

Targeted Synovial Fluid Proteomics for Biomarker Discovery in Rheumatoid Arthritis

Leticia Cano · Daniel G. Arkfeld

Published online: 23 May 2009
© Humana Press 2009

Abstract

Objective Rheumatoid arthritis (RA) is an autoimmune disease that targets the synovium. The autoantigens involved in the autoantibody responses in RA are unknown. A targeted proteomics approach was used to identify proteins in RA synovial fluid (SF) that are recognized by autoantibodies in RA sera.

Methods RA SF, depleted of abundant proteins, was fractionated by two-dimensional liquid chromatography (chromatofocusing followed by reverse phase HPLC). Protein arrays constructed from these fractions were probed with RA and normal control sera, and proteins within reactive fractions were identified by mass spectrometry. The reactivity of RA sera to an identified peptide was confirmed by ELISA.

Results RA sera specifically reacted to a SF fraction containing fibrin. Mass spectrometry analyses established the presence of a citrullinated arginine at position 271 of the fibrin fragment present in RA SF. A synthetic peptide corresponding to fibrin residues 259–287, containing the citrulline substitution at Arg 271, was recognized by 10 of 12 RA sera, but by two of 18 normal control sera and one of 10 systemic lupus erythematosus sera.

Conclusion Proteomics methodology can be used to directly characterize post-translational modifications in candidate autoantigens isolated from sites of disease activity. The finding that RA sera contain antibodies to the citrullinated fibrin 259–287 peptide may ultimately lead to improved diagnostic tests for RA and/or biomarkers for disease activity.

Electronic supplementary material The online version of this article (doi:10.1007/s12014-009-9028-1) contains supplementary material, which is available to authorized users.

L. Cano (✉)
Division of Immunology, Beckman Research Institute of the
City of Hope National Medical Center,
Duarte, CA 91010, USA
e-mail: canol@nhlbi.nih.gov

D. G. Arkfeld
Division of Rheumatology, Department of Medicine,
University of Southern California Keck School of Medicine and
the Los Angeles County and University of Southern California
Medical Center,
Los Angeles, CA 90033, USA

Present address:

L. Cano
Laboratory of Applied Mass Spectrometry, National Heart,
Lung and Blood Institute, National Institutes of Health,
Bethesda, MD, USA

Keywords Rheumatoid arthritis · Synovial fluid ·
Clinical proteomics · Biomarker discovery · Citrullination ·
Fibrinogen · ELISA, protein macroarrays ·
Autoimmune disease · Autoantibodies · Autoantigens ·
Mass spectrometry · Post-translational modification

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that targets the joints and affects 0.8% of the adults worldwide [1, 2]. Chronic joint inflammation leads to cartilage and bone destruction, resulting in loss of function. Many self-antigens have been implicated in the triggering and/or maintenance of autoreactive lymphocyte responses in RA [3–5]. Nevertheless, there remains an uncertainty as to how disease is caused and maintained [6]. Aberrant post-translational modifications of self-proteins may play a role in breaking T and B cell tolerance, leading to autoimmunity [7–9]. Of particular interest to the clinical management of

RA are the anti-citrulline antibodies [10], which can predict both development [11, 12] and severity of disease [13, 14]. Citrullination is the post-translational modification (deimination) of arginine to citrulline catalyzed by protein arginyl deiminase (PAD) enzymes [15]. This conversion changes the charge of the site from a positive to a neutral and increases the mass of the amino acid by 1 Da. The difference in charge may cause protein unfolding [16], thereby exposing novel epitopes.

The current diagnostic test for anti-citrulline antibodies employs a cyclic citrullinated peptide (CCP), yet the citrulline residues on synovial joint proteins that are target(s) of anti-citrulline antibodies *in vivo* have not been precisely defined. Autoantigens, which exist in citrullinated forms include fibrinogen [17–19] (which was initially thought to be filaggrin [20, 21]), vimentin [22–24], collagen type I [25, 26], collagen type II [26–28], fibronectin [29], and alpha-enolase [30]. The presence of citrulline-modified fibrinogen alpha (FIBA) and beta chains in RA synovial tissue or fluid has been reported [17, 19].

The goal of this project was to develop a method to discover novel RA autoantigens using a targeted proteomic analysis. We and others have reasoned that autoantigens might be enriched in RA SF and specifically recognized by autoantibodies in RA sera. Previous autoantigen and biomarker discovery projects have employed one of several approaches to fractionate biological sample preparations, including two-dimensional PAGE, miniaturized chips with diverse surfaces to promote differential protein binding, and multidimensional LC–MS/MS [31]. Often these fractionation approaches were combined with immunoblotting with patient sera. Although they yielded some novel information, such methods were complicated by the wide dynamic range of protein concentrations in serum and SF, which obscures identification of potentially informative proteins in minor abundance.

To separate and probe SF proteins, we used a method that was previously used to characterize cancer antigens [32], which included two-dimensional liquid chromatography, protein arrays, and high-resolution mass spectrometry. Depletion of abundant serum proteins followed by protein fractionation via two-dimensional liquid chromatography increased the likelihood of identifying the lower abundance proteins. Protein arrays constructed from fractionated SF were probed with RA and control sera to identify biologically significant fractions, and further analyses were performed only on those targeted fractions. It is important to note that the arrays were constructed from clinical samples that contain proteins with their post-translational modifications acquired *in vivo*. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) was then used to identify immunogenic protein fragments and their post-translational

modifications. Through this targeted proteomics approach, we have identified a citrulline-modified Arg 271 residue, within a fibrin alphaC domain fragment stably present in RA SF, as a target of autoantibodies in RA sera.

Materials and Methods

Patient Samples

All samples were obtained using IRB approved protocols and all patients consented to be part of the study. SF and sera were obtained from patients receiving care in the outpatient Rheumatology clinics at the Los Angeles County and University of Southern California Medical Center. RA and systemic lupus erythematosus (SLE) patients were diagnosed according to established clinical criteria [33, 34]. All patients had to have a clinical diagnosis of RA as defined by rheumatologists at academic center. The sampling was on consecutive RA patients from LA County hospital who had a joint effusion that was aspirated. Due to use of biologic agents, much less effusions are seen in the clinic. Patient population was mostly Hispanic, approximately 80%, with active RA stage 2 to 4 with most probably falling into 2 or 3 but data not collected so this is speculative. Comorbidities were not examined. All samples processed within 4 h. Control sera were obtained from healthy volunteers at the City of Hope General Clinical Research Center. SF samples were diluted 1:5 in PBS, centrifuged to remove cellular debris, and stored at -80°C . There are no viscosity issues when the SF is diluted 1:5 in PBS. Blood samples were allowed to clot overnight at 4°C . The next day, the blood was centrifuged and the top layer of serum was transferred into new tubes. Serum samples were stored at -80°C until use.

Protein Fractionation

A multiple affinity removal column (Agilent Technologies, Wilmington, DE, USA) was used to remove six abundant proteins (albumin, IgG, antitrypsin, IgA, transferrin, and haptoglobin) from SF. The procedure removed 85–90% of the total protein mass, which increased the probability of detecting the lower abundance proteins. Depletion was performed according to manufacturer's protocol. After the depletion, samples were desalted by use of a 5-kDa MWCO spin filter (Amicon Ultra-15, Millipore Corp., Bedford, MA, USA). Reactivity of RA sera to proteins in the 5-kDa filtrate was not detected. Protein concentration of the desalted SF was determined by RCDC protein assay (BioRad Laboratories, Hercules, CA, USA).

SF proteins were separated by 2D-HPLC, chromatofocusing, and reverse phase (RP) HPLC. A Beckman PF2D

System (Beckman Coulter, Inc., Fullerton, CA, USA) with a PF2D kit (column and buffers) was used for the first-dimension separation. Approximately 5 mg of proteins was separated in the pH range 8.5 to 4.0. After loading, the sample was washed in start buffer for 20 min, eluant buffer for 75 min, and then 1 M sodium chloride buffer for 45 min. The column was then washed overnight in water. Fractions were collected in increments of 5 min or 0.2 pH units, whichever came first, into a cooled deep well 96-well plate. This separation was reproducible as performed according to manufacturers' supplied protocol. The second-dimension separation was performed on a Vydac C4 column (5 μ m, 300 A, 2.1 \times 250 mm) using the following program: hold for 12 min at 5% buffer B; 5% to 95% buffer B in 25 min; hold at 95% buffer B for 8 min. The flow rate was 0.25 ml/min. Fractions were collected between 14 and 45 min at every 2 min into standard 96-well plates using a fraction collector.

The pooled SF second-dimension separations were performed on a Vydac C4 column (10 μ m, 300 A, 4.6 \times 250 mm) using a gradient of 2% to 98% buffer B in 60 min and a flow rate of 1 ml/min. Corresponding fractions that eluted in the same pH range from four first-dimension runs were combined to ensure enough material was obtained for analysis by mass spectrometry. Buffer A consisted of 0.1% TFA. Buffer B consisted of 0.1% TFA in 90% acetonitrile. A Beckman System Gold 126 equipped with a model 168 diode array detector (Beckman Coulter, Inc.) was used to perform the separations. Fractions were collected every minute. Samples were frozen and lyophilized to dryness.

Protein Arrays

Custom arrays were hand made using a VP 409 replicator with 96 pins each holding 100 nl of fluid (V&P Scientific, Inc., San Diego, CA, USA). The selected HPLC fractions were resuspended in ~200 μ l of 6 M urea/sodium bicarbonate pH 8.0. The microtiter plates were slowly rocked for about 30 min to facilitate protein solubility. The replicator was dipped into a 96-well plate, and the fluid (~100 nl) retained on the tips of the pins was transferred to a nitrocellulose membrane. Each fraction was arrayed in triplicate. To serve as a positive control for serum antibody reactivity, an influenza vaccine preparation also was arrayed. After drying overnight in a laminar flow hood, the arrayed membranes were blocked overnight in a non-fat dried milk solution and subsequently were rinsed twice in TBS (20 mM Tris-HCl, 500 mM NaCl, pH 7.5). The arrays were incubated with a 1:200 dilution of RA or control sera for 1 h at room temperature. After rinsing twice in TBS, the arrays were incubated with a 1:100,000 dilution of HRP-conjugated F(ab')₂ goat anti-human IgG + IgM + IgA secondary antibodies (Jackson ImmunoResearch Laborato-

ries, Inc., West Grove, PA, USA). The arrays were washed twice with 3% newborn calf serum/0.05% Tween/TBS, twice with a 0.05% Tween/TBS, twice with TBS, and twice with water. ECL Plus Western Blotting Detection Reagents (GE Healthcare, Piscataway, NJ, USA) were used as the detection agent. The arrays were imaged on a Typhoon 9410 (GE Healthcare) using the following conditions: Laser (457 nm), Emission filter (520BP40), Focal Plane (Platen), Pixel Size (50 μ m), Sensitivity (Normal). Different voltages were used to scan the images but typically 500v was used for most of the images. To distinguish background noise from foreground signal by a statistical method that complemented the visual inspection of the protein arrays, the median filter smoothing technique was applied to the imaged signals as described [35].

Mass Spectrometry

Approximately 80% of the solubilized RP-HPLC fraction was digested with trypsin (Promega, Madison, WI, USA). Approximately 5% of the digested material was analyzed by LC/MS/MS. Analyses were performed on a Thermo Finnigan LTQ-FT linear ion trap-Fourier transform mass spectrometer (Thermo Electron Corporation, San Jose, CA, USA) coupled to an Eksigent nanoLC-2D capillary HPLC system (Eksigent Technologies, LLC, Dublin, CA, USA). Samples were loaded onto a 300 μ m \times 5 mm C18 trapping column (Dionex Corporation, Sunnyvale, CA, USA) and then eluted through a lab-built 75 μ m \times 10 cm analytical column packed with 3 μ m C18 Pursuit resin (Varian, Inc., Palo Alto, CA, USA). The gradient for the trapping column was 100% A for 5 min using a flow rate of 10 μ l/min. The gradient for the analytical column was 2% to 35% B in 45 min, 35% to 50% B in 4 min, and 50% to 95% B in 2 min using a flow rate of 0.2 μ l/min. High-resolution full-scale mass spectra were acquired in the Fourier transform ion cyclotron resonance (FT-ICR) section of the mass spectrometer while fragment ion (MS/MS) spectra were obtained from the linear ion trap section. Fragmentation was performed using a collision energy setting of 35. Dynamic exclusion was set at 15 s.

Monoisotopic peaks and peptide charge states were determined during acquisition by the Xcalibur acquisition software using the high-resolution Fourier transform mass spectrometry (FTMS) spectra. Protein identifications were made by SEQUEST [36]. SEQUEST searches were performed with the following parameters: use of the 10/17/08 release of the SwissProt database (downloaded from <ftp://ftp.ncbi.nlm.nih.gov/blast/db>), monoisotopic masses, partial trypsin cleavage, 2 amu peptide and fragment tolerance, and automatic charge state determination. The SwissProt database was filtered to include only entries containing _HUMAN as a parameter. Peptide hits were

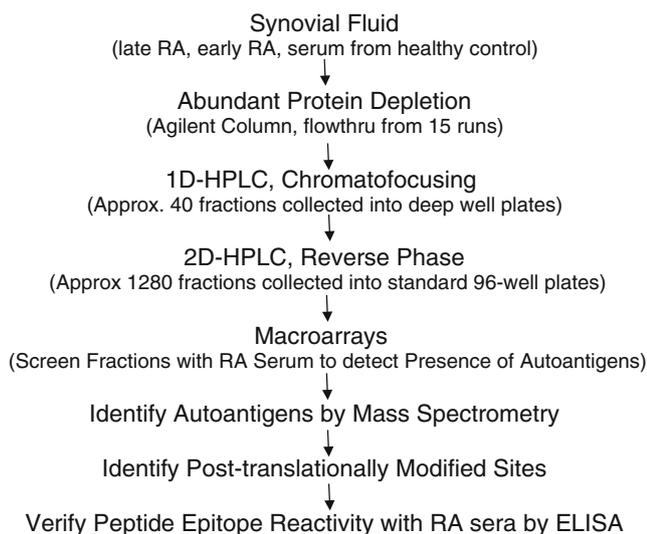


Fig. 1 Proteomic Strategy to Identify autoantigens present in synovial fluid of RA patients

filtered using the following criteria: DeltaCn greater than or equal to 0.08, XC greater than or equal to 1.8 for peptides having +1 charge, 2.5 for peptides with a +2 charge, and 3.5 for peptides having a +3 charge and the peptide must be to a protein with probability score less than 0.0001 used [37]. Peptides meeting these criteria were further analyzed. Although the LTQ-FT has routine mass accuracy of 2 ppm, it is set to perform MS/MS on the most abundant peak in an isotope envelope, rather than the monoisotopic mass. This frequency results in errors of 1 or 2 mass units for large peptides. Because SEQUEST and extract_msn do not correct for this error, it was necessary to set peptide mass tolerance to 2.5 to account

for errors in precursor assignment. Fragment ion tolerance was set at 0.0. The searches were performed assuming both trypsin and no enzyme specificity. Spectra were hand sorted to identify and verify post-translational modifications (PTM). All charge states and mass were manually verified using the high-resolution FTMS data. All spectra corresponding to candidate autoantigens were manually verified.

Synthetic Peptides

The FIBA 259–287 peptides with and without the citrulline substitution for arginine 271 (MELERPGGNEITR*GGST-SYGTGSETESPR) were synthesized in the City of Hope Peptide Synthesis Core Facility. The synthesized profilaggrin 619–631 peptide (SFSSQDR*DSQAQS) contained a citrulline for arginine substitution at position 625. Peptides were dissolved in 0.1% TFA and purified two to three times on a C18 reverse phase column. Concentration was determined by amino acid analysis.

