Supplementary Material

NMR based serum metabolomics discriminates Takayasu Arteritis from Healthy Individuals

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Appendix I:

Unambiguous assignments of various metabolites were obtained using two-dimensional *J*-resolved (JRES), TOCSY (total proton-proton correlation spectroscopy) and HSQC (heteronuclear single quantum correlation) spectra (acquired on three-to-four serum samples) and spiking experiments using standard chemicals.

Homonuclear 2D *J*-resolved spectra (jresgpprqf) were recorded in magnitude mode using quadrature phase (QF)- with water pre-saturation during recycle delay (RD) of 2 sec [32]. 16K data points were collected along direct proton (F_2) dimension with spectral width of 16 ppm, whereas, along indirect J- Couplings (F_1) dimension, 80 points (increments) corresponding to spectral width of 78 Hz (~0.0976 ppm) were collected and for each F_1 increment, 16 transients were acquired. Prior to Fourier transform, free induction decay (FID) signals were weighted in both dimensions by a sine-bell function and zero-filled in the F_1 dimension to 256 data points. The spectra were tilted by 45° to provide orthogonality of the chemical shift and coupling constant axes and subsequently symmetrized about the F_1 axis.

Two-dimensional ^{1}H-^{1}H TOCSY (dipsi2esgpph) and sensitivity enhanced ^{1}H–^{13}C HSQC (hsqcetgp) spectra were acquired in phase sensitive mode using time proportional phase incrementation (TPPI). 2D TOCSY spectrum was recorded using 2048 data points along direct dimension (F_2) and 512 increments along indirect dimension (F_1) with 16 transients per increment and a spectral width of 12 ppm in both dimensions. The FIDs were weighted using a sine–bell-squared function in both dimensions and zero filled to 2048 and 4096 data points, respectively, in the F_1 and F_2 dimensions prior to FT. For HSQC experiment, the RD between successive pulse sequence cycles was 1.5 sec, while for TOCSY experiment (with mixing time of 80 ms), the RD between successive pulse cycles was 3.0 sec. Spin-lock was achieved by a DIPSI2 pulse sequence train [33] during the TOCSY mixing time. 2D HSQC spectrum was recorded with inverse ^{13}C detection and using ^{13}C decoupling during acquisition using GARP-1 [29]. A RD of 2.0 sec was used between successive pulse sequence cycles and a refocusing delay equal to $1/(4*{}^{1}J_{C-H}$ =1.75 ms) was employed. 512 x 128 complex t_2 (^{1}H) and t_1(^{13}C) data points with 96 scans per increment were acquired with spectral widths of 12 and 165 ppm, respectively, in the ^{1}H and ^{13}C dimensions. The FIDs were weighted using a sine-bell-squared function in both dimensions and zero filled to 1024 and 2048 data points, respectively, in the F_1 and F_2 dimensions prior to FT. After FT, the final spectrum was manually phase corrected and ^{1}H and ^{13}C dimensions were referenced to lactate methyl protons and carbon, respectively, at 1.31 and 22.5 ppm.

Appendix II:

Quantification of metabolites:

Due to improved baseline and metabolite detection in the CPMG spectra, these were chosen for quantitative measurements by spectral integration, assuming that any reduction in signal intensity due to relaxation effects would be consistent across samples, thus not affecting the evaluation of relative changes in metabolite levels. The peak of identified metabolite and standard TSP were integrated and the concentrations of metabolites were estimated using the following formula:

$$[C]_X = [C]_{TSP} \frac{N_{TSP}.I_X}{N_X.I_{TSP}}$$

where $[C]_X$ and $[C]_{TSP}$ are the concentration of metabolites and pre-calibrated known concentration of TSP, respectively. $[I]_X$ and $[I]_{TSP}$ are the NMR signal integration of metabolites and TSP, respectively. N_X is the number of protons per molecule giving rise to the integrated signal and N_{TSP}= 9.

