Autoimmune Response to Amyloidogenic Transthyretin in Juvenile Idiopathic Arthritis

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Supplement Figures legends

Figure S1: Global analysis of the human synovial fluid proteome from seven JIA patients determined by 1DE SDS–PAGE coupled with nanoLC tandem MS/MS.

A) Pie chart representing molecular functions of the human synovial fluid from seven JIA patients created with the IPA (ingenuity pathway analysis) and the GO annotation tools at the Gene Ontology Consortium (http://beta.geneontology.org/). The graphic display was generated from the total of 392 proteins shown in the Supplement Table 1.

B) Histograms display of the major canonical biochemical pathways created with the IPA (ingenuity pathway analysis) using the total proteome of the JIA synovial fluid shown in Supplement table 1 at a statistical significance $P<0.05$ (the higher the "–log (p)" value the more matches between the experimentally determined molecules and those predicted in molecular pathway from the IPA).

Figure S2: **The peptidomic analysis of the human synovial fluid from seven JIA and two control patients obtained by nanoLC-MS/MS on LTQ/Orbitrap Velos mass spectrometer.**

A) The comparative base chromatograms (MS^1/MS^2) for the two controls (C1 and C2) and for the seven JIA patients (P31-P37) of one out of the three nanoLC-MS/MS experiments (one dimensional nanoUPLC-MS/MS with HCD/ETD combined analysis on Orbitrap, full scan).

B) The 2D-heat map (retention time vs. m/z) of a typical nanoLC-separation for a selected JIA patient (P7) showing in blue spots the experimental MS/MS data and in red spots the sequenced peptides using "PEAKS Peptide De novo sequencing" algorithm with their protein ID assigned by "PEAKS DB-Peptide identification" module. The separation took 90 minutes and the m/z range was 300-1500. The two dimensional graph shows the part of the separation where most of the peptides eluted. The spot intensity in the graph is represented by gray-scale changes, with the black being the most intense signal, and white the lowest signal. The map is displayed at a noise level of 500.

Figure S3: Histogram displays of the total number of unique endogenous peptides derived from the degradation of the complement and the fibrinogens proteins (A) and the proteases/inhibitors of the proteases (JIA vs controls). The enhanced degradation of the proteome from the synovial fluid of JIA patients correlates with the increased number of sequenced endogenous peptides. **(C) Histograms display of the major canonical biochemical pathways generated from the IPA analysis of the whole protein substrates generating the peptidome from the synovial fluid of JIA and control patients** (Proteomic data used to generate the major biochemical pathways are reported in Supplement Table S2f)**.**

Figure S4 anti TTR antibodies in the synovial fluid and sera of JIA and controls.

Quantification of TTR autoantibodies, as detected by ELISA, present in the a) synovial fluid and b) sera from patients with JIA and controls (data are compiled from 3 separate ELISA with each patient's samples run in quadruplicate). Data were analyzed by one-way ANOVA ($p < 0.005$) and Tukey test. Number on top of each bar correspond to patient ID as outlined in Supplement Table S1

Figure S5 TTR aggregates in the synovial fluid of JIA.

a and b) Western blot analysis of TTR proteins present in the synovial fluid of controls and JIA patients run on a (a) native gel or (b) SDS-PAGE (representative gel out of 2 runs). TTR monomers (15kDa), dimers (30 kDa), tetramers (60 kDa) and aggregates (above 60 kDa) are visible in JIA patients, when run on a native gel. Number on top of each lane correspond to patient ID as outlined in Supplement Table S1. To avoid protein aggregation, induced by freeze-thaw, SF samples were run immediately upon collection; this explains the out-of order number in which the samples were run which depended on each patient clinical appointment schedule..

Figure S6 Patients' specific sequences of JIA (top) and controls (middle) aligned to the Transthyretin reference exon genome (NM 000371.3, bottom). No genomic variations at the nucleotide level are observed.

Supplement Table S1: Clinical Data for JIA patients and Controls

Supplement Table S2: Proteomic Profiling of Synovial Fluid from Control and JIA patients

Supplement Table S3: MS/MS analysis of the Control and JIA Peptidome

Supplement Table S4: Ingenuity Pathway Analysis of the Synovial Fluid JIA and Control peptidome