ELISA

Maleic anhydride coated ELISA plates (Pierce Biotechnology, Inc., Rockford, IL, USA) were incubated overnight with 100 μ l of 12.5 μ g/ml peptide in PBS. The plates were blocked overnight in a dried milk solution, washed twice in TBS, and incubated overnight in a 1:20 dilution of patient sera. After rinsing two times in TBS, the plates were incubated in a 1:10,000 dilution of HRP-conjugated F(ab')₂ goat anti-human IgG + IgM + IgA secondary antibodies for 1 h. The plates were then rinsed 6 times in TBS. For color development 150 μ l ABTS (Pierce Biotechnology) was

Fig. 2 Fraction containing candidate autoantigen detected on second-dimension RP-HPLC. Fractions from the SF separation were spotted in a protein array, which was used to determine which fractions contained an autoantigen. Arrow points patient synovial fluid fraction that reacted with RA patient serum

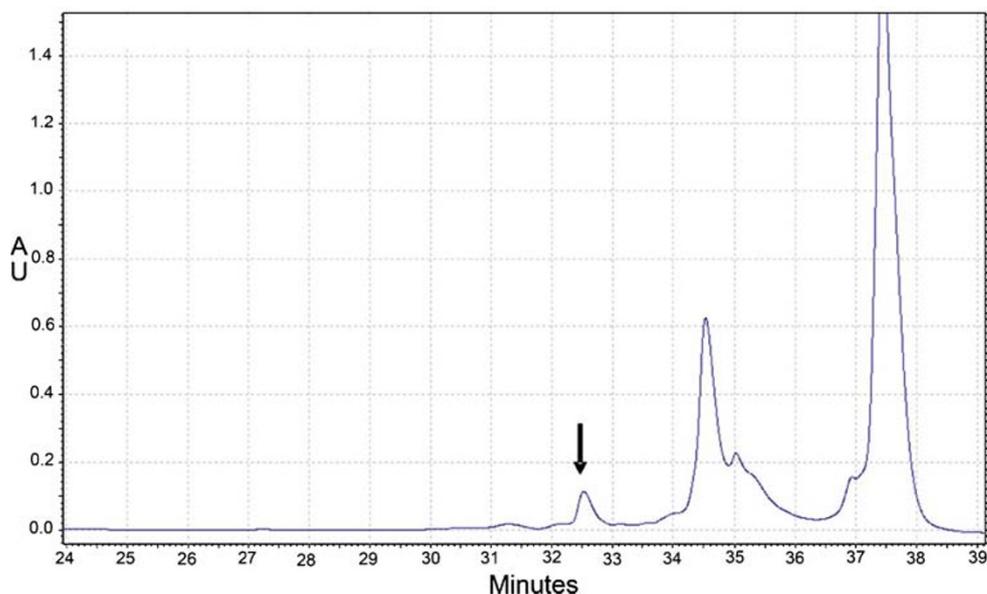
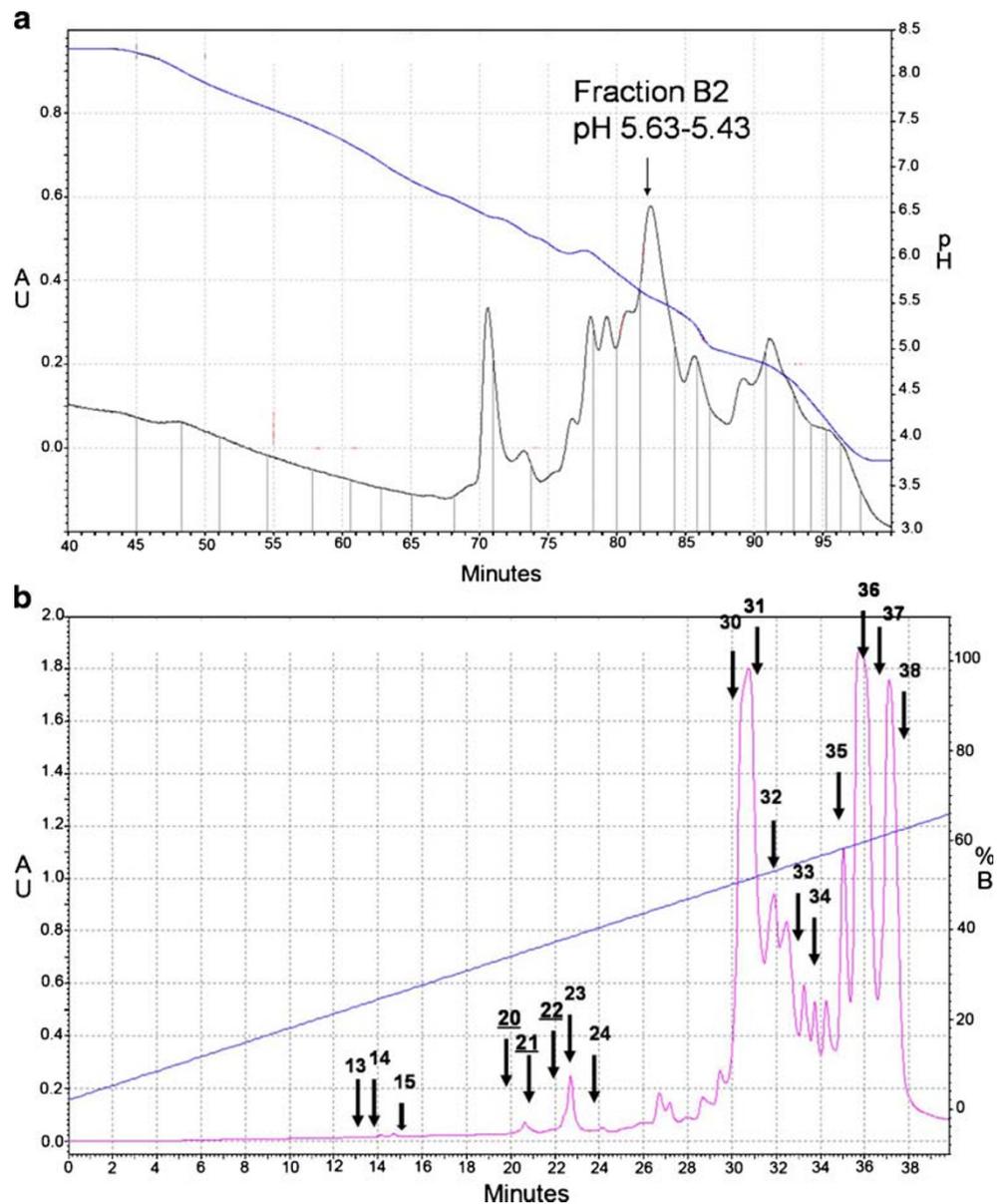


Fig. 3 a Representative HPLC chromatogram for the first-dimension chromatofocusing separation of the abundant protein depleted SF sample. The *diagonal line* indicates the observed pH gradient. The fractions corresponding to the elution range of pH 5.63–5.45 from four runs were combined for the second-dimension separation. **b** HPLC chromatogram for the second-dimension reverse phase separation. This sample was separated on a Beckman System Gold HPLC coupled to a diode array detector. The *diagonal line* indicates the solvent gradient (%B). The fraction (between 20 and 25 min) corresponding to the region that tested positive in the protein array assay was selected for further characterization by mass spectrometry



added to the wells and incubated for 30 min. To stop the reaction, 100 μ l of 1% SDS was added. The absorbance at 405 nm was read on a plate reader.

Results

A targeted proteomic approach was performed to identify autoantigens in RA SF. The experimental design is diagrammed in Fig. 1. An immunodepletion column was first used to remove six abundant serum proteins (albumin, antitrypsin, haptoglobin, IgA, IgG, and transferrin). The depleted SF was then fractionated by chromatofocusing HPLC (provided as Supplement Fig. 1) and reverse phase

HPLC (provided as Supplement Fig. 2). This protein fractionation strategy increased the chance of identifying the lower abundance proteins by separating them from the higher abundance proteins. The second-dimension fractions were used to construct protein arrays on nitrocellulose membranes, which were used to test for the presence of autoantigens by analyzing differential reactivity of RA and control sera. We focused on one second-dimension fraction resulting from the separation of the first-dimension fraction eluting at pH 5.63–5.45 (Fig. 2) that tested positive when probed with RA serum but negative when probed with normal control serum. This was the only fraction that tested positive in which a peak was detected on the HPLC chromatographs. Although there was sufficient material for

Table 1 Proteins identified in the B2 second-dimension RP fractions

	P (pro)	Score	MW	Hits
Fraction 13 proteins				
K1C9_HUMAN Keratin, type I cytoskeletal 9	3.93E-13	60.40	62,091.8	6
PRG4_HUMAN Proteoglycan-4	5.32E-13	40.33	15,0983.2	5
GRP78_HUMAN 78 kDa glucose-regulated protein	1.57E-09	10.25	72,288.5	1
ALBU_HUMAN Serum albumin	2.20E-09	40.24	69,321.6	5
TYB4_HUMAN Thymosin beta-4	1.25E-07	30.23	5,049.5	6
K2C1_HUMAN Keratin, type II cytoskeletal 1	2.44E-07	96.26	65,977.9	10
K1C10_HUMAN Keratin, type I cytoskeletal 10	2.80E-07	30.25	59,474.8	3
Fraction 14 proteins				
K1C9_HUMAN Keratin, type I cytoskeletal 9	1.00E-30	110.27	62,091.8	13
PRG4_HUMAN Proteoglycan-4	1.22E-14	60.34	150,983.2	11
K2C6C_HUMAN Keratin, type II cytoskeletal 6C	1.33E-14	10.31	59,988.4	2
K2C1_HUMAN Keratin, type II cytoskeletal 1	2.93E-11	126.39	65,977.9	15
K1C10_HUMAN Keratin, type I cytoskeletal 10	7.90E-10	10.26	59,474.8	1
ALBU_HUMAN Serum albumin	2.28E-09	10.22	69,321.6	2
TTHY_HUMAN Transthyretin	3.41E-08	20.26	15,877.1	4
BASP_HUMAN Brain acid soluble protein 1	5.02E-07	20.25	22,680.0	2
EFHD2_HUMAN EF-hand domain-containing protein D2	1.42E-06	10.19	26,680.5	1
K22E_HUMAN Keratin, type II cytoskeletal 2 epidermal	1.16E-05	10.21	65,825.4	1
APOA2_HUMAN Apolipoprotein A-II	3.90E-04	10.15	11,167.9	1
Fraction 15 proteins				
PRG4_HUMAN Proteoglycan-4	1.13E-11	50.33	150,983.2	8
K1C10_HUMAN Keratin, type I cytoskeletal 10	3.63E-10	30.27	59,474.8	3
K1C9_HUMAN Keratin, type I cytoskeletal 9	9.80E-08	30.41	62,091.8	3
FIBA_HUMAN Fibrinogen alpha chain	7.51E-07	30.22	94,914.3	6
K2C1_HUMAN Keratin, type II cytoskeletal 1	6.51E-06	70.26	65,977.9	7
PMS2_HUMAN Mismatch repair endonuclease PMS2	2.41E-05	10.19	95,737.1	1
APOA1_HUMAN Apolipoprotein A-I	3.14E-05	30.13	30,758.9	7
K22E_HUMAN Keratin, type II cytoskeletal 2 epidermal	1.45E-04	10.20	65,825.4	1
S10A6_HUMAN Protein S100-A6	4.94E-04	10.13	10,173.3	1
Fraction 20 proteins				
K2C6C_HUMAN Keratin, type II cytoskeletal 6C	3.02E-12	20.30	59,988.4	3
FIBA_HUMAN Fibrinogen alpha chain	4.08E-12	408.35	94,914.3	87
K2C1_HUMAN Keratin, type II cytoskeletal 1	2.09E-11	106.30	65,977.9	12
APOA4_HUMAN Apolipoprotein A-IV	4.79E-11	20.25	45,371.5	3
K1C10_HUMAN Keratin, type I cytoskeletal 10	1.88E-10	30.23	59,474.8	3
K1C9_HUMAN Keratin, type I cytoskeletal 9	4.42E-10	60.31	62,091.8	6
ALBU_HUMAN Serum albumin	5.87E-08	20.21	69,321.6	3
OSTP_HUMAN Osteopontin	7.36E-07	10.22	35,401.3	1
APOA1_HUMAN Apolipoprotein A-I	1.23E-05	20.20	30,758.9	2
K22E_HUMAN Keratin, type II cytoskeletal 2 epidermal	6.83E-05	10.22	65,825.4	1
Fraction 21 proteins				
K1C9_HUMAN Keratin, type I cytoskeletal 9	1.00E-30	70.31	62,091.8	8
K22E_HUMAN Keratin, type II cytoskeletal 2 epidermal	5.55E-15	30.28	65,825.4	4
K2C6C_HUMAN Keratin, type II cytoskeletal 6C	1.11E-14	10.29	59,988.4	2
K2C1_HUMAN Keratin, type II cytoskeletal 1	6.48E-13	100.38	65,977.9	12
FIBA_HUMAN Fibrinogen alpha chain	8.06E-12	428.33	94,914.3	87
OSTP_HUMAN Osteopontin	2.97E-09	70.25	35,401.3	14
K1C10_HUMAN Keratin, type I cytoskeletal 10	8.09E-09	40.24	59,474.8	4

Table 1 (continued)

	P (pro)	Score	MW	Hits
ADIPO_HUMAN Adiponectin	2.01E-08	10.22	26,397.0	2
APOA1_HUMAN Apolipoprotein A-I	3.54E-08	110.20	30,758.9	15
K2C5_HUMAN Keratin, type II cytoskeletal 5	4.79E-08	10.25	62,340.0	1
HEP2_HUMAN Heparin cofactor 2	1.86E-07	20.26	57,034.3	6
A2MG_HUMAN Alpha-2-macroglobulin	4.97E-07	10.22	163,174.3	2
HPT_HUMAN Haptoglobin	2.91E-06	62.22	45,176.6	11
APOB_HUMAN Apolipoprotein B-100	1.20E-05	10.17	515,241.6	2
APOA4_HUMAN Apolipoprotein A-IV	1.46E-05	20.19	45,371.5	2
CLUS_HUMAN Clusterin	5.35E-05	10.16	52,461.1	1
K2C75_HUMAN Keratin, type II cytoskeletal 75	1.47E-04	16.20	59,468.0	2
CD99_HUMAN CD99 antigen	1.73E-04	10.16	18,836.3	1
Fraction 22 proteins				
K22E_HUMAN Keratin, type II cytoskeletal 2 epidermal	1.00E-30	130.30	65,825.4	17
K1C9_HUMAN Keratin, type I cytoskeletal 9	6.66E-15	80.38	62,091.8	9
K2C1_HUMAN Keratin, type II cytoskeletal 1	5.66E-14	226.35	65,977.9	34
HPT_HUMAN Haptoglobin	1.12E-13	98.26	45,176.6	25
FIBA_HUMAN Fibrinogen alpha chain	1.22E-11	588.31	94,914.3	115
K1C10_HUMAN Keratin, type I cytoskeletal 10	2.69E-11	200.34	59,474.8	34
K2C6C_HUMAN Keratin, type II cytoskeletal 6C	4.30E-11	50.31	59,988.4	7
FETUA_HUMAN Alpha-2-HS-glycoprotein	2.71E-09	10.21	39,299.7	1
PLMN_HUMAN Plasminogen	3.35E-09	20.28	90,510.2	5
K2C5_HUMAN Keratin, type II cytoskeletal 5	7.18E-08	20.22	62,340.0	3
K1C14_HUMAN Keratin, type I cytoskeletal 14	9.30E-08	20.17	51,589.5	2
APOA1_HUMAN Apolipoprotein A-I	1.80E-07	158.21	30,758.9	78
LBR_HUMAN Lamin-B receptor	7.18E-07	10.18	70,658.2	1
UPAR_HUMAN Urokinase plasminogen activator surface receptor	8.12E-07	10.24	36,953.3	1
FINC_HUMAN Fibronectin	8.99E-07	30.22	262,439.5	4
ECM1_HUMAN Extracellular matrix protein 1	1.83E-06	20.18	60,635.4	2
K2C6A_HUMAN Keratin, type II cytoskeletal 6A	1.85E-06	10.13	60,008.3	1
TMOD3_HUMAN Tropomodulin-3	2.02E-06	10.20	39,570.3	1
K1C24_HUMAN Keratin, type I cytoskeletal 24	3.28E-06	10.16	55,053.5	1
OSTP_HUMAN Osteopontin	9.62E-06	30.24	35,401.3	6
K1C17_HUMAN Keratin, type I cytoskeletal 17	1.05E-05	30.17	48,076.1	3
APOB_HUMAN Apolipoprotein B-100	2.09E-05	10.15	515,241.6	1
K2C75_HUMAN Keratin, type II cytoskeletal 75	2.46E-05	36.21	59,468.0	4
CFAB_HUMAN Complement factor B	2.82E-05	40.22	85,478.6	6
DEF3_HUMAN Neutrophil defensin 3	6.01E-05	10.14	10,238.2	2
HEP2_HUMAN Heparin cofactor 2	2.17E-04	10.25	57,034.3	1
Fraction 23 proteins				
K2C6C_HUMAN Keratin, type II cytoskeletal 6C	1.55E-14	10.35	59,988.4	3
FIBA_HUMAN Fibrinogen alpha chain	2.36E-11	180.27	94,914.3	23
K2C1_HUMAN Keratin, type II cytoskeletal 1	4.97E-11	110.35	65,977.9	12
IBP6_HUMAN Insulin-like growth factor-binding protein 6	6.63E-11	20.26	25,306.2	3
K2C5_HUMAN Keratin, type II cytoskeletal 5	2.42E-10	10.31	62,340.0	1
K1C9_HUMAN Keratin, type I cytoskeletal 9	2.71E-10	110.33	62,091.8	12
K1C10_HUMAN Keratin, type I cytoskeletal 10	2.05E-09	50.30	59,474.8	5
APOA1_HUMAN Apolipoprotein A-I	7.38E-08	100.23	30,758.9	31
TTHY_HUMAN Transthyretin	1.41E-07	40.28	15,877.1	8
APOC3_HUMAN Apolipoprotein C-III	7.99E-07	10.25	10,845.5	1

