these data are the findings of hK2 protein secretion and up-regulation by androgens and progestins in

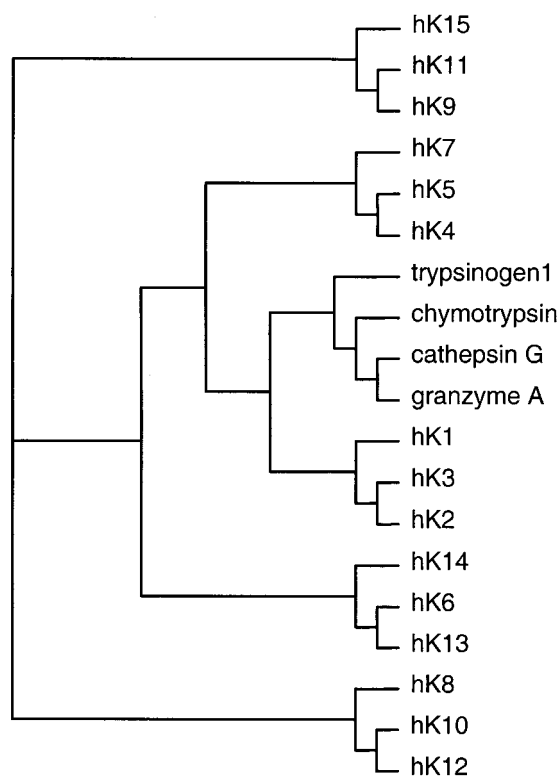


FIG. 4. Dendrogram of the predicted phylogenetic tree for the 15 kallikrein proteins and a few other related serine proteases. The neighbor-joining method was used to align these proteins. The classical kallikreins (hK1, hK2, and PSA) were grouped together; other kallikreins and serine proteases were separated in different groups, as shown. For full protein names, please see Table 2 and abbreviation footnote.

the breast cancer cell lines BT-474, T47-D, and MFM223 (134). Although the KLK2 gene promoter is not exclusively functional in the prostate, gene therapy protocols have used it for prostate cancer therapy (139).

The KLK4 gene was found to be up-regulated by androgens in the prostatic carcinoma cell line LNCaP (70) and by androgens and progestins in the breast carcinoma cell line BT-474 (71). The mode of regulation of KLK2 and KLK4 genes appears to be very similar to the mode of regulation of PSA (KLK3). Stephenson *et al.* (72) have identified putative androgen response elements in the proximal promoter region of the KLK4 gene (up to 553 bp from the transcription initiation site). However, such putative AREs have not been functionally tested, and no data have been published as yet on the characterization of possible enhancer regions further upstream from the proximal KLK4 promoter.

For the remaining 11 human kallikrein genes that have been recently identified, in none of them was the promoter functionally tested for the presence for hormone response elements (HREs). Most studies regarding hormonal regulation of these new genes have been performed with the breast carcinoma cell line BT-474 and, in some cases, with the prostatic carcinoma cell line LNCaP and other breast carcinoma cell lines. It is clear that for 10 of 11 genes under discussion (KLK5-KLK15), transcription is affected by steroid hormones, with the selectivities and potencies shown in Table 5.

Most genes appear to be up-regulated by estrogens, androgens, and progestins but with different potencies. It is possible that some of these genes are hormonally regulated through indirect mechanisms, involving *trans*-acting elements (140).

Clearly, there is a need to functionally characterize the promoter and enhancer regions of these genes to understand better the mechanism of transcriptional and posttranscriptional regulation by steroid hormones.

VII. Tissue Expression of Kallikreins

KLK1 gene expression is highest in the pancreas, kidney, and salivary glands (9). The other two classical kallikrein genes, KLK3 and KLK2, were thought, for many years, to be expressed exclusively in the prostate (39–42, 46, 141, 142). By using highly sensitive immunological techniques (143), RT-PCR technology (144) as well as immunohistochemistry (145), it has now been demonstrated unequivocally that both KLK3 and KLK2 genes are expressed in diverse tissues but at relatively much lower concentrations than prostatic tissues (55–57, 146–148). Especially, hK3 (PSA) and hK2 proteins and mRNA have been found in significant amounts in the female breast and at lower levels in many other tissues (Table 6). KLK4 also appears to have prostatic-restricted expression (70) but by RT-PCR, it was demonstrated that it is also expressed in breast and other tissues (71, 72). None of the remaining kallikreins is tissue-specific, although certain genes are preferentially expressed in breast (*e.g.*, KLK5, KLK6, KLK10, KLK13), skin (KLK5, KLK7, KLK8), central nervous system (KLK6, KLK7, KLK8, KLK9, KLK14), salivary glands (almost all kallikreins), etc. A diagrammatic representation of expression of all these kallikreins in human tissues is shown in Fig. 5. Most data have been generated by RT-PCR.

