Supporting Information

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SI Materials and Methods

Collection of Prostatic Corpora Amylacea and Calculi. Grossly visible PC were collected with sterile forceps during tissue processing for surgical pathology. CA were collected by fine-needle aspiration of the prostate into sterile 1X PBS. The aspirated material (which included both CA and prostate-derived cells) then was passed through a 100-μm nylon mesh strainer. The CA remaining in the mesh filter were collected for further processing. All CA and PC specimens for protein analysis were washed once in 1X PBS, incubated at room temperature in 1X PBS with 0.1% SDS (to wash away any cellular debris on the outer surface) for 1 h, washed twice with 1X PBS, and then stored at 4 °C until protein extraction. CA/PC collected for infrared spectroscopy were stored at −20 °C until they were sent for analysis.

Processing Excised Gel Bands for In-Gel LC/MS/MS Analyses. SDS PAGE gels were transferred to a sterile Petri dish, and gel bands of interest were excised with a sterile scalpel. A control gel band excised from a lane containing only sample loading buffer was included for each band analyzed to control for contamination. Excised bands were placed in a microcentrifuge tube that had been rinsed with HPLC-grade methanol. Bands were washed twice in 50% HPLC-grade methanol for 10 min, then all liquid was removed, and samples were stored at −20 °C until they were submitted for mass spectrometry analysis.

Mass Spectrometry Analyses. Proteins in solution were reduced and alkylated with iodacetamide and proteolyzed with sequencing-grade modified porcine trypsin (Promega). Proteins in gel bands were proteolyzed with trypsin (Promega) as described previously (1). Protein identification by LC/MS/MS analysis of peptides was performed using an LTQ ion trap mass spectrometer (Thermo Fisher Scientific) or a QSTAR/Pulsar mass spectrometer (Applied Biosystems/MDX Sciex) interfaced with a 2D nanoLC system (Eksigent). Peptides were fractionated by reverse-phase HPLC on a 75-μm × 100-mm C18 column with a 10-μm emitter using a 0%–60% acetonitrile/0.5% formic acid gradient over 30 min at 300 nl/min. Peptide sequences were identified using Mascot software (www.matrixscience.com) to search the National Center for Biotechnology Information nonredundant database with acquired fragmentation data. Identified sequences were confirmed by manually inspecting fragmentation spectra.

Positive Controls for Western Blot Analyses. Positive controls for Western blot analyses included purified human milk and neutrophil lactoferrin (Sigma L0520 and L6793, respectively). Peripheral blood white blood cells were prepared from 8 ml of whole peripheral blood after lysis of red blood cells with ACK lysis buffer (Quality Biological, Inc.). Prostate tissue biopsies (peripheral zone) were obtained from a radical prostatectomy specimen and stored at −20 °C until protein extraction. White blood cell, prostate tissue, and cell line protein extracts (LNCaP, PC3, fibroblasts) were processed in an identical manner as follows: cells were washed twice with 1X PBS and then resuspended in RIPA buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1% Triton X-100, 0.5% Na-Deoxycholate, 0.1% SDS, 1 mM EDTA) with 1X HALT Protease Inhibitor Mixture (Pierce). Samples were allowed to solubilize for 30 min. at 4 °C and then were sonicated briefly. The resulting lysate was centrifuged at 16,000 × *g* for 5 min, the supernatant was removed, and protein was quantitated using the standard bicinchoninic acid (BCA) assay (Pierce). Prostatic fluid from a radical prostatectomy specimen was diluted in 1X PBS with 1X HALT Protease Inhibitor Mixture, and protein was quantified by BCA assay.