Table 1 (continued)

	P (pro)	Score	MW	Hits
CLUS_HUMAN Clusterin	9.57E-07	30.20	52,461.1	5
K22E_HUMAN Keratin, type II cytoskeletal 2 epidermal	1.13E-06	10.21	65,825.4	1
CFAB_HUMAN Complement factor B	1.29E-06	60.23	85,478.6	10
PLMN_HUMAN Plasminogen	1.69E-06	10.25	90,510.2	1
K2C75_HUMAN Keratin, type II cytoskeletal 75	1.01E-04	16.21	59,468.0	2
ACTS_HUMAN Actin, alpha skeletal muscle	1.21E-04	10.15	42,023.9	1
STP2_HUMAN Nuclear transition protein 2	5.46E-04	10.19	15,630.8	1
Fraction 24 proteins				
K2C6C_HUMAN Keratin, type II cytoskeletal 6C	1.00E-30	10.33	59,988.4	2
K22E_HUMAN Keratin, type II cytoskeletal 2 epidermal	2.22E-15	40.25	65,825.4	4
K2C1_HUMAN Keratin, type II cytoskeletal 1	8.66E-14	170.28	65,977.9	18
K1C9_HUMAN Keratin, type I cytoskeletal 9	5.47E-12	80.34	62,091.8	9
FIBA_HUMAN Fibrinogen alpha chain	4.36E-10	410.31	94,914.3	93
IBP6_HUMAN Insulin-like growth factor-binding protein 6	1.62E-09	10.24	25,306.2	2
APOC3_HUMAN Apolipoprotein C-III	2.04E-09	20.27	10,845.5	4
FHR4_HUMAN Complement factor H-related protein 4	2.82E-09	20.28	37,300.4	10
FINC_HUMAN Fibronectin	7.99E-09	110.25	262,439.5	15
CO3_HUMAN Complement C3	1.44E-08	30.23	187,029.3	3
KNK1_HUMAN Kininogen-1	1.81E-08	10.21	71,912.1	3
AHNK_HUMAN Neuroblast differentiation-associated protein AHNK	2.21E-08	144.21	628,705.2	17
CFAI_HUMAN Complement factor I	3.09E-08	10.22	65,676.7	1
TTHY_HUMAN Transthyretin	3.45E-08	70.27	15,877.1	13
PLMN_HUMAN Plasminogen	6.87E-08	20.26	90,510.2	3
CLUS_HUMAN Clusterin	1.57E-07	10.21	52,461.1	1
EMIL2_HUMAN EMILIN-2	1.82E-07	10.23	115,544.0	1
UPAR_HUMAN Urokinase plasminogen activator surface receptor	2.83E-07	10.25	36,953.3	1
APOA1_HUMAN Apolipoprotein A-I	6.53E-07	100.23	30,758.9	23
PRIO_HUMAN Major prion protein	7.31E-07	10.21	27,643.2	2
HRG_HUMAN Histidine-rich glycoprotein	1.84E-06	10.19	59,540.9	1
FHR1_HUMAN Complement factor H-related protein 1	2.60E-06	30.17	37,637.0	3
CFAB_HUMAN Complement factor B	3.71E-06	80.21	85,478.6	10
HPT_HUMAN Haptoglobin	6.41E-06	38.18	45,176.6	7
FHR3_HUMAN Complement factor H-related protein 3	9.61E-06	20.16	37,298.8	2
K1C10_HUMAN Keratin, type I cytoskeletal 10	1.00E-05	20.19	59,474.8	2
RETBP_PANTR Plasma retinol-binding protein	1.19E-05	30.18	22,995.3	4
GDIS_HUMAN Rho GDP-dissociation inhibitor 2	1.48E-05	20.22	22,973.6	2
NHERF_HUMAN Ezrin–radixin–moesin-binding phosphoprotein 50	4.74E-05	20.16	38,844.6	2
IBP3_HUMAN Insulin-like growth factor-binding protein 3	9.03E-05	40.18	31,653.8	7
ACTS_HUMAN Actin, alpha skeletal muscle	2.25E-04	10.15	42,023.9	1
K2C75_HUMAN Keratin, type II cytoskeletal 75	2.54E-04	16.21	59,468.0	2
HSPB1_HUMAN Heat shock protein beta-1	3.19E-04	10.15	22,768.5	1
APOC2_HUMAN Apolipoprotein C-II	4.13E-04	10.16	11,276.8	1
ADAM8_HUMAN ADAM 8	5.40E-04	10.15	88,615.7	1
Fraction 30 proteins				
HEMO_HUMAN Hemopexin	5.65E-16	604.31	51,643.3	307
CFAI_HUMAN Complement factor I	1.11E-15	300.33	65,676.7	79
FETUB_HUMAN Fetuin-B	3.33E-15	80.26	42,028.0	14
AMBP_HUMAN AMBP protein	2.00E-14	20.24	38,974.0	2
IGKC_HUMAN Ig kappa chain C region	7.99E-14	30.27	11,601.7	11

Table 1 (continued)

	P (pro)	Score	MW	Hits
TTHY_HUMAN Transthyretin	1.11E-13	150.29	15,877.1	44
TRFE_HUMAN Serotransferrin	2.02E-13	40.33	76,999.7	4
RETBP_PANTR Plasma retinol-binding protein	4.00E-13	70.32	22,995.3	33
VTDB_HUMAN Vitamin D-binding protein	9.50E-13	20.27	52,929.1	3
CO7_HUMAN Complement component C7	3.29E-12	80.22	93,457.3	10
FIBG_HUMAN Fibrinogen gamma chain	1.25E-11	120.29	51,478.9	27
FIBA_HUMAN Fibrinogen alpha chain	1.75E-11	70.28	94,914.3	8
ITIH4_HUMAN Inter-alpha-trypsin inhibitor heavy chain H4	2.39E-11	80.29	103,261.2	22
ALBU_HUMAN Serum albumin	4.44E-11	716.29	69,321.6	473
APOC3_HUMAN Apolipoprotein C-III	1.08E-10	38.26	10,845.5	6
FIBB_HUMAN Fibrinogen beta chain	2.14E-10	130.29	55,892.2	25
TENX_HUMAN Tenascin-X	7.70E-10	10.21	464,165.9	1
A2MG_HUMAN Alpha-2-macroglobulin	2.49E-09	10.17	163,174.3	1
HBB_HUMAN Hemoglobin subunit beta	6.11E-09	28.22	15,988.3	3
FETUA_HUMAN Alpha-2-HS-glycoprotein	1.00E-08	20.26	39,299.7	2
APOA1_HUMAN Apolipoprotein A-I	2.09E-08	110.23	30,758.9	34
HPTR_HUMAN Haptoglobin-related protein	3.73E-08	10.18	38,982.7	1
K2C1_HUMAN Keratin, type II cytoskeletal 1	4.44E-08	10.34	65,977.9	2
CERU_HUMAN Ceruloplasmin	4.89E-08	10.22	122,127.6	1
HBA_HUMAN Hemoglobin subunit alpha	6.40E-08	10.23	15,247.9	3
PCOC1_HUMAN Procollagen C-endopeptidase enhancer 1	2.70E-07	30.16	47,942.0	7
CO3A1_HUMAN Collagen alpha-1(III) chain	4.23E-07	20.21	138,479.2	2
LAC_HUMAN Ig lambda chain C regions	5.62E-07	20.23	11,229.5	9
HPT_HUMAN Haptoglobin	7.82E-07	38.18	45,176.6	13
CATB_HUMAN Cathepsin B	1.21E-06	20.21	37,796.8	2
MUCB_HUMAN Ig mu heavy chain disease protein	1.89E-06	38.19	43,030.3	4
IGHA2_HUMAN Ig alpha-2 chain C region	4.73E-06	10.13	36,485.1	2
COR1A_HUMAN Coronin-1A	6.19E-06	10.23	50,993.9	1
TETN_HUMAN Tetranectin	9.66E-06	30.24	22,552.3	5
IGHM_HUMAN Ig mu chain C region	1.93E-05	20.26	49,275.6	3
CFAB_HUMAN Complement factor B	2.98E-05	40.18	85,478.6	4
CAPG_HUMAN Macrophage-capping protein	3.70E-05	40.20	38,493.6	6
IGHD_HUMAN Ig delta chain C region	4.73E-05	10.14	42,227.3	1
IGHA1_HUMAN Ig alpha-1 chain C region	1.52E-04	10.18	37,630.7	1
ATS9_HUMAN ADAMTS-9	4.44E-04	10.15	216,415.5	1
Fraction 31 proteins				
CFAB_HUMAN Complement factor B	1.00E-30	296.35	85,478.6	74
CO8A_HUMAN Complement component C8 alpha chain	1.00E-30	104.27	65,121.0	18
TTHY_HUMAN Transthyretin	1.11E-16	340.32	15,877.1	103
CERU_HUMAN Ceruloplasmin	1.11E-16	80.31	122,127.6	10
RETBP_PANTR Plasma retinol-binding protein	1.11E-16	70.31	22,995.3	31
HEMO_HUMAN Hemopexin	2.22E-16	236.31	51,643.3	85
K1C9_HUMAN Keratin, type I cytoskeletal 9	4.44E-16	20.39	62,091.8	2
HBA_HUMAN Hemoglobin subunit alpha	4.44E-15	40.29	15,247.9	5
S10AC_HUMAN Protein S100-A12	6.66E-15	10.30	10,568.5	1
CO8G_HUMAN Complement component C8 gamma chain	7.77E-15	60.26	22,263.6	11
IGKC_HUMAN Ig kappa chain C region	9.99E-15	70.30	11,601.7	53
ITIH4_HUMAN Inter-alpha-trypsin inhibitor heavy chain H4	1.44E-14	468.31	103,261.2	188
IGHA1_HUMAN Ig alpha-1 chain C region	5.46E-12	80.32	37,630.7	35

Table 1 (continued)

	P (pro)	Score	MW	Hits
APOC2_HUMAN Apolipoprotein C-II	5.67E-12	60.28	11,276.8	14
FIBB_HUMAN Fibrinogen beta	7.98E-12	210.39	55,892.2	78
AMBP_HUMAN AMBP protein	8.25E-12	40.25	38,974.0	5
CLUS_HUMAN Clusterin	1.08E-11	148.28	52,461.1	26
HV3P_HUMAN Ig heavy chain V-III region TEI	1.38E-11	10.28	12,794.4	4
ALBU_HUMAN Serum albumin	1.48E-11	370.28	69,321.6	160
FIBG_HUMAN Fibrinogen gamma chain	1.71E-11	240.30	51,478.9	46
KV1D_HUMAN Ig kappa chain V-I region CAR	2.53E-11	10.26	11,696.3	3
AACT_HUMAN Alpha-1-antichymotrypsin	3.63E-11	10.21	47,620.6	1
LAC_HUMAN Ig lambda chain C regions	5.29E-11	60.26	11,229.5	16
IGHA2_HUMAN Ig alpha-2 chain C region	5.55E-11	120.26	36,485.1	44
HPT_HUMAN Haptoglobin	8.17E-11	110.29	45,176.6	25
ENOB_HUMAN Beta-enolase	1.28E-10	10.24	46,957.4	1
ENOA_HUMAN Alpha-enolase	1.31E-10	30.26	47,139.4	3
APOA1_HUMAN Apolipoprotein A-I	1.45E-10	330.30	30,758.9	111
CFAI_HUMAN Complement factor I	1.53E-10	118.28	65,676.7	23
COR1A_HUMAN Coronin-1A	1.97E-10	64.25	50,993.9	8
HBD_HUMAN Hemoglobin subunit delta	2.66E-10	78.23	16,045.3	12
FETUA_HUMAN Alpha-2-HS-glycoprotein	6.50E-10	20.27	39,299.7	4
DIAC_HUMAN Di-N-acetylchitobiase	9.02E-10	20.20	43,731.6	2
A2AP_HUMAN Alpha-2-antiplasmin	9.62E-10	40.24	54,531.2	4
KV3H_HUMAN Ig kappa chain V-III region CLL	1.61E-09	18.23	14,266.2	4
SHBG_HUMAN Sex hormone-binding globulin	2.19E-09	60.24	43,751.9	6
ENO1B_HUMAN Alpha-enolase, lung specific	2.99E-09	10.21	49,446.4	2
AIF1_HUMAN Allograft inflammatory factor 1	3.56E-09	10.23	16,692.6	1
FRIH_HUMAN Ferritin heavy chain	5.57E-09	60.25	21,212.3	8
FIBA_HUMAN Fibrinogen alpha chain	7.21E-09	98.28	94,914.3	17
IGHD_HUMAN Ig delta chain C region	7.77E-09	30.23	42,227.3	6
LUM_HUMAN Lumican	1.22E-08	60.30	38,404.8	14
GELS_HUMAN Gelsolin	2.34E-08	20.21	85,644.3	2
HBB_HUMAN Hemoglobin subunit beta	2.70E-08	50.22	15,988.3	14
COF1_HUMAN Cofilin-1	3.91E-08	10.26	18,490.7	1
KV1S_HUMAN Ig kappa chain V-I region Wes	4.88E-08	10.21	11,600.7	1
MUCB_HUMAN Ig mu heavy chain disease protein	5.93E-08	56.22	43,030.3	15
LV3B_HUMAN Ig lambda chain V-III region LOI	9.13E-08	20.24	11,927.8	6
FETUB_HUMAN Fetuin-B	9.28E-08	60.27	42,028.0	8
K2C1_HUMAN Keratin, type II cytoskeletal 1	1.53E-07	46.37	65,977.9	6
IGHM_HUMAN Ig mu chain C region	2.18E-07	30.23	49,275.6	5
MMP1_HUMAN Interstitial collagenase	2.78E-07	30.21	53,972.8	3
COTL1_HUMAN Coactosin-like protein	3.14E-07	10.23	15,935.0	1
KV3G_HUMAN Ig kappa chain V-III region GOL	3.86E-07	10.21	11,823.0	4
CATB_HUMAN Cathepsin B	3.96E-07	10.16	37,796.8	1
S10A9_HUMAN Protein S100-A9	4.68E-07	10.19	13,233.5	1
THIO_HUMAN Thioredoxin	4.91E-07	10.18	11,729.7	4
TRFE_HUMAN Serotransferrin	5.57E-07	50.26	136,191	6
A2MG_HUMAN Alpha-2-macroglobulin	6.03E-07	20.21	163,174.3	3
ANT3_HUMAN Antithrombin-III	6.29E-07	10.21	52,569.0	1
KPYM_HUMAN Pyruvate kinase isozymes M1/M2	7.06E-07	10.24	57,900.2	1
PROF1_HUMAN Profilin-1 (Profilin I)	8.10E-07	30.20	15,044.6	3

Table 1 (continued)

