Original Article

Methionine Adenosyltransferase II Alpha Upregulation Correlates with Plasma Chitotriosidase Activity in *Helicobacter pylori*-Negative Gastric Cancer: A Hypothesis-Generating Pilot Study of a Candidate Tissue Biomarker

Fatemeh Babajani[1], PhD candidate; Mohammad Reza Kalhori[2], PhD; Hadi Mozafari[1,3], PhD

[1]Department of Clinical Biochemistry, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran;
[2]Fertility and Infertility Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran;
[3]Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

Correspondence:
Hadi Mozafari, PhD;
Department of Clinical Biochemistry, School of Medicine, Kermanshah University of Medical Sciences, Daneshgah St., Shahid Shiroudi Blvd., Postal code: 6714-869914, Kermanshah, Iran
Email: hmozafari@kums.ac.ir
Received: 23 February 2026
Revised: 01 June 2026
Accepted: 03 July 2026

What's Known

- Methionine adenosyltransferase 2 alpha is a key enzyme in one-carbon metabolism that synthesizes S-adenosylmethionine and is frequently upregulated in various cancers. Chitotriosidase is an enzyme secreted by activated macrophages and serves as a biomarker of macrophage activation in inflammatory diseases.

What's New

- This study is the first to demonstrate that methionine adenosyltransferase 2 is significantly upregulated in *Helicobacter pylori*-negative gastric cancer tissues with high diagnostic accuracy (area under the curve=0.848). Additionally, significant positive correlations were found between plasma chitotriosidase activity and the tumoral expression of both methionine adenosyltransferase 2 and chitotriosidase.

Abstract

Background: Gastric cancer (GC) remains a major global health threat. Chronic inflammation is a key driver of gastric carcinogenesis, influencing both the tumor microenvironment and cellular metabolism. This study aimed to evaluate Methionine Adenosyltransferase II Alpha *(MAT2A)* and Chitotriosidase *(CHIT1)* expression in *H. pylori*-negative GC, assess MAT2A as a biomarker, and explore correlations with plasma CHIT1 activity and clinical features.

Methods: In this hypothesis-generating pilot case-control study conducted in Kermanshah (September 2024 to July 2025), 27 tumor tissues and 27 matched adjacent noncancerous tissues from GC patients were analyzed for expression of *MAT2A* and *CHIT1* using qRT-PCR. *Helicobacter pylori*-positive patients were excluded from the study. For CHIT1 activity, plasma samples were obtained from the same 27 GC patients and 32 healthy control individuals. CHIT1 activity was assessed by a fluorometric method. Paired *t* test, independent *t* test, and Pearson's test were used.

Results: *MAT2A* was significantly upregulated in tumor tissues compared to matched adjacent noncancerous tissue (fold change=3.863, P<0.001). The Area under the curve (AUC) for *MAT2A* expression was 0.848, with a sensitivity of 74.1% and specificity of 81.5% at the optimal cut-off value of 7.78 (AUC=0.848±0.05, 95% CI=0.7472-0.9497). The *CHIT1* expression (fold change=1.347, P=0.221) and activity in the patient group (106.67±48.33 nmol/mL/h) compared to the healthy control (92.16±34.01 nmol/mL/h) (P=0.183) showed no significant differences between groups. Significant positive correlations were found between CHIT1 activity and the expression of both *MAT2A* (r=0.32, P=0.042) and *CHIT1* (r=0.30, P=0.048).

Conclusion: Our results showed that MAT2A can act as a candidate tissue biomarker in gastric carcinogenesis. A weak but significant positive correlation was found between *MAT2A* expression and CHIT1 activity, suggesting a possible link between tumor metabolism and systemic macrophage activation. Therefore, selective targeting of the MAT2A pathway may be a promising approach for GC treatment. However, further functional and mechanistic studies are required to validate this therapeutic potential.

Please cite this article as: Babajani F, Kalhori MR, Mozafari H. Methionine Adenosyltransferase II Alpha Upregulation Correlates with Plasma Chitotriosidase Activity in *Helicobacter pylori*-Negative Gastric Cancer: A Hypothesis-Generating Pilot Study of a Candidate Tissue Biomarker. Iran J Med Sci. doi: 10.30476/ijms.2026.110866.4671.