Immunohistochemistry. Slides containing sections of FFPE prostate tissue were steamed for 20 min in citrate-based unmasking solution for antigen retrieval (Vector Laboratories) (for lactoferrin staining) or treated with protease (Subtilisin, Sigma 82490) at a concentration of 1 mg/ml for 7 min at room temperature (for calprotectin staining). Slides were incubated with either the same anti-human lactoferrin antibody used for Western blotting (Sigma, catalog # L3262) at 1:2000 or with monoclonal mouse anti-human calprotectin (Dako, MAC 387, catalog #M0747) (2) at 1:300. Staining was visualized using 3,3′-Diamino-benzidine (Sigma FAST 3,3′-Diamino-benzidine tablets), and slides were counterstained with hematoxylin.

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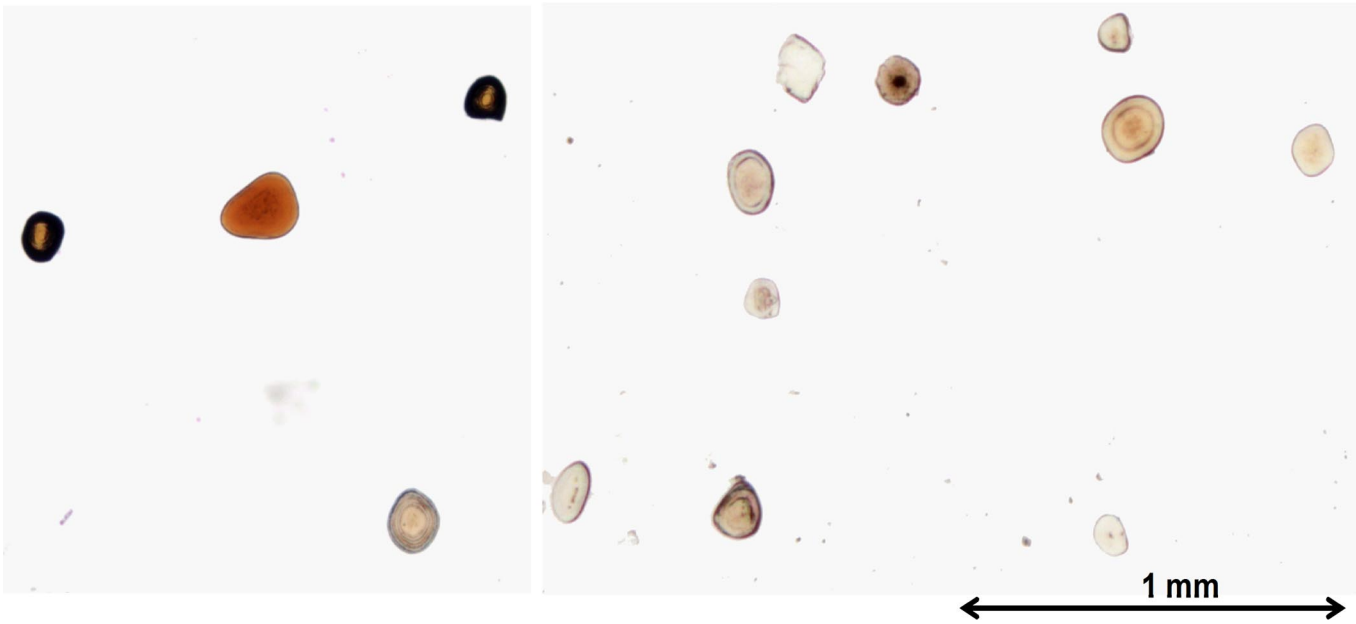

Fig. S1. Examples of isolated corpora amylacea submitted for infrared spectroscopy analyses.