	P (pro)	Score	MW	Hits
CO8B_HUMAN Complement component C8 beta chain	8.76E-07	30.17	67,003.5	3
PCOC1_HUMAN Procollagen C-endopeptidase enhancer 1	1.74E-06	20.19	47,942.0	2
HV3J_HUMAN Ig heavy chain V-III region HIL	1.97E-06	10.19	13,557.2	3
SAMP_HUMAN Serum amyloid P-component	2.29E-06	10.16	25,371.1	1
KV3I_HUMAN Ig kappa chain V-III region VG	2.90E-06	20.25	12,567.3	4
CAPG_HUMAN Macrophage-capping protein	3.08E-06	10.20	38,493.6	1
APOC3_HUMAN Apolipoprotein C-III	4.71E-06	20.21	10,845.5	3
CO7_HUMAN Complement component C7	5.24E-06	48.24	93,457.3	6
ADIPO_HUMAN Adiponectin	7.98E-06	10.22	26,397.0	1
KV1V_HUMAN Ig kappa chain V-I region BAN	9.70E-06	10.21	11,832.8	1
HV1G_HUMAN Ig heavy chain V-I region V35	1.01E-05	10.15	13,000.4	1
KV4C_HUMAN Ig kappa chain V-IV region B17	2.11E-05	20.16	14,956.5	2
TETN_HUMAN Tetranectin	2.36E-05	10.18	22,552.3	1
APOD_HUMAN Apolipoprotein D	7.83E-05	20.13	21,261.8	4
IGLL1_HUMAN Immunoglobulin lambda-like polypeptide 1	8.60E-05	28.19	22,948.6	3
IGJ_HUMAN Immunoglobulin J chain	8.94E-05	10.13	15,584.6	1
HV3R_HUMAN Ig heavy chain V-III region TUR	1.01E-04	10.22	12,423.3	3
SAA4_HUMAN Serum amyloid A-4 protein	1.78E-04	10.16	14,797.3	2
HV1C_HUMAN Ig heavy chain V-I region ND	1.93E-04	10.13	16,493.0	1
HPTR_HUMAN Haptoglobin-related protein	1.94E-04	10.18	38,982.7	1
LV3A_HUMAN Ig lambda chain V-III region SH	5.30E-04	10.17	11,385.6	2
CO1A1_HUMAN Collagen alpha-1(I) chain	5.86E-04	10.15	138,826.8	1
MBOA2_HUMAN Membrane-bound O-acyltransferase domain-containing	7.03E-04	10.11	59,488.3	1
ZA2G_HUMAN Zinc-alpha-2-glycoprotein	8.67E-04	10.14	33,850.9	1
Fraction 32 proteins				
TTHY_HUMAN Transthyretin	1.00E-30	350.34	15,877.1	114
HBA_HUMAN Hemoglobin subunit alpha	1.00E-30	90.31	15,247.9	15
CO8B_HUMAN Complement component C8 beta chain	1.00E-30	10.26	67,003.5	1
CFAB_HUMAN Complement factor B	7.77E-15	220.34	85,478.6	52
ITIH2_HUMAN Inter-alpha-trypsin inhibitor heavy chain H2	8.88E-15	10.29	106,369.8	1
CO8G_HUMAN Complement component C8 gamma chain	9.99E-15	30.25	22,263.6	5
K1C9_HUMAN Keratin, type I cytoskeletal 9	9.99E-15	20.38	62,091.8	2
CERU_HUMAN Ceruloplasmin	1.11E-14	60.28	122,127.6	7
CLUS_HUMAN Clusterin	1.67E-14	190.29	52,461.1	57
G3P_HUMAN Glyceraldehyde-3-phosphate dehydrogenase	2.11E-14	50.33	36,030.4	5
CBPN_HUMAN Carboxypeptidase N catalytic chain	3.55E-14	20.26	52,253.4	3
RETBP_PANTR Plasma retinol-binding protein	4.00E-14	60.32	22,995.3	34
AMBP_HUMAN AMBP protein	4.00E-14	30.27	38,974.0	4
ENOA_HUMAN Alpha-enolase	5.33E-14	148.35	47,139.4	43
IGKC_HUMAN Ig kappa chain C region	5.77E-14	60.29	11,601.7	66
HEMO_HUMAN Hemopexin	2.15E-13	136.31	51,643.3	41
ANT3_HUMAN Antithrombin-III	7.48E-13	316.31	52,569.0	59
S10AC_HUMAN Protein S100-A12	1.80E-12	40.36	10,568.5	8
HBD_HUMAN Hemoglobin subunit delta	2.46E-12	206.30	16,045.3	77
KLKB1_HUMAN Plasma kallikrein	2.60E-12	50.24	71,322.8	6
ENOB_HUMAN Beta-enolase	2.71E-12	38.26	46,957.4	9
SHBG_HUMAN Sex hormone-binding globulin	5.08E-12	90.29	43,751.9	23
ITIH4_HUMAN Inter-alpha-trypsin inhibitor heavy chain H4	6.78E-12	110.29	103,261.2	25
S10A8_HUMAN Protein S100-A8	7.06E-12	20.31	10,827.7	5

Table 1 (continued)

	P (pro)	Score	MW	Hits
APOC2_HUMAN Apolipoprotein C-II	9.10E-12	100.29	11,276.8	30
PGRP2_HUMAN N-acetylmuramoyl-L-alanine amidase	1.26E-11	60.29	62,178.0	8
KVID_HUMAN Ig kappa chain V-I region CAR	1.76E-11	10.25	11,696.3	3
KVIS_HUMAN Ig kappa chain V-I region Wes	2.20E-11	10.23	11,600.7	2
APOM_HUMAN Apolipoprotein M	2.21E-11	70.25	21,239.4	11
HBB_HUMAN Hemoglobin subunit beta	2.64E-11	208.31	15,988.3	131
IGHA1_HUMAN Ig alpha-1 chain C region	3.97E-11	80.26	37,630.7	29
LAC_HUMAN Ig lambda chain C regions	6.32E-11	60.27	11,229.5	19
CFAI_HUMAN Complement factor I	6.39E-11	116.28	65,676.7	21
KV4C_HUMAN Ig kappa chain V-IV region B17	7.16E-11	20.26	14,956.5	3
THRB_HUMAN Prothrombin	1.36E-10	40.28	69,992.2	5
APOA1_HUMAN Apolipoprotein A-I	1.44E-10	530.26	30,758.9	302
CFAD_HUMAN Complement factor D	2.12E-10	30.22	27,015.9	4
IGHA2_HUMAN Ig alpha-2 chain C region	2.79E-10	118.23	36,485.1	36
FIBB_HUMAN Fibrinogen beta chain	2.86E-10	100.27	55,892.2	16
HPT_HUMAN Haptoglobin	3.59E-10	130.32	45,176.6	28
SAMP_HUMAN Serum amyloid P-component	4.06E-10	60.21	25,371.1	12
KV3H_HUMAN Ig kappa chain V-III region CLL	5.94E-10	10.22	14,266.2	2
APOA2_HUMAN Apolipoprotein A-II	7.84E-10	60.31	11,167.9	7
HSP71_PONAB Heat shock 70 kDa protein 1	8.30E-10	10.24	70,009.2	1
CO8A_HUMAN Complement component C8 alpha chain	9.20E-10	20.25	65,121.0	2
S10A9_HUMAN Protein S100-A9	1.43E-09	40.25	13,233.5	7
ALBU_HUMAN Serum albumin	2.15E-09	262.26	69,321.6	70
VASP_HUMAN Vasodilator-stimulated phosphoprotein	2.32E-09	10.23	39,805.1	1
K2C1_HUMAN Keratin, type II cytoskeletal 1	2.40E-09	26.41	65,977.9	4
FIBA_HUMAN Fibrinogen alpha chain	2.63E-09	30.23	94,914.3	3
ADIPO_HUMAN Adiponectin	3.36E-09	10.22	26,397.0	1
FIBG_HUMAN Fibrinogen gamma chain	1.33E-08	70.27	51,478.9	14
ENO1B_HUMAN Alpha-enolase, lung specific	1.53E-08	50.29	49,446.4	9
LV4C_HUMAN Ig lambda chain V-IV region Hil	1.59E-08	10.25	11,509.6	2
IGHG3_HUMAN Ig gamma-3 chain C region	2.06E-08	10.18	41,260.4	1
COR1A_HUMAN Coronin-1A	2.58E-08	10.24	50,993.9	1
CO4A_HUMAN Complement C4-A	3.08E-08	20.24	192,649.5	2
KV3G_HUMAN Ig kappa chain V-III region GOL	3.61E-08	10.21	11,823.0	3
IGHD_HUMAN Ig delta chain C region	3.83E-08	20.19	42,227.3	3
FRIH_HUMAN Ferritin heavy chain	3.91E-08	10.21	21,212.3	1
IGHM_HUMAN Ig mu chain C region	5.37E-08	20.23	49,275.6	13
LUM_HUMAN Lumican	5.61E-08	80.26	38,404.8	17
PSA6_RAT Proteasome subunit alpha type-6	6.04E-08	20.17	27,381.8	2
MUCB_HUMAN Ig mu heavy chain disease protein	1.04E-07	58.23	43,030.3	12
LV3B_HUMAN Ig lambda chain V-III region LOI	1.17E-07	30.24	11,927.8	6
CO1A1_HUMAN Collagen alpha-1(I) chain	1.76E-07	10.15	138,826.8	1
APOE_HUMAN Apolipoprotein E	1.87E-07	20.19	36,131.8	3
CAPG_HUMAN Macrophage-capping protein	2.00E-07	10.19	38,493.6	1
LMNA_HUMAN Lamin-A/C	2.15E-07	30.20	74,094.8	4
A2MG_HUMAN Alpha-2-macroglobulin	3.81E-07	60.19	163,174.3	7
IGLL1_HUMAN Immunoglobulin lambda-like polypeptide 1	4.24E-07	38.22	22,948.6	5
CFAH_HUMAN Complement factor H	6.11E-07	10.14	139,004.4	1
GELS_HUMAN Gelsolin	1.31E-06	20.23	85,644.3	2

Table 1 (continued)

	P (pro)	Score	MW	Hits
HV1G_HUMAN Ig heavy chain V-I region V35	1.50E-06	10.16	13,000.4	1
CO3_HUMAN Complement C3	1.59E-06	30.22	187,029.3	3
FETUB_HUMAN Fetuin-B	1.64E-06	10.26	42,028.0	1
UBP15_HUMAN Ubiquitin carboxyl-terminal hydrolase 15	3.10E-06	10.20	112,347.6	1
GPX3_HUMAN Glutathione peroxidase 3	3.23E-06	60.20	25,386.0	6
FETUA_HUMAN Alpha-2-HS-glycoprotein	4.85E-06	20.18	39,299.7	2
APOA4_HUMAN Apolipoprotein A-IV	5.76E-06	10.19	45,371.5	1
FGFP2_HUMAN Fibroblast growth factor-binding protein 2	1.29E-05	20.18	24,565.2	2
APOC1_HUMAN Apolipoprotein C-I	2.47E-05	10.12	9,326.1	1
APOD_HUMAN Apolipoprotein D	2.86E-05	10.12	21,261.8	1
HV3P_HUMAN Ig heavy chain V-III region TEI	2.88E-05	10.20	12,794.4	3
GDIS_HUMAN Rho GDP-dissociation inhibitor 2	3.11E-05	10.18	22,973.6	1
TENA_HUMAN Tenascin	3.65E-05	10.16	240,710.7	1
HV1B_HUMAN Ig heavy chain V-I region HG3	3.81E-05	10.17	12,937.3	1
TRFE_HUMAN Serotransferrin	4.38E-05	66.22	76,999.7	7
PROF1_HUMAN Profilin-1	4.39E-05	10.22	15,044.6	1
KV3I_HUMAN Ig kappa chain V-III region VG	8.00E-05	20.25	12,567.3	3
APOB_HUMAN Apolipoprotein B-100	8.41E-05	20.21	515,241.6	2
LV3A_HUMAN Ig lambda chain V-III region SH	8.52E-05	10.21	11,385.6	1
APOC3_HUMAN Apolipoprotein C-III	8.88E-05	10.16	10,845.5	1
PRDX6_HUMAN Peroxiredoxin-6	9.49E-05	10.14	25,019.2	1
DOPO_HUMAN Dopamine beta-hydroxylase	1.01E-04	20.16	69,020.8	2
KV1V_HUMAN Ig kappa chain V-I region BAN	1.47E-04	10.19	11,832.8	2
TRIM7_HUMAN Tripartite motif-containing protein 7	2.81E-04	10.15	56,595.0	6
SAA4_HUMAN Serum amyloid A-4 protein	3.43E-04	20.16	14,797.3	4
Fraction 33 proteins				
HBA_HUMAN Hemoglobin subunit alpha	1.00E-30	350.39	15,247.9	127
PGRP2_HUMAN N-acetylmuramoyl-L-alanine amidase	1.11E-15	268.33	62,178.0	53
CFAB_HUMAN Complement factor B	1.11E-15	180.34	85,478.6	30
RETBP_PANTR Plasma retinol-binding protein	1.11E-15	70.32	22,995.3	30
APOA1_HUMAN Apolipoprotein A-I	8.88E-15	648.34	30,758.9	410
S10AC_HUMAN Protein S100-A12	1.22E-14	20.35	10,568.5	3
CO3_HUMAN Complement C3	2.11E-14	468.34	187,029.3	76
HEMO_HUMAN Hemopexin	6.33E-14	178.29	51,643.3	30
TTHY_HUMAN Transthyretin	1.45E-13	220.33	15,877.1	49
CFAI_HUMAN Complement factor I	2.96E-13	88.28	65,676.7	14
ENOA_HUMAN Alpha-enolase	3.60E-13	50.33	47,139.4	7
CERU_HUMAN Ceruloplasmin	3.69E-13	60.33	122,127.6	6
K2C1_HUMAN Keratin, type II cytoskeletal 1	3.94E-13	76.34	65,977.9	9
ITIH2_HUMAN Inter-alpha-trypsin inhibitor heavy chain H2	1.32E-12	50.33	106,369.8	6
ENOB_HUMAN Beta-enolase	1.48E-12	10.25	46,957.4	3
CO8G_HUMAN Complement component C8 gamma chain	3.98E-12	20.20	22,263.6	3
ANT3_HUMAN Antithrombin-III	4.03E-12	268.30	52,569.0	39
IGKC_HUMAN Ig kappa chain C region	4.14E-12	100.29	11,601.7	54
IGHA1_HUMAN Ig alpha-1 chain C region	1.07E-11	90.29	37,630.7	32
KV1D_HUMAN Ig kappa chain V-I region CAR	1.20E-11	20.26	11,696.3	3
APOM_HUMAN Apolipoprotein M	1.29E-11	60.25	21,239.4	8
CO4A_HUMAN Complement C4-A	1.48E-11	200.28	192,649.5	25
PSA2_HUMAN Proteasome subunit alpha type-2	1.55E-11	60.29	25,882.3	8

Table 1 (continued)

	P (pro)	Score	MW	Hits
HV3R_HUMAN Ig heavy chain V-III region TUR	2.17E-11	20.25	12,423.3	4
KV1R_HUMAN Ig kappa chain V-I region WEA	2.35E-11	26.28	11,832.8	8
HBB_HUMAN Hemoglobin subunit beta	3.15E-11	190.27	15,988.3	85
HBD_HUMAN Hemoglobin subunit delta	4.14E-11	228.29	16,045.3	66
GSTP1_HUMAN Glutathione S-transferase P	7.69E-11	30.25	23,341.0	4
LV4C_HUMAN Ig lambda chain V-IV region Hil	8.74E-11	20.26	11,509.6	4
FIBA_HUMAN Fibrinogen alpha chain	9.51E-11	20.26	94,914.3	2
APOC2_HUMAN Apolipoprotein C-II	1.01E-10	100.28	11,276.8	17
LAC_HUMAN Ig lambda chain C regions	1.09E-10	50.27	11,229.5	14
S10A9_HUMAN Protein S100-A9	1.16E-10	128.28	13,233.5	31
CLUS_HUMAN Clusterin	2.11E-10	218.30	52,461.1	47
SAMP_HUMAN Serum amyloid P-component	2.58E-10	10.19	25,371.1	2
HV3P_HUMAN Ig heavy chain V-III region TEI	4.78E-10	10.26	12,794.4	3
KV3H_HUMAN Ig kappa chain V-III region CLL	6.52E-10	10.21	14,266.2	2
PERM_HUMAN Myeloperoxidase	6.57E-10	10.21	83,815.0	1
ALBU_HUMAN Serum albumin	6.70E-10	190.27	69,321.6	44
SPB10_HUMAN Serpin B10	7.84E-10	30.21	45,374.0	3
CO6A1_HUMAN Collagen alpha-1(VI) chain	7.93E-10	20.26	108,462.0	3
A2GL_HUMAN Leucine-rich alpha-2-glycoprotein	8.29E-10	50.25	38,154.1	8
IGHA2_HUMAN Ig alpha-2 chain C region	1.05E-09	94.24	36,485.1	28
ITIH1_HUMAN Inter-alpha-trypsin inhibitor heavy chain H1	1.10E-09	30.26	101,325.8	3
KV1S_HUMAN Ig kappa chain V-I region Wes	1.21E-09	10.23	11,600.7	1
APOA2_HUMAN Apolipoprotein A-II	1.22E-09	100.28	11,167.9	13
CATD_HUMAN Cathepsin D	1.30E-09	32.22	44,523.7	4
A2MG_HUMAN Alpha-2-macroglobulin	1.34E-09	120.23	163,174.3	16
SHBG_HUMAN Sex hormone-binding globulin	1.66E-09	10.29	43,751.9	1
KV4C_HUMAN Ig kappa chain V-IV region B17	1.69E-09	30.24	14,956.5	5
TRFE_HUMAN Serotransferrin	4.52E-09	20.21	76,999.7	2
ITIH4_HUMAN Inter-alpha-trypsin inhibitor heavy chain H4	6.22E-09	110.26	103,261.2	16
MUCB_HUMAN Ig mu heavy chain disease protein	6.34E-09	68.25	43,030.3	20
S10A8_HUMAN Protein S100-A8	6.64E-09	20.25	10,827.7	2
HSP71_PONAB Heat shock 70 kDa protein 1	6.79E-09	20.27	70,009.2	2
HPT_HUMAN Haptoglobin	7.42E-09	132.26	45,176.6	24
KV3G_HUMAN Ig kappa chain V-III region GOL	7.50E-09	10.23	11,823.0	2
AFAM_HUMAN Afamin	2.07E-08	70.24	69,024.1	7
FIBB_HUMAN Fibrinogen beta chain	2.09E-08	70.24	55,892.2	8
K1C9_HUMAN Keratin, type I cytoskeletal 9	2.09E-08	40.33	62,091.8	4
TKT_HUMAN Transketolase	2.24E-08	40.21	67,834.9	5
IGHM_HUMAN Ig mu chain C region	2.76E-08	50.25	49,275.6	9
GELS_HUMAN Gelsolin	3.10E-08	10.23	85,644.3	1
FIBG_HUMAN Fibrinogen gamma chain	3.14E-08	50.19	51,478.9	5
IGHD_HUMAN Ig delta chain C region	3.27E-08	20.19	42,227.3	3
ADIPO_HUMAN Adiponectin	4.87E-08	10.23	26,397.0	1
PRDX2_HUMAN Peroxiredoxin-2	5.29E-08	50.21	21,878.2	6
LV3B_HUMAN Ig lambda chain V-III region LOI	7.16E-08	20.22	11,927.8	4
VASP_HUMAN Vasodilator-stimulated phosphoprotein	1.37E-07	30.25	39,805.1	5
PSA6_RAT Proteasome subunit alpha type-6	2.97E-07	10.17	27,381.8	1
ENO1B_HUMAN Alpha-enolase, lung specific	6.65E-07	10.26	49,446.4	1
HPTR_HUMAN Haptoglobin-related protein	6.93E-07	10.20	38,982.7	1