It is clear that there is frequent coexpression of many kallikreins in the same tissues, and this may point to a functional relationship. For example, it has been shown that hK3 and hK2 are regulated by similar mechanisms (134) (see also previous section) and that they are frequently coexpressed in tissues and body fluids (146–148). *In vitro* data have demonstrated that hK2, which has trypsin-like activity, can activate the proform of PSA (149–151). Other functional relationships between members of the kallikrein gene family have not been demonstrated as yet.

VIII. Variants of Kallikrein Transcripts

A relatively large number of variant transcripts have already been identified for the classic and the new human kallikrein genes (Table 7). The functional and diagnostic importance of these transcripts has not as yet been studied in detail. It will be interesting to examine whether any of these transcripts are specific for certain disease states or tissues. Although other forms of some kallikreins in serum have already been described (*e.g.*, kallikreins bound to proteinase inhibitors, internally clipped kallikreins, circulating proforms, etc.), these will not be described in this review. Excellent accounts of these forms and their clinical signifi-

TABLE 4. Proteins encoded by kallikrein genes

Kallikrein	Length of pre-proenzyme	Length of signal peptide (cleavage) ^a	Length of activation peptide/cleavage ^a	Length of mature protein	Amino acid of substrate binding pocket	Reference
hK1	262	17 (A ↓ A)	7 (R ↓ I)	238	D	34, 35, 43, 49
hK2	261	17 (A ↓ V)	7 (R ↓ I)	237	D	43, 49, 94
hK3	261	17 (A ↓ A)	7 (R ↓ I)	237	S	43, 49, 95–97
hK4	254	26 (G ↓ S)	4 (Q ↓ I)	224	D	70–72
hK5	293	29 (A ↓ N)	37 (R ↓ I)	227	D	80, 81
hK6	244	16 (A ↓ E)	5 (K ↓ L)	223	D	73, 74, 82, 83
hK7	253	22 (G ↓ E)	7 (K ↓ I)	224	N	84, 85, 107
hK8	260	28 (A ↓ Q)	4 (K ↓ V)	228	D	86, 98
hK9	251	19 (A ↓ D)	3 (R ↓ A)	229	G	67
hK10	276	33 (A ↓ A)	9 (R ↓ L)	234	D	76
hK11	250	18 (G ↓ E)	3 (R ↓ I)	229	D	88, 89, 100
hK12	248	17 (A ↓ A)	4 (K ↓ I)	227	D	77
hK13	277	20 (S ↓ Q)	5 (K ↓ V)	252	D	78
hK14	251	18 (S ↓ Q)	6 (K ↓ I)	227	D	90
hK15	256	16 (A ↓ Q)	5 (K ↓ L)	235	E	69

^a Most are predicted; need verification by experiment.

TABLE 5. Hormonal regulation of human kallikreins

Gene	Systems tested	HREs ^a	Functionally tested?	Up-regulating hormone(s)	Reference
KLK1	<i>In vivo</i> humans, rodents	Putative	No	Uncertain	19
KLK2	LNCaP; BT-474, T-47D	Yes (2 AREs)	Yes	Androgen, progestin	134–137
KLK3	LNCaP, BT-474, T-47D	Yes (3 AREs)	Yes	Androgen, progestin	118–125 133–134
KLK4	LNCaP; BT-474	Putative (AREs)	No	Androgen, progestin	70–72
KLK5	BT-474	NS (not studied)		Estrogen, progestin > androgen	80
KLK6	BT-474	NS		Estrogen, progestin > androgen	83
KLK7	BT-474	NS		Estrogen > glucocorticoid	85
KLK8	Not studied			–	
KLK9	BT-474	NS		Estrogen, progestin > androgen	67
KLK10	BT-474	Not found		Estrogen > androgen > progestin	140
KLK11	BT-474	NS		Estrogen, glucocorticoid	89
KLK12	LNCaP, BT-474, T-47D	NS		Androgen, progestin > estrogen but in BT-474, estrogen > androgen > progestin	77
KLK13	BT-474	NS		Androgen, progestin > estrogen	78
KLK14	Not studied				
KLK15	LNCaP		No	Androgen > progestin, estrogen	69

LNCaP, Prostate carcinoma cell line; BT-474, T-47D, breast carcinoma cell lines.

^a Hormone response elements.

cance already exist (43, 47–49, 152–158). It should be emphasized that, in general, the putative proteins encoded by these variant transcripts have not been isolated. By open reading frame analysis, it has been predicted that most transcripts will produce truncated proteins due to frameshifts originating from deleted exons. More details on these variant transcripts and the predicted encoded proteins can be found in the literature cited in Table 7 (34, 69, 77, 78, 86, 94, 96–98, 137, 159–165).

