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Porcine kallikrein gene family: Genomic structure, mapping, and differential expression analysis

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Abstract

Kallikreins belong to a family of serine proteases that are widespread throughout living organisms, expressed in diverse tissue-specific patterns, and known to have highly diverse physiological functions. The 15 human and 24 mouse kallikreins have been implicated in pathophysiology of brain, kidney, and respiratory and reproductive systems and often are used as cancer biomarkers. To better elucidate the structure and evolutionary origin of this important gene family in the pig, we have constructed a contiguous BAC clone-derived physical map of the porcine kallikrein gene region and have fully sequenced a BAC clone containing 13 kallikrein genes, 11 of which are novel. Radiation hybrid mapping assigns this kallikrein-gene-rich region to porcine chromosome 6. Phylogenetic and percent identity plot-based analyses revealed strong structure and order conservation of kallikreins among four mammalian species. Reverse transcriptase-polymerase chain reaction-based expression analysis of porcine kallikreins showed a complex expression pattern across different tissues with the thymus being the only tissue expressing all 13 kallikrein genes. [The sequence data described in this paper has been submitted to GenBank under Accession No. AC149292].

Keywords: Kallikrein; Porcine; Pig; Serine proteases; Radiation hybrid mapping; RH mapping; Comparative genomics; Physical mapping

Kallikreins are members of a multigene family of serine proteases that are widespread throughout living organisms [1]. They have a high degree of substrate specificity and demonstrate highly diverse physiological functions [2]. Kallikrein gene expression occurs in various tissues and biological fluids, suggesting that they may play roles in fertilization, digestion, regulation of blood flow, blood coagulation, inflammatory responses, endothelial cell migration, leukocyte aggregation, tissue remodeling, tumor-cell invasion and apoptosis, and disease-related specialized enzymatic activities [3–6].

Tissue kallikreins are broadly divided into two groups. Kallikreins with kininogenase activity that can release bioactive peptides from precursor molecules are described as classical kallikreins and those that reside in the same chromosomal region with highly conserved gene and protein structure to the classical kallikreins are described as nonclassical kallikreins [6]. All known classical and nonclassical kallikreins within a species show a high degree of conservation at both gene and protein levels and they colocalize to the same chromosomal locus [2,4,6].

Based on the conservation of protein structure and the enzymatic activities among serine proteases, it is hypothesized that all known serine proteases arose from a common ancestor through gene and/or chromosomal duplication during the course of evolution, resulting in their members being clustered within the genome [1,4]. The relatively similar sizes of the two rodent kallikrein gene families suggest that they may have been amplified in the rodent lineage before the divergence of rat and mouse. However, the differing number of gene family members among rodents may be due to close linkage of the family that results in gene family expansion and contraction by unequal crossing-over [7].

Comparisons of the kallikrein family members among species reveal that there is a greater level of conservation within species than among orthologous genes across species,

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suggesting that recombination has occurred within a species leading to concerted evolution [7].

Fifteen kallikrein genes have been identified from diverse tissues in humans. They reside on the same locus on chromosome 19q13.3–q13.4 [4,8] and several are implicated in breast, ovarian, and other human cancers [9]. Kallikrein 2 (KLK2) and KLK3 have been identified as candidate biomarkers for tumors and other prostatic diseases [10].

The mouse kallikrein gene family, located on a ~310-kb region of chromosome 7 and the largest family of kallikreins known to date, consists of 24–37 members. Among those, 14–26 may encode active proteins while the remaining are pseudogenes [11–13].

The functional diversity among kallikreins is emphasized by their diverse, tissue-specific expression patterns. Tissue kallikreins through their multifunctional roles are involved in (patho-)physiology of brain, kidney, respiratory, gastrointestinal, and reproductive system [14]. The diverse expression pattern of human kallikreins has led to the suggestion that the functional role of this enzyme family is dependent upon cell type, indicating that, apart from the kininogenase activity found in human KLK1, kallikreins may have the ability to process and activate growth factors and peptide hormones [4].