Table 1 (continued)

	P (pro)	Score	MW	Hits
IGHG3_HUMAN Ig gamma-3 chain C region	1.04E-06	20.18	41,260.4	2
G3P_HUMAN Glyceraldehyde-3-phosphate dehydrogenase	1.35E-06	20.22	36,030.4	3
KLKB1_HUMAN Plasma kallikrein	2.39E-06	10.18	71,322.8	1
ANXA1_HUMAN Annexin A1	2.64E-06	10.20	38,690.0	1
ANXA3_HUMAN Annexin A3	3.79E-06	24.18	36,352.7	3
AMPL_HUMAN Cytosol aminopeptidase	4.31E-06	10.14	56,130.9	1
S100P_HUMAN Protein S100-P	4.65E-06	10.18	10,393.2	1
MMP8_HUMAN Neutrophil collagenase	4.76E-06	30.23	53,378.6	3
APOE_HUMAN Apolipoprotein E	5.02E-06	50.21	36,131.8	6
HBG1_PANTR Hemoglobin subunit gamma-1	8.24E-06	20.16	16,130.3	2
KV1V_HUMAN Ig kappa chain V-I region BAN	9.89E-06	10.22	11,832.8	1
APOA4_HUMAN Apolipoprotein A-IV	1.25E-05	60.19	45,371.5	7
KV3I_HUMAN Ig kappa chain V-III region VG precursor	1.41E-05	20.23	12,567.3	3
HV3A_HUMAN Ig heavy chain V-III region TRO	1.43E-05	10.16	13,463.5	1
THBG_HUMAN Thyroxine-binding globulin	1.94E-05	10.20	46,294.7	1
K1C14_HUMAN Keratin, type I cytoskeletal 14	2.73E-05	10.13	51,589.5	1
IGLL1_HUMAN Immunoglobulin lambda-like polypeptide 1	3.37E-05	38.21	22,948.6	5
K1C10_HUMAN Keratin, type I cytoskeletal 10	5.01E-05	30.19	59,474.8	3
LV3A_HUMAN Ig lambda chain V-III region SH	8.87E-05	10.18	11,385.6	1
CRP_HUMAN C-reactive protein	2.33E-04	20.18	25,022.7	2
CBPN_HUMAN Carboxypeptidase N catalytic chain	2.42E-04	10.24	52,253.4	1
CAPG_HUMAN Macrophage-capping protein	2.55E-04	20.16	38,493.6	2
SAA4_HUMAN Serum amyloid A-4 protein	2.71E-04	20.14	14,797.3	2
MMP3_HUMAN Stromelysin-1	4.23E-04	10.13	53,943.4	1
GDIS_HUMAN Rho GDP-dissociation inhibitor 2	6.94E-04	20.25	22,973.6	2
HV3V_HUMAN Ig heavy chain V-III region GAR	8.20E-04	10.13	13,079.2	1
Fraction 34 proteins				
CO3_HUMAN Complement C3	1.00E-30	576.33	187,029.3	99
HBA_HUMAN Hemoglobin subunit alpha	1.00E-30	230.37	15,247.9	91
TTHY_HUMAN Transthyretin	1.00E-30	150.33	15,877.1	28
CFAB_HUMAN Complement factor B	3.33E-15	120.35	85,478.6	20
PGRP2_HUMAN N-acetylmuramoyl-L-alanine amidase	8.88E-15	290.34	62,178.0	52
IGKC_HUMAN Ig kappa chain C region	1.11E-14	70.29	11,601.7	40
APOA1_HUMAN Apolipoprotein A-I	1.89E-14	862.32	30,758.9	660
ENOB_HUMAN Beta-enolase	2.34E-13	10.24	46,957.4	1
ENOA_HUMAN Alpha-enolase	4.31E-13	40.24	47,139.4	4
RETBP_PANTR Plasma retinol-binding protein	7.37E-13	58.31	22,995.3	20
KV4C_HUMAN Ig kappa chain V-IV region B17	8.39E-13	20.26	14,956.5	3
IGHA1_HUMAN Ig alpha-1 chain C region	9.03E-13	110.27	37,630.7	28
QSOX1_HUMAN Sulfhydryl oxidase 1	1.02E-12	60.29	82,525.7	6
HV3R_HUMAN Ig heavy chain V-III region TUR	1.09E-12	10.28	12,423.3	2
MMP8_HUMAN Neutrophil collagenase	1.19E-12	60.26	53,378.6	9
CO4A_HUMAN Complement C4-A	4.03E-12	190.32	192,649.5	21
ITIH1_HUMAN Inter-alpha-trypsin inhibitor heavy chain H1	4.04E-12	110.34	101,325.8	16
APOL1_HUMAN Apolipoprotein-L1	4.42E-12	10.28	43,947.0	1
CO6A1_HUMAN Collagen alpha-1(VI) chain	8.68E-12	40.32	108,462.0	6
LAC_HUMAN Ig lambda chain C regions	1.47E-11	50.28	11,229.5	20
CFAI_HUMAN Complement factor I	1.60E-11	66.24	65,676.7	10
ALBU_HUMAN Serum albumin	2.54E-11	150.26	69,321.6	27

Table 1 (continued)

	P (pro)	Score	MW	Hits
CBPB2_HUMAN Carboxypeptidase B2	3.13E-11	10.23	48,381.5	1
APOA2_HUMAN Apolipoprotein A-II	4.64E-11	118.31	11,167.9	24
HEMO_HUMAN Hemopexin	9.46E-11	100.31	51,643.3	16
S10A9_HUMAN Protein S100-A9	2.64E-10	136.27	13,233.5	33
HV3P_HUMAN Ig heavy chain V-III region TEI	3.69E-10	10.26	12,794.4	1
ITIH2_HUMAN Inter-alpha-trypsin inhibitor heavy chain H2	3.86E-10	30.29	106,369.8	3
GSTP1_HUMAN Glutathione S-transferase P	4.12E-10	30.26	23,341.0	3
PERM_HUMAN Myeloperoxidase	4.13E-10	90.26	83,815.0	10
FIBB_HUMAN Fibrinogen beta chain	4.90E-10	60.27	55,892.2	7
HBG1_PANTR Hemoglobin subunit gamma-1	5.83E-10	30.22	16,130.3	4
ANT3_HUMAN Antithrombin-III	7.22E-10	50.30	52,569.0	8
HBD_HUMAN Hemoglobin subunit delta	8.76E-10	108.31	16,045.3	20
ILEU_HUMAN Leukocyte elastase inhibitor	9.91E-10	10.24	42,714.8	1
KV1V_HUMAN Ig kappa chain V-I region BAN	1.08E-09	10.21	11,832.8	1
MUCB_HUMAN Ig mu heavy chain disease protein	1.48E-09	78.23	43,030.3	14
IGHA2_HUMAN Ig alpha-2 chain C region	1.79E-09	98.22	36,485.1	28
APOC2_HUMAN Apolipoprotein C-II	2.18E-09	30.26	11,276.8	3
IGHM_HUMAN Ig mu chain C region	2.74E-09	40.24	49,275.6	9
ANXA1_HUMAN Annexin A1	3.24E-09	80.27	38,690.0	13
CLUS_HUMAN Clusterin	4.75E-09	10.22	52,461.1	2
KV3H_HUMAN Ig kappa chain V-III region CLL	5.07E-09	10.25	14,266.2	1
HBB_HUMAN Hemoglobin subunit beta	6.68E-09	80.27	15,988.3	18
IGHD_HUMAN Ig delta chain C region	7.74E-09	20.19	42,227.3	2
FIBA_HUMAN Fibrinogen alpha chain	9.00E-09	20.27	94,914.3	2
ANXA3_HUMAN Annexin A3	9.98E-09	48.24	36,352.7	5
IGHG3_HUMAN Ig gamma-3 chain C region	1.14E-08	10.19	41,260.4	1
A2AP_HUMAN Alpha-2-antiplasmin	1.51E-08	20.24	54,531.2	3
APOM_HUMAN Apolipoprotein M	1.72E-08	30.25	21,239.4	3
CO6A3_HUMAN Collagen alpha-3(VI) chain	1.86E-08	34.23	343,337.4	4
K2C1_HUMAN Keratin, type II cytoskeletal 1	3.05E-08	20.35	65,977.9	3
FIBG_HUMAN Fibrinogen gamma chain	3.23E-08	20.22	51,478.9	2
LV4C_HUMAN Ig lambda chain V-IV region Hil	3.65E-08	10.26	11,509.6	3
CO8G_HUMAN Complement component C8 gamma chain	3.98E-08	30.22	22,263.6	4
APOE_HUMAN Apolipoprotein E	4.02E-08	60.27	36,131.8	8
APOA4_HUMAN Apolipoprotein A-IV	4.26E-08	146.23	45,371.5	22
A2MG_HUMAN Alpha-2-macroglobulin	4.73E-08	90.22	163,174.3	9
LV3B_HUMAN Ig lambda chain V-III region LOI	6.46E-08	20.24	11,927.8	2
KV3G_HUMAN Ig kappa chain V-III region GOL	8.44E-08	10.24	11,823.0	1
KV1S_HUMAN Ig kappa chain V-I region Wes	1.74E-07	10.28	11,600.7	1
HPTR_HUMAN Haptoglobin-related protein	2.32E-07	10.20	38,982.7	1
PRDX2_HUMAN Peroxiredoxin-2	4.68E-07	40.22	21,878.2	5
K1C10_HUMAN Keratin, type I cytoskeletal 10	7.41E-07	20.19	59,474.8	2
ITIH4_HUMAN Inter-alpha-trypsin inhibitor heavy chain H4	7.44E-07	100.25	103,261.2	11
A1AT_HUMAN Alpha-1-antitrypsin	9.34E-07	10.18	46,707.1	1
HMOX1_HUMAN Heme oxygenase 1	1.60E-06	10.20	32,798.0	1
IGJ_HUMAN Immunoglobulin J chain	1.88E-06	10.15	15,584.6	2
ACTB_HUMAN Actin, cytoplasmic 1	3.59E-06	30.19	41,709.7	3
CFAH_HUMAN Complement factor H	5.24E-06	10.16	139,004.4	1
AFAM_HUMAN Afamin	5.26E-06	10.19	69,024.1	1

Table 1 (continued)

	P (pro)	Score	MW	Hits
TRFE_HUMAN Serotransferrin	5.69E-06	10.19	76,999.7	1
A2GL_HUMAN Leucine-rich alpha-2-glycoprotein	6.14E-06	20.21	38,154.1	2
CO9_HUMAN Complement component C9	7.89E-06	20.21	63,132.8	2
KV3I_HUMAN Ig kappa chain V-III region VG	9.13E-06	20.24	12,567.3	2
KV2F_HUMAN Ig kappa chain V-II region RPMI 6410	1.13E-05	10.13	14,697.4	1
MMP3_HUMAN Stromelysin-1	2.47E-05	10.17	53,943.4	1
HPT_HUMAN Haptoglobin	2.94E-05	74.19	45,176.6	13
S10A8_HUMAN Protein S100-A8	2.99E-05	10.19	10,827.7	3
HV1B_HUMAN Ig heavy chain V-I region HG3	5.54E-05	10.16	12,937.3	1
S11Y_HUMAN Putative S100 calcium-binding protein H_NH0456N16.1	6.65E-05	10.20	11,501.9	1
GGH_HUMAN Gamma-glutamyl hydrolase	7.09E-05	40.19	35,941.2	4
SAMP_HUMAN Serum amyloid P-component	2.25E-04	30.14	25,371.1	3
PROF1_HUMAN Profilin-1	3.64E-04	10.23	15,044.6	1
KV1F_HUMAN Ig kappa chain V-I region EU	5.68E-04	10.16	11,780.8	1
S100P_HUMAN Protein S100-P	6.18E-04	10.18	10,393.2	1
TRIM7_HUMAN Tripartite motif-containing protein 7	8.38E-04	10.16	56,595.0	2
Fraction 35 proteins				
A2AP_HUMAN Alpha-2-antiplasmin	1.00E-30	50.30	54,531.2	9
HBA_HUMAN Hemoglobin subunit alpha	5.55E-15	50.32	15,247.9	8
APOA1_HUMAN Apolipoprotein A-I	5.83E-15	1,010.32	30,758.9	1,258
CO3_HUMAN Complement C3	1.44E-14	190.35	187,029.3	28
A2MG_HUMAN Alpha-2-macroglobulin	1.44E-14	60.37	163,174.3	6
ACTB_HUMAN Actin, cytoplasmic 1	2.23E-13	20.34	41,709.7	2
IGKC_HUMAN Ig kappa chain C region	2.95E-13	30.25	11,601.7	22
S10A6_HUMAN Protein S100-A6	3.49E-13	30.26	10,173.3	4
K2C1_HUMAN Keratin, type II cytoskeletal 1	1.93E-12	20.25	65,977.9	2
FRIL_HUMAN Ferritin light chain	3.90E-12	14.27	20,007.1	3
APOA2_HUMAN Apolipoprotein A-II	1.08E-11	110.31	11,167.9	17
TTHY_HUMAN Transthyretin	1.16E-11	70.30	15,877.1	14
LAC_HUMAN Ig lambda chain C regions	4.68E-11	20.30	11,229.5	13
PGRP2_HUMAN N-acetylmuramoyl-L-alanine amidase	5.23E-11	30.29	62,178.0	3
HV3P_HUMAN Ig heavy chain V-III region TEI	9.68E-11	10.26	12,794.4	1
HBD_HUMAN Hemoglobin subunit delta	3.71E-10	28.26	16,045.3	3
CO4A_HUMAN Complement C4-A	6.90E-10	40.25	192,649.5	4
IGHA2_HUMAN Ig alpha-2 chain C region	1.08E-09	58.22	36,485.1	13
MUCB_HUMAN Ig mu heavy chain disease protein	1.21E-09	48.26	43,030.3	6
ILEU_HUMAN Leukocyte elastase inhibitor	1.50E-09	30.23	42,714.8	3
KV1D_HUMAN Ig kappa chain V-I region CAR	2.61E-09	10.27	11,696.3	3
KAIN_HUMAN Kallistatin	3.73E-09	50.22	48,511.2	6
HEMO_HUMAN Hemopexin	4.23E-09	30.23	51,643.3	3
S10A8_HUMAN Protein S100-A8	5.29E-09	10.24	10,827.7	1
ALBU_HUMAN Serum albumin	7.79E-09	40.24	69,321.6	5
HBB_HUMAN Hemoglobin subunit beta	1.31E-08	30.26	15,988.3	4
APOA4_HUMAN Apolipoprotein A-IV	2.29E-08	40.23	45,371.5	9
IGHA1_HUMAN Ig alpha-1 chain C region	2.35E-08	40.27	37,630.7	5
CFAI_HUMAN Complement factor I	2.62E-08	20.23	65,676.7	2
ITIH1_HUMAN Inter-alpha-trypsin inhibitor heavy chain H1	5.56E-08	20.23	101,325.8	2
KV3H_HUMAN Ig kappa chain V-III region CLL	7.79E-08	10.25	14,266.2	1
APOE_HUMAN Apolipoprotein E	1.90E-07	20.27	36,131.8	3

Table 1 (continued)