IX. Association of Kallikreins with Human Diseases

As already mentioned, the only enzyme with efficient kininogenase activity, among the human kallikrein family members, is hK1. The biological effects of this enzyme, and of plasma kallikrein, are mediated mainly by kinin release. Kinin binds to specific G protein-coupled cell surface receptors to mediate diverse biological functions. The kallikrein-kinin system is involved in many disease processes, including inflammation (9), hypertension (166), renal disease (167, 168), pancreatitis (169), and cancer (170–174). A recent book summarizes elegantly the physiology, molecular biology,

and pathophysiology of the kallikrein-kinin system and its association to various disease processes (175).

Among all other kallikreins, the best studied, by far, is PSA (hK3) and especially, its application to prostate cancer diagnostics. A comprehensive volume on PSA as a tumor marker has been recently published (176). The extensive literature on PSA and prostate cancer does not warrant further discussion in this review.

Although PSA concentration is generally elevated in the serum of prostate cancer patients, one less known and usually not well understood finding is PSA down-regulation in prostate cancer tissue, in comparison to normal or hyperplastic prostatic tissues (177–182). Furthermore, it has been demonstrated that lower tissue PSA concentration is associated with more aggressive forms of prostate cancer (182, 183). These data agree with those published for breast cancer, where it was found that PSA is down-regulated in cancerous breast tissues, in comparison to normal or hyperplastic breast tissues, and in more aggressive forms of breast cancer. Patients with PSA-positive tumors usually have earlier disease stage, live longer, and relapse less frequently (184–186). Furthermore, it was found that lower PSA levels in nipple as-

TABLE 6. Tissue expression of human kallikreins

Gene	Tissue expression ^a		Reference
	Highest	Other tissues	
KLK1	Pancreas, kidney, salivary glands	Sweat glands, intestine, CNS, ^b neutrophils, uterus, prostate, testis, breast, placenta	7, 9
KLK2	Prostate	Breast, thyroid, salivary glands	46, 147, 148
KLK3	Prostate	Breast, thyroid, salivary glands, lung, trachea	39–45, 54–57
KLK4	Prostate	Breast, thyroid, testis, uterus, adrenal, colon, spinal cord	70–72
KLK5	Breast, brain, testis, skin	Salivary glands, thymus, CNS, prostate, thyroid, trachea	80, 81
KLK6	CNS, breast, kidney, uterus	Salivary gland, spleen, testis	73, 74, 82, 83
KLK7	Skin, CNS, kidney, breast	Salivary glands, thymus, uterus, thyroid, placenta, trachea, testis, ovary	84, 85, 107
KLK8	CNS, skin, ovary		86, 98
KLK9	Thymus, testis, CNS, trachea	Breast, prostate, salivary glands, ovary, skin	67
KLK10	Breast, ovary, testis, prostate	Small intestine, lung, colon, pancreas, uterus, CNS, salivary glands, trachea	76
KLK11	Brain, skin, salivary gland, stomach, uterus, lung, thymus, prostate, spleen, liver, small intestine, trachea	Heart, fetal liver, breast, thyroid, skeletal muscle	88, 89
KLK12	Salivary glands, stomach, uterus, trachea, prostate, thymus, lung, colon, brain, breast, thyroid	Testis, pancreas, small intestine, spinal cord	77
KLK13	Breast, prostate, salivary glands, testis	Lung, heart, thymus, adrenal, colon, thyroid, trachea	78
KLK14	CNS	Breast, thyroid, uterus, thymus, colon, spleen, placenta, small intestine, kidney, bone marrow	90
KLK15	Thyroid, salivary glands, prostate	Adrenal, colon, testis, kidney	69

^a Most data have been produced by RT-PCR technology.

^b CNS, central nervous system.

pirate fluid of women are associated with higher risk for developing breast cancer (187). Other published data suggest that PSA may be a tumor suppressor (188), an inducer of apoptosis (188), a negative regulator of cell growth (189), and an inhibitor of angiogenesis (190, 191) and bone resorption (192, 193). These data have recently been reviewed (194).

Another set of investigations suggests that PSA may be associated with unfavorable prognosis/outcomes in breast, prostate, and other cancers. More specifically, it was found that breast tumors with higher PSA content do not respond well to tamoxifen therapy (195). Further, patients with breast tumors, which produce PSA after stimulation by medroxyprogesterone acetate (a synthetic progestin/androgen), have a worse prognosis than patients with tumors that do not produce PSA (196). A number of reports have indicated that PSA may cleave insulin-like growth factor binding protein-3, thus liberating insulin-like growth factor I (IGF-I), which is a mitogen for prostatic stromal and epithelial cells (197–199). PSA may activate latent transforming growth factor- β (TGF β), stimulate cell detachment and facilitate tumor spread (200). Like other serine proteases, PSA may mediate proteolysis of basement membrane, leading to invasion and metastasis (201).