Supplement Table S5: Degradome Analysis of the JIA and Control peptidome

Supplement Table S6: Analysis of the Proteome eluted from the immunocomplexes isolated from JIA and Control Synovial Fluids
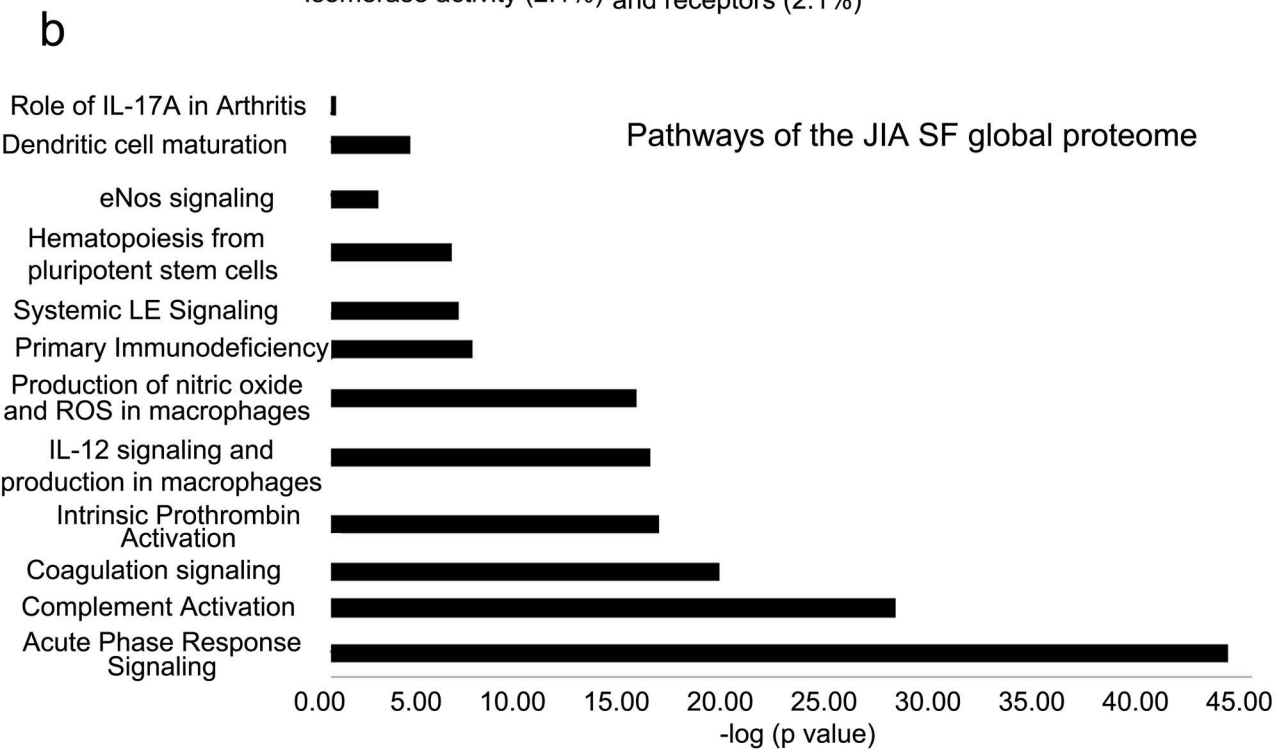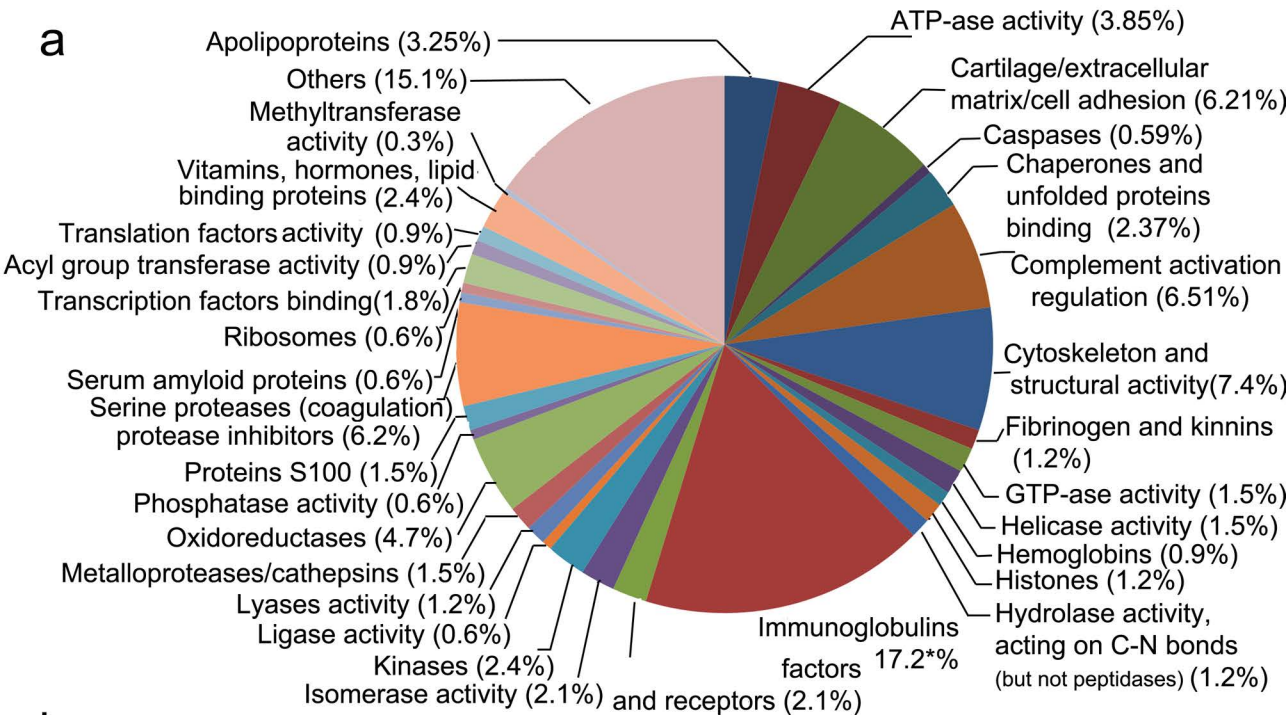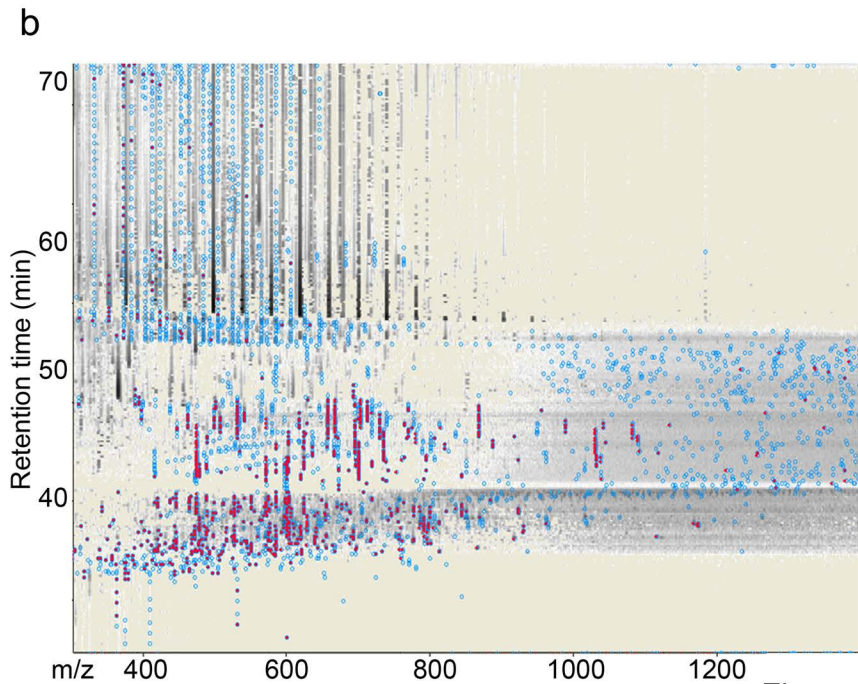
a

