

Human tissue kallikrein gene family: a rich source of novel disease biomarkers

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The organization of the human tissue kallikrein gene family has now been fully elucidated. This family contains 15 genes encoding secreted serine proteases, which share significant homologies at both the DNA and amino acid level. Two members of the human kallikrein gene family, prostate-specific antigen and human kallikrein 2, have already found important clinical application as prostate cancer biomarkers. In this review, we examine the diagnostic and prognostic value of the 15 human kallikrein genes and proteins. It is clear that at least a few members show promise of becoming novel cancer and other disease biomarkers.

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The completion of the human genome project will facilitate the identification of all human genes. The application of powerful new technologies – including DNA and protein microarrays and mass spectrometry – will enable researchers to study large numbers of genes and proteins simultaneously, for the purpose of identifying novel molecules suitable for diagnostics and therapeutics. It is projected that these findings will revolutionize the way we diagnose, monitor and treat human disease.

Recently, the complete genomic organization of the human tissue kallikrein gene family and the identification of 15 members, which share significant similarities at both the DNA and amino acid level, have been described [1–4]. For some of these members, highly sensitive and specific immunoassays have already been developed. These methods are suitable for measuring secreted proteins in serum and other biological fluids. While prostate-specific antigen (PSA) and human glandular kallikrein 2 (hK2) have already found clinical applicability as prostate cancer biomarkers, many other kallikreins are currently under investigation. We have strong indications that some new kallikreins may constitute valuable biomarkers for diverse diseases, including cancer and Alzheimer's disease.

In this review, we will briefly describe the reported utility of kallikrein genes and proteins for diagnosis, prognosis, prediction of therapeutic response and monitoring of patients with various diseases, with a special emphasis on cancer.

What is a kallikrein?

The term 'kallikrein' was introduced in the 1930s to describe proteolytic enzymes that can release small vasoactive peptides from high molecular weight precursors. There are two categories of kallikrein enzymes. Plasma kallikrein is encoded by a single gene on chromosome 4. This enzyme (a serine protease) releases the vasoactive peptide bradykinin from a high molecular weight precursor synthesized in the liver [5]. The human tissue kallikreins are a family of genes localized on chromosome 19, which also encode for serine protease enzymes. One of these enzymes (pancreatic/renal kallikrein) releases lysyl-bradykinin (kallidin) from a low molecular weight protein precursor. This review will focus only on the human tissue kallikrein gene family; plasma kallikrein will not be discussed further.

Based on the original definition of kallikreins, which is based on the kininogenase activity of these enzymes, only pancreatic/renal kallikrein fulfils this criterion. Until a few years ago,

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another two enzymes, hK2 and human kallikrein 3 (PSA), were also classified as members of the human tissue kallikrein gene family, based on a number of significant homologies and similarities with pancreatic/renal kallikrein. More recently, other genes, encoding for similar enzymes, are also classified as members of the human kallikrein gene family. This classification is not based on the functional definition of kallikreins, but on other criteria, as exemplified in the next section. Based on the newer definition, the number of genes that are included in the human tissue kallikrein gene family has now been increased to 15.

The expansion of the human tissue kallikrein gene family and the identification of new genes has led to the conclusion that the human gene family, originally thought to be much smaller than similar families found in rodents, is now as large as the homologous families found in rat and mouse [1-6].

Human tissue kallikrein gene family

A schematic diagram showing the human tissue kallikrein gene locus on chromosome 19q13.4 is shown in FIGURE 1. All known kallikrein genes map within an approximately 300 kb region and the lengths of the genes, the distances between them as well as the direction of transcription have now been accurately defined [1-4,6]. Telomeric from the last kallikrein gene identified (KLK14), we cloned a gene that belongs to the Siglec multigene family [7]. This finding suggests that this area defines the end of the kallikrein gene family and the beginning of another family (the Siglec family of genes) [7-9]. Centromeric from the KLK1 gene, we have identified a novel gene, named 'testicular acid phosphatase' (ACPT), which is not a kallikrein and appears to indicate the end of the kallikrein gene family from this end

[Yousef *et al.*, unpublished data; see Genbank accession #AF321918]. Thus, we suggest that between the two nonkallikrein genes identified in this region (ACPT and Siglec-9), there are 15 kallikrein genes, which are tandemly aligned, as shown in FIGURE 1.

