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Circulatory histidine levels as predictive indicators of disease activity in takayasu arteritis

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Abstract

Background and objective: Quantitative assessment of disease activity is important for effective management of patients with autoimmune inflammatory diseases (AIDs) including Takayasu arteritis (TA). Histidine supplementation alleviates inflammation and has strong anti-oxidative effects as well. The present study aims to evaluate the diagnostic potential of circulatory histidine for predicting disease activity in TA.

Methods: The serum metabolic profiles on 98 TA-patients and 77 normal controls (NC) samples were measured at high-resolution 800 MHz NMR spectrometer employing standard 1D-^{1}H-CPMG NMR experiments. The NMR spectral data were processed and concentrations of histidine and other circulatory metabolites were estimated with respect to formate (as an internal reference) and compared using ANOVA based on Tukey's multiple comparison test and statistical significance was considered at *P*-value < 0.05. The correlations of histidine with plasma CRP and ESR levels were evaluated using Spearman-*r* method. Data were expressed as median (interquartile-range [IQR]).

Results: Histidine levels were significantly decreased in active TA patients (23.90; IQR:16.10) compared to both inactive TA patients (35.50, IQR:24.30) and NC (42.80, IQR:22.10), whereas there was no significant difference between inactive TA and NC. For TA patients, the histidine levels correlated negatively with clinical markers of inflammation, that is, ESR (r = -0.19, P < .078) and with the CRP (r = -0.26, P < .013). Further, the receiver-operating-characteristic (ROC) curve analysis was performed to test the diagnostic potential of histidine for differentiating active from inactive disease. The area under the ROC curve (AUROC) value equal to 0.65 [95% CI = 0.54-0.76] revealed its moderate discriminatory ability. Compared to other circulatory metabolites, the discriminatory performance of histidine was also found to be in the moderate range (highest AUROC-value of 0.76 was found for glutamine-to-glucose ratio (QGR).

Conclusion: The study demonstrated the altered circulatory histidine levels in TA patients that may serve as a surrogate marker for improving the diagnostic screening of active and inactive TA patients.

Abbreviations: AUROC, area under ROC curve; CI, confidence interval; CPMG, Carr–Purcell–Meiboom–Gill; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HTR, histidine to tyrosine ratio; IQR, interquartile range; ITAS, Indian Takayasu Clinical Activity Score; ITAS, Indian Takayasu Clinical Activity Score; LGR, lactate to glucose ratio; mTOR, mechanistic target of rapamycin; NC, normal control; NEFA, nonesterified fatty acids; NMR, nuclear magnetic resonance; PTR, phenylalanine to tyrosine ratio; QGR, glutamine to glucose ratio; ROC, receiver operating characteristic curve; SLC15A4, Solute Carrier Family 15 Member 4; TA, Takayasu arteritis

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1 | INTRODUCTION

Takayasu arteritis (TA) is a large vessel vasculitis with a predilection for the aorta and its major branches primarily affecting young Asian women.[1,2] A major challenge in the management of the vasculitis is differentiating damage from disease activity.[3,4] The importance of this lies firstly in determining the treatment course with the need for intensification of immunosuppression in those with persistent disease activity and secondly in ensuring the success of interventional procedures as the rate of complications rises to sevenfold in those with active disease at the time of the procedure.[1] The conventional markers of inflammation like erythrocyte sedimentation rate (ESR) and CRP (C-Reactive protein) are poor correlates of disease activity in TA.[2] The poor reliability of ESR and CRP to composite indices based on history, physical examination, and inflammatory biomarkers such as Indian Takayasu Activity Score (ITAS) and Disease Extent Index-Takayasu Arteritis (DEI·Tak).[3,4] However, such scores ultimately rely on the physician's discretion thus necessitating the need for more objective markers. Imaging modalities are being explored but the utility remains questionable owing to mixed results, further the associated risks of radiation exposure and cost due to the need for serial tests.[5] The lack of appropriate outcome measures hampers the treat to target approach in the management of the disease and thus calls for wider exploration into establishing easy-to-use, cost-effective, and reliable biomarkers.

Previous work from our group has focused on clinical metabolomics to determine serum metabolic disturbances associated with TA and that the serum metabolic profiling has the potential to differentiate active from inactive TA patients.[6,7] Further, considering active glutaminolysis and dampened glycolysis as the potential hallmarks of active inflammation, the circulatory Glutamine/Glucose ratio (QGR) was evaluated for screening TA patients with active disease.[8] Though many factors are involved in the pathogenesis of inflammatory diseases and of them; oxidative stress is the major contributor to the establishment of chronic inflammation.[9] Histidine is an anti-inflammatory amino acid and is a precursor of histamine. Both clinical and preclinical data suggest that histidine has strong anti-oxidative and anti-inflammatory effects.[10–12] A recent randomized controlled trial study performed on obese women revealed that histidine supplementation improves insulin resistance reduces BMI, fat mass, and serum non-esterified fatty acids (NEFA) through suppressing inflammation and oxidative stress.[13,14] Based on this, we hypothesized that the lower circulatory histidine levels can serve as a surrogate marker for the assessment of disease activity in TA and can be used for monitoring treatment response and so deciding clinical treatment appropriately.

2 | MATERIALS AND METHODS

2.1 | Patient selection and sample collection

Serum samples were obtained from 98 patients diagnosed with TA (based on the 1990 American College of Rheumatology (ACR) classification criteria[15] attending the Department of Clinical Immunology and Rheumatology at SGPGIMS, Lucknow. The study protocol was approved by the Institutional Research Ethics Committee, SGPGIMS, Lucknow, India and samples were collected with informed consent from the patients and stored with permission. Demographic parameters, clinical symptoms, and physical examination findings of patients were recorded. Serological tests including complete blood count, ESR, CRP, creatinine, serum transaminases were performed within 3 days of blood sampling. For comparison, the serum samples of 77 normal healthy control subjects were collected. All the patients were evaluated for disease activity based on Indian Takayasu Clinical Activity Score (ITAS2010) combined with circulatory ESR [ITAS-ESR].[4] The cohort of TA patients was prospectively evaluated and divided into active and inactive groups based on the described cutoff value of ITAS-ESR $\geq$ 4.[4] The serum samples were extracted as described previously[16] and stored in aliquots of 250 µL each at -80°C until NMR data acquisition.