Table S1. Characteristics and disease associations of non-prostatic corpora amylacea (CA) and calculi in humans

Organ System	Composition	Disease Association(s)	Size	Frequency[a]
		Corpora amylacea		
Central nervous system[1];optic nerve, retina (CA)	Polyglucosan; (87.9% hexose)[2]/stress proteins; AGEs[b]; S100 proteins (brain CA)[3]	Neurodegeneration (Lafora body disease, adult polyglucosan disease, Bielschowsky body disease)[1]	2–20 μM	Common; up to 100% postpuberty (central nervous system)[1]
Cervix (CA[4]/psammoma body[5])	Calcium/unknown	Associated with gynecological (cervical, ovarian, uterine) malignancies, particularly in postmenopausal women[5]	Variable	Rare ($<$ 0.1%)[6]
Heart (CA)[1]/basophilic degeneration	Polyglucosan[7]	Unknown	2–20 μM	Common, up to 100% post 60 years of age[7]
Lung (CA)	β2-microglobin[8]; polysaccaride[9]; surfactant apoprotein[10]	Unknown	30–200 μM	Rare (3.8%)[9]
Thyroid (CA[1]/psammoma body)	Calcium/unknown	Associated with papillary thyroid carcinoma[11]	20 μM	Rare in normal thyroid (0.05%–1.6%), large increase in papillary thyroid carcinoma ($\sim$ 60%)[12–16]
Uterus[8] (CA)	Unknown	Unknown	Variable	Up to 22% in the elderly[8]
		Calculi/lithiasis		
Bladder stone	Calcium oxalate/calcium phosphate[17]/neutrophil-related proteins[18]	Urachal carcinoma[17]; schistosomiasis	Variable	Infrequent[19]
Breast (Microcalcifications)	Apatite/calcite/oxalate[20]	May be indicative of ductal carcinoma in situ[21]	Variable	26%–43%[21, 22]
Gallbladder stone	Cholesterol/cholesterol hydrates/calcium carbonates[23]	Biliary dysfunction	Variable	10%–15%[24]
Kidney stone	Calcium oxalate/apatite[25]/ neutrophil-related proteins[26]	Urinary dysfunction	Highly variable	10%–15%[27]
Lung (pulmonary alveolar microlithiasis)	Calcium phosphate[28]	Hereditary pulmonary alveolar microlithiasis	$\geq$ 50 μM	Very rare[29]
Pancreatic stone	Calcium carbonate/ lithostathine (pancreatic stone protein), Lactoferrin[30]	Pancreatitis; associated with pancreas carcinoma[31, 32]	Variable	Very rare[31]
Salivary gland (sialolithiasis)	Hydroxyapatite[33]	Sialolithiasis	1–55 mm	1% at autopsy[33]
Testicles (microlithiasis)	Hydroxyapatite/collagen[34]	Testicular malignancy[35]	20 μM	0.6%–9%[36]

Note: This table does not include deposits associated with systemic amyloidosis. Please see refs. 37, and 38 for a good review of those disorders.

[a]Frequency reported as estimated % of individuals in the population.

[b]Advanced glycation end-product.

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Table S2. Identification of peptide hits in total protein analysis of extracted CA/PC proteins as determined by LC/MS/MS