	P (pro)	Score	MW	Hits
ITIH4_HUMAN Inter-alpha-trypsin inhibitor heavy chain H4	2.81E-07	30.21	103,261.2	3
CFAB_HUMAN Complement factor B	3.03E-07	30.24	85,478.6	4
IGHM_HUMAN Ig mu chain C region	3.85E-07	30.23	49,275.6	5
LV3B_HUMAN Ig lambda chain V-III region LOI	1.97E-06	10.25	11,927.8	2
CO6A3_HUMAN Collagen alpha-3(VI) chain	2.12E-06	44.24	343,337.4	6
HV3R_HUMAN Ig heavy chain V-III region TUR	2.31E-06	10.20	12,423.3	1
HPT_HUMAN Haptoglobin	4.31E-06	20.18	45,176.6	3
FIBA_HUMAN Fibrinogen alpha chain	1.10E-05	10.21	94,914.3	1
LV4C_HUMAN Ig lambda chain V-IV region Hil	2.21E-05	10.22	11,509.6	1
APOM_HUMAN Apolipoprotein M	3.10E-05	10.24	21,239.4	1
PLMN_HUMAN Plasminogen	4.83E-05	10.22	90,510.2	1
RETBP_PANTR Plasma retinol-binding protein	5.79E-05	20.15	22,995.3	3
Fraction 36 proteins				
CO3_HUMAN Complement C3	1.00E-30	220.30	187,029.3	29
IGHA2_HUMAN Ig alpha-2 chain C region	1.00E-30	70.34	36,485.1	20
CO4B_HUMAN Complement C4-B	2.22E-15	10.33	192,671.6	1
HBA_HUMAN Hemoglobin subunit alpha	5.55E-15	50.33	15,247.9	8
ACTB_HUMAN Actin, cytoplasmic 1	7.77E-15	30.32	41,709.7	4
A2MG_HUMAN Alpha-2-macroglobulin	2.22E-14	100.32	163,174.3	17
APOA1_HUMAN Apolipoprotein A-I	6.08E-14	1,012.33	30,758.9	1,155
A2AP_HUMAN Alpha-2-antiplasmin	1.15E-13	136.34	54,531.2	20
HEMO_HUMAN Hemopexin	1.15E-13	50.21	51,643.3	5
ITIH1_HUMAN Inter-alpha-trypsin inhibitor heavy chain H1	1.77E-13	10.24	101,325.8	1
K1C9_HUMAN Keratin, type I cytoskeletal 9	3.15E-13	10.38	62,091.8	1
KAIN_HUMAN Kallistatin	6.87E-13	140.28	48,511.2	26
PLMN_HUMAN Plasminogen	1.32E-12	30.32	90,510.2	3
APOA2_HUMAN Apolipoprotein A-II	3.83E-12	248.32	11,167.9	167
KV4C_HUMAN Ig kappa chain V-IV region B17	3.96E-12	10.28	14,956.5	1
IGKC_HUMAN Ig kappa chain C region	3.97E-12	40.26	11,601.7	23
RETBP_PANTR Plasma retinol-binding protein	5.60E-12	40.31	22,995.3	7
K2C1_HUMAN Keratin, type II cytoskeletal 1	1.01E-11	30.37	65,977.9	4
APOA4_HUMAN Apolipoprotein A-IV	1.79E-11	70.28	45,371.5	14
LAC_HUMAN Ig lambda chain C regions	6.60E-11	30.30	11,229.5	13
KV1D_HUMAN Ig kappa chain V-I region CAR	6.89E-11	10.29	11,696.3	1
HV3P_HUMAN Ig heavy chain V-III region TEI	7.58E-11	10.30	12,794.4	1
HBB_HUMAN Hemoglobin subunit beta	3.96E-10	70.27	15,988.3	13
FRIL_HUMAN Ferritin light chain	4.07E-10	36.27	20,007.1	7
TTHY_HUMAN Transthyretin	4.79E-10	50.31	15,877.1	6
CFAB_HUMAN Complement factor B	7.78E-10	20.25	85,478.6	2
AMBP_HUMAN AMBP protein	1.37E-09	10.25	38,974.0	1
ALBU_HUMAN Serum albumin	1.37E-09	70.24	69,321.6	9
MUCB_HUMAN Ig mu heavy chain disease protein	3.44E-09	38.22	43,030.3	6
KV3H_HUMAN Ig kappa chain V-III region CLL	4.18E-09	10.24	14,266.2	1
A1AT_HUMAN Alpha-1-antitrypsin	4.96E-09	20.23	46,707.1	2
APOL1_HUMAN Apolipoprotein-L1	5.95E-09	30.29	43,947.0	4
CO6A3_HUMAN Collagen alpha-3(VI) chain	7.12E-09	48.23	343,337.4	6
S10A9_HUMAN Protein S100-A9	8.28E-09	10.26	13,233.5	1
CFAI_HUMAN Complement factor I	1.04E-08	46.24	65,676.7	5
IGHG3_HUMAN Ig gamma-3 chain C region	1.24E-08	10.17	41,260.4	1

Table 1 (continued)

	P (pro)	Score	MW	Hits
FIBA_HUMAN Fibrinogen alpha chain	1.61E-08	20.22	94,914.3	2
APOE_HUMAN Apolipoprotein E	3.40E-08	20.27	36,131.8	3
IGHA1_HUMAN Ig alpha-1 chain C region	6.15E-08	30.24	37,630.7	5
IGHM_HUMAN Ig mu chain C region	1.50E-07	40.24	49,275.6	8
ACTS_HUMAN Actin, alpha skeletal muscle	2.46E-07	50.23	42,023.9	6
TALDO_HUMAN Transaldolase	2.88E-07	20.19	37,516.5	2
ANT3_HUMAN Antithrombin-III	1.16E-06	10.22	52,569.0	1
CO4A_HUMAN Complement C4-A	6.92E-06	26.18	192,649.5	3
CO8G_HUMAN Complement component C8 gamma chain	1.20E-05	10.20	22,263.6	1
HPT_HUMAN Haptoglobin	1.99E-05	28.19	45,176.6	4
ILEU_HUMAN Leukocyte elastase inhibitor	2.13E-05	20.23	42,714.8	2
IGJ_HUMAN Immunoglobulin J chain	2.93E-05	10.16	15,584.6	1
LV3B_HUMAN Ig lambda chain V-III region LOI	3.63E-05	10.20	11,927.8	1
M6PBP_HUMAN Mannose-6-phosphate receptor-binding protein 1	3.95E-04	10.22	47,018.0	1
PROF1_HUMAN Profilin-1	7.14E-04	10.20	15,044.6	1
Fraction 37 proteins				
IGHA1_HUMAN Ig alpha-1 chain C region	1.00E-30	70.29	37,630.7	17
K2C1_HUMAN Keratin, type II cytoskeletal 1	1.11E-16	60.39	65,977.9	7
ACTB_HUMAN Actin, cytoplasmic 1	5.55E-16	150.32	41,709.7	19
K1C9_HUMAN Keratin, type I cytoskeletal 9	1.11E-15	44.38	62,091.8	6
APOB_HUMAN Apolipoprotein B-100	1.33E-15	398.38	515,241.6	53
A2AP_HUMAN Alpha-2-antiplasmin	3.33E-15	222.33	54,531.2	32
PLMN_HUMAN Plasminogen	4.44E-15	50.33	90,510.2	8
CO3_HUMAN Complement C3	9.88E-15	352.35	187,029.3	49
APOE_HUMAN Apolipoprotein E	7.77E-14	50.29	36,131.8	5
IGKC_HUMAN Ig kappa chain C region	1.58E-13	70.30	11,601.7	30
LKHA4_HUMAN Leukotriene A-4 hydrolase	1.75E-13	50.26	69,241.3	5
PGRP2_HUMAN <i>N</i> -acetylmuramoyl-L-alanine amidase	3.96E-13	20.27	62,178.0	2
APOL1_HUMAN Apolipoprotein-L1	4.22E-13	150.31	43,947.0	23
A2MG_HUMAN Alpha-2-macroglobulin	5.13E-13	380.37	163,174.3	58
ANGT_HUMAN Angiotensinogen	2.56E-12	50.31	53,120.6	5
CO4A_HUMAN Complement C4-A	5.35E-12	36.27	192,649.5	4
KAIN_HUMAN Kallistatin	6.59E-12	40.30	48,511.2	5
APOA2_HUMAN Apolipoprotein A-II	8.45E-12	174.33	11,167.9	68
APOA1_HUMAN Apolipoprotein A-I	8.67E-12	912.32	30,758.9	918
PLTP_HUMAN Phospholipid transfer protein	2.02E-11	30.26	54,704.7	3
KV4C_HUMAN Ig kappa chain V-IV region B17	2.83E-11	20.26	14,956.5	3
S10A6_HUMAN Protein S100-A6	2.88E-11	10.25	10,173.3	1
CO5_HUMAN Complement C5	2.96E-11	110.25	188,185.3	11
RETBP_PANTR Plasma retinol-binding protein	3.46E-11	50.29	22,995.3	12
HBA_HUMAN Hemoglobin subunit alpha	5.76E-11	30.20	15,247.9	5
ITIH1_HUMAN Inter-alpha-trypsin inhibitor heavy chain H1	6.33E-11	10.30	101,325.8	1
LAC_HUMAN Ig lambda chain C regions	8.48E-11	50.30	11,229.5	15
KV1D_HUMAN Ig kappa chain V-I region CAR	1.34E-10	20.26	11,696.3	4
HEMO_HUMAN Hemopexin	1.43E-10	88.25	51,643.3	11
CO6A3_HUMAN Collagen alpha-3(VI) chain	1.90E-10	38.22	343,337.4	5
HV3R_HUMAN Ig heavy chain V-III region TUR	2.35E-10	10.26	12,423.3	1
ITIH2_HUMAN Inter-alpha-trypsin inhibitor heavy chain H2	3.54E-10	100.25	106,369.8	15
TTHY_HUMAN Transthyretin	3.86E-10	30.34	15,877.1	4

Table 1 (continued)

	P (pro)	Score	MW	Hits
HBD_HUMAN Hemoglobin subunit delta	4.49E-10	38.25	16,045.3	6
AMBP_HUMAN AMBP protein	7.76E-10	10.24	38,974.0	1
CO8G_HUMAN Complement component C8 gamma chain	8.36E-10	30.23	22,263.6	4
IGHA2_HUMAN Ig alpha-2 chain C region	4.57E-09	68.25	36,485.1	13
ALBU_HUMAN Serum albumin	4.66E-09	60.24	69,321.6	7
HBB_HUMAN Hemoglobin subunit beta	5.88E-09	40.27	15,988.3	6
FIBB_HUMAN Fibrinogen beta chain	7.26E-09	10.24	55,892.2	1
CFAI_HUMAN Complement factor I	1.06E-08	46.21	65,676.7	5
MUCB_HUMAN Ig mu heavy chain disease protein	1.38E-08	58.24	43,030.3	11
LV3B_HUMAN Ig lambda chain V-III region LOI	1.87E-08	10.23	11,927.8	1
POSTN_HUMAN Periostin	2.21E-08	34.25	93,255.4	5
KV3H_HUMAN Ig kappa chain V-III region CLL	2.88E-08	10.24	14,266.2	1
S10A9_HUMAN Protein S100-A9	3.71E-08	20.17	13,233.5	2
IGHM_HUMAN Ig mu chain C region	4.69E-08	30.24	49,275.6	7
KV3G_HUMAN Ig kappa chain V-III region GOL	5.54E-08	10.22	11,823.0	1
FIBA_HUMAN Fibrinogen alpha chain	7.08E-08	20.26	94,914.3	2
CO2_HUMAN Complement C2	7.13E-08	20.22	83,214.4	2
LDHB_HUMAN L-lactate dehydrogenase B chain	8.55E-08	10.18	36,615.2	1
PEDF_HUMAN Pigment epithelium-derived factor	9.52E-08	30.17	46,313.4	3
AACT_HUMAN Alpha-1-antichymotrypsin	1.12E-07	10.27	47,620.6	1
ITIH4_HUMAN Inter-alpha-trypsin inhibitor heavy chain H4	1.20E-07	20.22	103,261.2	3
ANT3_HUMAN Antithrombin-III	1.79E-07	10.27	52,569.0	1
KV1V_HUMAN Ig kappa chain V-I region BAN	2.30E-07	10.22	11,832.8	1
ACTS_HUMAN Actin, alpha skeletal muscle	2.70E-07	96.22	42,023.9	14
APOA4_HUMAN Apolipoprotein A-IV	3.06E-07	30.18	45,371.5	4
HRG_HUMAN Histidine-rich glycoprotein	8.44E-07	10.18	59,540.9	1
ADIPO_HUMAN Adiponectin	8.48E-07	10.23	26,397.0	1
HV3P_HUMAN Ig heavy chain V-III region TEI	1.03E-06	10.26	12,794.4	1
SAMP_HUMAN Serum amyloid P-component	2.09E-06	10.17	25,371.1	1
TALDO_HUMAN Transaldolase	2.15E-06	30.20	37,516.5	5
KV3I_HUMAN Ig kappa chain V-III region VG	6.88E-06	10.23	12,567.3	2
VINC_HUMAN Vinculin	8.61E-06	30.23	123,721.9	3
LDHA_HUMAN L-lactate dehydrogenase A chain	1.07E-05	10.15	36,665.4	1
IGJ_HUMAN Immunoglobulin J chain	1.38E-05	10.15	15,584.6	1
HPT_HUMAN Haptoglobin	2.95E-05	64.24	45,176.6	7
FA12_HUMAN Coagulation factor XII	3.00E-05	10.23	67,774.1	1
LV4C_HUMAN Ig lambda chain V-IV region Hil	3.06E-05	10.20	11,509.6	1
PROF1_HUMAN Profilin-1	1.12E-04	10.21	15,044.6	1
HV3A_HUMAN Ig heavy chain V-III region TRO	1.46E-04	10.16	13,463.5	1
BGH3_HUMAN Transforming growth factor-beta-induced protein ig-h3	1.76E-04	10.18	74,634.1	1
CSN8_HUMAN COP9 signalosome complex subunit 8	1.78E-04	10.16	23,211.0	1
O52A5_HUMAN Olfactory receptor 52A5	3.20E-04	10.12	35,931.3	1
Fraction 38 proteins				
A2AP_HUMAN Alpha-2-antiplasmin	1.11E-16	108.31	54,531.2	15
A2MG_HUMAN Alpha-2-macroglobulin	2.22E-15	538.38	163,174.3	88
APOA1_HUMAN Apolipoprotein A-I	2.55E-14	350.33	30,758.9	389
IGHA1_HUMAN Ig alpha-1 chain C region	3.00E-14	40.25	37,630.7	16
PGRP2_HUMAN N-acetylmuramoyl-L-alanine amidase	6.44E-14	10.29	62,178.0	3
ACTB_HUMAN Actin, cytoplasmic 1	1.98E-13	90.32	41,709.7	13

Table 1 (continued)