2.2 | Sample preparation for NMR experiments

Before starting NMR experiments, the stored serum samples (250 µL in each case) were thawed and mixed with 250 µL of sodium phosphate buffer of strength 50 mM (0.9% saline, pH 7.4 and prepared in 100% D_2O). The samples were centrifuged at 16 278 $\times$ g for 5 min and then 450 µL of each sample volume was transferred to 5 mm NMR tubes (Wilmad Glass, USA). The NMR tube filling of 4.0 cm (as required for Bruker spectrometer) was achieved by inserting a co-axial insert (Wilmad, with stem length 50 mm). The co-axial NMR tube inserted separately contained 1.0 mM TSP (sodium salt of 3-trimethylsilyl-(2,2,3,3-d4)-propionic acid) dissolved in deuterium oxide (D_2O) that served as an external reference. Deuterium oxide (D_2O) and sodium salt of trimethylsilylpropionic acid-d4 (TSP) used for NMR spectroscopy were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.3 | NMR measurements

The NMR spectra were acquired on a Bruker Avance-III NMR spectrometer operating at 800.12 MHz frequency and equipped with Cryoprobe (Bruker BioSpin GmbH, Rheinstetten, Germany) following the procedure and parameters described previously.[6,7] Briefly, the standard CPMG (Carr–Purcell–Meiboom–Gill) spin-echo pulse sequence (cpmgpr1d, from Bruker library) with water presaturation to suppress the water signal and a total spin-spin relaxation delay of five seconds was used. In total, 128 transients with 64 K data points were recorded over a spectral width of 20 ppm, resulting in an acquisition time per scan of 15 min. For quantitative profiling of serum metabolites, we used the commercial software CHENOMX (v8.2, Edmonton, Canada) as described previously.[8] First, the spectrum was opened in its PROCESSOR module and was phased manually, baseline corrected, and calibrated with respect to format peak at 8.43 ppm. Formate has been

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TABLE 1 Clinical and demographic details of active and inactive TAKAYASU arteritis patients

Parameter	Active TA (ITAS-A ≥4)	Inactive TA (ITAS-A ≤ 3)	*t*-Test (*P*-value)	NC
Number (Female: Male)	45 (35:10)	53 (44:9)		77 (64:13)
Age (in Years)[#]	27 [22-35]	27 [23-37]	.60	27.9 [23-32][$]
Duration of Symptoms (in Years)[#]	5 [2-9]	3 [1-6]		
ESR (mm at one Hour)	57.3 ± 29.1	41.4 ± 26.3	.0064↑[*]	
CRP mg/dl)[¥]	6.1 ± 12.6	1.16 ± 1.34	.0093↑[*]	
Immunosuppression (yes)[^]	33	37		
ITAS[¥]	8.5 ± 5.5	0.2 ± 0.7	<.0001↑****	
ITAS-A using CRP[¥]	9.3 ± 5.0	1.3 ±1.3[†]	<.0001↑****	
ITAS-A using ESR[¥]	10.6 ± 5.4[+]	1.6 ±1.0[†]	<.0001↑****	
Angiographic Classification	I	12	9	
	II	4	5	
	III	3	2	
	IV	5	2	
	V	21	34	

[#]median [IQR, 25th-75th percentile], [¥]mean ± SD.
[†]Detail for one sample not available (inactive group); +1- only ITAS used as ESR/CRP was not available; [††]for five patients ITAS-A (CRP) was used as ESR was not available,.
[^]immunosuppression at the time of enrollment into the study,.
*for *P*-value < .05 | **P-value < .001 | ***P-value < .0001 | ****P-value < .00001.
[$]The normal control group was also age-matched to active and inactive TA groups (with *P*-values equal to .26 and .06, respectively).

used as an internal reference and concentration set to 10 micromolar, that is, nearly close to the detection limit of 800 MHz NMR spectrometer. The resulted spectra were then analyzed using PROFILER-Module of CHENOMX and concentrations of selected metabolites (including histidine) were estimated in all the 98 serum samples of TA patients and 77 control serum samples.

2.4 | Statistical analysis

Categorical variables were compared between the two groups based on the two-tail test using SPSS Statistics software (version 11.2, IBM). All continuous variables were described either as mean ± SD or median [interquartile range (IQR)]. The variables between the two groups were compared using Student's *t*-test. The multivariate statistical analysis (i.e. comparison of estimated relative concentrations of circulatory serum metabolites) were performed employing Random Forest (RF) classification algorithm (a supervised machine learning tool).[17] RF classification analysis was performed using the Statistical analysis module of MetaboAnalyst (https://www.metaboanalyst.ca).[18,19] To validate the diagnostic potential of marker metabolites, the receiver operating characteristic curve (ROC) analysis was performed using Biomarker module of web-based software tool MetaboAnalyst (https://www.metaboanalyst.ca).[18,19] The area under the ROC curve (AUROC), 95% confidence intervals (CIs), and *P*-values were computed for the assessment of sensitivity and specificity of a given metabolic feature as a diagnostic test for differentiating active and inactive TA patients. As described previously,[20] the AUROC value less than 0.5 indicates that the test does not have any discriminatory ability (i.e. it is like a random guess), while a value between 0.60 and 0.9 indicates moderate discrimination and a value greater than 1.0 indicates excellent discriminatory potential. Statistical significance was defined at $P < 0.05$ (estimated based on *t*-test). The correlation analysis between concentration levels of circulatory metabolites and clinical parameters were performed using professional software tool GraphPadPrism-7 and evaluated based on the Spearman correlation coefficient(r).