These confusing clinical data are due to differences in methodology, purity, and source of PSA preparations used, selection of patients, etc. Furthermore, the lack of knowledge of the biological pathways in which PSA is participating poses significant difficulties in interpreting these clinical observations, as further exemplified in a recent commentary (194).

Human glandular kallikrein 2 (hK2) appears to be a new, promising biomarker for prostatic carcinoma (43). It is clear that the diagnostic value of hK2 measurement in serum is not

superior to PSA; hK2 may aid in the differential diagnosis between prostate cancer and benign prostatic hyperplasia (57–66) as well as in the identification of organ-confined *vs.* non-organ-confined disease (202). Immunohistochemical studies have shown that prostate cancer tissue produces more hK2 than normal or hyperplastic tissue (203, 204). However, recent quantitative data demonstrate that hK2 concentration, although to a lesser extent than PSA, is also decreased in cancerous tissue, in comparison to adjacent normal tissue (181). Although hK2 has been detected in breast and other tissues (146–148), no studies have as yet been performed to examine its biological action or its value as a breast disease biomarker.

Although it has been shown that KLK4 expression is relatively high in prostate (70, 71), there are no reports describing association or usefulness of this kallikrein in prostatic disease. It will be worthwhile to examine the possible clinical value of this kallikrein as a biomarker in prostatic and other diseases. Recently, KLK4 was found to be overexpressed in a subset of ovarian tumors (205).

A single report describes overexpression of KLK5 in ovarian carcinomas and association with less favorable clinical outcomes (206). Further, KLK6 appears to be dramatically down-regulated at metastatic breast cancer sites and up-regulated in a subset of primary breast and ovarian tumors (73). These data should be interpreted with caution since the number of patients was small and the techniques used were qualitative. Additionally, Little *et al.* (74) suggested that KLK6 may be amyloidogenic and may play a role in the development of Alzheimer's disease by cleaving amyloid precursor proteins. Recently, a number of newly cloned aspartyl proteinases were also shown to be amyloidogenic (207). The connection between various types of proteases and

