Background: Kallikreins (KLKs) are a group of 15 secreted serine proteases. Some KLKs are established or candidate cancer biomarkers, but for most the physiological function is unknown. We characterized the protein and mRNA abundance patterns of all 15 KLKs in multiple panels of human tissues and biological fluids.

Methods: We used sensitive and specific sandwich-type ELISAs for each KLK. Reverse transcription PCR was used for transcript amplification. Multiple panels of human tissue extracts (adult and fetal) were tested, along with various biological fluids.

Results: Quantitative protein expression data on 7 sets of adult and 3 sets of fetal tissues were collected for all 15 KLKs. KLKs were also quantified in the following biological fluids: seminal plasma, breast milk, follicular fluid, breast cyst fluid, breast cancer cytosol, amniotic fluid, ovarian cancer ascites, cerebrospinal fluid, cervicovaginal fluid, and urine. The data were used to generate heat maps of KLK concentrations in tissues and fluids and categorize KLK abundance as highly restricted (KLK2 and KLK3 in prostate), restricted (KLK5 in skin, salivary gland, breast, and esophagus; KLK6 in brain and central nervous system; KLK7 in esophagus, heart, liver, and skin; KLK8 in breast, esophagus, skin, and tonsil; KLK13 in esophagus and tonsil), or wide (KLKs 1, 4, 9, 10, 11, 12, 14, and 15).

Conclusions: Quantitative KLK concentrations in tissues and fluids aid in the elucidation of KLK function, and coexpression patterns provide clues for KLK participation in proteolytic cascades.

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sitive ELISAs to examine global KLK abundance patterns in human tissues and biological fluids.

**Materials and Methods**

**Tissue Extracts**

Seven adult and 3 fetal tissue sets were examined. Fetus 2 was female; the sexes of the other 2 fetuses were unknown. All tissues were collected at autopsy, a maximum of 24 h after death, and were stored at −80 °C until use. Adults were 50- to 70-year-old individuals who died from heart failure or myocardial infarction, and fetuses were spontaneously aborted at gestational ages of 13–18 weeks. We prepared tissue extracts by pulverizing 0.2 g of each tissue in liquid nitrogen into fine powders. We added extraction buffer (2 mL of 50 mmol/L Tris-HCl buffer, pH 8.0, containing 150 mmol/L NaCl, 5 mmol/L EDTA, and 10 mL/L NP-40 surfactant), with and without proteinase inhibitors, to the powders and incubated the mixture on ice for 30 min with vortex-mixing every 10 min. We centrifuged the mixtures at 14 000g at 4 °C for 30 min. The supernatants were collected and stored at −20 °C until use. Our procedures have been approved by the institutional review boards of Mount Sinai Hospital and the University Health Network, Toronto, Canada.

**Biological Fluids**

The biological fluids were leftovers of samples submitted for routine biochemical testing, or collected with informed consent and institutional review board approval, and stored at −80 °C until use. Amniotic fluids were collected between 15 and 23 weeks of gestation. Cervicovaginal fluid (CVF) samples were from healthy women between 20 and 30 years of age. Follicular fluids were collected from women under gonadotropin stimulation during in vitro fertilization. Urine samples were collected from 6 men and 6 women and verified as negative for signs of infection.

**KLK-Specific ELISAs**

All ELISAs used in this study were sandwich type, with one antibody used for capture and another used for detection. We used 3 classes of immunoassays—monoclonal-monoclonal, monoclonal-polyclonal, or polyclonal-polyclonal configurations. In Table 1 of the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol53/issue8, we present the type and sources of all antibodies used in the ELISAs. All assays were highly specific, having <1% cross-reactivity with noncognate KLKs.

**Monoclonal-Monoclonal ELISA Configuration, Version 1**

This assay follows the same procedure described above, up until addition of the detection antibody. Then we added 100 μL polyclonal antiserum diluted 1000-fold in assay buffer and incubated the plates for 1 h as above. We washed the plates 6 times, added 100 μL alkaline phosphatase–conjugated goat antirabbit antibody diluted 3000-fold in assay buffer to each well, and incubated them for 45 min. We added substrate and developing solution and read the plates as described above.