3 | RESULTS

The study involved 98 TA patients (45 with active disease and 53 with inactive disease similarly as described previously).[7] The patients details and presented in Table 1. Figure 1A compares the circulatory levels of histidine between active and inactive TA patients with respect to normal control (NC) subjects. Clearly evident from the Figure that active TA patients have significantly low levels of circulatory histidine compared to both inactive TA patients and NC subjects; whereas there was no significant difference between inactive TA patients and NC subjects. The median histidine levels in active TA patients were 23.9 (IQR:16.10) as compared to 35.5 (IQR: 24.3) for inactive TA patients and 42.8 (IQR: 22.10) in normal control subjects. Circulating histidine levels showed a negative correlation with conventional inflammatory parameters i.e. CRP (r = -0.26; *P*-value = .013) and ESR (r = -0.19; *P*-value = .078) (Figure 1B,C). Further, the receiver operating

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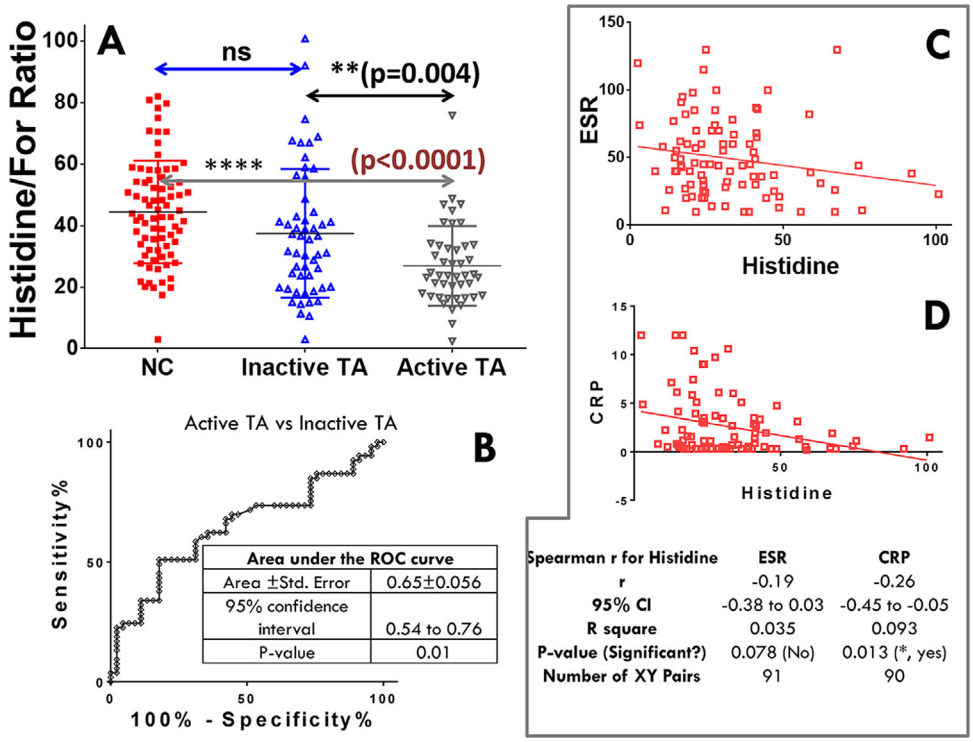

FIGURE 1 **(A)** Box plot showing a comparison of circulatory histidine levels in active and inactive TA patients with respect to normal healthy control. **(B)** Receiver operating characteristic (ROC) curve analysis performed for evaluating the diagnostic potential of Histidine. The area under the ROC curve (AUC, with 95% confidence interval (CI), and *P*-values) is shown in gray to highlight the diagnostic value of circulatory histidine for differentiating active and inactive TA patients. **(C, D)** Graphs showing Spearman-correlation for Histidine with erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels

characteristic (ROC) curve analysis was performed to evaluate its diagnostic utility. The estimated area under the ROC curve (AUROC) value of 0.65 with 95% CI = 0.54-0.76 (*P*-value < .05) suggested that the circulatory histidine levels have moderate discrimination ability (Figure 1D).

3.1 | Relevance of Histidine as a predictive biomarker

In order to establish the utility of histidine as a reliable clinical marker of disease activity compared to other circulatory metabolic features, we further performed random forest (RF) classification analysis that is a machine learning tool suitable for identifying predictive biomarkers (features) from higher-dimensional metabolomics data.[21,22] The relative concentrations of specific metabolic features profiled in this study are tabulated in Table 2. Figure 2A shows the RF cumulative error rates measured for each class using ensemble of classification trees. Clearly evident from the Figure is that the estimated serum metabolic profiles of TA patients are distinctively different compared to normal control subjects. Figure 2B shows the mean decrease in accuracy (MdAcc) that measures the importance of each variable to the predicted RF classification model. The general idea is to permute the values of each variable and measure the decrease in the accuracy of the model as described previously.[21,22] Clearly evident that the circulatory HTR levels are highly discriminatory followed by histidine in the RF classification model. Further, the estimated metabolic concentrations were compared between active and inactive TA patient's with respect to the normal control (NC) group and the results are summarized in Figure 3. Compared to inactive TA patients, the circulatory levels of the majority of amino acids (including alanine, glutamine, glutamate, etc.), creatine, choline, and citrate were found significantly decreased in active TA patients and. These metabolic changes were found well consistent with our previous reports[7,8,23] suggesting their augmented utilization to regulate various immune-inflammatory functions required for immune-mediated inflammatory processes. We further confirmed these metabolic features for their significant differences between the study groups using univariate statistical analysis tools such as ANOVA and Student's *t*-test. The results of these analyses are provided in the Supporting Information (See Figure S1 and Tables S1 and S2).