Keywords ● Stomach neoplasms ● Genetics, Methionine adenosyltransferase ● Chitotriosidase ● *Helicobacter pylori* ● Biomarkers

Copyright: ©Iranian Journal of Medical Sciences. This is an open-access article distributed under the terms of the Creative Commons Attribution-NoDerivatives 4.0 International License. This license allows reusers to copy and distribute the material in any medium or format in unadapted form only, and only so long as attribution is given to the creator. The license allows for commercial use.

Introduction

Gastric cancer (GC) remains a global health challenge, ranking as the fifth most common malignancy and the fourth leading cause of cancer-related mortality worldwide.[1] Despite advances in diagnostic and therapeutic strategies, the prognosis for advanced-stage disease remains disappointing. Thus, it is also important to further understand its molecular pathogenesis and identify reliable biomarkers for early diagnosis and novel therapeutic targeting.

The carcinogenesis of GC is a multifactorial process, with chronic inflammation serving as a critical driver.[2] This inflammatory condition orchestrates a cascade of oxidative stress, genetic instability, and epigenetic reprogramming that propels malignant transformation.[2] The key cellular mediators of this inflammation are Chitotriosidase (CHIT1), an enzyme secreted by activated macrophages,[3] and Methionine Adenosyltransferase II Alpha (MAT2A), a central metabolic enzyme that catalyzes the synthesis of S-adenosylmethionine (SAM), the universal methyl donor for DNA and histone methylation.[4]

MAT2A has emerged as a fundamental enzyme in cellular metabolism and epigenetics. It catalyzes the synthesis of SAM, the primary methyl donor for many methylation reactions, including those modifying DNA and histones.[5] Given the high demand for SAM in rapidly proliferating cancer cells, *MAT2A* is frequently upregulated in various malignancies, such as liver, colorectal, and pancreatic cancers.[6, 7] Its role in maintaining one-carbon metabolism and supporting nucleotide synthesis positions it as a central metabolic node for cancer cell survival and growth.[8] Importantly, SAM generated by MAT2A serves as the essential substrate for DNA methyltransferases (DNMTs) and histone methyltransferases (HMTs).[4] Consequently, upregulation of *MAT2A* and the subsequent increase in SAM availability can lead to aberrant epigenetic alterations, including hypermethylation of tumor suppressor gene promoters and repressive histone methylation marks such as H3K27me3. These epigenetic changes are known to contribute to gastric carcinogenesis by silencing key differentiation genes and maintaining a proliferative state.[9, 10] Therefore, MAT2A may link cellular metabolism to epigenetic reprogramming in gastric cancer. However, its expression pattern, diagnostic utility, and functional significance in GC remain largely unexplored.

On the other hand, CHIT1, an enzyme secreted by activated macrophages, has been implicated in a range of inflammatory conditions.[11] Its role in cancer, however, remains less clearly defined. Although emerging evidence suggests that CHIT1 may contribute to tumor-associated macrophage (TAM) activity, inflammatory signaling, and tumor progression, its expression and potential biological significance within the gastric tumor microenvironment remain poorly characterized.[3] Furthermore, the correlation between tumor tissue gene expression of *CHIT1* and its systemic enzymatic activity in plasma in cancer patients is not well understood.

Most studies on MAT2A and CHIT1 in GC have been conducted on unselected patient populations, without considering *H. pylori* infection status. Given that *H. pylori* infection profoundly affects gastric inflammation, immunity, and carcinogenesis, the role of these molecular markers may differ in *H. pylori*-negative patients. However, no study to date has specifically investigated *MAT2A* and *CHIT1* expression in *H. pylori*-negative GC. Therefore, this subgroup remains understudied, and the diagnostic and prognostic value of these markers in this specific population is unknown.

The present study aimed to investigate the expression of MAT2A and CHIT1 in tumor tissues compared to matched adjacent noncancerous tissues in patients with *H. pylori*-negative GC. Furthermore, we evaluated the diagnostic potential of MAT2A as a tissue biomarker and assessed the correlation between tumoral gene expression of *MAT2A* and *CHIT1* and systemic plasma CHIT1 activity. This study also aimed to explore the relationship between these molecular markers and patients' clinicopathological characteristics.

Materials and Methods

Patients

In this case-control study, a total of 27 GC patients were enrolled. For tissue gene expression analysis, paired tissue samples including tumor tissues and matched adjacent noncancerous tissues (marginal tissues) were obtained from the same 27 GC patients. The adjacent noncancerous tissues served as the control group for gene expression analysis. The samples were collected from the Iran Tumor Bank at Tehran University of Medical Sciences, following established inclusion and exclusion criteria. Initially, an internal medicine specialist identified lesions in new patients through endoscopy. For a definitive diagnosis, biopsy samples were taken from the gastric tissue during surgery, with final results provided by a pathologist. Patients who had undergone chemotherapy or radiotherapy were excluded.