**Monoclonal-Polyclonal ELISA Configuration, Version 2**

These assays follow the same procedure described above, up until addition of the detection antibody. Then we added 100 μL alkaline phosphatase–conjugated goat antirabbit antibody diluted 3000-fold in assay buffer to each well, and incubated them for 15 min with continuous shaking. We added 100 μL developing solution (1 mmol/L Tris, 0.4 mL/L NaOH, 2 mL/L TbCl3, and 3 mL/L EDTA) to each well and mixed for 1 min. We measured fluorescence with a time-resolved fluorometer, the Cyberfluo 615 Immunoanalyzer (MDS-Nordion). Calibration and data reduction were performed automatically, as described elsewhere (12).

**Polyclonal-Polyclonal ELISA Configuration**

This assay follows the same procedure as the monoclonal-moniclonal assay configuration, but with use of an affinity-purified polyclonal antibody kindly provided by Dr. Julie Chao at the Medical University of South Carolina.
TOTAL RNA EXTRACTION AND PCR FOR KLKS
We performed total RNA extraction from tissues and reverse transcription (RT)-PCR for each of the KLKs using previously described conditions (13–15). The primers used and expected product lengths are listed in Table 2 of the online Data Supplement.

RESULTS
TISSUE EXTRACTS
Seven adult and 3 fetal sets of human tissue extracts were assayed for KLK protein concentrations using specific ELISAs for each KLK. Representative data for KLK1, 3, and 6 are shown in Figs. 1–3. Data for all other KLKs are presented as supplementary information (see Figs. 1–12 in the online Data Supplement). Color-coded summaries of global KLK protein concentrations in adult and fetal tissues and fluids are shown in Fig. 4. KLK mRNA levels are shown in Fig. 5.

KLK1 was most abundant in the pancreas and salivary gland, followed by the colon and small intestine. Lower concentrations were found in the esophagus, kidney, lymph node, prostate, stomach, thyroid, ureter, and vagina. In fetal tissues, the highest concentrations were seen in the pancreas and colon.

KLK2 and 3 were found primarily in the prostate. KLK3 was detected only in the fetal adrenal gland. Low concentrations of KLK4 were found in a wide variety of tissues, adult and fetal.

KLK5 was most abundant in skin (adult and fetal), with lower concentrations in the breast, esophagus, and salivary gland. KLK6 was most abundant in the brain and spinal cord. Relatively low concentrations of KLK6 were found in a wide array of other adult and fetal tissues.

The highest concentrations of KLK7 were found in skin (adult and fetal). Relatively high concentrations of KLK7 were also found in the esophagus and heart. KLK8 was highly abundant in the esophagus, skin (adult and fetal), and tonsil, with lower concentrations in the adrenal gland (adult and fetal), breast, kidney (adult and fetal), fetal liver, salivary gland, and vagina. The highest concentrations of KLK9 were seen in the heart (adult and fetal) and fetal cartilage. KLK9 was also found in a wide variety of adult and fetal tissues.

KLK10 was found at high concentrations in the tonsil (adult and fetal). Lower concentrations were seen in the brain, cervix, esophagus, fallopian tube, lung (adult and fetal), salivary gland, fetal thymus, trachea (adult and fetal), and vagina. KLK11 was most abundant in the prostate and testis. The highest concentrations of KLK12 were found in bone marrow and bone, followed by adult and fetal colon and stomach. KLK12 was also found at relatively moderate concentrations in a variety of other adult tissues. KLK13 was found at high concentrations in the esophagus and tonsil. Lower concentrations were seen in the cervix, salivary gland, and vagina. KLK13 was also found at relatively low concentrations in several other adult and fetal tissues.

Highest concentrations of KLK14 were found in fetal skin and cartilage. KLK14 was also found in an array of other tissues, the most prominent being breast, skin, and vagina. KLK15 is most abundant in the breast, adult and fetal skin, and fetal stomach.

The quantitative data on global KLK concentrations in adult and fetal tissues are further presented in Tables 3, A and B, through 17, A and B, in the online Data Supplement.