Compared to NC subjects, the circulatory HTR levels were significantly decreased in TA patients (irrespective of disease activity) compared to NC subjects (Figure 3) and this might be related to differentially active pro-inflammatory pathways in TA. Well consistent to this, the phenylalanine to tyrosine ratio (PTR, which is an indicative of oxidative stress)[24] was found to be elevated in TA patients as compared to NC subjects suggesting elevated oxidative stress in TA. However, the circulatory HTR and PTR levels were comparable between active and inactive TA patients suggesting that their lack of utility in the assessment of disease activity (Figure 3). Among

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TABLE 2 The concentrations of 30 distinctive serum metabolites estimated based on 1D ^{1}H CPMG NMR spectra (reported as mean ± standard deviation (SD). Additionally, seven metabolic ratios (HTR: Histidine-to-tyrosine ratio, PTR: Phenylalanine-to-tyrosine ratio; ALR: Alanine-to-lactate ratio; QGR: Glutamine-to-glucose ratio; EQR: Glutamate-to-glutamine ratio; LPR: Lactate to pyruvate ratio; BTR: Branched chain amino acids-to-tyrosine ratio) were also evaluated and compared between the study groups

		NC	Inactive TA	Active	*P*-value showing if the
#	Metabolite	Median (Q1-Q3): IQR	Median (Q1-Q3): IQR	Median (Q1-Q3): IQR	linear trend is significant?
1	3HB	6.30(4.30-11.60):7.30	14.90(9.20-24.60):15.40	12.30(4.90-19.70):14.80	.0007***
2	Acetate	27.50(20.00-33.40):13.40	21.30(18.10-33.20):15.10	22.60(15.20-34.00):18.80	.11
3	Acetone	9.60(5.70-13.40):7.70	12.30(8.30-19.40):11.10	9.40(4.90-17.40):12.50	.0217*
4	Alanine	229.60(156.30-280.60):124.30	185.10(112.70-292.00):179.30	145.00(101.40-194.30):92.90	<.0001****
5	Aspartate	20.90(16.70-26.80):10.10	20.60(12.90-33.90):21.00	18.30(10.40-23.20):12.80	.3097
6	Betaine	20.20(12.80-29.90):17.10	18.50(11.80-28.60):16.80	16.80(12.00-24.80):12.80	.2624
7	Choline	15.70(10.90-21.80):10.90	20.30(11.70-27.90):16.20	11.10(7.70-17.20):9.50	.02*
8	Citrate	27.80(18.70-41.50):22.80	30.20(18.80-53.00):34.20	17.70(11.80-33.60):21.80	.0207*
9	Creatine	12.70(7.80-16.60):8.80	15.10(9.30-26.20):16.90	9.60(5.60-14.70):9.10	.23
10	Creatinine	15.70(9.70-18.90):9.20	17.80(11.30-25.20):13.90	13.20(11.30-20.40):9.10	.51
11	Glucose	1.35(0.78-1.77):0.98	1.40(0.79-2.09):1.31	1.30(0.97-1.99):1.03	.27
12	Glutamate	68.40(43.50-109.80):66.30	45.20(32.40-65.30):32.90	41.70(26.50-68.80):42.30	<.0001****
13	Glutamine	170.30(102.80-242.60):139.80	186.30(103.30-256.70):153.40	124.30(91.40-166.10):74.70	.02*
14	Glycerol	35.65(22.08-60.40):38.33	67.50(37.50-109.60):72.10	59.00(34.70-70.30):35.60	.06
15	Glycine	153.70(114.30-199.80):85.50	139.60(96.70-188.80):92.10	107.20(84.40-151.50):67.10	.0023**
16	Isoleucine	26.50(20.50-33.20):12.70	25.90(18.10-36.60):18.50	20.90(15.40-27.30):11.90	<.075
17	Lactate	1.34(1.11-1.73):0.62	1.37(0.80-2.15):1.36	0.75(0.59-1.47):0.88	.0033**
18	Leucine	43.30(31.60-58.20):26.60	43.60(25.60-67.10):41.50	32.50(23.10-50.30):27.20	.0512
19	Methionine	9.30(5.60-11.80):6.20	10.80(7.10-16.30):9.20	7.80(5.10-11.10):6.00	.359
20	DMG	1.70(1.20-2.70):1.50	2.40(1.60-3.80):2.20	2.10(0.90-3.30):2.40	.217
21	Phenylalanine	11.70(7.30-15.10):7.80	17.90(8.70-29.90):21.20	11.70(5.70-23.60):17.90	.163
22	Proline	81.40(50.80-102.90):52.10	86.30(57.00-135.70):78.70	66.70(37.40-91.20):53.80	.1347
23	Pyruvate	10.80(5.60-17.90):12.30	15.50(7.20-31.80):24.60	13.30(4.40-20.30):15.90	.561
24	Succinate	3.30(2.70-4.70):2.00	3.70(2.40-4.80):2.40	3.40(1.70-4.80):3.10	.467
25	Threonine	73.20(47.10-92.70):45.60	67.80(42.50-94.50):52.00	45.60(26.80-69.60):42.80	.0019**
26	Tyrosine	16.50(10.70-21.80):11.10	19.10(11.40-24.40):13.00	13.80(9.50-19.00):9.50	.2654
27	Valine	86.50(62.90-110.80):47.90	82.30(54.60-110.40):55.80	59.50(43.80-77.60):33.80	.0059**
28	Myo-Inositol	14.30(10.40-18.00):7.60	13.50(8.20-17.95):9.75	11.90(9.00-15.00):6.00	.1975
29	GPC	17.90(13.80-22.50):8.70	22.60(17.70-28.90):11.20	20.00(15.60-27.70):12.10	.0656
30	Histidine	42.80(32.10-54.20):22.10	35.50(20.10-44.40):24.30	23.90(17.30-33.40):16.10	<.0001****
31	HTR	2.69(2.34-3.11):0.78	1.91(1.62-2.21):0.59	1.93(1.47-2.24):0.77	<.0001****
32	PTR	0.71(0.59-0.86):0.27	0.99(0.71-1.12):0.41	0.89(0.44-1.29):0.85	.006**
33	ALR	155.10(119.63-189.54):69.91	136.69(108.82-179.42):70.60	161.58(119.22-207.14):87.92	.7582
34	QGR	137.67(112.33-170.61):58.28	124.35(99.30-167.07):67.77	90.22(70.69-110.12):39.43	<.0001****
35	EQR	0.54(0.29-0.70):0.41	0.27(0.21-0.35):0.14	0.32(0.22-0.55):0.33	.03*
36	LPR	0.13(0.09-0.21):0.12	0.07(0.05-0.13):0.08	0.07(0.05-0.15):0.10	.0105*
37	BTR	10.36(8.70-11.36):2.67	8.69(7.52-9.42):1.90	8.65(8.09-9.81):1.72	.0002***