Apolipoproteins (3.25%)
Others (15.1%)
Methyltransferase activity (0.3%)
Vitamins, hormones, lipid binding proteins (2.4%)
Translation factors activity (0.9%)
Acyl group transferase activity (0.9%)
Transcription factors binding (1.8%)
Ribosomes (0.6%)
Serum amyloid proteins (0.6%)
Serine proteases (coagulation) protease inhibitors (6.2%)
Proteins S100 (1.5%)
Phosphatase activity (0.6%)
Oxidoreductases (4.7%)
Metalloproteases/cathepsins (1.5%)
Lyases activity (1.2%)
Ligase activity (0.6%)
Kinases (2.4%)
Isomerase activity (2.1%)

ATP-ase activity (3.85%)
Cartilage/extracellular matrix/cell adhesion (6.21%)
Caspases (0.59%)
Chaperones and unfolded proteins binding (2.37%)
Complement activation regulation (6.51%)
Cytoskeleton and structural activity (7.4%)
Fibrinogen and kinnins (1.2%)
GTP-ase activity (1.5%)
Helicase activity (1.5%)
Hemoglobins (0.9%)
Histones (1.2%)
Hydrolase activity, acting on C-N bonds (but not peptidases) (1.2%)

Immunoglobulins factors 17.2*%
and receptors (2.1%)

b

Pathways of the JIA SF global proteome

Role of IL-17A in Arthritis
Dendritic cell maturation
eNos signaling
Hematopoiesis from pluripotent stem cells
Systemic LE Signaling
Primary Immunodeficiency
Production of nitric oxide and ROS in macrophages
IL-12 signaling and production in macrophages
Intrinsic Prothrombin Activation
Coagulation signaling
Complement Activation
Acute Phase Response Signaling

-log (p value)

Figure S1
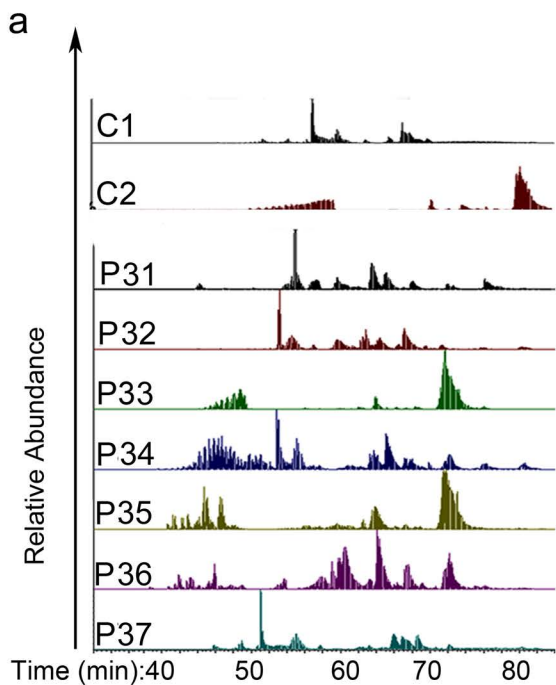
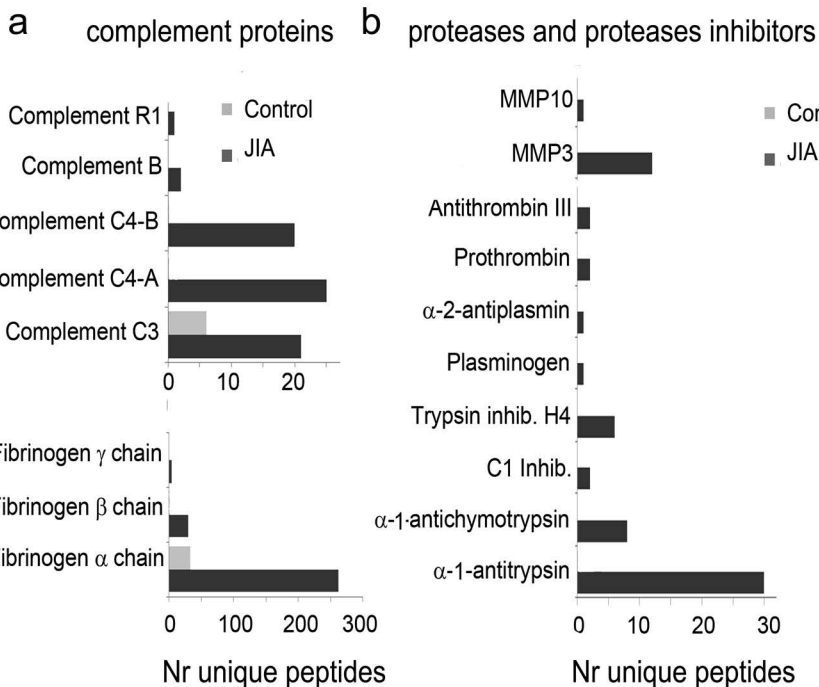Figure S2