Figure S1: The assignment of peaks corresponding to serum metabolite L-arginine confirmed using spiking method following sequential addition of L-arginine solution prepared in saline D_2O (all purchased from Sigma-Aldrich) into control serum sample. The spiking experiments clearly revealed that the Arginine concentration is very low (i.e. below the NMR detection limit, possibly below 50 μM).

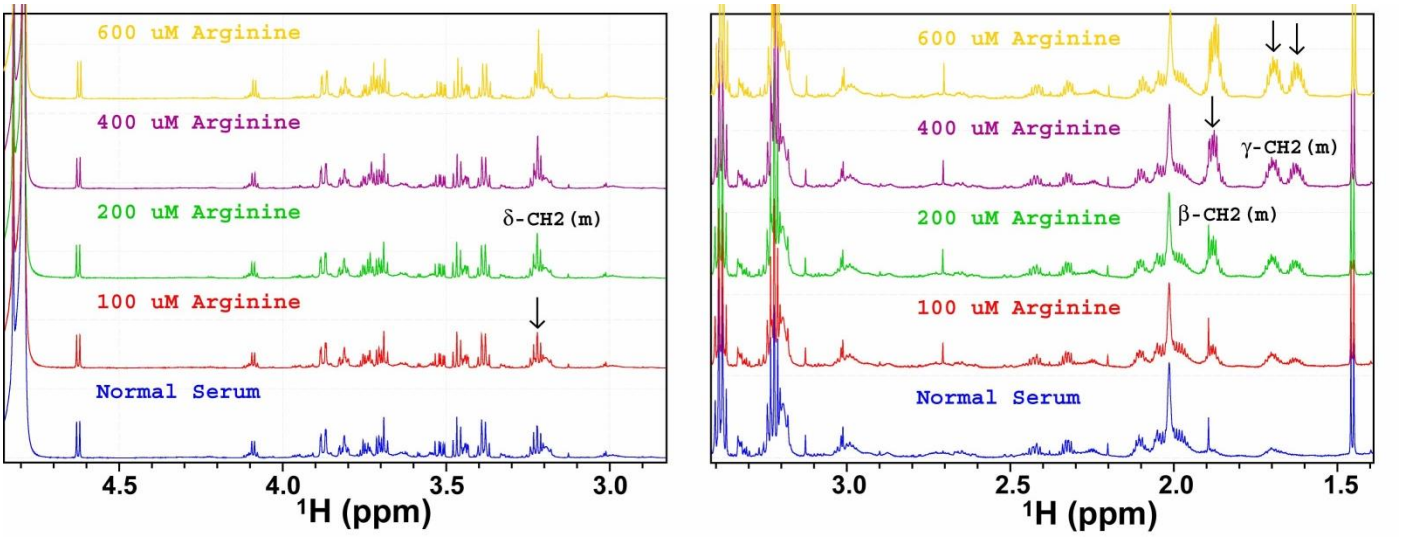


Figure S2: The assignment of peaks corresponding to serum metabolite L-Isoleucine identified using spiking method following sequential addition of L- Isoleucine solution prepared in saline D_2O (all purchased from Sigma-Aldrich) into control serum sample. The spiking experiments clearly revealed that the Isoleucine is present in moderately high concentration in serum (i.e. above the NMR detection limit, possibly above the 50 μM).