Additionally, due to the known impact of *H. pylori* on inflammation and GC, samples positive for this bacterium were also excluded. Inclusion criteria for healthy control individuals were: no prior history of any malignancy, no acute or chronic inflammatory diseases, no history of gastrointestinal disorders, and no history of surgery or hospitalization. Exclusion criteria were: history of any malignancy, active infection or inflammatory disease, known autoimmune disorders, pregnancy or lactation, and use of immunosuppressive drugs within the past 6 months. All tissues were tested for *H. pylori*, which was reported by the tumor bank (through real-time PCR and histopathological methods) to ensure uniformity across samples. For plasma enzyme activity measurement, peripheral blood samples were collected from the same 27 GC patients. Additionally, blood samples were collected from 32 healthy individuals to serve as a separate healthy control group for plasma chitotriosidase activity measurement. Thus, two distinct control groups were used in this study: matched adjacent noncancerous tissues (n=27) for gene expression analysis, and healthy individuals (n=32) for plasma enzyme activity analysis. Five mL peripheral blood was collected from each subject, mixed with EDTA, and then plasma and cell pellets were kept at −80 °C until the time of analysis.

Based on previous studies[10, 12] evaluating *MAT2A* expression in GC using quantitative reverse transcription PCR (qRT-PCR) and considering the hypothesis-generating pilot nature of this study, the sample size was calculated using the standard formula for two independent means. Using data from previous studies, with α=0.05 (Z=1.96) and power=90% (Z=1.28), the minimum required sample size was 13 per group. To account for potential sample loss and biological variability, we enrolled 27 patients per group, which is consistent with previous studies. Using the formula for sample size calculation in case-control studies:

$$n = \frac{(z_{1-\alpha/2} + z_{1-\beta})^2(\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2}$$

Informed consent was obtained from all GC patients and from all healthy control individuals before participation in the study. The present study was approved by the Research Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran (IR.KUMS.MED.REC.1403.021).

Total RNA Extraction, cDNA Synthesis, and qRT-PCR

The gastric tissue was first homogenized in a mortar with liquid nitrogen, and total RNA was extracted using the Trizol solution (YT9065, Yekta Tajhiz Azma Company, Iran). The RNA was then dissolved in DEPC-treated water (CinnaGen Company, Iran), and the integrity of the extracted RNA was assessed by electrophoresis on a 1% agarose gel. RNA concentration and purity were measured using a NanoDrop spectrophotometer. All samples had appropriate concentrations for subsequent cDNA synthesis. The absorbance ratio (A260/A280) for all RNA samples ranged between 1.8 and 2.0, indicating acceptable purity without significant protein or phenol contamination. In continuation of the work, the RNA was prepared for subsequent analysis. cDNA synthesis was performed with a Parstous Company cDNA Synthesis Kit (A101162, Parstous Company, Iran), which included an RT primer mix containing optimized oligo-dT, random primers, and reverse transcriptase. qRT-PCR was conducted using a real-time cycler in a clinical biochemistry laboratory with a Corbett 6000 Real-Time PCR thermocycler (Qiagen Company, Australia) and RealQ Plus 2x Master Mix Green without ROX (A323402, Ampliqon Company, Denmark). The properties and sequences of the primers (Pishgam Biotech Company, Iran) are shown in table 1. The amplification efficiency of each primer pair (β-actin, MAT2A, and CHIT1) was determined by constructing standard curves using serial dilutions of cDNA. All primer pairs exhibited amplification efficiencies close to 100% (β-actin: 98.5%, MAT2A: 99%, CHIT1: 99.2%). The expression levels of the target genes were measured relative to the housekeeping gene β-actin. Finally, relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

The Plasma CHIT1 Activity

The CHIT activity was measured using a fluorimetric method based on the protocol described by Hollak and others.[13] In this procedure, 5 µL of plasma was combined with 100 µL of a 0.022 mmol/L solution of 4-methylumbelliferyl β-D-N,N′,N″-triacetylchitotrioside (4MU-chitotrioside) (Sigma-Aldrich Company, USA) in McIlvaine's buffer. The mixture was then incubated at 37 °C for 30 min in a dark environment. The reaction was terminated by adding 1395 µL of 0.2 M Na_2CO_3/glycine buffer with a pH of 10.5. The resulting fluorescence was measured using a Biotek Synergy H1m Microplate Reader (Biotek Company, USA) with excitation at 366 nm and emission at 448 nm. Enzyme activities were calculated against a calibration curve of 4-methylumbelliferone (4-MU) for each assay.