	P (pro)	Score	MW	Hits
K2C1_HUMAN Keratin, type II cytoskeletal 1	3.56E-13	130.36	65,977.9	16
APOL1_HUMAN Apolipoprotein-L1	1.43E-12	80.29	43,947.0	10
PLMN_HUMAN Plasminogen	1.47E-12	20.31	90,510.2	3
IGKC_HUMAN Ig kappa chain C region	5.07E-12	60.29	11,601.7	43
HV3R_HUMAN Ig heavy chain V-III region TUR	5.17E-12	10.29	12,423.3	2
K1C10_HUMAN Keratin, type I cytoskeletal 10	5.49E-12	80.31	59,474.8	9
TLN1_HUMAN Talin-1	5.71E-12	40.28	269,596.3	5
APOB_HUMAN Apolipoprotein B-100	6.46E-12	498.35	515,241.6	82
HBA_HUMAN Hemoglobin subunit alpha	2.90E-11	50.30	15,247.9	8
CO3_HUMAN Complement C3	2.99E-11	480.30	187,029.3	69
KV4C_HUMAN Ig kappa chain V-IV region B17	5.13E-11	40.24	14,956.5	5
LV4C_HUMAN Ig lambda chain V-IV region Hil	7.27E-11	10.24	11,509.6	1
LAC_HUMAN Ig lambda chain C regions	1.33E-10	20.28	11,229.5	17
APOA2_HUMAN Apolipoprotein A-II	1.34E-10	120.32	11,167.9	20
TTHY_HUMAN Transthyretin	2.49E-10	60.23	15,877.1	8
HEMO_HUMAN Hemopexin	2.93E-10	60.25	51,643.3	15
S10A6_HUMAN Protein S100-A6	4.78E-10	20.22	10,173.3	2
ACTS_HUMAN Actin, alpha skeletal muscle	1.07E-09	28.20	42,023.9	4
RETBP_PANTR Plasma retinol-binding protein	1.47E-09	100.30	22,995.3	31
ENOA_HUMAN Alpha-enolase	1.49E-09	30.24	47,139.4	3
ANT3_HUMAN Antithrombin-III	2.72E-09	20.25	52,569.0	2
HBB_HUMAN Hemoglobin subunit beta	3.32E-09	50.25	15,988.3	12
CFAB_HUMAN Complement factor B	3.59E-09	40.23	85,478.6	4
S10A9_HUMAN Protein S100-A9	3.63E-09	20.21	13,233.5	2
K1C9_HUMAN Keratin, type I cytoskeletal 9	4.51E-09	90.40	62,091.8	13
CO8G_HUMAN Complement component C8 gamma chain	5.21E-09	30.21	22,263.6	3
CO5_HUMAN Complement C5	6.03E-09	160.23	188,185.3	17
DCTN2_HUMAN Dynactin subunit 2	9.83E-09	30.20	44,203.9	3
CO6A3_HUMAN Collagen alpha-3(VI) chain	1.06E-08	10.19	343,337.4	1
MUCB_HUMAN Ig mu heavy chain disease protein	1.58E-08	68.23	43,030.3	23
CO4A_HUMAN Complement C4-A	1.67E-08	62.23	192,649.5	7
IGHA2_HUMAN Ig alpha-2 chain C region	1.83E-08	100.23	36,485.1	20
IGHM_HUMAN RecName: Full=Ig mu chain C region	2.08E-08	30.23	49,275.6	9
HRG_HUMAN Histidine-rich glycoprotein	2.20E-08	20.23	59,540.9	2
6PGD_HUMAN 6-phosphogluconate dehydrogenase, decarboxylating	2.72E-08	10.23	53,106.0	1
SAMP_HUMAN Serum amyloid P-component	2.99E-08	30.20	25,371.1	4
ITIH4_HUMAN Inter-alpha-trypsin inhibitor heavy chain H4	5.84E-08	30.22	103,261.2	3
TKT_HUMAN Transketolase	7.66E-08	20.19	67,834.9	3
BGH3_HUMAN Transforming growth factor-beta-induced protein ig-h3	1.10E-07	40.20	74,634.1	4
ALBU_HUMAN Serum albumin	1.25E-07	130.25	69,321.6	18
SPB9_HUMAN Serpin B9	1.27E-07	80.19	42,376.4	8
HBD_HUMAN Hemoglobin subunit delta	1.52E-07	28.19	16,045.3	3
APOA4_HUMAN Apolipoprotein A-IV	2.42E-07	40.20	45,371.5	4
APOE_HUMAN Apolipoprotein E	2.58E-07	20.25	36,131.8	2
HPT_HUMAN Haptoglobin	2.74E-07	102.23	45,176.6	18
CFAI_HUMAN Complement factor I	2.85E-07	56.23	65,676.7	8
ADIPO_HUMAN Adiponectin	4.68E-07	10.23	26,397.0	1
ANGT_HUMAN Angiotensinogen	4.90E-07	30.22	53,120.6	3
IGLL1_HUMAN Immunoglobulin lambda-like polypeptide 1	7.41E-07	10.19	22,948.6	1

Table 1 (continued)

	P (pro)	Score	MW	Hits
ENO1B_HUMAN Alpha-enolase, lung specific	9.50E-07	20.24	49,446.4	2
PEDF_HUMAN Pigment epithelium-derived factor	9.80E-07	46.18	46,313.4	5
KV3G_HUMAN Ig kappa chain V-III region GOL	1.03E-06	10.19	11,823.0	2
KAIN_HUMAN Kallistatin	1.10E-06	10.20	48,511.2	1
ENOB_HUMAN Beta-enolase	1.18E-06	10.23	46,957.4	2
S10A8_HUMAN Protein S100-A8	1.41E-06	10.22	10,827.7	1
KV1D_HUMAN Ig kappa chain V-I region CAR	2.42E-06	20.24	11,696.3	2
LV3B_HUMAN Ig lambda chain V-III region LOI	2.43E-06	30.24	11,927.8	4
IGJ_HUMAN Immunoglobulin J chain	3.40E-06	10.16	15,584.6	1
ITIH2_HUMAN Inter-alpha-trypsin inhibitor heavy chain H2	4.00E-06	40.20	106,369.8	4
K22E_HUMAN Keratin, type II cytoskeletal 2 epidermal	6.60E-06	20.23	65,825.4	2
HV3P_HUMAN Ig heavy chain V-III region TEI	8.90E-06	10.26	12,794.4	3
PSME1_HUMAN Proteasome activator complex subunit 1	9.97E-06	20.15	28,705.0	2
PLTP_HUMAN Phospholipid transfer protein	1.10E-05	40.20	54,704.7	5
CO2_HUMAN Complement C2	1.43E-05	10.23	83,214.4	1
KV3H_HUMAN Ig kappa chain V-III region CLL	1.66E-05	20.19	14,266.2	2
TETN_HUMAN Tetranectin	1.82E-05	10.18	22,552.3	1
LV3A_HUMAN Ig lambda chain V-III region SH	1.87E-05	10.21	11,385.6	2
LDHA_HUMAN L-lactate dehydrogenase A chain	1.89E-05	10.19	36,665.4	1
LDHB_HUMAN L-lactate dehydrogenase B chain	2.04E-05	30.22	36,615.2	3
C1QB_HUMAN Complement C1q subcomponent subunit B	3.63E-05	10.23	26,442.4	1
ILEU_HUMAN Leukocyte elastase inhibitor	5.71E-05	10.17	42,714.8	1
IPSP_HUMAN Plasma serine protease inhibitor	7.70E-05	10.16	45,672.8	1
IGHD_HUMAN Ig delta chain C region	1.11E-04	10.17	42,227.3	1
FIBB_HUMAN Fibrinogen beta chain	2.02E-04	10.18	55,892.2	1
IGHG3_HUMAN Ig gamma-3 chain C region	2.10E-04	10.16	41,260.4	1
HV3V_HUMAN Ig heavy chain V-III region GAR	2.52E-04	10.17	13,079.2	1
HPTR_HUMAN Haptoglobin-related protein	2.62E-04	10.20	38,982.7	1
C1QC_HUMAN Complement C1q subcomponent subunit C	2.92E-04	10.19	25,757.1	1
KV3I_HUMAN Ig kappa chain V-III region VG	3.01E-04	10.20	12,567.3	1
PROF1_HUMAN Profilin-1	3.49E-04	10.17	15,044.6	1
K1C25_HUMAN Keratin, type I cytoskeletal 25	7.85E-04	10.15	49,287.4	1

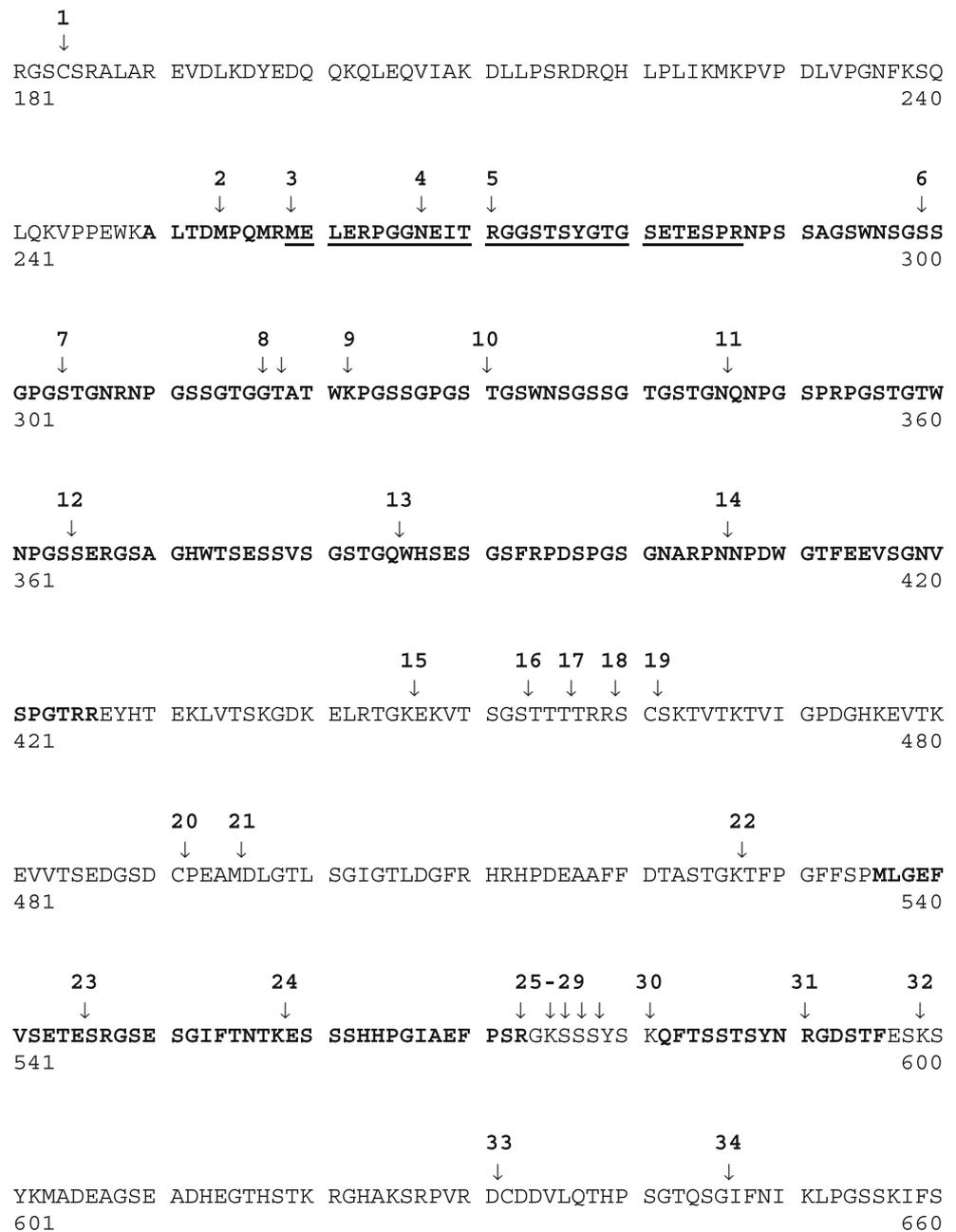
the protein array analysis, the mass spectrometry analysis did not yield a protein identification. Both the protein arrays and HPLC chromatographs appear to be more sensitive at detecting proteins than the mass spectrometry.

In order to obtain enough material for mass spectrometry analysis, SF from nine different patients was pooled (total volume of 50 ml) and used for a large-scale procedure. After protein depletion, approximately 20 mg of depleted SF material was used to perform four first-dimension chromatofocusing runs (Fig. 3a). The fractions that eluted at pH 5.63–5.45 were combined to perform one second-dimension RP-HPLC run (Fig. 3b). Fractions corresponding to the region that tested positive for RA serum binding in the protein array were digested with trypsin and analyzed by LC/MS/MS. SEQUEST searches were performed using the SwissProt database limiting the search to tryptic peptides.

Table 1 lists the proteins found in these fractions and the corresponding peptides are included as supplementary material (Supplement Table 1).

The candidate autoantigen specifically detected by the RA serum was estimated to be in fractions 20–22. Amongst other proteins, these fractions contained fibrinogen, a known autoantigen that can be citrullinated *in vivo*. Fibrinogen alpha (FIBA_HUMAN, SwissProt Accession # P02671) was identified in fraction 20 with nine unique peptide hits (15% sequence coverage) and in fraction 22 with 18 unique peptides (24% sequence coverage). Altogether, peptides were only found originating from the center of the FIBA protein (amino acids 250–599) corresponding to the alphaC domain of fibrin (amino acids 239–629). Figure 4 shows the sequence of the fibrin alphaC domain with the amino acids identified by mass spectrometry.

Fig. 4 The fibrin alphaC domain (239–629) with the regions identified by mass spectrometry in *bold* and the FIBA 259–287 *underlined*. Arrows point to modifications



etry in bold and arrows pointing to any modifications. Peptides from the center region were not identified due a lysine-/arginine-rich area followed by two cross-linked amino acids (Ser461–Ser491).

Careful examination of the mass spectra assigned to arginine-containing FIBA peptides led to the assignment of a citrullinated peptide corresponding to FIBA 259–287. The calculated mass obtained from the mass spectrum (Fig. 5) was 1 Da higher than the calculated mass for the unmodified peptide. The lower resolution MS/MS spectrum obtained with the ion trap part of the LTQ-FT did not allow determination of the exact location of the modification. However, a number of other fragments corresponding to

parts of the peptide (Table 2) were observed in the spectra obtained with the ion cyclotron resonance (ICR) analyzer of the LTQ-FT, which provided precise mass measurements. There are four sites on the FIBA 259–287 peptide that could possibly be modified resulting in a mass shift of +1 Da. The peptide contains three arginines that can be citrullinated and an asparagine that can be deaminated to form aspartic acid (Fig. 5). All fragments that did not show the expected tryptic cleavage at Arg 271 showed observed masses that were 1 Da higher than the expected mass for the unmodified form. This is consistent with the known failure of trypsin to cleave citrullinated Arg residues. Thus, only conversion of Arg 271 to citrulline is

Fig. 4 (continued)

1. Cys184, disulfide link to Cys223 in FIBB
2. Met254, oxidized
3. Met259, oxidized
4. Asn267, methylation
5. Arg271, citrullination
6. Ser299 -> G conflict
7. Ser304 -> G conflict
8. Gly317,Thr318 -> SG
9. Lys322, link to Gln41 in alpha-2- antiplasmin
10. Thr331 -> A
11. Gln347. link to Lys
12. Ser364, phosphorylation
13. Gln385, link to Lys
14. Asn406, methylated
15. Lys446 -> E
16. Ser453 glycosylation site
 - a. S -> N
 - b. N-Linked (GLCNAC...)
17. Thr456 -> A
18. Ser460, phosphorylation
19. Ser461 linked to Ser491
20. Ser491 linked to Ser461
21. Met495, oxidized
22. Lys527 linked to Gln
23. E545 -> V
24. Lys558 linked to Gln
25. Arg573 -> C
 - a. R -> L
26. Lys575 linked to Gln
27. Ser576 phosphorylation
28. Ser577 phosphorylation
29. Ser 578 phosphorylation
30. Lys581 linked to Gln
31. Arg591 cleavage site tryptase beta1
32. Lys599 linked to Gln
33. Splicing variant, DCDDVLQTHPSGTQ -> GIHTSPLGKPSLSP
34. Splicing variant, Missing

consistent with observed mass values of this FIBA fragment in RA SF and the failure of trypsin to cleave at that site.

As a final proof that the citrullination site was correctly assigned, the peptide corresponding to residues 259–287 was synthesized with and without the citrulline in position 271 (designated 271X and 271R, respectively). Both the charge state distribution in the electrospray spectrum and the fragment masses in the MS/MS spectrum of the 3+ charge state of 271X matched spectral data obtained with the sample isolated from RA SF.

To establish that the citrullinated FIBA 259–287 peptide was recognized specifically by RA patient sera, the two FIBA 259–287 synthetic peptides were tested in an ELISA. An additional citrullinated synthetic peptide, corresponding to profilaggrin 619–631 (FIL) with a citrulline substitution at Arg 625, was included as a control. The immobilized peptides were incubated with sera from RA, SLE, or healthy controls, followed by detection of bound antibodies by HRP-conjugated anti-human IgG, IgA, and IgM antibodies and a colorimetric assay. Graphs depicting the

ELISA optical density readings are shown in Fig. 6. Of 18 healthy control sera tested, two reacted to the 271R peptide, two reacted to the 271X peptide, and one reacted to the FIL peptide. Of the 12 RA sera tested, four reacted to the 271R peptide, 10 reacted to the 271X peptide, and three reacted to the FIL peptide. Of the 10 SLE sera tested, one patient reacted to all three peptides. The number of sera that reacted exclusively to the 271X peptide, and not with the 271R nor FIL peptides, was 5/12 RA sera, 0/18 healthy sera, and 0/10 SLE sera. These results provide evidence that antibodies in a subset of RA sera bind specifically to the citrulline residue at position 271 of the FIBA alphaC domain found in RA SF.