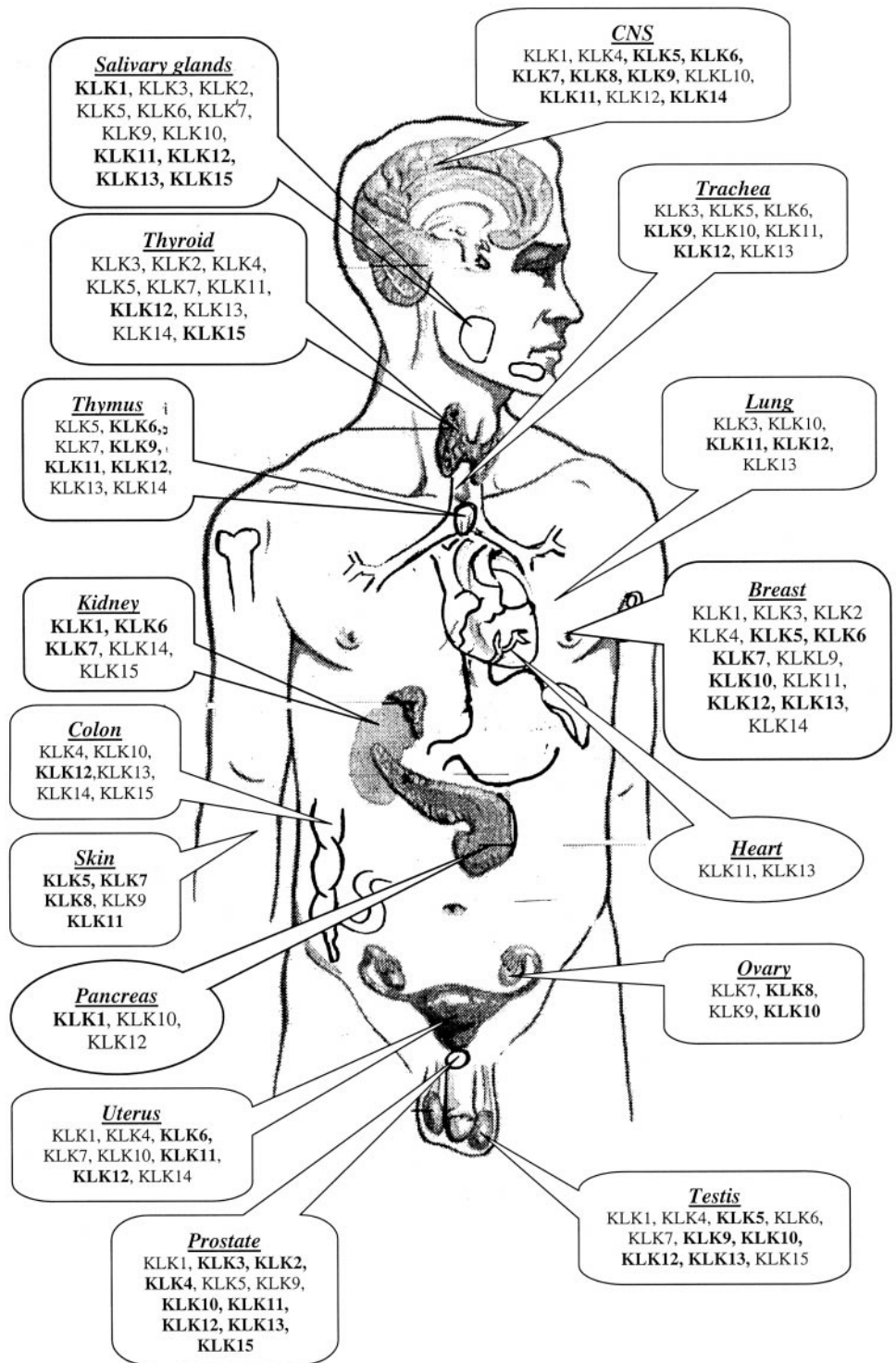


FIG. 5. Schematic representation of tissue kallikrein expression in various tissues. Higher level of expression is shown in **bold**. For more information and discussion, see text and Table 6.

this disease is still ill-defined. The connections of KLK7 with skin diseases, including pathological keratinization and psoriasis, have already been reported (75, 208). KLK7 was also found to be overexpressed in a subset of ovarian carcinomas (107). There are reports describing connection of KLK8 expression with diseases of the central nervous system, including epilepsy (209–212), injury (213, 214), and learning disturbances (215). Another report describes KLK8 overex-

pression in a subset of ovarian carcinomas (98). Although KLK10 has been shown to be a breast cancer tumor suppressor in animal models (76, 99), there is no report as yet describing prognostic or diagnostic value of KLK10 in breast carcinomas. Recently, KLK10 was found to be down-regulated in more aggressive forms of prostate cancer (216). Preliminary data suggest that KLK12, KLK13, and KLK14 may be down-regulated in a subset of breast carcinomas (77, 78,

90) while KLK15 may be overexpressed in more aggressive forms of prostate cancer (69).

The associations of kallikreins to human diseases are summarized in Table 8. Clearly, except for hK1, hK2, and hK3, the literature is quite limited and the value of the new kallikreins as disease biomarkers is just starting to be examined. Since most studies thus far used small numbers of clinical samples and qualitative methodologies, the data should be interpreted with caution. The knowledge that these kallikreins are secreted proteins supports the idea that they likely circulate in blood and that their concentration may be altered in cer-

tain human diseases, including cancer. The experience with hK3 (PSA) and hK2 in prostate cancer may be used to exploit other cancers, including those of breast, ovarian, lung, etc. These possibilities deserve further investigation.

X. Physiological Functions

Among the 15 new human kallikrein genes, only 3 have been assigned to a specific biological function (Fig. 6). hK1 exerts its biological activity mainly through the release of lysyl-bradykinin (kallidin) from low molecular weight kininogen. However, the diverse expression pattern of hK1 has led to the suggestion that the functional role of this enzyme may be specific to different cell types (7, 22). Apart from its kininogenase activity, tissue kallikrein has been implicated in the processing of growth factors and peptide hormones (217–220) in light of its presence in pituitary, pancreas, and other tissues. As summarized by Bhoola *et al.* (7), hK1 has been shown to cleave pro-insulin, low density lipoprotein, the precursor of atrial natriuretic factor, prorenin, vasoactive intestinal peptide, procollagenase, and angiotensinogen. Kallikreins, in each cell type, may possess single or multiple functions, common or unique, but Bhoola *et al.* (7) suggest that the release of kinin should still be considered the primary effect of hK1 (7).

The physiological function of hK2 protein has been examined only recently, with the availability of preparations of recombinant origin, which are essentially free of hK3 (PSA)

TABLE 7. Variant transcripts of human kallikreins

Gene	No. of known variant transcripts	GenBank accession no.	References
KLK1	3	Not available (N/A)	34, 159, 160
KLK2	6	AF188746	94, 137, 161
KLK3	5	M21896 M21897	96, 97, 162, 163
KLK4	2	N/A	Our unpublished data ^a
KLK7	4	AF166330	85
KLK8	5	AB010780 AF095743 AF251125	86, 98, 164
KLK11	2	AF164623 AB041036	89, 100
KLK12	3	AF135025	77
KLK13	7	AF135024	78, 165
KLK15	3	AF242195	69

^a Also, J. Clements, personal communication.