The genomic organization of each one of these kallikrein genes is very similar [2]. We have also found that all these genes and proteins exhibit many other similarities, as summarized in BOX 1. In short, all genes encode for putative secreted serine proteases and have five coding exons of similar lengths. All genes share significant sequence homologies at both the DNA and amino acid level and many of them are regulated by steroid hormones. Despite these similarities, the tissue expression of these genes varies considerably. Some genes are expressed in one or very few tissues, while others are abundantly expressed in most tissues. Detailed tissue expression data can be found elsewhere [1,2,6].

In order to simplify communication, an international group of scientists working in the field has established uniform nomenclature for the kallikrein genes and their encoded proteins. In TABLE 1, we present the official nomenclature for each one of these genes (as approved by the human gene nomenclature committee) along with names originally assigned by individual investigators [10]. In this manuscript, the official nomenclature for these genes and proteins is used throughout.

Why are kallikreins good candidate disease biomarkers?

Among all kallikrein genes, at least three of them have very restricted tissue expression. PSA (KLK3) [11], human glandular kallikrein (KLK2) [12] and prostatic acid phosphatase (KLK4) [13,14] genes are tandemly localized and are highly expressed in the prostate, but are expressed to a much lower extent in other tissues [2,6,13-15]. This

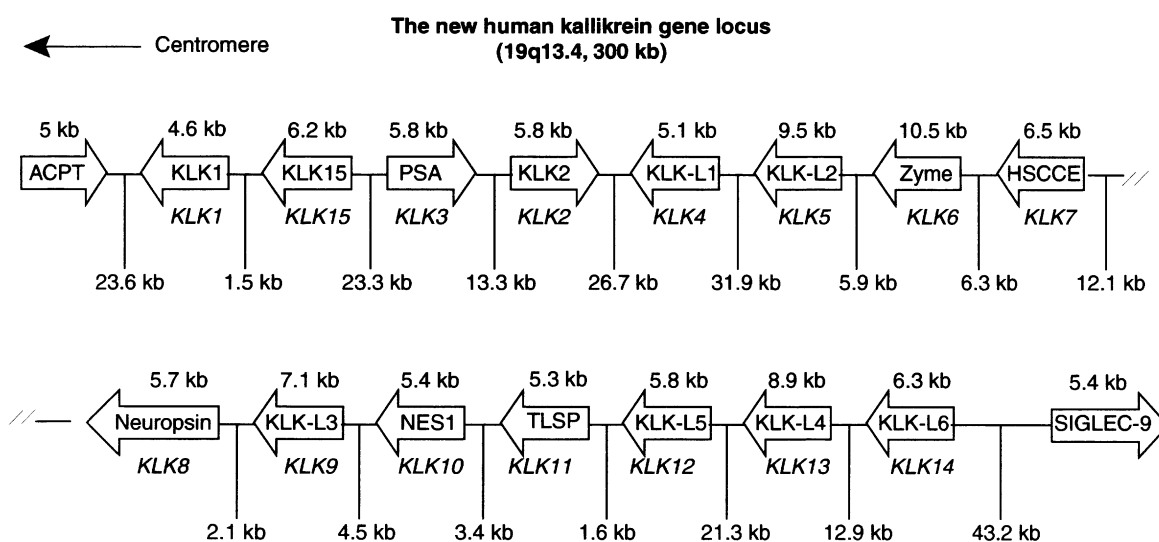


Figure 1. An approximate 300 kb region of contiguous genomic sequence around chromosome 19q13.4. The direction of transcription of each gene is illustrated by the arrows. Arrows represent genes and contain the gene names; their genomic length, in base pairs (bp) is shown above each gene. Official names for these genes are shown underneath the arrows. Distances between genes in bp are shown between arrows. The Siglec-9 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.