a complement proteins

b proteases and proteases inhibitors

c Pathways of the JIA SF global degradome
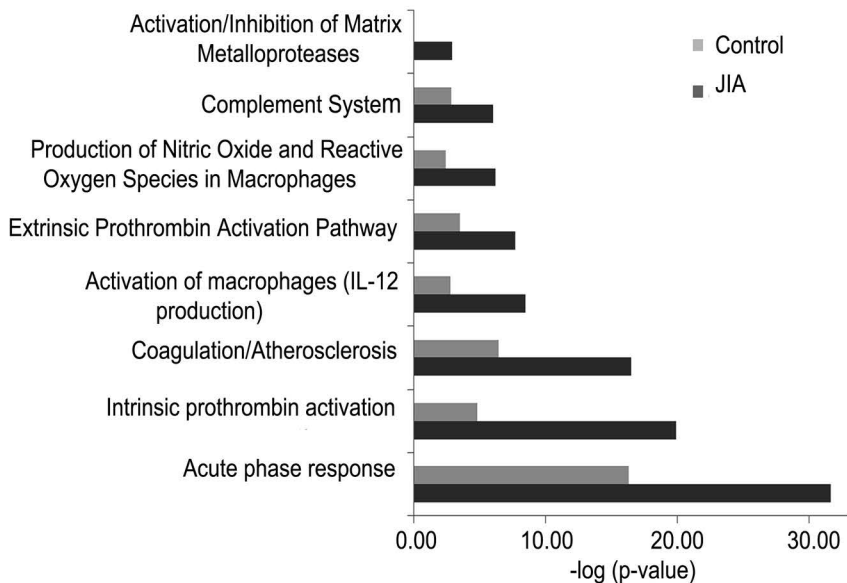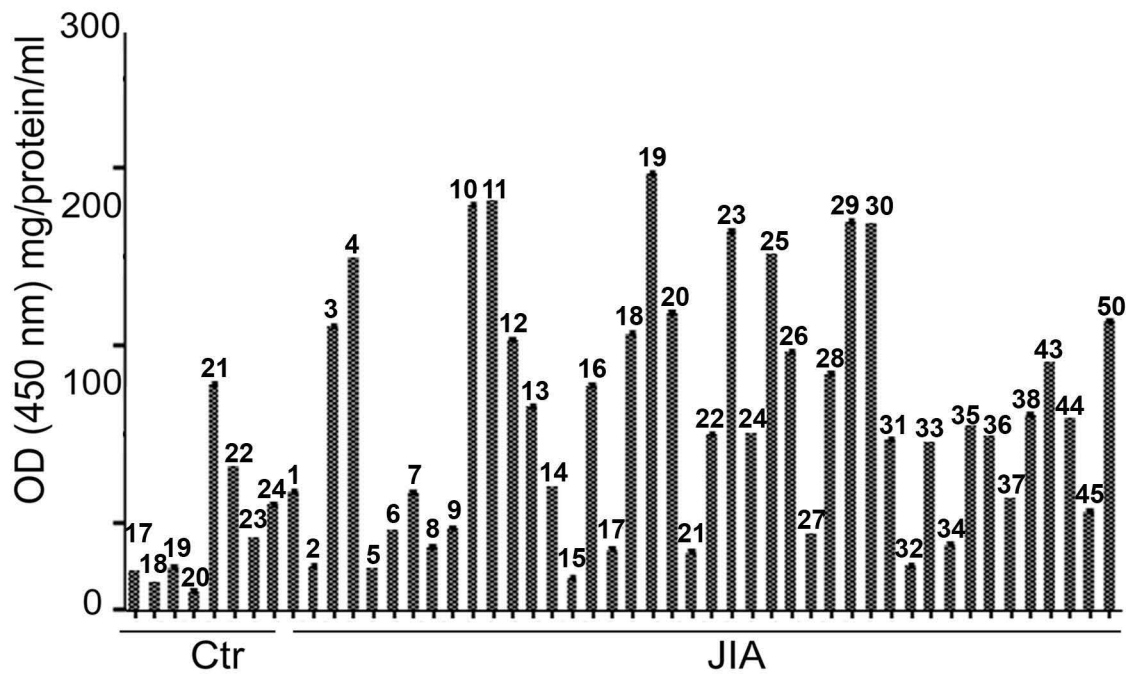
Figure S3

Synovial Fluid (anti TTR Ab)

Sera (anti TTR Ab)

Figure S4

A

19 20 21 22 23 24 | 6 5 15 17 2 7 | 14 9 11 4 28 | 21 27 | 29 23 24 16 19 26 18 30 | 12 20 13 22 10 | 31 32 33 34 35 36 37 | 38 39 | 25 3

1236
1038
720
480
242
140
66
20

250
100
75
50
37
15

B

Ctr   JIA

Figure S5

Exon 2　　Exon 4　Stop　　　　　　　　　5' UTR

Cases
n=25

Controls
n=16

TTR
reference

134bp　　　　　　457bp

206bp　　　　　　206bp

Transthyretin

Exon 1　　　　　　Exon 3

7,170

AGAACGCTA

AGAACGCTA
AGAACGCTA
AGAACGCTA
AGAACGCTA
AGAACGCTA
AGAATGCTA
AGAACGCTA
AGAACGCTA
AGAACGCTA
AGAACGCTA
AGAATGCTA
AGAATGCTA
AGAACGCTA
AGAACGCTA
AGAACGCTA
AGAACGCTA
AGAACGCTA
AGAACGCTA

AGAACGCTA

C/T
hg19
Chr18:29,178,899

Figure S6