Discussion

Biomarker analysis consists of three phases: discovery, verification, and validation [38, 39]. Discovery is performed by a thorough analysis of a few samples with hopes of identifying many candidate biomarkers to be used for

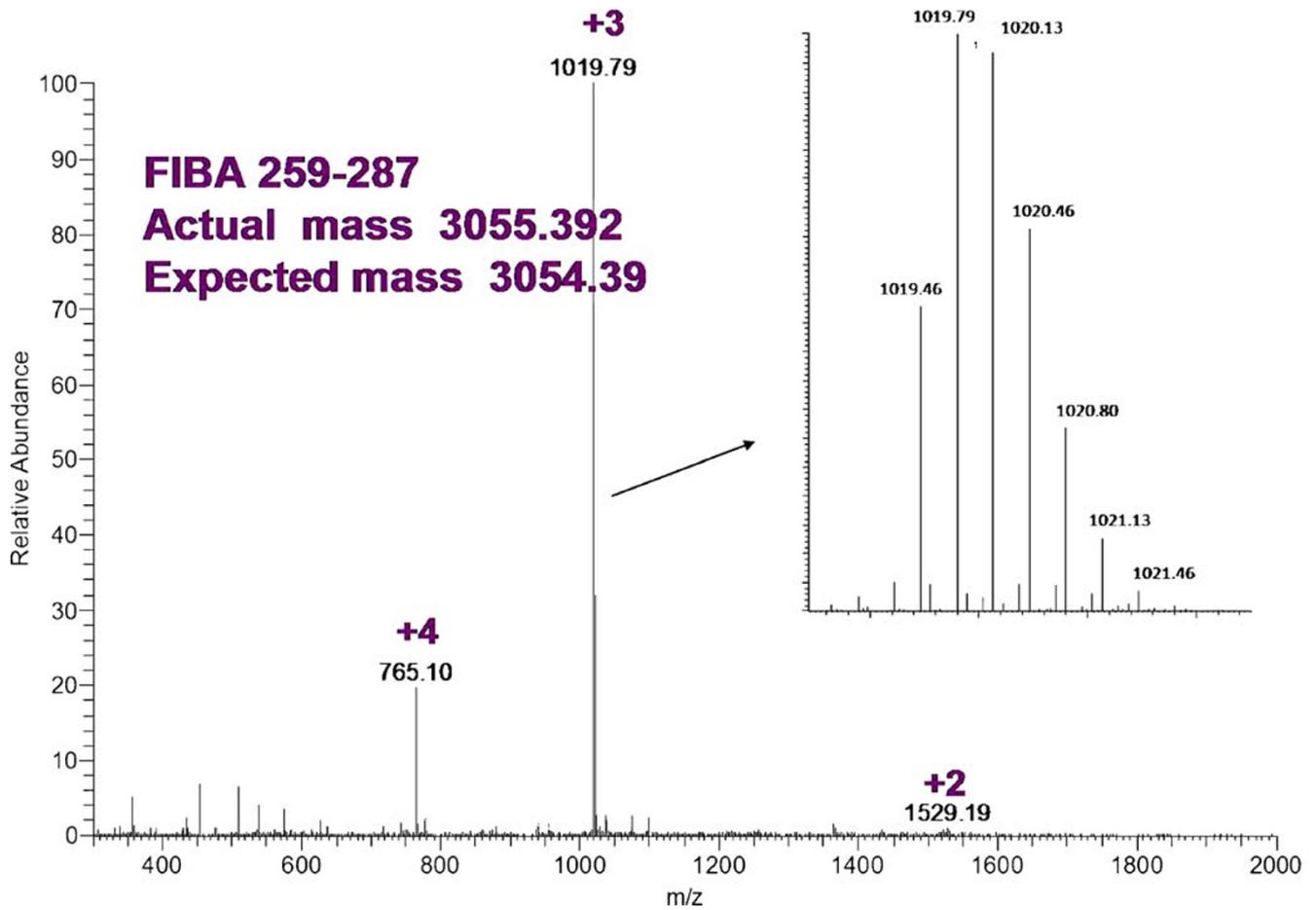


Fig. 5 Electrospray mass spectra of the citrullinated FIBA 259–287 peptide. The *upper spectrum* shows the observed mass value for each of the indicated charge states. The *inset* shows the isotope distribution for the 3+ charge state

Table 2 The sequence for the citrullinated peptide along with other fragments for which high-resolution MS and MS/MS spectra were obtained

z	M(obs)	M(pep)	dM	T	Peptide	aa#	Mods
2	1500.742	1500.74	0	F	MELERPGGNEITR	259-271	
3	1516.743	1500.74	16	F	MELERPGGNEITR	259-271	oxidation
2	1344.644	1344.63	0	P	MELERPGGNEIT	259-270	
2	1360.634	1344.63	16	P	MELERPGGNEIT	259-270	oxidation
4	3053.396	3054.39	1	F	MELERPGGNEITRGGSTSYGTGSETESPR	259-287	citrullination
3	3055.38	3054.39	1	F	MELERPGGNEITRGGSTSYGTGSETESPR	259-287	citrullination
2	1240.66	1240.66	0	P	LERPGGNEITR	261-271	
2	1240.66	1240.66	0	P	LERPGGNEITR	261-271	
3	2795.29	2794.31	1	P	LERPGGNEITRGGSTSYGTGSETESPR	261-287	citrullination
2	1127.576	1127.57	0	P	ERPGGNEITR	262-271	
2	2398.044	2396.08	2	F	PGGNEITRGGSTSYGTGSETESPR	264-287	deamidation & citrullination
3	2423.088	2299.03	124	P	GGNEITRGGSTSYGTGSETESPR	265-287	acetylation
2	2071.926	2070.95	1	P	EITRGGSTSYGTGSETESPR	268-287	citrullination
3	2071.935	2070.95	1	P	EITRGGSTSYGTGSETESPR	268-287	citrullination
2	1571.67	1571.67	0	F	GGSTSYGTGSETESPR	272-287	

The dM indicates the nominal mass difference between the expected and observed mass for the unmodified precursor ion. Possible sites of post-translational modification are indicated in red. Only citrullination of the Arg residue is consistent with the observed mass values and the failure of trypsin to cleave at that site

of citrullinated fibrin/fibrinogen in SF. If our study had been performed using RA plasma as the source of autoantigens, we would not have been successful at identifying autoantigens. Identifying the best biomarker source is crucial in biomarker discovery.

It is possible that our study failed to detect additional immunogenic citrulline sites on FIBA as well as other molecules. The failure of trypsin to cleave at citrulline residues often results in large peptides that are difficult to characterize by mass spectrometry. Other autoantigens could be present at very low concentrations, lost due to non-specific binding during chromatography, did not bind to the nitrocellulose, did not bind in the right conformation to the nitrocellulose, are heavily modified or glycosylated, were undetected by the antibodies in the macroarrays, and/or were not completely digested by trypsin. For these and many more reasons, the proteins could have escaped detection by the macroarray or mass spectrometry experiments. This study was designed to identify proteins so if the autoantigens are carbohydrate, lipid, or another type of molecule then they would have also been undetected. The chromatographs are more sensitive at detecting proteins than the mass spectrometry. However, since small chromatography peaks yielded no protein identifications, the nature of the molecule that produced the peak is unknown.

The role for the fibrin alphaC domain fragment in RA pathogenesis may be complex. Soluble citrullinated fibrinogen and fibrin degradation products have been found in RA SF but not RA plasma, suggesting that there are high levels of active PAD enzymes in RA SF [19, 45]. Fibrin deposits in the joints of RA patients are widely observed and have been hypothesized to be the cause of pannus formation [46]. The fibrin deposits in the joints allow the fibrin molecules to remain in an inflammatory environment for a prolonged period of time, which could facilitate post-translational citrulline modification [5]. The alphaC domain fragments are the first to be released during fibrinolysis so they are constantly being generated [47]. If stably present, such degraded alphaC fragments could readily be taken up by antigen presenting cells and chronically displayed to the immune system. Hence, a degradation product of an aberrantly modified self-protein may be an RA autoantigen.

This discovery of post-translationally modified immunogenic epitopes present on self-proteins *in vivo* may contribute to improved diagnostic tests for RA. With the data obtained from a limited number of patients, the citrullinated 271X peptide seems to have similar sensitivity (83.3%) as the commercially available CCP2 test. Since antibodies to CCP have been shown to be present prior to disease-onset development [11, 12], there is a possibility that the citrullinated 271X peptide can also predict disease. Studies determining when autoantibodies to the citrullinated 271X are produced need to be conducted. Whether a

test using the native peptide offers any improvement over the commercially available tests remains to be shown. Irreversible joint damage can occur early in the disease process [48], so early diagnosis and aggressive treatment is vital to the preservation of joint function. Autoantibodies specific for citrullinated epitopes are predominant in early RA patients with high-grade joint inflammation and clinical manifestations predicting development of severe erosive disease [43, 49]. One promising diagnostic tool to define clinically distinct subsets of RA patients is antigen microarray profiling of autoantibodies, an assay in which known autoantigens are arrayed on slides, which are probed with patient sera [43]. The method described here, which can be used to identify epitopes on proteins that are modified in inflamed synovial tissue *in situ*, will lead to additional information about autoantigens that will help to increase the power of such diagnostic autoantigen arrays.

In summary, proteomic analysis defined an immunogenic citrulline-containing epitope, within the fibrin alphaC domain fragment, as an autoantigen present in RA SF. This study provides further validation that citrullinated fibrinogen is an autoantigen in RA. The strategy used in this project should be useful for identification of novel autoantigens in RA and other autoimmune diseases.

Acknowledgements The authors would like to thank Drs. Andrew J. Alpert, Alex Kurosky, Paul Plotz, and Henry M. Fales for reviewing the manuscript, Dr. Robert Nathan for discussions on array analysis, Dr. Leonid Medved for providing the structure of the alphaC domain, and the patients and volunteers who donated the serum and SF samples.

References

1. Feldmann M, Brennan FM, Maini RN. Rheumatoid arthritis. *Cell*. 1996;85:307–10.
2. Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature*. 2003;423(6937):356–61.
3. Corrigall VM, Panayi GS. Autoantigens and immune pathways in rheumatoid arthritis. *Crit Rev Immunol*. 2002;22(4):281–93.
4. Boekel MA, et al. Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. *Arthritis Rheum*. 2002;4:87–93.
5. Gaalen Fv, et al. The devil in the details: the emerging role of anticitrulline autoimmunity in rheumatoid arthritis. *J Immunol*. 2005;175:5575–80.
6. Firestein G. Immunologic mechanisms in the pathogenesis of rheumatoid arthritis. *J Clin Rheumatol*. 2005;11:S39–44.
7. Utz PJ, Gensler TJ, Anderson P. Death, autoantigen modifications, and tolerance. *Arthritis Res Ther*. 2000;2:101–14.
8. Vossenaar ER, Venrooij WJv. Citrullinated proteins: sparks that may ignite the fire in rheumatoid arthritis. *Arthritis Res Ther*. 2004;6:107–11.
9. Plotz P. The autoantibody repertoire: searching for order. *Nat Rev Immunol*. 2003;3:73–8.

10. Schellekens GA, et al. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J Clin Invest.* 1998;101:273–81.
11. Rantapää-Dahlqvist S, et al. Antibodies against cyclic citrullinated peptide and iga rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum.* 2003;48(10):2741–9.
12. Nielen MMJ, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis. *Arthritis Rheum.* 2004;50(2):380–6.
13. Lundberg K, et al. Citrullinated proteins have increased immunogenicity and arthritogenicity and their presence in arthritic joints correlates with disease severity. *Arthritis Res Ther.* 2005;7:R458–67.
14. Mil AHvdH-v, et al. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Res Ther.* 2005;7:R949–58.
15. Suzuki A, Yamada R, Chang X, Tokuiro S, et al. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat Genet.* 2003;34:395–402.
16. Tarcsa E, et al. Protein unfolding by peptidylarginine deiminase. *J Biol Chem.* 1996;271(48):30709–16.
17. Masson-Bessière C, et al. The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the a- and b-chains of fibrin. *J Immunol.* 2001;166:4177–84.
18. Skriner K, et al. Association of citrullinated proteins with synovial exosomes. *Arthritis Rheum.* 2006;54(12):3809–14.
19. Takizawa Y, et al. Citrullinated fibrinogen detected as a soluble citrullinated autoantigen in rheumatoid arthritis synovial fluids. *Ann Rheum Dis.* 2006;65:1013–20.
20. Girbal-Neuhausser E, et al. The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are posttranslationally generated on various sites of (Pro) filaggrin by deimination of arginine residues. *J Immunol.* 1999;162:585–94.
21. Sebbag M, et al. The antiperinuclear factor and the so-called antikeratin antibodies are the same rheumatoid arthritis-specific autoantibodies. *J Clin Invest.* 1995;95:2672–9.
22. Vossenaar ER, et al. Rheumatoid arthritis specific anti-Sa antibodies target citrullinated vimentin. *Arthritis Res Ther.* 2004;6:R142–50.
23. Bang H, et al. Mutation and citrullination modifies vimentin to a novel autoantigen for rheumatoid arthritis. *Arthritis Rheum.* 2007;56(8):2503–11.
24. Tilleman K, et al. Synovial detection and autoantibody reactivity of processed citrullinated isoforms of vimentin in inflammatory arthritides. *Rheumatology (Oxford).* 2008;47(5):597–604.
25. Suzuki A, et al. Anti-citrullinated collagen type I antibody is a target of autoimmunity in rheumatoid arthritis. *Biochem Biophys Res Commun.* 2005;333:418–26.
26. Koivula MK, et al. Sensitivity and specificity of autoantibodies binding to citrullinated carboxyterminal telopeptides of types I and II collagens in an early arthritis series. *Rheumatology (Oxford).* 2008;47(5):656–9.
27. Burkhardt H, et al. Humoral immune response to citrullinated collagen type II determinants in early rheumatoid arthritis. *Eur J Immunol.* 2005;35:1643–52.
28. Yoshida M, et al. Autoimmunity to citrullinated type II collagen in rheumatoid arthritis. *Mod Rheumatol.* 2006;16(5):276–81.
29. Chang X, et al. Citrullination of fibronectin in rheumatoid arthritis synovial tissue. *Rheumatology.* 2005;44(11):1374–1382.
30. Kinloch A, et al. Identification of citrullinated α -enolase as a candidate autoantigen in rheumatoid arthritis. *Arthritis Res Ther.* 2005;7:R1421–9.
31. Hueber W, Robinson WH. Proteomic biomarkers for autoimmune disease. *Proteomics.* 2006;6:4100–4115.
32. Yan F, et al. Protein microarrays using liquid phase fractionation of cell lysates. *Proteomics.* 2003;3:1228–35.
33. Arnett FC, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum.* 1988;31(3):315–24.
34. Hochberg MC, et al. The American College of Rheumatology 1991 revised criteria for the classification of global functional status in rheumatoid arthritis. *Arthritis Rheum.* 1992;35(5):498–502.
35. Smith DD, et al. Median filter algorithm for estimating the threshold of detection on custom protein arrays. *Biotechniques.* 2006;41:74–8.
36. Yates JRd, Eat J, McCormack AL, Schieltz D. Method to correlate tandem mass spectra of modified peptides to amino acid sequences in the protein database. *Anal Chem.* 1995;67:1426–36.
37. Chen EI, et al. Large scale protein profiling by combination of protein fractionation and multidimensional protein identification technology (MudPIT). *Mol Cell Proteomics.* 2006;5:53–6.
38. Rifai N, Gillette MA, Carr SA. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat Biotechnol.* 2006;24(8):971–83.
39. Zolg W. The proteomic search for diagnostic biomarkers: lost in translation? *Mol Cell Proteomics.* 2006;5(10):1720–6.
40. Chang X, Yamada R, Suzuki A, Sawada T, Yoshino S, Tokuiro S, et al. Localization of peptidylarginine deiminase 4 (PADI4) and citrullinated protein in synovial tissue of rheumatoid arthritis. *Rheumatology (Oxford).* 2005;44(1):40–50.
41. Nakayama-Hamada M, et al. Comparison of enzymatic properties between hPADI2 and hPADI4. *Biochem Biophys Res Commun.* 2005;327:192–200.
42. Kubota K, Yoneyama-Takazawa T, Ichikawa K. Determination of sites citrullinated by peptidylarginine deiminase using ^{18}O stable isotope labeling and mass spectrometry. *Rapid Commun Mass Spectrom.* 2005;19:683–8.
43. Hueber W, et al. Antigen microarray profiling of autoantibodies in rheumatoid arthritis. *Arthritis Rheum.* 2005;52(9):2645–55.
44. Sebbag M, et al. Epitopes of human fibrin recognized by the rheumatoid arthritis-specific autoantibodies to citrullinated proteins. *Eur J Immunol.* 2006;36:2250–63.
45. Sinz A, et al. Mass spectrometric proteome analyses of synovial fluids and plasmas from patients suffering from rheumatoid arthritis and comparison to reactive arthritis or osteoarthritis. *Electrophoresis.* 2002;23:3445–56.
46. Sánchez-Pernaute O, et al. A fibrin based model for rheumatoid synovitis. *Ann Rheum Dis.* 2003;62:1135–8.
47. Henschen-Edman AH. Fibrinogen non-inherited heterogeneity and its relationship to function in health and disease. *Annals NY Acad Sci.* 2001;936:580–93.
48. Scott D. Radiological progression in established rheumatoid arthritis. *J Rheumatol Suppl.* 2004;69:55–65.
49. Gaalen FAv, et al. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis. *Arthritis Rheum.* 2004;50(3):709–15.