TABLE 8. Association of kallikreins to human disease

	Disease	Effect/use	Reference
KLK1	Inflammation; sepsis; pancreatitis; bone metabolism; heart disease; renal disease; cancer	Mediation by bradykinin or lysyl-bradykinin	9, 166–178
KLK2	Prostate cancer Breast cancer	Biomarker for diagnosis, monitoring, prognosis Expressed in breast cancer but prognostic/diagnostic value not as yet examined	43, 57–66, 202 146–148
KLK3	Prostate cancer Breast cancer	Biomarker for diagnosis and monitoring Inducer of apoptosis; decreases cell proliferation; inhibitor of angiogenesis Favorable prognostic indicator; down-regulated in more aggressive disease	42–53 188–191 184–187
KLK4	Ovarian cancer	Overexpression in a subset of more aggressive ovarian tumors	205
KLK5	Ovarian cancer	Overexpression in a subset of more aggressive ovarian tumors	206
KLK6	Breast cancer Ovarian cancer Alzheimer's disease	Down-regulation at metastatic sites and up-regulation in a subset of primary tumors Up-regulation in a subset of ovarian tumors Has amyloidogenic potential	73 73 74
KLK7	Pathological keratinization Psoriasis Ovarian cancer	Overexpression in lichen planus and benign oval keratosis Overexpression Overexpression; may be involved in tumor growth and metastasis	208 75 107
KLK8	CNS injury Kindling epilepsy Ovarian cancer	Increased KLK8 expression Increased KLK8 expression Overexpression in a subset of tumors	213, 214 209–212 98
KLK10	Breast cancer Prostate cancer	Down-regulation; KLK10 may be a tumor suppressor Down-regulated in more aggressive prostate cancer	76, 99 216
KLK12	Breast cancer	Down-regulated in a subset of breast tumors	77
KLK13	Breast cancer	Down-regulated in a subset of breast and testicular tumors	78
KLK14	Breast cancer	Down-regulation	90
KLK15	Prostate cancer	Overexpressed in more aggressive forms	69