Kallikreins activate a wide range of substrates and growth factors [15], suggesting that they are involved in many integral processes of early embryonic development such as regulation of local blood flow, angiogenesis, tissue invasion, and mitogenesis [14]. In the rat, the kallikrein–kininogen–kinin system is activated in the ovary during ovulation [16]. In the mouse five kallikrein genes are implicated in playing a major role in uterine physiological functions and during embryo implantation [15,17,18]. These findings in rodents, together with the roles of kallikreins in embryo attachment, implantation, and placentation in humans [19], support the notion that the kallikrein–kininogen–kinin system also plays an important role during pregnancy in the pig.

Unfortunately, little is known about the structural organization and expression of the porcine kallikrein gene family. Except for KLK4 (Accession No. U76256) and KLK1 (Accession No. NM_001001911), no other porcine tissue kallikreins have been identified thus far. As a first step toward elucidating the evolution and regulation of the porcine kallikrein gene family, we have isolated and characterized its genomic region by constructing a BAC clone-based physical map of the greater kallikrein gene region, mapped it to porcine chromosome 6q12-q21, and sequenced the entire porcine kallikrein gene region revealing 11 novel porcine tissue kallikrein genes. We also have profiled the expression of kallikrein genes across a battery of porcine tissues and conducted in-depth comparative and phylogenetic analyses.

Results

Physical mapping

As a first step toward isolation and functional analysis of the kallikrein gene family in the pig, we developed overgo probes for all known human kallikrein exonic sequences and identified eight clones that contained putative kallikrein gene fragments (CH242-003F18, CH242-069G11, CH242-117C13, CH242-266E15, CH242-329F12, CH242-338F22, CH242-366F08, and CH242-370C15). Clone CH242-051O23 was selected as a random negative control. A PCR assay developed using porcine KLK4 sequence confirmed that seven clones contained KLK4 gene. Insert ends of the selected BAC clones were isolated using vectorette PCR end rescue approach and sequenced. Sequence tagged sites (STSs) were developed from clone insert ends and were used to assemble the BAC clones into a contig. Hybridization experiments were also carried out on individual BAC clones using individual overgo probes to identify the number of kallikrein genes present on each clone. The BAC end-specific PCR assay and the overgo-based hybridization experiments with probes developed against human kallikrein sequences resulted in the construction of a BAC clone-based physical map of the porcine greater kallikrein gene region (Fig. 1). The assembled contig of the porcine kallikrein region containing eight BAC clones provides ordering information for 12 STSs. Of the 12 STSs, 7 correspond to BAC insert ends, 4 to new tissue kallikrein genes in the pig, and 1 to the previously identified porcine KLK4 gene.

To further test the relationship between individual clones in the contig and to identify a minimal tiling set of clones for sequencing the greater kallikrein region of the pig, all eight BAC clones that represent the kallikrein gene region were subjected to restriction-enzyme-based fingerprint analysis [20]. The resulting data were analyzed by FPC [21], which yielded one contig along with estimated sizes of individual BACs. The relative order and orientation of the FPC-generated, fingerprint-based contig and the PCR/hybridization-based contig were identical. Based on the length of all nonredundant restriction fragments the total length of the contig is ~375 kb.

Identification of novel kallikreins

Based on the physical map constructed and the data generated through overgo-based hybridization experiments and restriction-enzyme-based fingerprint analysis, clone CH242-69G11 was selected to contain all porcine tissue kallikrein genes identified by us so far. Clone CH242-69G11 was sequenced to completion (Accession No. AC149292) and was analyzed using GENSCAN analysis software to predict the coding sequences and peptides present in the BAC clone. The GENSCAN results suggested that the BAC clone was sequenced to have 13 kallikrein genes (Fig. 2) (KLK1, KLK4, KLK5, KLK6, KLK7, KLK8, KLK9, KLK10, KLK11, KLK12, KLK13, KLK14, and KLK15), among which 11 have not been previously reported. The presence of 13 kallikrein genes in the generated sequence was further confirmed by analysis with the software package Spidey. All kallikreins were observed to be transcribed from telomere to centromere direction.

Physical mapping of porcine kallikrein genes

A PCR assay was developed for porcine KLK4 and radiation hybrid mapping was performed using INRA-Minnesota 7000