BIOLICAL FLUIDS
KLKs were quantified in biological fluids using ELISAs. Representative data for KLK1, 3, and 6 are shown in Figs. 1–3, and a summary for all KLKs is shown in Fig. 4C. Quantitative data for all KLKs and all fluids are presented as supplementary information (see Figs. 1–12 and Tables 3C through 17C in the online Data Supplement).

KLK1 was found at high concentrations in urine, with lower concentrations in seminal plasma, saliva, and CVF. Relatively high concentrations of KLK2 were found in seminal plasma, with trace amounts in several other fluids tested. Very high concentrations of KLK3 were found in seminal plasma, with relatively low concentrations in urine. KLK4 was primarily found in seminal plasma, with lower concentrations in breast milk and urine.

Breast milk, breast cyst fluid, ovarian cancer ascites, and CVF contained the highest concentrations of KLK5, with lower concentrations found in seminal plasma, follicular fluid, breast cancer cytosol, amniotic fluid, saliva, cerebrospinal fluid (CSF), and urine. KLK6 was detected at high concentrations in breast milk, CSF, and CVF, with lower concentrations in breast cyst fluid, ascites, and saliva. KLK7 was found at highest concentrations in CVF, with lower concentrations in breast milk, seminal plasma, breast cancer cytosol, ascites, and saliva. The highest concentrations of KLK8 were found in breast milk and CVF. KLK9 was found primarily in breast milk, with low concentrations in seminal plasma, amniotic fluid, and CSF.

KLK10 was found at high concentrations in CVF and saliva, with lower concentrations in breast cyst fluid and ovarian cancer ascites. The highest concentrations of KLK11 were found in seminal plasma and CVF, with relatively low concentrations in all other fluids tested. High levels of KLK12 were found in CVF, with moderate concentrations in breast milk, breast cancer cytosol, saliva, and urine. KLK13 was found at highest concentrations in CVF, followed by seminal plasma and saliva. KLK14 was most abundant in CVF, with lower concentrations in seminal plasma, amniotic fluid, and saliva. KLK15 was found primarily in CVF and breast milk, with lower concentrations in seminal plasma, breast cancer cytosol, and saliva.

RT-PCR
For comparative purposes, we also assessed global KLK levels by RT-PCR in one adult tissue set. The data are

4 Human genes: KLK, kallikrien.
summarized in Fig. 5. We classified the abundance by semiquantitative scoring, based on visual comparison of band intensities of ethidium bromide–stained agarose gels.

**TISSUE SPECIFICITY OF KLK EXPRESSION**

Based on the quantitative data of KLK protein concentrations in diverse adult tissues, we separated KLK levels into 3 categories, as follows. Very restricted [present at
comparatively high concentrations in 1 tissue, with lower concentrations (<1%) in other tissues], restricted [present at comparatively high concentrations in 2–4 tissues, with lower concentrations (<20%) in other tissues], and wide (comparatively high concentrations in 5 or more tissues). The data are shown in Table 1.

**Discussion**

The protein abundance patterns of the KLKs can be divided into 3 classes; highly restricted, restricted, and wide (Table 1). Our most pertinent findings are presented in Figs. 1–5. In general, good concordance between KLK transcript and protein concentrations was observed; in some cases, however, discrepancies between mRNA transcript and protein abundance existed. These discrepancies are most likely due to degradation of KLK proteins, or because KLKs are secreted and therefore may not be present in high abundance in cytosols where their concentrations were measured.
We have confirmed previous findings on KLK1 mRNA and protein concentrations (16, 17) in tissues. Consistent with KLK1 expression in the salivary gland and prostate, and with previous studies (18), we found relatively high concentrations of KLK1 in saliva and seminal plasma. We also found high concentrations of KLK1 in urine, consistent with previous findings (19).

KLK2 and KLK3 are highly abundant in the prostate and found in seminal plasma, as expected. They both participate in seminal liquefaction (20). KLK2 and KLK3 concentrations in other tissues are much lower. Given that KLK2 is a known activator of KLK3, KLK2 and KLK3 coexpression suggests participation in a common cascade in the prostate and other tissues (20). KLK2 and KLK3 were
found in breast milk, breast cytosol, breast cyst fluid, saliva, and urine but at much lower concentrations than in seminal plasma.