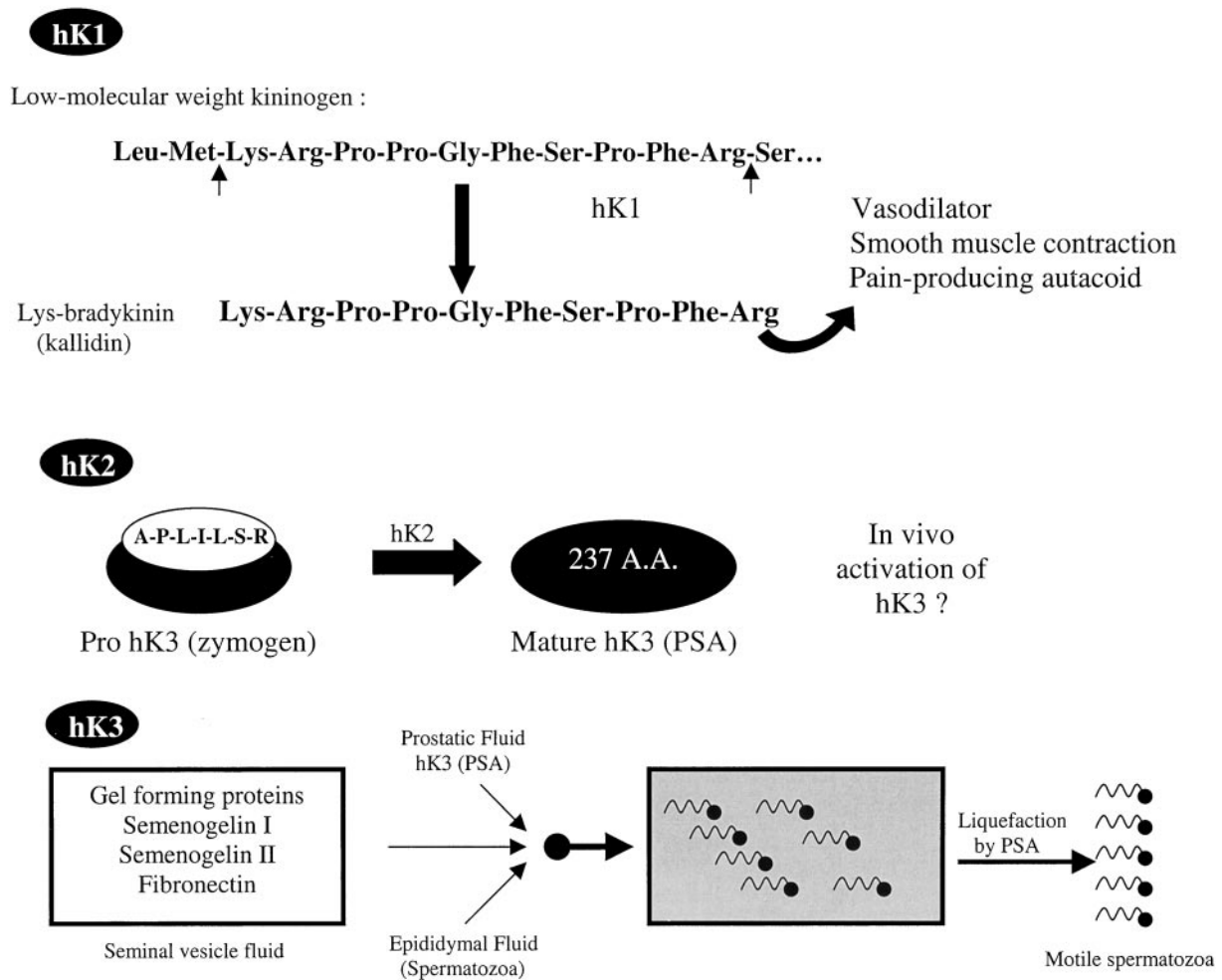


FIG. 6. Biological functions of the three classical kallikreins. hK1 cleaves low molecular weight kininogen and releases lysyl-bradykinin which mediates pleiotropic effects. Human glandular kallikrein 2 activates the pro-form of PSA. Other possible biological functions and substrates of hK2 are described in the text. hK3 (PSA) cleaves semenogelins and fibronectin and mediates seminal clot liquefaction, which increases the motility of spermatozoa. [Adapted with permission from H. G. Rittenhouse *et al.*: *Crit Rev Clin Lab Sci* 35:275–368, 1998 (43). © CRC Press.]

or other kallikrein contaminations (221–224). Three independent groups have reported activation of the pro-form of PSA by hK2 (Fig. 6) (149–151) with a process that is very similar to the autoactivation of hK2 (removal of 7 amino acids) (225). The study of substrate specificities between hK1 and hK2 reveals important differences (106, 226) suggesting that the two proteins have different natural substrates, a notion that is supported by the finding of very low kininogenase activity of hK2 in comparison to hK1 (14, 15). Seminal plasma hK2 was found to be able to cleave semenogelin I and semenogelin II but at different cleavage sites and at a lower efficiency than PSA (227). Since the amount of hK2 in seminal plasma is much lower than PSA (1–5%), the contribution of hK2 in the process of seminal clot liquefaction is expected to be relatively small (43).

In any biological fluid thus far studied, hK3 (PSA) and hK2 were found to coexist (146–148), suggesting a possible functional relationship along the lines described above. Furthermore, a role of hK2 in regulating growth factors, through IGFBP-3 proteolysis, has been suggested (228).

Recently, hK2 was found to activate the zymogen or

single-chain form of urokinase-type plasminogen activator (uPA) *in vitro* (229). Since uPA has been implicated in the promotion of cancer metastasis, hK2 may be part of this pathway in prostate cancer.

While both hK1 and hK2 have trypsin-like enzymatic activities, hK3 has chymotrypsin-like substrate specificity (230–233). Since PSA is present at very high levels in seminal plasma, most studies focused on its biological activity within this fluid. Lilja (234) has shown that PSA hydrolyzes rapidly both semenogelin I and semenogelin II, as well as fibronectin, resulting in liquefaction of the seminal plasma clot after ejaculation (234) (Fig. 6). Several other potential substrates for PSA have been identified, including IGFBP-3 (197, 199), TGF β (200), basement membrane (201), PTH-related peptide (192, 193), and plasminogen (191). The physiological relevance of these findings is still not clear.

hK3 is now known to be found at relatively high levels in nipple aspirate fluid (187, 235), breast cyst fluid (236–240), milk of lactating women (241), amniotic fluid (242), and tumor extracts (184–186). It is thus very likely that hK3 has biological extraprostatic functions in breast and other tissues

TABLE 9. Future directions in kallikrein research

Physiology	Identification of physiological substrates and metabolic pathways in various tissues Delineate mode of regulation—functional characterization of gene promoters Mode of activation and participation in cascade pathways Secretion and deactivation; binding to proteinase inhibitors/serpins Characterization and physiology of enzyme isoforms in different tissues
Pathobiology	Differential expression (overexpression/underexpression) between normal and diseased tissue Tumor promotion/metastasis or tumor suppressor activity Aberrant/ectopic expression, mutation. Activation or inactivation of other effector molecules (growth factors; peptide hormones; cytokines)
Diagnostics	Development of analytical tools (highly sensitive and specific immunoassays) Measurement in biological fluids (especially serum or plasma) Diagnosis; monitoring; prognosis; prediction of therapeutic response; population screening Tumor localization
Therapeutics	Overexpression; underexpression by using external modulators (<i>e.g.</i> , steroid hormone agonists/antagonists) Serine protease inhibitors; activators Immunotherapy; vaccination Tissue-specific delivery of therapeutic agents by using gene promoters

and may also play a role during fetal development (243). These possibilities merit further investigation.

Among all other human kallikreins, some have been connected to physiological processes and pathological conditions (as described in *Section IX*) but none has been assigned to cleave a specific substrate. Human kallikrein enzymes, with the exception of hK1, hK2, and hK3, are not commercially available and the study of their biological function has not as yet been published. Below, we will attempt to formulate some functional hypotheses for the human kallikreins.