Box 1. Similarities between members of the human tissue kallikrein gene family.

- All genes localize to the same chromosomal region (19q13.4)
- All genes encode for putative serine proteases with a conserved catalytic triad (histidine, aspartic acid and serine in the appropriate positions)
- All genes have five coding exons (some members contain one or more 5'- untranslated exons)
- Coding exon sizes are similar or identical
- Intron phases fully conserved among all 15 human members and among members of the rodent kallikrein gene families⁵
- All genes have significant sequence homologies at the DNA and amino acid levels (40–80%)
- Many of these genes are regulated by steroid hormones

⁵Intron phase refers to the location of the intron within the codon. Intron phase I: Intron occurs after the first nucleotide of the codon; II: Intron occurs after the second nucleotide; 0: Intron occurs between codons.

restricted tissue expression and secretion into biological fluids, qualifies them as good markers for prostatic diseases. For PSA and hK2 – where experience is considerable – it is known that concentration increases in biological fluids and especially serum are not usually observed in nonprostatic diseases [11,12]. Among other kallikreins, tissue expression is more ubiquitous, although some of them are predominantly expressed in only a few tissues [1,2].

The elevation of serum concentration of these biomarkers in cancer is likely. This is due to the increased vasculature (angiogenesis) of cancerous tissues, the destruction of the glandular architecture of the tissues involved and the subsequent leakage of these proteins into the general circulation [11–13,16]. Contrary to previous beliefs, the most widely studied kallikrein tumor marker, PSA, is not overexpressed in cancer cells; its expression is lower in cancer, in comparison to normal prostatic epithelial cells [17]. It is possible that the concentration of other kallikreins may be increased in serum, due to gene overexpression, as well as to increased diffusion of these molecules into the general circulation.

For kallikreins that immunological assays have already been developed, e.g., PSA, hK2, hK6 and hK10, it has been established that their serum concentration is easily measurable in normal subjects (this is true only for males for PSA and hK2). These kallikreins have also been found in various biological fluids, including milk, nipple aspirate fluid, breast cyst fluid, seminal plasma, amniotic fluid and cerebrospinal fluid. The clinical applicability of kallikrein measurements is further discussed.

Current applications of kallikrein analysis

In TABLES 2–9 published data on the measurement of kallikrein proteins in biological fluids or tissue extracts for the purpose of disease diagnosis, monitoring, prognosis or subclassification are summarized. Studies related to prognostic and predictive value of kallikrein mRNA analysis in tissues have also been included. It is clear from these data that at least a few kallikreins have

already found important clinical applications, while some other members show promising potential. The availability of sensitive analytical methods will allow the examination of the remaining kallikreins as potential disease biomarkers.

It is possible that some kallikreins may become valuable therapeutic targets when the biological pathways that are involved are delineated. For example, the enzymatic activity of these serine proteases may initiate biological events (e.g., tumor invasion, activation of hormones, growth factors, other enzymes, receptors or cytokines, amyloid formation) or terminate biological events (e.g., inhibition of angiogenesis, inactivation of growth factors, hormones, enzymes, cytokines or receptors). Once known, these events could be manipulated

Table 1. Approved new nomenclature for human kallikreins.

New gene symbol ⁵	Previous gene symbol(s)	New protein symbol	Other protein names/symbols
KLK1	KLK1	hK1	Pancreatic/renal kallikrein, hPRK
KLK2	KLK2	hK2	Human glandular kallikrein 1, hGK-1
KLK3	KLK3	hK3	Prostate-specific antigen, PSA
KLK4	PRSS17, KLK-L1, KLK4	hK4	Protease, KLK-L1 protein, EMSP1
KLK5	KLK-L2	hK5	KLK-L2 protein, HSCTE
KLK6	PRSS9	hK6	Zyme, Protease M, neurosin
KLK7	PRSS6	hK7	HSCCE
KLK8	PRSS19	hK8	Neurosin, ovasin, TADG-14
KLK9	KLK-L3	hK9	KLK-L3 protein
KLK10	PRSSL1, NES1	hK10	NES1 protein
KLK11	PRSS20	hK11	TLSP/hippostasin
KLK12	KLK-L5	hK12	KLK-L5 protein
KLK13	KLK-L4	hK13	KLK-L4 protein
KLK14	KLK-L6	hK14	KLK-L6 protein
KLK15	-	hK15	-