Table 1: Primers sequences and their characteristics

Gene	Primers	GC%	Tm	Product length
β actin				
Forward	5'-CATGTACGTTGCTATCCAGGC-3'	52.38	59.13	250
Reverse	5'-CTCCTTAATGTCACGCACGAT-3'	47.62	58.46	
CHIT1				
Forward	5'-CTGCATCATGGTGCGGTC-3'	61.11	58.90	156
Reverse	5'-CAAGGCAAGGCTGAGAGC-3'	61.1	58.41	
MAT2A				
Forward	5'-ACAATCTACCACCTACAGCCAGT-3'	47.83	61.08	248
Reverse	5'-GCATAAGAGACCTGAACAAGAACC-3'	45.83	59.61	

CHIT1: Chitotriosidase; MAT2A: Methionine adenosyl transferase II alpha

Table 2: Demographic information of patients with gastric cancer

Characteristic	Categorization	n (%)
Age	≤65	8 (29.6%)
	>65	19 (70.4%)
Alcohol	Drinker	6 (22.2%)
	Non-drinker	21 (77.8%)
Smoking status	Non smoker	21 (77.8%)
	Smoker	6 (22.2%)
Family history	Yes	12 (44.4%)
	No	15 (55.6%)

Statistical Analysis

Statistical analysis was performed using SPSS version 16.0 (IBM, USA), GraphPad Prism version 8 (GraphPad Software, Inc., USA), and RStudio version 4.4.2 (Posit Software, PBC, USA). The normality of data distribution was assessed using the Shapiro-Wilk test. For tissue gene expression comparisons, tumor and matched adjacent noncancerous tissues were obtained from the same patients (dependent groups). The paired *t* test was used to compare *MAT2A* and *CHIT1* expression between tumor and marginal tissues. For plasma CHIT1 activity, GC patients and healthy controls represented independent groups. Therefore, the independent *t* test was used to compare enzyme activity between the two groups. Pearson's test was used to examine the correlation between groups. Receiver Operating Characteristic (ROC) curve analysis was performed to evaluate the diagnostic potential of MAT2A mRNA expression. The AUC, 95% confidence interval (CI), and P value were calculated. To externally validate our findings, we analyzed *MAT2A* expression in the TCGA stomach adenocarcinoma (STAD) dataset using the UALCAN platform.[14] Analysis of 415 gastric tumor tissues and 34 normal tissues confirmed significantly higher *MAT2A* expression in tumor tissues than normal controls (P<0.001), which is consistent with our qRT-PCR results. All data were presented as mean±SD, with a significance threshold set at P<0.05.

Results

Demographic and Clinicopathological Information of Patients

A total of 59 individuals participated in the present study. Therefore, the healthy control group consisted of 32 individuals, which explains the difference in numbers between cases (n=27) and healthy controls (n=32). The mean age of the GC patients was 67.19±11.07 years, while the mean age of healthy control subjects was 69.50±7.28 years. In addition, the mean age was 69.53±9.07 years for males and 67.31±9.35 years for females. The difference in age between the two groups was not statistically significant.

The demographic information of patients, including age, history of alcohol and smoking, and family history of the disease, is shown in table 2. In addition, the patients' frequency of pathological information, including tumor size, histology, tumor grading, vascular and lymphatic invasion, tumor staging (primary tumor, lymph node involvement, and metastasis), and TNM stage, is presented in table 3.

Expression of MAT2A and CHIT1 in Gastric Tumor Tissues

The expression of *MAT2A* was significantly upregulated in tumor tissues compared to the marginal tissues (P<0.001, figure 1A).

In contrast, the expression level of *CHIT1* in tumor tissues did not show a statistically significant difference when compared to the marginal tissues (P=0.221, figure 1B).