Abbreviations : SD, standard deviation; 3HB, 3-hydroxybutyrate; DMG, Dimethylglycine; GPC, Glycerophosphocholine; PTR, Phenylalanine-to-tyrosine ratio; LPR, Lactate-to-Pyruvate ratio; ALR, Alanine-to-lactate ratio; EQR, Glutamate-to-glutamine ratio; QGR, Glutamine-to-glucose ratio; BTR, Branched chain amino acid to tyrosine ratio (ie, [Leucine+Isoleucine+Valine]/Tyrosine). ***Note*** : The values reported are in micro-molar except for Lactate and glucose ($^{\$}$) for which the values are reported in mM. All the values are estimated using formate as an internal reference (considering its concentration equal to 10 μM). The symbol asterisk "*" represents the metabolic change is statistically significant as per the ANOVA *t*-test (criterion for significance is *P*-value < .05). (Note: these are adjusted *P*-values to control the false discovery rate below .05). The symbol asterisks "*", "**", "***", and "****" represent the statistical significance at the level of *P*-value less than .01, .001, .0001, and .00001, respectively.

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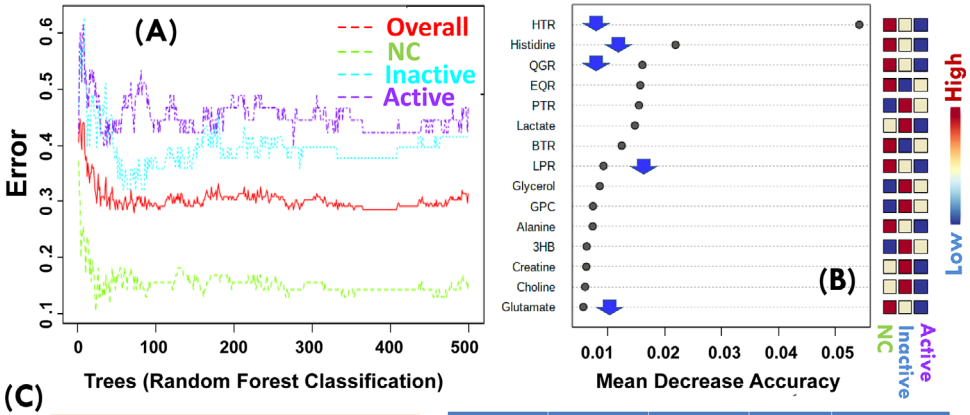

	NC	Inactive	Active	Class.error
NC	65	7	5	0.16
Inactive	9	31	13	0.42
Active	4	17	24	0.47

FIGURE 2 Random Forest (RF) classification analysis performed using Statistical analysis module of MetaboAnalyst. **(A)** Cumulative error rates measured for each class using RF machine learning algorithm. The overall error rate is shown as the red line and other color lines represent the error rates for each class as indicated. **(B)** Significant features were identified by ranking of mean decrease accuracy extracted with RF analysis when the features are permuted. **(C)** The out-of-bag (OOB) error for the model was found to be 31.4% suggesting its moderate prediction accuracy, that is, 68.6%

all the three aromatic amino acids (AAAs, ie, Histidine, Phenylalanine, Tyrosine), only histidine was found to be significantly different between active and inactive TA patients (Figure 3). Further, the various NMR-based circulatory parameters (such as Histidine, Phenylalanine, Tyrosine, PTR, and HTR) were evaluated for their correlation with clinical parameters CRP, ESR and ITAS (See **ESM,** Figure S2). Of five circulatory features, the circulatory metabolite histidine was found showing the highest value of correlation with clinical parameters used to assess the disease activity in TAKAYASU arteritis. The finding further strengthens our proposed hypothesis that immune-mediated active inflammation involves augmented utilization of histidine in the active TA patients compared to that in inactive TA patients.

4 | DISCUSSION

In this study exploring the utility of histidine levels in differentiating active from inactive disease, we found that circulating histidine levels were significantly depressed in TA patients with active disease and showed an inverse correlation with commonly used biomarkers in clinical practice like ESR and CRP.

Histidine is a semi-essential amino acid that exerts anti-inflammatory action through its imidazole ring that scavenges Reactive Oxygen Species (ROS).[10,25] It serves as a precursor for histamine that is an inflammatory peptide stored in the secretory granules of leucocytes and plays an important role in acute inflammation. Histidine supplementation in obese women has shown a decline in inflammatory markers like Tumor Necrosis Factor-alpha and Interleukin (IL)-6 through suppression of nuclear factor kappa- B (NF-kB) in adipocytes.[26] Its shown to negatively regulate IL-8 production through NF-kB in in-vitro studies in intestinal epithelial cells.[27] In patients with chronic kidney disease, histidine negatively correlated with CRP and IL-6.[28] Likewise, low histidine levels have been seen in active Rheumatoid Arthritis (RA) exhibiting a negative correlation with 28-joint disease activity score based on erythrocyte sedimentation rate (DAS28-ESR).[29] Histidine supplementation has shown benefit in animal models of Inflammatory Bowel disease[30] however an older trial in RA did not show a significant benefit.[31] Similarly, the metabolic profile of patients with Systemic Lupus Erythematosus has also revealed a decline in amino acids such as histidine, choline, and phosphocholine representing increased oxidative stress and altered protein metabolism.[23,32] We demonstrate similar findings in our study with low histidine levels in patients with active TA that is also an inflammatory disease. This inverse correlation of low histidine with inflammation can be explained on the basis of the anti-inflammatory action of histidine, or the converse, inflammation itself may result in low levels of circulating amino acids.[33] Like histidine, other amino acids also showed a similar significant decline in the active group as compared to the inactive group of patients (Table 2, Figure 3) that may imply excessive mobilization toward the generation of pro-inflammatory mediators and inflammatory cytokines.[23] Inflammation can induce oxidative stress and chronic oxidative stress is thought to have an important role in the pathogenesis of autoimmune diseases.[34] The elevated PTR levels in TA patients are indicative of oxidative stress[35] and this may have an important role in the pathogenesis of TA. Inflammation is also associated with suboptimal glucose metabolism and resultant shunting of alternative sources of energy like amino acids for energy generation.[8,32,33,36] High concentration of branched-chain amino acids has been reported to promote oxidative stress and inflammation[37]; however, contrary to this, we found significantly lower levels of valine, isoleucine, and leucine in the active group suggesting chronic disease-related energy wasting.[23,38] Since histidine levels solely did not have