**KLK4**

KLK4 was not highly abundant in any of the tissues tested, compared with other KLKs. We confirmed previous findings of low KLK4 abundance in the brain, breast, cervix, liver, prostate, salivary gland, and thyroid (21). We also confirmed previous findings (21) of KLK4 secretion into seminal plasma and urine.

**KLK5**

KLK5 is known to play a role in skin desquamation (7). We found highest concentrations of KLK5 in adult and fetal skin. KLK5 has been shown to be differentially regulated in testicular and lung cancer, at the mRNA level (22, 23) We found moderate KLK5 concentrations in breast and testis and low concentrations in lung.

**KLK6**

Highest KLK6 concentrations were in the brain and spinal cord, with moderate to high concentrations in the breast, fallopian tube, kidney, lung, and salivary gland, as reported (24). KLK6 was previously found in seminal plasma, breast milk, breast cancer cytosol, breast cyst

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Fig. 4. KLK protein concentrations in tissue extracts and biological fluids. KLK concentrations are indicated by color codes according to the scale on the bottom of each Fig. (A), adult tissues; (B), fetal tissues; and (C), biological fluids.

Table 1. Abundance patterns of KLKs, categorized according to concentrations in adult tissues.

<table>
<thead>
<tr>
<th>Very restricted (tissue)</th>
<th>Tissue abundance restricted (tissue)</th>
<th>Wide</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLK2 (prostate)</td>
<td>KLK5 (skin, salivary, breast, esophagus)</td>
<td>KLK1</td>
</tr>
<tr>
<td>KLK3 (prostate)</td>
<td>KLK6 (brain/central nervous system)</td>
<td>KLK4</td>
</tr>
<tr>
<td></td>
<td>KLK7 (esophagus, heart, liver, skin)</td>
<td>KLK9</td>
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<tr>
<td></td>
<td>KLK8 (breast, esophagus, skin, tonsil)</td>
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<td>KLK13 (esophagus, tonsil)</td>
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fluid, amniotic fluid, ovarian cancer ascites, and CSF (25). We confirmed these findings and report high concentrations of KLK6 in CVF and traces of KLK6 in follicular fluid, saliva, and urine.

KLK7
KLK7 is involved in skin desquamation (26). We found high KLK7 concentrations in adult and fetal skin. We also confirmed previous findings of KLK7 presence in the esophagus, kidney, lung, and salivary gland (27). Relatively high concentrations of KLK7 were found in the adult heart at both the mRNA and protein level. This finding was not previously reported.

We have also confirmed previous findings of KLK7 secretion into seminal plasma, breast milk, amniotic fluid, ovarian cancer ascites, saliva, CSF, and urine (27). In addition, we found high concentrations of KLK7 in CVF.

KLK8
We confirmed KLK8 presence in the ovary, esophagus, kidney, salivary gland, skin, tonsil, breast, and cervix, as reported (28). KLK8 expression in fetal kidney and skin were also confirmed (28). KLK8 has been previously detected in breast milk and amniotic fluid (28). We have confirmed KLK8 secretion in these 2 fluids and have also detected KLK8 in ovarian cancer ascites, saliva, CVF, and CSF.

KLK9
KLK9 has previously been shown to be expressed in the brain, liver, lung, small intestine, spinal cord, thymus,
trachea, prostate, testis, and breast at the mRNA level (29) and primarily expressed in the testis and seminal vesicle at the protein level (30). We confirmed KLK9 protein presence in the brain, liver, small intestine, spinal cord, trachea, prostate, testis, and breast. In addition, KLK9 was found in a wide range of other tissues, both adult and fetal, in concordance with our mRNA findings.

The secretion of KLK9 into biological fluids has not been studied to date. Here, we report KLK9 presence in breast milk and at lower concentrations in amniotic fluid, CSF, ovarian cancer ascites, saliva, breast cancer cytosols, seminal plasma, and urine.