Protein	Ions Score[a]	# of Peptide Matches[a]	Accession #	Mass[b]	% Coverage[a]	Frequency (%)
Lactoferrin	3519	32	AAA36159	78346	61	8/8 (100)
Myeloperoxidase	1418	12	NP_000241	83815	21	8/8 (100)
S100 calcium-binding protein A9 (calprotectin)	592	5	NP_002956	13234	67	8/8 (100)
S100 calcium-binding protein A8 (calprotectin)	223	4	NP_002955	10828	39	8/8 (100)
Prostate specific antigen (PSA)	519	5	AAA60193	28840	39	6/8 (75)
Serum albumin	436	9	CAA23754	69321	18	6/8 (75)
Beta-hemoglobin	1041	10	NP_000509	15988	81	5/8 (63)
Prostatic acid phosphatase (PAP)	313	5	AAH08493	44487	16	5/8 (63)
Defensin HNP2 or HNP3 (alpha)	187	2	1DFN_A	3490	60	5/8 (63)
N-acylsphingosine amidohydrolase (acid ceramidase) 1	182	3	BAD9650	44663	9	5/8 (63)
Secretory leukocyte peptidase inhibitor (SLPI)	74	1	NP_003055	14316	9	5/8 (63)
Neutrophil gelatinase-associated lipocalin (NGAL, lipocalin 2)	501	4	NP_005555	22574	34	4/8 (50)
Agrin (AGRN)	410	5	NP_940978	214706	4	4/8 (50)
Beta-2 microglobulin	305	2	CAA23830	12791	29	4/8 (50)
Immunoglobulin heavy chain VHDJ	267	3	CAC10223	41287	10	4/8 (50)
Azurocidin 1 (AZU1)	231	5	CAA41601	2691	34	4/8 (50)
Apolipoprotein D (APOD)	214	2	AAB32200	27975	10	4/8 (50)
Beta-microseminoprotein	161	1	1209281A	10644	17	4/8 (50)
Tomoregulin (TMEFF2)	128	2	NP_057276	41401	6	4/8 (50)
Immunoglobulin kappa light chain	223	1	AAA87674	23442	9	3/8 (38)
Bactericidal/permeability-increasing protein (BPI)	183	1	AAH40955	53846	3	3/8 (38)
Ribonuclease, RNase A family, 3 (eosinophil cationic protein)	149	3	NP_002926	18373	23	3/8 (38)
Cathepsin G	124	4	1CGH_A	25423	14	3/8 (38)
Lysozyme C	119	2	P79239	16596	27	3/8 (38)
Complement component C3	89	1	AAA85332	187046	<1	3/8 (38)
Prosaposin (PSAP)	62	1	CAG33027	58058	1	3/8 (38)
Insulin-like growth factor binding protein 5	429	2	NP_000590	30550	14	2/8 (25)
Orosomucoid 1	340	3	P02763	23497	21	2/8 (25)
Thrombospondin, type I, domain-containing 4	336	5	NP_079093	112377	5	2/8 (25)
TIMP metallopeptidase inhibitor 1 (TIMP-1)	251	4	P49061	23198	38	2/8 (25)
Phospholipase A2, group IIA	203	1	NP_000291	16072	12	2/8 (25)
CD177 (neutrophil-specific antigen 1)	181	2	BAE93254	44296	9	2/8 (25)
Granulin	123	2	AAA58617	63529	4	2/8 (25)
Complement component 9	120	1	NP_001728	63133	4	2/8 (25)
Growth differentiation factor 15	112	2	Q99988	34147	10	2/8 (25)
Nephronectin (NPNT)	105	1	NP_001028219	61866	2	2/8 (25)
Fibronectin 1	97	2	AAI17177	239471	1	2/8 (25)
Follistatin-like 1	96	2	Q62356	34516	8	2/8 (25)
Medullasin (elastase 2, neutrophil)	91	2	BAA00128	25432	7	2/8 (25)
Prostate stem cell antigen (PSCA)	77	1	AAQ89271	12474	14	2/8 (25)
Collagen, type I, alpha 1	74	1	AAH36531	138926	1	2/8 (25)
Complement component C4A	60	1	AAB59537	193541	<1	2/8 (25)
Ribonuclease 4 (RNase 4)	59	1	AAA96750	13816	9	2/8 (25)
S100 calcium-binding protein A12	59	1	NP_005612	10569	19	2/8 (25)
E-cadherin (uvomorulin)	53	1	CAA79356	97456	1	2/8 (25)

[a]For multiple matches, the highest score observed in a single case is shown.

[b]Nominal mass (M_r).