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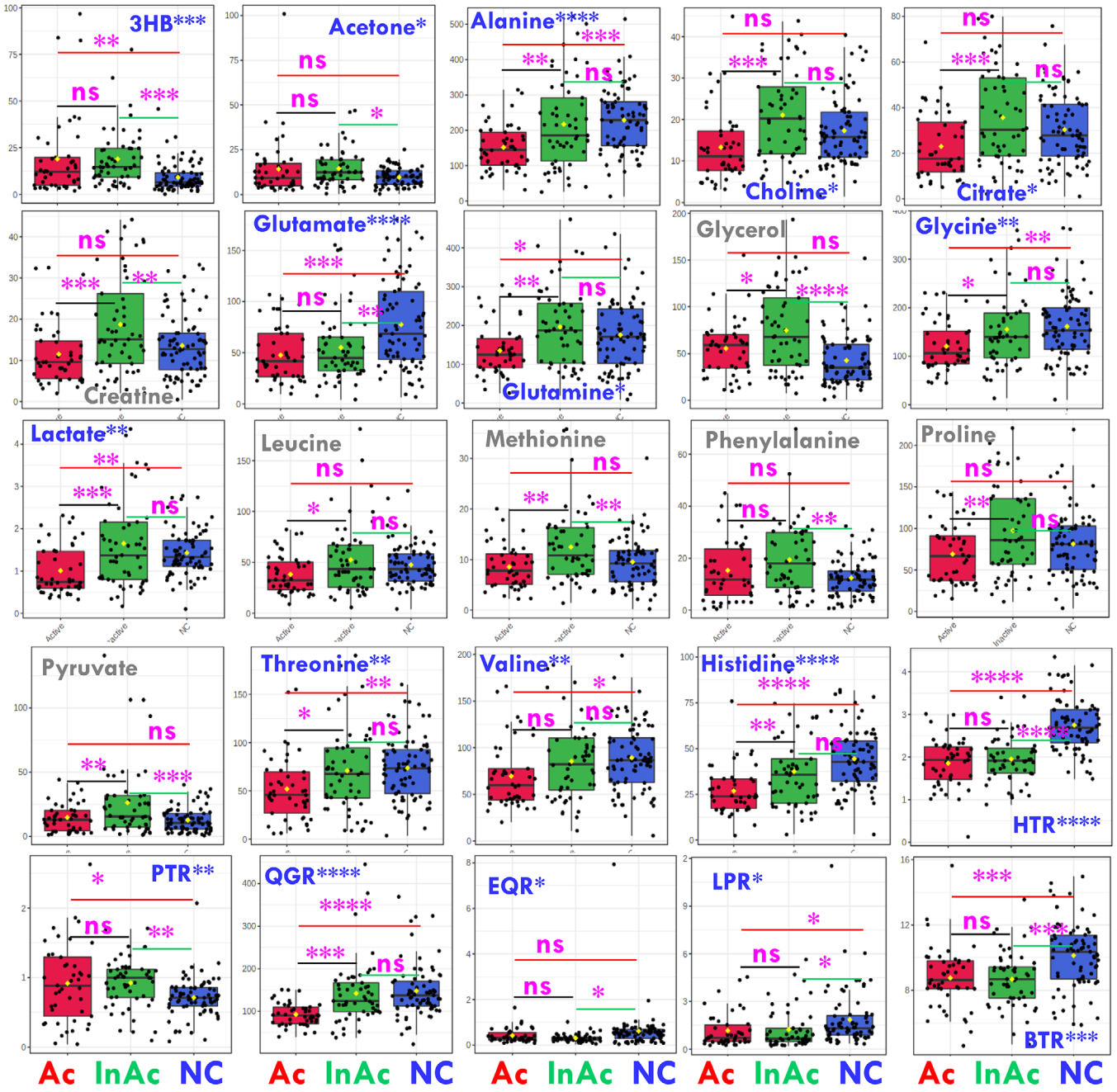

FIGURE 3 The box-cum-whisker plots were obtained for various metabolic concentrations (as per **Table 2**) **and** evaluated for statistically significant changes employing univariate ANOVA-based multiple comparison testing (selected Post-Hoc Analysis is Fisher's LSD). The symbol asterisks "*", "**", "***", and "****" represent the statistical significance at the level of *P*-value (adjusted for false discovery rate, FDR) less than .01, .001, .0001, and .00001, respectively. In each box plot, the box denote interquartile range, horizontal line inside the box denote the median, and bottom and top boundaries of boxes are 25th and 75th percentiles, respectively. Lower and upper whiskers are 5th and 95th percentiles, respectively. As three study groups (ie, normal control, inactive disease, and active disease) have a linear trend of health, we further evaluated the significance of this trend using the linear trend estimation method. The box plot labels in blue represent statistically significant linear metabolic trends between column mean and right-to-left column order (i.e. NC to inactive to active disease). The explicit values are shown in **Table 2**

the sole reliability in differentiating active from inactive disease, it is imperative to explore composite scores or ratios[36] to improve its diagnostic utility for clinical practice. The AUROC values of eight circulatory metabolites were found to be higher than the AUROC value of Histidine (ie, 0.650 with *P*-value = .003); these were as following: Glutamine-to-glucose ratio (QGR; AUROC = 0.76; *P*-value = 5.70E-5), Choline (AUROC: 0.710; *P*-value = 2.7508E-4), Citrate (AUROC: 0.694, *P*-value = 7.0799E-4); Lactate (AUROC: 0.690; *P*-value = 5.9401E-4), Creatine AUROC: 0.684; *P*-value = 7.1206E-4), Glutamine (AUROC: 0.676; *P*-value = 8.9467E-4), Methionine (AUROC: 0.670;

P-value = .001954), Alanine (AUROC: 0.651; *P*-value = .0022172). The results of the ROC analysis are summarized in the Supporting Information (Figure S3 and Table S3).