**KLK10**

We confirmed previous findings of KLK10 presence in the cervix, fallopian tube, liver, lung, salivary gland, and skin (31), in concordance with our own mRNA findings and previous findings (32). We were unable to confirm KLK10 expression in breast tissue by our criteria (at least 50% of all tissues tested should be positive), although KLK10 was found in 2 of 5 breast tissues examined, with a mean value of 600 ng/g (data not shown). We found KLK10 to be relatively highly abundant in the adult brain and tonsil, which has not been previously shown.

We confirmed previous results of KLK10 in breast milk, seminal plasma, amniotic fluid, CSF, and ovarian cancer ascites (31). In this study, highest concentrations of KLK10 were found in CVF, followed by saliva, findings not previously reported.

**KLK11**

As previously shown (33) KLK11 protein is most abundant in the prostate, and is secreted at high amounts into seminal plasma. High concentrations of KLK11 were found in diverse tissues and fluids, including the lung, which has previously been reported at the mRNA level (34). We also found relatively high concentrations of KLK11 in CVF. Lower concentrations were detected in breast milk and ascites from ovarian cancer patients, which has not been previously shown, although KLK11 has been proposed as an ovarian cancer biomarker (33).

**KLK12**

The KLK12 protein expression pattern has not been characterized; however, 1 study reported KLK12 expression in microvascular endothelial cells (35). We show that KLK12 is present in a wide number of adult tissues, but is restricted to the fetal colon, albeit at relatively high concentrations. KLK12 concentrations are high in CVF and moderate in all other biological fluids tested.

**KLK13**

KLK13 was previously found in the breast, esophagus, kidney, prostate, salivary gland, skin, testis, thyroid, tonsil, trachea, ureter, and lung, with highest concentrations seen in esophagus and tonsil (36). We confirmed these findings and also confirmed a previous report on KLK13 secretion in seminal plasma (36). In addition, KLK13 was found at high concentrations in CVF and at moderate concentrations in saliva.

**KLK14**

We confirmed KLK14 presence in both adult and fetal skin. KLK14 has also been previously found in the breast, prostate, brain, lymph node, lung, testis, and stomach (37). We confirmed KLK14 concentrations in the breast, prostate, brain, lung, and stomach; however, we did not detect KLK14 in the lymph node or testis. KLK14 was found in a wide variety of other tissues, particularly of fetal origin, suggesting that KLK14 may play a developmental role. KLK14 was found primarily in CVF, with lower concentrations in seminal plasma and amniotic fluid (37).

**KLK15**

A previous study by our group showed very low concentrations of KLK15 protein in the thyroid gland, colon, and prostate, and secretion of KLK15 primarily into seminal plasma (38). Analysis of multiple tissues sets in this study has revealed that KLK15 is most abundant in the breast and fetal skin. Low concentrations of KLK15 were detected in the prostate, as published (38). We confirmed KLK15 secretion into seminal plasma and even higher concentrations in breast milk and CVF. KLK15 tissue protein concentrations are not always in concordance with KLK15 mRNA presence.

KLK15 was found at low concentrations in several tissues but more widely in fetal tissues than adult tissues, similar to KLK14.

**KLK Coexpression Patterns**

Our studies have revealed many tissues in which multiple KLKs are present. Previous studies reported KLK coexpression and involvement in proteolytic cascades in seminal plasma and skin (4–6, 11). Our results pinpoint other potential areas of KLK coexpression and, possibly, cooperation. These data are shown in Fig. 4. Many KLKs are found in the skin, salivary gland, prostate, central nervous system, and breast, as reported (Fig. 4A). Here, we report additional tissues, as shown in Fig. 4A. Coexistence in biological fluids is shown in Fig. 4C. These data should be valuable in future efforts to build or complement proteolytic cascade pathways involving several KLKs and cross-talks with other proteolytic systems. These data also complement previous findings of KLK coregulation by steroid hormones.

Grant/funding support: None declared.
Financial disclosures: None declared.
Acknowledgments: We thank Iacovos Michael and Nader Memari for providing RT-PCR data and Julie Chao, from the Medical University of South Carolina, for providing us with an immunoassay for KLK1.
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