⁵The order of the genes on chromosome 19q13.4 is shown in FIGURE 1. EMSP1: Enamel matrix serine proteinase 1; hGK-1: Human glandular kallikrein 1; HSCCE: Human stratum corneum chymotryptic enzyme; HSCTE: Human stratum corneum tryptic enzyme; KLK: Kallikrein; NES1: Normal epithelial cell-specific 1; PRK: Pancreatic/renal kallikrein; PSA: Prostate-specific antigen; PRSS: Protease, serine; PRSSL: Protease, serine-like; TADG-14: Tumor-associated differentially expressed gene-14; TLSP: Trypsin-like serine protease.

for therapeutic purposes by enzyme inhibitors or activators. Literature on these issues does not currently exist since the function of most kallikrein enzymes is not known at present.

Expert opinion

Currently, very few cancer biomarkers have had a major impact in clinical practice; a notable exception is PSA. One of the reasons for this low impact is that most of the current cancer biomarkers are not tissue- or cancer-specific; they are elevated in benign as well as in malignant diseases of many organs and they lack sensitivity and specificity for early disease diagnosis. For monitoring, the time-window from biochemical to clinical relapse is usually short (less than 1 year) and therapeutic approaches for treating relapsing disease are not generally very successful. It is clear that we need more specific and sensitive cancer biomarkers for early detection and more efficient monitoring of cancer.

It is anticipated with the completion of the human genome project, many new analytes will be examined as disease biomarkers. In our opinion, not a single biomarker will be effective for any disease (with some notable exceptions). It is conceivable that combination of a carefully selected panel of biomarkers will offer the required sensitivity and specificity. The advent of new, miniaturized, multiparametric testing (e.g., microarray technology) will likely facilitate the introduction of multiple tests for each disease and bioinformatic approaches (e.g., neural networks, pattern recognition and logistic regression) will bring about the required sensitivity and specificity.

The human kallikrein gene family has already contributed the best known cancer biomarker (PSA). Another new biomarker, hK2, has been tested and it shows promise of being a complementary test [12]. The new information presented here pinpoints the fact that a few other members of this gene family may have applicability as diagnostic, prognostic and predictive indicators in various cancers as well as in neurodegenerative disorders. The availability of reliable analytical methodologies for all members of the kallikrein gene family will facilitate further research. It is conceivable, that the kallikrein chip (multiparametric testing of all kallikreins simultaneously) as well as their combination with other biomarkers, may bring about a powerful diagnostic and prognostic multiparametric procedure.

Five-year view

Now that we know all the genes of the human kallikrein gene family, we predict that over the next 5 years, the following events will happen:

- We will have available purified recombinant proteins as well as monoclonal and polyclonal antibodies for all kallikrein proteins.
- We will have available highly sensitive immunoassay methodologies for quantifying all human kallikreins in biological fluids and especially in serum.
- We will be able to study the concentration levels of all these kallikreins in serum and other biological fluids from healthy individuals and patients with various diseases (e.g., cancer, neurodegenerative disorders, endocrinopathies). It is

conceivable that these investigations will lead to new diagnostic and prognostic/predictive tests.

- The availability of purified recombinant proteins will promote studies examining the physiology of these molecules, the discovery of their substrates and their mode of regulation. Biological pathways involving human kallikreins may be discovered.
- We will be able to identify the tissues expressing kallikreins, the types of cells that are secreting them and obtain clues for their function.
- It is conceivable that some kallikreins are involved – in a positive or a negative way – in pathways that lead to carcinogenesis or to other human diseases. Some of these enzymes may be suitable as therapeutic targets.