Important to be included here is that histidine is a precursor of histamine. Histamine and the immune cells that produce it (such as mast cells and basophils) are involved in various autoimmune diseases.[10,39] The histamine exerts its actions through four known receptors (H1R, H2R, H3R, and H4R) and of four, the histamine H4 receptor (H4R) has been shown to drive inflammatory responses in various clinical/preclinical models of autoimmune diseases.[39–41] Recently, a study has also established the coordination between histidine transporter SLC15A4 and mTOR-dependent inflammatory responses.[42] These various facts further strengthen the finding of this study that there is augmented utilization of histidine during immune-mediated active inflammatory condition and targeting of histamine H4 receptor (through designing novel antagonists) may be useful in treating autoimmune diseases including TA as proposed recently.[39,41]

5 | CONCLUDING REMARKS

It is the first study, to the best of our knowledge, exploring circulatory histidine levels in patients with TA. Compared to inactive TA patients, the histidine levels were significantly decreased in the sera of active TA patients. Further, the histidine levels in TA patients were found significantly correlated with clinical scores of disease activity suggesting that these might indicate disease activity in TA. Another important finding of the study is that the circulatory HTR levels are significantly decreased in TA patients (irrespective of disease activity) compared to NC subjects, whereas there is no significant difference in HTR levels between active and inactive TA patients. Therefore, it may serve as a useful biomarker for therapeutic monitoring in TA and opens avenues to limit histidine utilization as a therapeutic option. However, our study has several shortcomings. The most important being that it is a cross-sectional study and thus may fail to reflect the natural course of the disease. ITAS ESR may not be an ideal tool to categorize patients into active disease thus misclassification is a possibility. Nevertheless, this work represents an advance in biomedical science as it is exploring a new marker and suggests moving towards a composite clinical-score as there is no single marker that reliably identifies disease activity in TA. For this, we plan to validate these in a longitudinal manner, with the possible use of imaging and histopathology to assess disease activity in addition to clinical scoring.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: RM and DK. Ethical clearance, patient selection, and sample collection: RM, AJ, SK, and PM. Sample preparation and NMR data collection: UK and VK. Contributed reagents/materials/analysis tools: VK, AG. Data analysis, data interpretation, and preparation of Figures: UK, AG, and DK. Wrote the manuscript: DK, UK, AG, and PM.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study has been uploaded on ZENODO (https://zenodo.org/record/4559294) and is available without undue reservation for further studies on request to the corresponding author.

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REFERENCES

1. Saadoun D, Lambert M, Mirault T, et al. Retrospective analysis of surgery versus endovascular intervention in Takayasu arteritis: a multicenter experience. *Circulation*. 2012;125:813-819.
2. Cong XL, Dai SM, Feng X, et al. Takayasu arteritis: clinical features and outcomes of 125 patients in China. *Clinical rheumatology*. 2010;29:973-981.
3. Aydin SZ, Yilmaz N, Akar S, et al. Assessment of disease activity and progression in Takayasu 's arteritis with Disease Extent Index-Takayasu. *Rheumatology*. 2010;49:1889-1893.
4. Misra R, Danda D, Rajappa SM, et al. Development and initial validation of the Indian Takayasu Clinical Activity Score (ITAS2010). *Rheumatology*. 2013;52:1795-1801.
5. Barra L, Kanji T, Malette J, Pagnoux C. Imaging modalities for the diagnosis and disease activity assessment of Takayasu's arteritis: a systematic review and meta-analysis. *Autoimmunity Reviews*. 2018;17:175-187.
6. Guleria A, Misra DP, Rawat A, et al. NMR-based serum metabolomics discriminates Takayasu arteritis from healthy individuals: a proof-of-principle study. *J Proteome Res*. 2015;14:3372-3381.
7. Jain A, Kumar D, Guleria A, et al. NMR-based serum metabolomics of patients with Takayasu arteritis: relationship with disease activity. *J Proteome Res*. 2018;17:3317-3324.
8. Kumar U, Jain A, Guleria A, et al. Circulatory Glutamine/Glucose ratio for evaluating disease activity in Takayasu arteritis: a NMR based serum metabolomics study. *Journal of Pharmaceutical and Biomedical Analysis*. 2020;180:113080.
9. Profumo E, Buttari B, Rigano R. Oxidative stress in cardiovascular inflammation: its involvement in autoimmune responses. *International journal of inflammation*. 2011;2011.
10. Wade AM, Tucker HN. Antioxidant characteristics of L-histidine. *J Nutr Biochem*. 1998;9:308-315.