First, all kallikreins are predicted to be secreted proteases, and it is very likely that their biological function is related to their ability to digest one or more substrates. The diversity of expression in human tissues further suggests that they may act on different substrates in different tissues. Their enzymatic activity may initiate, by activation, or terminate, by inactivation, events mediated by other molecules, including hormones, growth factors, receptors, and cytokines. The parallel expression of many kallikreins in the same tissues further suggests that they may participate in cascade reactions similar to those established for the processes of digestion, fibrinolysis, coagulation, and apoptosis. The role of these enzymes in tumor metastasis, as suggested for other proteases (244, 245), should be further investigated.

XI. Future Directions

In Table 9, we summarize some areas that may be fruitful for future kallikrein research. We have already indicated that it will be important to identify the physiological substrates of these enzymes in different tissues and the metabolic pathways in which they participate. The mode of hormonal regulation has been extensively studied only for KLK3 and KLK2. It will be important to functionally characterize gene promoters in view of the preliminary knowledge that the expression of most of these proteases in breast and prostate cancer cell lines is affected by steroid hormones. In addition, the details of activation and deactivation of these enzymes are still obscure. For some of these genes, we already have

some information regarding differential expression between normal and diseased tissues. More data are needed. The possible mutational spectrum of these genes in cancer has not been examined.

The most successful clinical application of hK3 (PSA) is currently in the diagnosis and monitoring of prostate cancer. It is anticipated that all these serine proteases circulate in the peripheral blood since they are secreted proteins. It will be important to develop the tools necessary to allow specific and highly sensitive detection of these proteins in biological fluids. Once these tools are available, we should examine whether any of these enzymes have value for diagnosis, monitoring, prediction of therapeutic response, and population screening for diseases such as prostate, breast, ovarian, and other cancers. Applicability to nonmalignant diseases, *e.g.*, Alzheimer's disease, skin pathologies, and inflammatory, autoimmune, and other chronic diseases of many organs in which kallikreins are expressed, should also be examined. Some of these enzymes may be useful targets for tumor localization with specific binding reagents or for therapeutic interventions. If any of these enzymes are shown to participate in cancer metastasis, it may be useful to examine proteinase inhibitors for therapeutic applications. Other possibilities include the use of some of these genes and their promoters for tissue-specific delivery of gene therapy or for over- or underexpression, using exogenously administered modulators (*e.g.*, hormones or hormone blockers) that are known to affect their expression.

XII. Conclusions

In this review, we attempted to summarize the very latest progress in research related to the human kallikrein gene family. For many years, this family was thought to consist of only three genes. We have provided strong evidence suggesting that the human kallikrein gene family now includes at least 15 genes, which are tandemly localized on chromosome 19q13.4 and have significant similarities at both the gene and protein level. Genomic analysis of a large region around the human kallikrein gene locus allowed not only the

precise mapping of these genes but also the delineation of the genomic organization, the prediction of protein sequence and structure, the construction of phylogenetic trees, and the comparison of homologies between all human kallikreins. The diverse tissue expression patterns and the parallel expression of many kallikreins in the same tissues suggest multiple physiological roles as well as possible interactions between the kallikrein enzymes. Many fruitful avenues of investigation are now possible. Most kallikrein genes are regulated by steroid hormones. Protein sequence variation among the kallikreins suggests that each one of them interacts with a specific substrate or a very restricted number of substrates to mediate specific biological events. Much needs to be learned about the substrate specificity of these kallikreins in diverse tissues and the mediation of biological effects from their enzymatic action.

The human kallikrein gene family has contributed the best tumor marker ever developed (PSA). It is possible that other kallikrein members may have applicability as biomarkers in cancer and other chronic and acute diseases. Unfortunately, no methods currently exist to monitor the newly discovered kallikreins with high sensitivity and specificity. The emergence of these new technologies may eventually lead to novel clinical applications of kallikreins other than PSA. We hope that this update will facilitate new developments in this field and lead to practical applications in diverse human diseases.

Acknowledgments

We would like to thank the current and previous members of the Diamandis laboratory, as well as our numerous national and international collaborators who have contributed to the work described in this review.

Note Added in Proof

Since preparation of this review, a few important developments have occurred as follows: The publication of a draft form of the sequence of the human genome will facilitate further genomic analysis within and around the human kallikrein gene locus. A recent paper further summarizes tissue expression data of kallikreins by array analysis (246). Highly sensitive and specific immunoassays for hK6 (247) and hK10 (248) have been published. With these methods, it was found that hK6 may be a biomarker for Alzheimer's disease (249) and a circulating tumor marker for ovarian cancer (250) and that hK10 is a promising new serum tumor marker for ovarian cancer (251).

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