Key issues

- The human tissue kallikrein gene family contains 15 genes, which are tandemly localized on chromosome 19q13.4 and they all encode for secreted serine proteases.
- All genes and proteins share significant homologies at both the DNA and amino acid level.
- Two members of this family, PSA and hK2, have already found important clinical applicability as prostate cancer biomarkers.
- Sensitive immunoassays for quantifying a few other kallikreins (e.g., hK6 and hK10) have recently become available. These assays have revealed that hK6 and hK10 are abundantly expressed in many tissues and that these secreted products can be found in various biological fluids, including serum, milk, breast cyst fluid, nipple aspirate fluid, seminal plasma, amniotic fluid and cerebrospinal fluid.
- In preliminary studies, it has already been shown that the concentrations of hK6 and hK10 in serum are elevated in a large proportion of ovarian cancer patients and that these two kallikreins may have value for monitoring disease progression and regression.
- The concentration of hK6 in serum and cerebrospinal fluid may have value for diagnosis and monitoring of Alzheimer's disease.
- The availability of purified reagents and highly sensitive immunoassays for other kallikreins will allow examination of their value as potential biomarkers for cancer and other human diseases.
- The challenge over the next 5–10 years is to identify the biological function of these enzymes, their physiological substrates and the connection of overexpression, underexpression and mutation of these genes with the pathogenesis of various human diseases, including cancer and neurodegenerative disorders.

Information resources

Further information on kallikreins can be found at the Website: www.kallikreins.com.

Table 2. Clinical application of the KLK3 gene and its encoded protein (PSA).

Sample used	Method	Application	Ref.
Serum	Immunoassay	Population screening, diagnosis, prognosis, monitoring and molecular staging of prostate cancer; Diagnosis of breast cancer	[11,18] [19,20]
Breast cancer cytosolic extracts	Immunoassay; Immunohistochemistry	Prediction of response to megestrol acetate in breast cancer patients; Prognosis and prediction of therapeutic response of breast cancer	[21,22] [20,23–25]

Since there are many specialized reviews on PSA and its clinical applications, it will not be discussed in detail here.

Table 3. Clinical application of the KLK2 gene and its encoded protein (hK2).

Sample used	Method	Application	Ref.
Serum	Immunoassay	Diagnosis and monitoring of prostate cancer; Differential diagnosis between prostate cancer and benign prostate hyperplasia	[26,27] [28–30]
Serum/prostatic tissues	Immunoassay; Immunohistochemistry	Prognosis; aggressiveness of prostate cancer	[31–33]
Blood	RT-PCR	Molecular staging of prostate cancer	[34]
Breast cancer cytosolic extracts	Immunoassay	Prognostic value in breast cancer; relation to hormone receptors	[35]
Fluids	Immunoassay	Identification of hK2 in breast milk, breast cyst fluid, amniotic fluid and other fluids	[36,37]

Table 4. Clinical applications of the KLK4–5 genes and their encoded proteins.

Sample used	Method	Application	Ref.
<i>KLK4 (hK4)</i>			
mRNA from ovarian cancer tissues	RT-PCR	Ovarian cancer prognosis; overexpression of KLK4 in more aggressive cancers (late-stage); higher grade; shorter disease-free and overall survival	[own unpublished data]
<i>KLK5 (hK5)</i>			
mRNA from ovarian cancer tissues	RT-PCR	Ovarian cancer prognosis; overexpression of KLK5 in more aggressive cancers (late-stage); higher grade; shorter disease-free and overall survival	[38]

The hK4–5 protein has not as yet been measured in any biological fluid; no methods are available at present.

Table 5. Clinical applications of the KLK6 gene and its encoded protein (hK6).