Table 3: Frequency of clinical (pathological) information of patients

Characteristic	Categorization	n (%)
Tumor Size	≤3 cm	11 (40.7%)
	>3 cm	16 (59.3%)
Tumor Histology	Adenocarcinoma	20 (74.1%)
	Mucinous (Colloid) adenocarcinoma	7 (25.9%)
Histology grade	Grade I (Well differentiated)	7 (25.9%)
	Grade II (Moderately differentiated)	7 (25.9%)
	Grade III (Poorly differentiated)	11 (40.7%)
	Grade IV (Undifferentiated)	2 (7.4%)
Pathological T	T1, N0S	2 (7.4%)
	T1b	2 (7.4%)
	T2, N0S	3 (11.1%)
	T2a	1 (3.7%)
	T3	18 (66.7%)
	T4	1 (3.7%)
Pathological N	N0	7 (25.9%)
	N1	7 (25.9%)
	N2	9 (33.3%)
	N3	4 (14.8%)
TNM staging	Stage I, II	10 (37%)
	Stage III, IV	17 (63%)
Vascular invasion	Yes	21 (77.8%)
	No	6 (22.2%)
Lymphatic invasion	Yes	18 (66.7%)
	No	9 (33.3%)

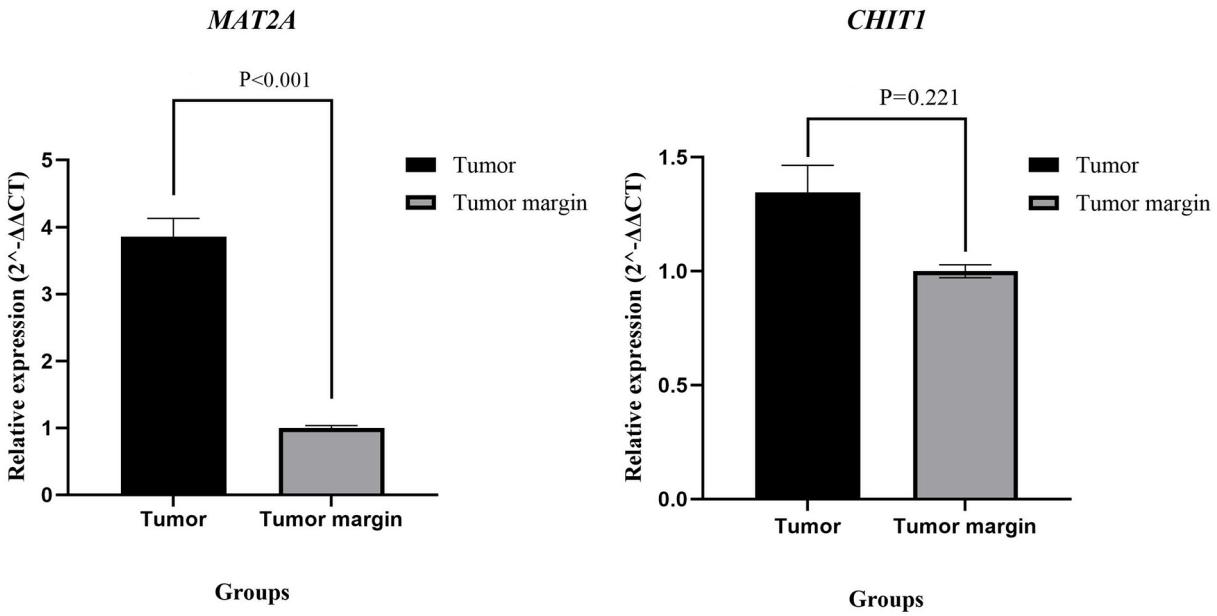


Figure 1: Gene expression analysis of *MAT2A* and *CHIT1* in gastric tissues is presented. Relative expression levels of *MAT2A* and *CHIT1* were measured by quantitative real-time PCR and calculated using the 2^-ΔΔCT method. *β-actin* was used as the housekeeping gene for normalization. (A) *MAT2A* expression is significantly higher in tumor tissues compared to matched adjacent noncancerous tissues (P<0.001, paired *t* test). (B) No significant difference was found in *CHIT1* expression between tumor and marginal tissues (P=0.221, paired *t* test). All data are presented as mean±SD.

Assessing MAT2A as Potential Biomarkers

In the present study, the ROC (Receiver operating characteristic) analysis was applied to investigate the mRNA expression of studied genes as potential biomarkers. As shown in figure 2, the AUC for MAT2A mRNA expression was 0.848 (95% CI: 0.7472-0.9497; P<0.001). At the optimal cut-off value of 7.78, the sensitivity was 74.1%, and specificity was 81.5% (Youden index=0.555).