11. Liu Wh, Liu Tc, Yin MC. Beneficial effects of histidine and carnosine on ethanol-induced chronic liver injury. *Food and chemical toxicology*. 2008;46:1503-1509.
12. Hasegawa S, Ichiyama T, Sonaka I, et al. histidine and glycine exhibit anti—inflammatory effects in human coronary arterial endothelial cells. *Clinical & Experimental Immunology*. 2012;167:269-274.
13. Sun X, Feng R, Li Y, et al. Histidine supplementation alleviates inflammation in the adipose tissue of high-fat diet-induced obese rats via the NF-κB-and PPARγ involved pathways. *British journal of nutrition*. 2014;112:477-485.
14. Feng RN, Niu YC, Sun XW, et al. Histidine supplementation improves insulin resistance through suppressed inflammation in obese women with the metabolic syndrome: a randomised controlled trial. *Diabetologia*. 2013;56:985-994.
15. Arend WP, Michel BA, Bloch DA, et al. The American College of Rheumatology 1990 criteria for the classification of Takayasu arteritis. *Arthritis Rheum*. 1990;33:1129-1134.
16. Gupta L, Guleria A, Rawat A, Kumar D, Aggarwal A. NMR-based clinical metabolomics revealed distinctive serum metabolic profiles in patients with spondyloarthritis. *Magnetic Resonance in Chemistry*. 2021;59:85-98.
17. Liaw A, Wiener M. Classification and regression by randomForest. *R news*. 2002;2:18-22.
18. Xia J, Sinelnikov IV, Han B, Wishart DS. MetaboAnalyst 3.0-making metabolomics more meaningful. *Nucleic acids research*. 2015;43:W251-W257.
19. Xia J, Psychogios N, Young N, Wishart DS. MetaboAnalyst: a web server for metabolomic data analysis and interpretation. *Nucleic acids research*. 2009;37:W652-W660.
20. Dubey D, Kumar S, Chaurasia S, et al. NMR-based serum metabolomics revealed distinctive metabolic patterns in reactive arthritis compared with rheumatoid arthritis. *Journal of proteome research*. 2018;18:130-146.
21. Chen T, Cao Y, Zhang Y, et al. Random forest in clinical metabolomics for phenotypic discrimination and biomarker selection. *Evidence-Based Complementary and Alternative Medicine*. 2013;2013.
22. Grissa D, Pétéra M, Brandolini M, Napoli A, Comte B. Pujos-Guillot, E. Feature selection methods for early predictive biomarker discovery using untargeted metabolomic data. *Frontiers in molecular biosciences*. 2016;3:30.
23. Suliman ME, Qureshi AR, Stenvinkel P, et al. Inflammation contributes to low plasma amino acid concentrations in patients with chronic kidney disease. *The American journal of clinical nutrition*. 2005;82:342-349.
24. Geisler S, Gostner JM, Becker K, Ueberall F, Fuchs D. Immune activation and inflammation increase the plasma phenylalanine-to-tyrosine ratio. *Pteridines*. 2013;24:27-31.
25. Peterson JW, Boldogh I, Popov VL, Saini SS, Chopra AK. Anti-inflammatory and antisecretory potential of histidine in Salmonella-challenged mouse small intestine. *Laboratory investigation; a journal of technical methods and pathology*. 1998;78:523-534.
26. Feng RN, Niu YC, Sun XW, et al. Histidine supplementation improves insulin resistance through suppressed inflammation in obese women with the metabolic syndrome: a randomised controlled trial. *Diabetologia*. 2013;56:985-994.
27. Son DO, Satsu H, Shimizu M. Histidine inhibits oxidative stress-and TNF-alpha-induced interleukin-8 secretion in intestinal epithelial cells. *FEBS letters*. 2005;579:4671-4677.
28. Watanabe M, Suliman ME, Qureshi AR, et al. Consequences of low plasma histidine in chronic kidney disease patients: associations with inflammation, oxidative stress, and mortality. *Am J Clin Nutr*. 2008;87:1860-1866.
29. Sasaki C, Hiraishi T, Oku T, et al. Metabolomic approach to the exploration of biomarkers associated with disease activity in rheumatoid arthritis. *PLoS One*. 2019;14:e0219400.
30. Andou A, Hisamatsu T, Okamoto S, et al. Dietary histidine ameliorates murine colitis by inhibition of proinflammatory cytokine production from macrophages. *Gastroenterology*. 2009;136:564-574.
31. Pinals RS, Harris ED, Burnett JB, Gerber DA. Treatment of rheumatoid arthritis with L-histidine: a randomized, placebo-controlled, double-blind trial. *The Journal of rheumatology*. 1977;4:414.
32. Kominsky DJ, Campbell EL, Colgan SP. Metabolic shifts in immunity and inflammation. *The Journal of Immunology*. 2010;184:4062-4068.
33. Fox CJ, Hammerman PS, Thompson CB. Fuel feeds function: energy metabolism and the T-cell response. *Nature Reviews Immunology*. 2005;5:844-852.
34. Kumagai S, Jikimoto T, Saegusa J. Pathological roles of oxidative stress in autoimmune diseases. *Rinsho byori The Japanese Journal of Clinical Pathology*. 2003;51:126-132.
35. Muhammed H, Kumar D, Dubey D, et al. Metabolomics analysis revealed significantly higher synovial Phe/Tyr ratio in reactive arthritis and undifferentiated spondyloarthropathy. *Rheumatology*. 2020;59:1587-1590.
36. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. *Immunity*. 2013;38:633-643.
37. Zhenyukh O, Civantos E, Ruiz-Ortega M, et al. High concentration of branched-chain amino acids promotes oxidative stress, inflammation and migration of human peripheral blood mononuclear cells via mTORC1 activation. *Free Radical Biology and Medicine*. 2017;104:165-177.
38. Holecek M. Branched-chain amino acids in health and disease: metabolism, alterations in blood plasma, and as supplements. *Nutrition & metabolism*. 2018;15:33.
39. Zhang M, Venable JD, Thurmond RL. The histamine H4 receptor in autoimmune disease. *Expert opinion on investigational drugs*. 2006;15:1443-1452.
40. Cowden JM, Yu F, Banie H, et al. The histamine H4 receptor mediates inflammation and Th17 responses in preclinical models of arthritis. *Annals of the rheumatic diseases*. 2014;73:600-608.
41. Zhang M, Thurmond RL, Dunford PJ. The histamine H4 receptor: a novel modulator of inflammatory and immune disorders. *Pharmacology & therapeutics*. 2007;113:594-606.
42. Kobayashi T, Shimabukuro-Demoto S, Yoshida-Sugitani R, et al. The histidine transporter SLC15A4 coordinates mTOR-dependent inflammatory responses and pathogenic antibody production. *Immunity*. 2014;41:375-388.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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