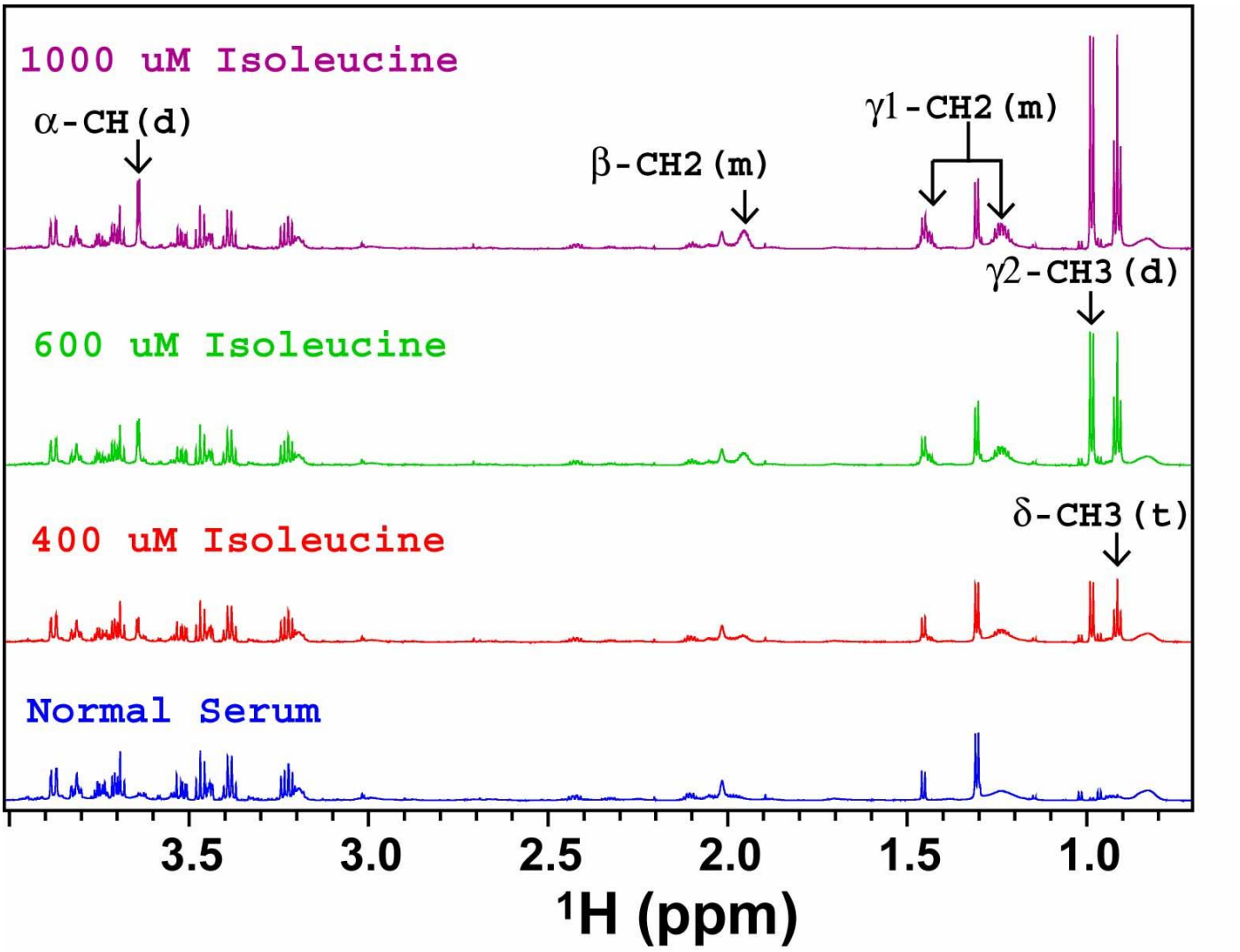


Figure S3: PLS-DA score plot generated from ^{1}H NMR spectra of serum samples obtained from the TA patients with medication and without medication.