Peptide hit(s) also were observed in a single case for the following proteins: uromodulin (Tamm-Horsfall glycoprotein), annexin A2, apolipoprotein E, actin, myosin, immunoglobulin lambda light chain, immunoglobulin J chain, S100 calcium-binding protein P, vasopressin-activated calcium-mobilizing receptor-1, nurse cell scavenger receptor 2, tropomyosin 1 alpha, fast skeletal myosin alkali, ubiquitin, BAZ1B, hemoglobin alpha-1 globin chain, hemoglobin subunit delta, alpha-1-antichymotrypsin, WAP four-disulfide core domain protein 2 precursor (major epididymis-specific protein E4), carbonic anhydrase I, transmembrane protease, serine 2 (TMPRSS2), alpha 2 macroglobulin, peroxiredoxin 2, haptoglobin, eukaryotic translation elongation factor 1 alpha 1, tetraspanin 6, brain abundant, membrane-attached signal protein 1, alpha-1-antitrypsin, kallikrein 11, alpha-1-microglobulin, ceruloplasmin (ferroxidase), lectin, galactoside-binding, soluble, 3 binding protein, MMP7, LOC124220 similar to common salivary protein 1, cystatin C, plasminogen activator, tissue, chitinase 3-like 1, vitronectin, transglutaminase 4 (prostate), defensin, alpha 4 (HNP4), Golgi apparatus protein 1, GAPDH, annexin A1, complement factor I, ADAM metallopeptidase with thrombospondin type 1 motif 1, cryptic, fibulin-1.

Table S3. Comparison of peptide hits for different types of CA/PC samples collected from the same patient

Patient 1 Stone 1	Ions Score	Patient 1 Stone 2	Ions Score
Lactoferrin[a]	459	Lactoferrin[a]	920
S100 calcium-binding protein A9 (calprotectin)[a]	208	S100 calcium-binding protein A9 (calprotectin)[a]	592
Defensin HNP2 or HNP3 (alpha)[a]	142	S100 calcium-binding protein A8 (calprotectin)[a]	214
S100 calcium-binding protein A8 (calprotectin)[a]	130	Defensin HNP2 or HNP3 (alpha)[a]	158
Lysozyme C[a]	118	Ribonuclease, RNase A family, 3 (eosinophil cationic protein)[a]	149
Ribonuclease, RNase A family, 3 (eosinophil cationic protein)[a]	91	Lysozyme C[a,b]	78
Prostate stem cell antigen (PSCA)[a,b]	68	Prostate stem cell antigen (PSCA)[a]	77
		Myeloperoxidase	76
		Secretory leukocyte peptidase inhibitor (SLPI)	67
Patient 2 PC		**Patient 2 CA**	
Lactoferrin[a]	1158	Lactoferrin[a]	1741
S100 calcium-binding protein A9 (calprotectin)[a]	402	S100 calcium-binding protein A9 (calprotectin)[a]	530
Immunoglobulin fragments[a]	223	Myeloperoxidase[a]	219
Myeloperoxidase[a]	212	Neutrophil gelatinase-associated lipocalin (NGAL, lipocalin 2)[a]	197
Defensin HNP2 or HNP3 (alpha)[a]	172	Immunoglobulin fragments[a]	188
Neutrophil gelatinase-associated lipocalin (NGAL, lipocalin 2)[a]	124	Defensin HNP2 or HNP3 (alpha)[a]	187
S100 calcium-binding protein A8 (calprotectin [a]	117	S100 calcium-binding protein A8 (calprotectin)[a]	170
Ribonuclease, RNase A family, 3 (eosinophil cationic protein)	89	Cathepsin G[a]	124
Medullasin (elastase 2), neutrophil[a]	85	Bactericidal/permeability-increasing protein (BPI)	116
Cathepsin G[a]	59	Medullasin (elastase 2), neutrophil[a]	91
Azurocidin 1 (AZU1)[b]	51	Serum albumin	87
		Prosaposin (PSAP)[b]	62
		Beta-hemoglobin	61
		Annexin A2[b]	53
		Vasopressin-activated calcium-mobilizing receptor-1[b]	51
		Complement component C3[b]	51
		Beta-microseminoprotein[b]	50
		Secretory leukocyte peptidase inhibitor (SLPI)[b]	48
		Prostate-specific antigen[b]	47
		S100 calcium-binding protein A12[b]	47
		Ubiquitin[b]	43

[a]Found in both samples from same patient.
[b]Single peptide match.