Plasma CHIT1 Activity

The mean plasma CHIT1 activity was higher in the patient group (106.67±48.33 nmol/mL/h)

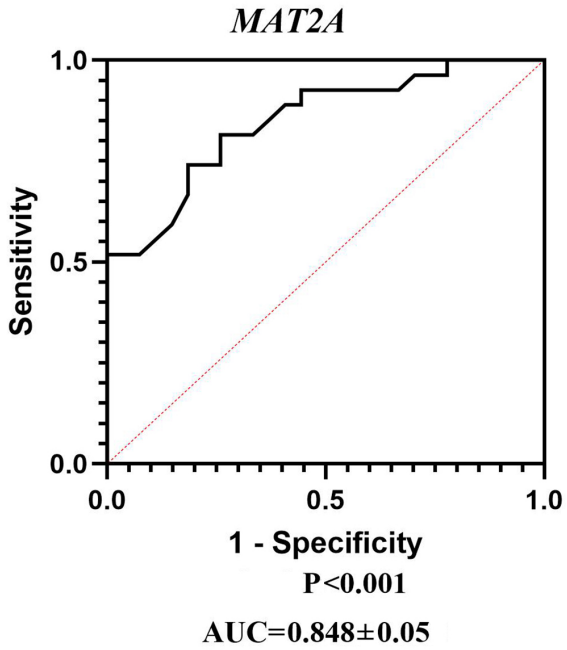


Figure 2: Evaluation of *MAT2A* as a potential tissue biomarker is presented using Receiver Operating Characteristic (ROC) curve analysis. The ROC curve demonstrates the diagnostic accuracy of *MAT2A* gene expression for distinguishing gastric tumor tissues from matched adjacent noncancerous tissues. The area under the curve (AUC) was 0.848 (95% CI: 0.7472-0.9497; P<0.001). At the optimal cut-off value of 7.78, the sensitivity was 74.1%, and the specificity was 81.5% (Youden index=0.555).

compared to the healthy control group (92.16±34.01 nmol/mL/h) (P=0.183), showing no significant differences between groups.

Correlation between Tumor Gene Expression and Plasma Enzyme Activity

As indicated in figure 3, a weak but statistically significant positive correlation was observed between the expression of tumor_MAT2A and plasma CHIT1 activity (r=0.32, P=0.042). Similarly, a significant correlation was found between Tumor_CHIT1 expression and plasma enzyme activity (r=0.30, P=0.048).

However, the correlation between the expression levels of the two genes within the tumor tissue, tumor_CHIT1 and tumor_MAT2A, was very weak and not significant (r=0.16). Moreover, we analyzed the correlation between MAT2A expression and clinicopathological features, including tumor stage (TNM), histologic grade, vascular invasion, and lymphatic invasion. No significant correlations were found between MAT2A expression and any of these parameters.

Discussion

In the present study, we found that *MAT2A* gene expression was significantly upregulated in tumor tissues of patients with *H. pylori*-negative GC, demonstrating high diagnostic accuracy. As well as, our finding of increased MAT2A expression could be consistent with the known role of this enzyme in epigenetics for rapidly proliferating cancer cells. We hypothesize that *MAT2A* upregulation may alter DNA and histone methylation patterns, but direct measurement of SAM levels or methylation status is needed to confirm this in future studies. Previous studies in cancers such as pancreas and liver have shown that MAT2A is critical for the synthesis of SAM, and SAM provides methyl groups for DNA and RNA methylation, a process that often leads to the silencing of tumor suppressor genes and the activation of tumorigenic pathways.[15, 16] Therefore, *MAT2A* upregulation may contribute to hypermethylation of tumor suppressor genes and, by modulating histone methylation patterns, it reprograms cell transcription. This possibly indicates that increased MAT2A expression could be an independent mechanism in tumorigenesis in these patients and could also make it a tissue biomarker.[17] The significant upregulation of *MAT2A* in our study suggests its involvement in critical oncogenic pathways beyond metabolic support.[18] In GC, possibly, overproduction of SAM guided by *MAT2A* primarily supports dysregulated DNA and histone methylation programs. Specifically, SAM serves as the primary methyl donor for DNA methyltransferases (DNMTs), potentially might play a role in the silencing of differentiation genes such as CDKN2A (p16) and MLH1, which can frequently implicated in gastric carcinogenesis.[9, 19]