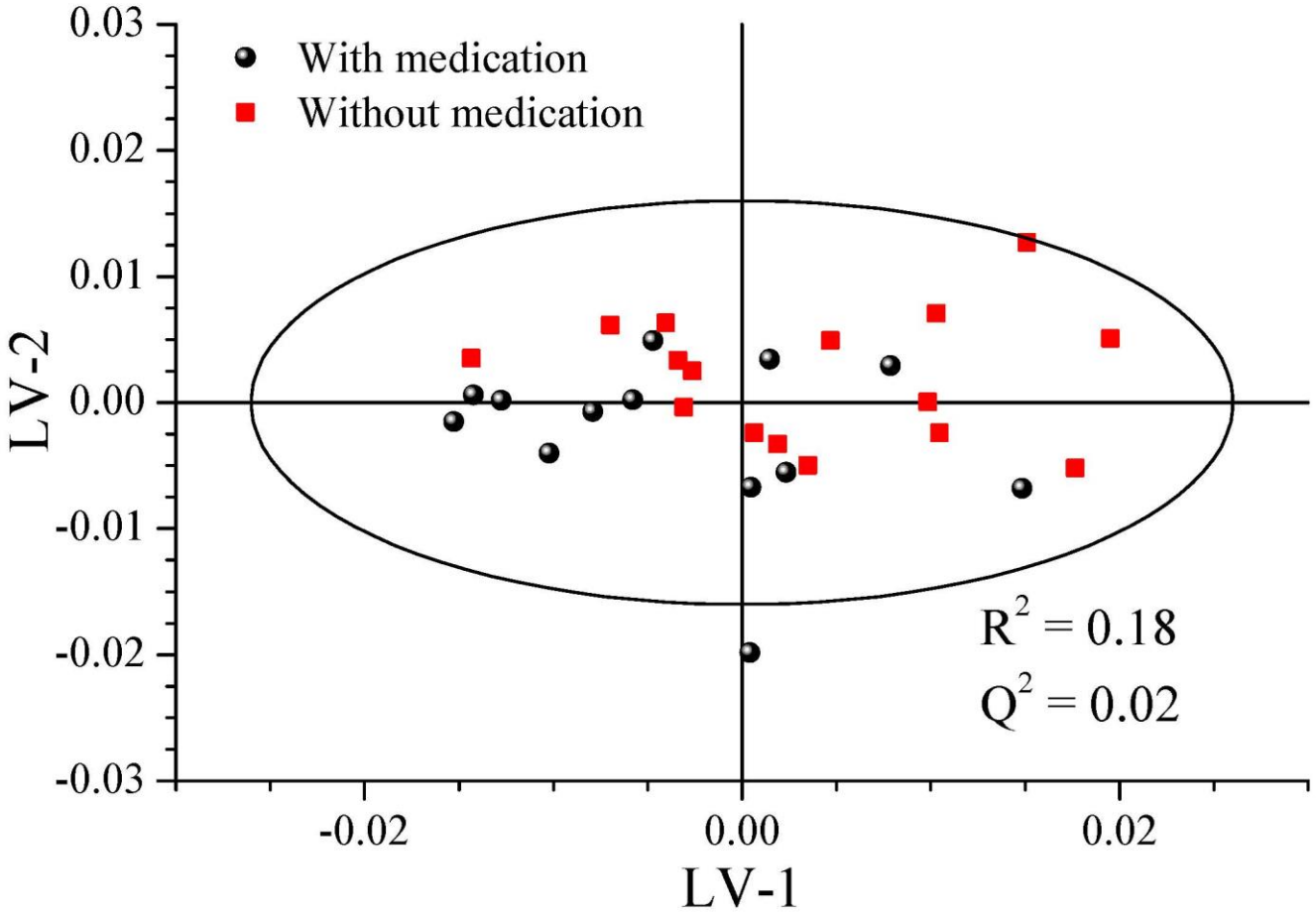
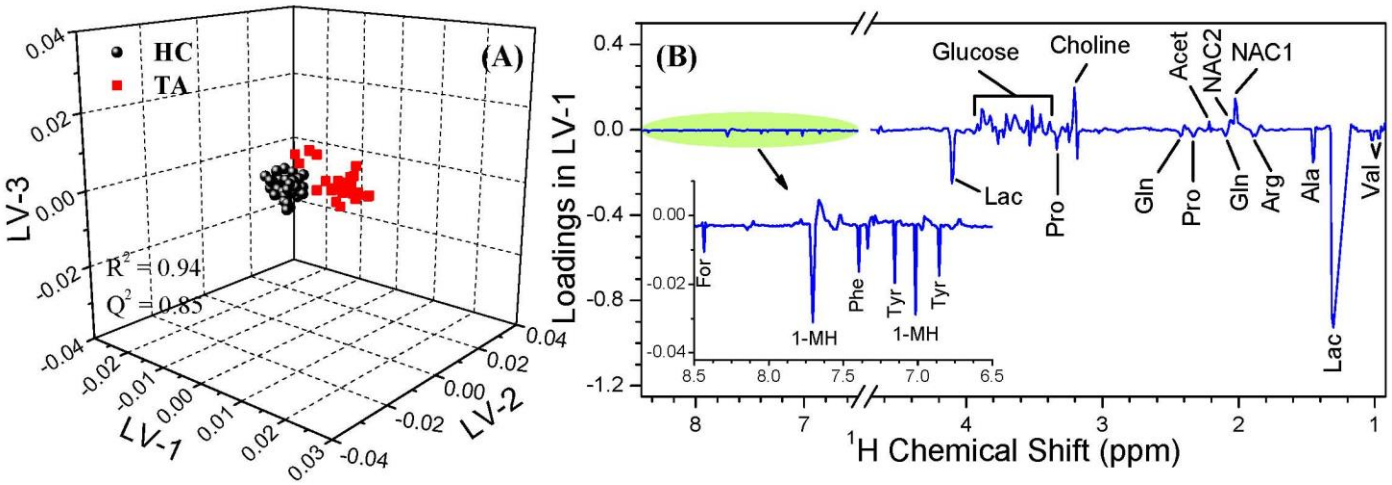