Sample used	Method	Application	Ref.
mRNA from breast, prostate and ovarian cancer cell lines and tissues	Northern blot	Higher level of message in some cell lines and tissues; No application specified	[39]
mRNA/protein from brain tissue	Transfection; RNA protection; Western blot; immunoprecipitation	Amyloidogenic potential of hK6	[40]
Various fluids	Immunoassay	Identification in biological fluids (milk, cerebrospinal fluid, nipple aspirate fluid, breast cyst fluid, serum)	[41]
Serum	Immunoassay	Diagnosis and monitoring of ovarian cancer	[42]
Breast tumor cytosols [§]	Immunoassay	Prognosis; association to hormone receptors	[own unpublished data]
Ovarian cancer cytosols	Immunoassay	Prognosis; higher levels associated with late-stage and decreased disease-free and overall patient survival	[own unpublished data]
Cerebrospinal fluid (CSF); brain tissue extracts; whole blood	Immunoassay	Diagnosis of Alzheimer's disease; higher levels of hK6 in CSF and whole blood and lower levels (all compared to normal controls) in brain tissue extracts of Alzheimer's disease patients	[43]

[§] hK6 concentration in breast cancer cytosolic extracts is negatively associated with estrogen and progesterone receptors (our unpublished data).

Table 6. Clinical applications of the KLK7 gene and its encoded protein (hK7).

Sample used	Method	Application	Ref.
Oral mucosa	Immunohistochemistry	Differential diagnosis of pathological keratinization	[44]
Epidermis	Immunohistochemistry	Upregulation in psoriasis	[45]
Ovarian cancer RNA and extracts	RT-PCR; Northern blots; Western blots; Immunohistochemistry	Overexpression of KLK7 mRNA and protein in the majority of ovarian tumors	[46]

Table 7. Clinical applications of the KLK8 gene and its encoded protein (hK8).

Sample used	Method	Application	Ref.
Rat brain	<i>In situ</i> hybridization	Overexpression in CNS injury	[47]
Mouse brain	<i>In situ</i> hybridization	Overexpression in kindling epilepsy	[48,49]
mRNA from ovarian cancer tissue	RT-PCR	Prognosis; higher expression is associated with lower grade and improved patient disease-free and overall survival	[50]

Table 8. Clinical applications of the KLK10 gene and its encoded protein (hK10).

Sample used	Method	Application	Ref.
mRNA and extracts from breast cancer tissue and cell lines	Northern blots; Western blots	Downregulation of KLK10 in cancer cell lines	[51,52]
Tissue extracts and fluids	Immunoassay	Identification of hK10 in various tissues and biological fluids (serum, breast milk, seminal plasma, amniotic fluid, breast and ovarian cancer tissue extracts)	[53]
Serum	Immunoassay	Diagnosis and monitoring of ovarian cancer	[54]
cancer cytosolic extracts	Immunoassay	Prognosis (negatively associated with estrogen and progesterone receptors)	[own unpublished data]
Ovarian cancer cytosolic/extracts	Immunoassay; Immunohistochemistry	Prognosis; high levels are associated with late-stage disease and decreased disease-free and overall patient survival	[own unpublished data]
Testicular tissue extracts	RT-PCR; immunohistochemistry	Prognosis; downregulation in cancer in comparison to normal tissues	[55]

Table 9. Clinical applications of the KLK12–15 genes and their encoded proteins.

Sample used	Method	Application	Ref.
<i>KLK12 (hK12)</i> mRNA from breast cancer	RT-PCR	Downregulation in a subset of breast tumors	[56]
<i>KLK13 (hK13)</i> mRNA from breast cancer	RT-PCR	Downregulation in a subset of breast tumors	[57]
<i>KLK14 (hK14)</i> mRNA from breast cancer	RT-PCR	Downregulation in a subset of breast tumors	[58]
<i>KLK15 (hK15)</i> mRNA from prostatic tissues	RT-PCR	Overexpression in more aggressive forms of prostate cancer	[59]

The hK12–15 protein has not as yet been measured in any biological fluid; no methods are available at present.

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