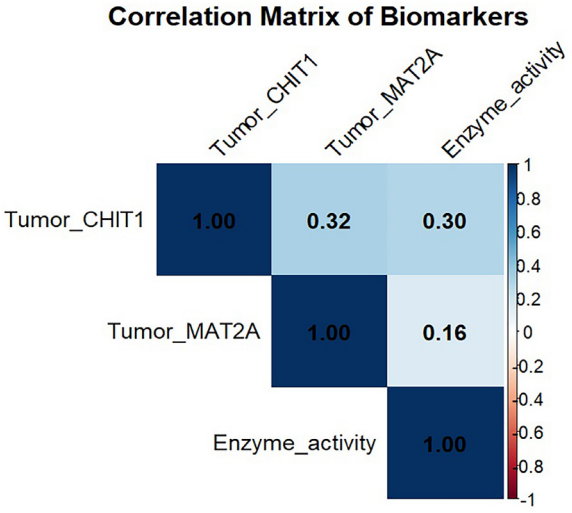


Figure 3: A correlation heatmap of the studied biomarkers is illustrated. The correlation matrix shows the relationships between tumor gene expression (Tumor_CHIT1 and Tumor_MAT2A) and plasma CHIT1 activity. The color intensity and the numbers within the cells represent the correlation coefficient (r). R software (version 4.4.2) was used to generate the correlation diagram.

Concurrently, SAM availability regulates histone methyltransferases and may repress H3K27me3 markers at the promoters of key differentiation genes. Furthermore, MAT2A is a crucial regulator of the polyamine synthesis pathway. SAM is decarboxylated to provide an aminopropyl group for the synthesis of spermidine and spermine. These polyamines are essential for cell growth and are often elevated in cancer. Therefore, *MAT2A* overexpression in GC may simultaneously guide both the epigenetic alterations and the high polyamine levels required for sustained tumor growth.

We speculate that this potential epigenetic silencing, coordinated by MAT2A, could promote an undifferentiated state.[10] In addition, Ma and others demonstrated that MAT2A protects GC cells from ferroptosis through the MAT2A-ACSL3 pathway and reported increased MAT2A expression in GC tissues, which is consistent with our findings.[10] However, our study extends these observations in several ways. We evaluated the diagnostic potential of MAT2A as a possible tissue biomarker, which was not investigated by Ma and others. In addition, we demonstrated a significant correlation between tumoral MAT2A expression and systemic CHIT1 activity, suggesting a possible link between tumor metabolism and macrophage activation. Additionally, our study specifically focused on *H. pylori*-negative GC patients, a subgroup not specifically examined in Ma and others' study. On the other hand, Ma and others provided protein-level validation (Western blot and immunohistochemistry) and functional data (ferroptosis assays), which our study lacks. These differences in study design and sample characteristics should be considered when comparing the results of these two studies.

In addition, the expression level of CHIT1 in tumor tissues did not show a statistically significant difference compared to the marginal tissues. Further, the lack of significant changes in tissue expression of CHIT1, a macrophage-dependent enzyme, could possibly indicate that the cancerous epithelial cells themselves are not the main source of this molecule.[20] This observation aligns with a growing body of evidence indicating the complex and non-linear role of CHIT1 in oncology. For instance, a recent pilot study by Nechita and others also reported no significant difference in baseline plasma CHIT1 activity between gastric adenocarcinoma patients undergoing curative-intent surgery versus those with advanced disease suited for palliative procedures.[21] This convergence suggests that absolute levels of CHIT1 expression or activity may not, by themselves, serve as a sensitive standalone diagnostic or staging biomarker for GC, likely due to the influence of more complex regulatory mechanisms and patient-specific factors.