Figure S4: (A) PLS-DA score plot obtained after excluding the signals of lipids resonances i.e. 0.8-0.92, 1.20-1.29, 1.96-2.00, 2.71-2.80 and 5.2-5.38 ppm regions during the multivariate statistical analysis and (B) corresponding loading plot between healthy control and TA patients to demonstrate the change in metabolites between the HC and TA groups. Positive and negative loadings represent elevated and decreased serum levels of metabolites in TA group compared to the HC.



Supplementary Table S1: Evaluation of statistical significance of quantified metabolites as observed in the ^{1}H NMR spectra of control and TA patients. Integral area of metabolites resonance was used for statistical comparison among groups. *P*-values less than 0.05 were considered as significant.

S. No.	Name of Metabolites	HC (n=39) Median (Range)	TA (n=29) Median (Range)	*p*-values HC Vs TA
1.	Valine	1.500 (0.900-3.030)	0.970 (0.140-1.520)	<0.001
2.	Alanine	3.730 (1.950-6.830)	2.220 (0.510-4.420)	<0.001
3.	Glutamine	2.200 (0.890-3.430)	1.630 (0.160-2.680)	0.011
4.	Citrate	0.413 (0.260-0.790)	0.325 (0.240-0.710)	0.262
5.	Creatinine	1.190 (0.770-2.350)	0.922 (0.580-1.540)	0.905
6.	Choline	6.110 (3.300-6.950)	6.670 (5.130-9.970)	<0.001
7.	Proline	0.692 (0.370-1.070)	0.600 (0.050-0.880)	<0.001
8.	Lactate	5.300 (2.760-10.890)	3.760 (0.380-9.540)	<0.001
9.	1-MH	0.178 (0.090-0.360)	0.114 (0.010-0.270)	0.001
10.	Tyrosine	0.287 (0.140-0.670)	0.180 (0.020-0.620)	0.293
11.	Formate	0.052 (0.020-0.200)	0.024 (0.000-0.120)	0.02
12.	Lipid-0.87	19.22 (6.740-53.070)	13.99 (2.060-23.520)	<0.001
13.	Lipid-1.26	33.07 (7.470-99.180)	17.70 (2.570-43.780)	<0.001
14.	Lipid-5.30	7.240 (4.240-26.60)	4.650 (2.650-21.030)	0.009
15.	Lipid-2.74	2.450 (1.190-7.640)	1.780 (0.260-3.240)	<0.001