Moreover, notable correlations were observed, such that tumor expression of both *MAT2A* and *CHIT1* genes independently showed a positive correlation with the level of systemic activity of the enzyme CHIT1. This positive correlation between tumor CHIT1 expression and plasma enzyme levels could strengthen the hypothesis that the infiltrating macrophage population in the microtumor environment may be the source of this enzyme, even in the absence of a major inflammatory stimulus.[22] To further clarify this relationship, future studies focusing on immunohistochemical staining to accurately identify and quantify CHIT1-expressing macrophages in tumor tissue, as well as to measure the protein levels of this enzyme, seem necessary. Moreover, this correlation may indicate a complex interaction between tumor metabolism and the host immune system.[23] One possible reason could be that the altered metabolic state in the tumor, characterized by increased MAT2A expression, alters the tumor microenvironment in a way that affects the activity and behavior of tumor-resident immune cells, including macrophages.[24] Furthermore, increased SAM levels in the tumor environment probably influence methylation pathways in immune cells, leading to polarization of macrophages towards a pre-tumorigenic M2 phenotype. These modulated macrophages secrete factors such as CHIT1, creating an inflammatory and immunosuppressive environment that contributes to tumor growth and spread. Weiwei Yu and others demonstrated that SAM production is essential for the activation of inflammatory macrophages, and the metabolic pathways synergistically guide epigenetic reprogramming. As a result, these modulated macrophages may secrete higher levels of CHIT1, which is reflected in the systemic circulation.[25] The correlation between tumoral MAT2A expression and plasma CHIT1 activity was weak but statistically significant ($r=0.32$, $P=0.042$). This finding suggests a possible association between tumor metabolism and systemic macrophage activation. However, causation cannot be inferred from this correlational data. Further functional studies are required to establish any causal relationship between MAT2A expression and CHIT1 activity.

Although future studies are necessary to precisely define the signaling pathways downstream of SAM and to characterize the dynamics of macrophage infiltration and

differentiation in the microtumor environment, the present findings provide a basis for developing novel therapeutic strategies. On the other hand, the selective targeting of the MAT2A/SAM pathway may simultaneously inhibit cancer cell growth by disrupting epigenetic programming and alter the tumor immunological environment to an antitumor state. Therefore, this strategy may be a promising approach in the treatment of this specific subtype of gastric cancer. However, it is important to note that our conclusions regarding therapeutic potential are based on correlative data. Future functional studies, including *MAT2A* knockdown or inhibition in gastric cancer cell lines, as well as *in vivo* animal models, are necessary to establish the causal role of *MAT2A* in gastric carcinogenesis and to validate its therapeutic targeting.

The limitations of this study included that gene expression was measured only at the mRNA level, and protein expression was not evaluated. Therefore, future studies incorporating immunohistochemistry or western blotting are warranted to confirm our findings at the protein level. In addition, the study focused on *H. pylori*-negative gastric cancer patients, and the results may not apply to *H. pylori*-positive cases. The sample size was relatively small (27 patients), which may limit the generalizability of our findings. Our study did not directly measure SAM levels or DNA/histone methylation status. Therefore, the proposed epigenetic mechanisms remain hypothetical and require direct experimental validation in future studies. We acknowledge that this study was designed as a hypothesis-generating pilot investigation and was not intended to provide mechanistic insights into pathogenesis or evidence for therapeutic targeting. Our findings are presented as preliminary data that generate hypotheses for future research. Mechanistic studies (including *MAT2A* knockdown or inhibition in gastric cancer cell lines, direct measurement of SAM levels, and assessment of methylation status) and therapeutic studies are required to further explore the role of *MAT2A* in GC. Despite these limitations, our study provides novel insights into the role of MAT2A as a potential tissue biomarker in *H. pylori*-negative GC and reports for the first time a significant correlation between tumoral MAT2A expression and systemic chitotriosidase activity.

Conclusion

In conclusion, this hypothesis-generating pilot study demonstrates that *MAT2A* is significantly upregulated in *H. pylori*-negative GC tissues compared to matched adjacent noncancerous tissues. MAT2A shows potential as a candidate tissue biomarker with acceptable diagnostic accuracy. A weak but significant positive correlation was observed between tumoral MAT2A expression and plasma CHIT1 activity ($r=0.32$, $P=0.042$). These findings generate the hypothesis that *MAT2A* may play a role in *H. pylori*-negative GC and that a possible link exists between tumor metabolism and systemic macrophage activation. However, further validation in larger cohorts and functional studies are required to confirm these preliminary findings and to explore the underlying mechanisms.

Acknowledgment

This article is derived from the doctoral dissertation of Fatemeh Babajani.

Authors' Contribution

F.B and M.R.K.: Methodology, investigation, and writing original draft; H.M.: Supervision, data curation, conceptualization, writing-reviewing and editing, formal analysis, software, validation, and investigation. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Declaration of AI

The authors confirm that no AI-based tools were used in the conduct of this study or in the preparation of the manuscript.

Conflicts of Interest: None declared.

References

1 Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71:209-49. doi: 10.3322/caac.21660. PubMed PMID: 33538338.

2 Jaroenlapnopparat A, Bhatia K, Coban S. Inflammation and Gastric Cancer. Diseases. 2022;10. doi: 10.3390/diseases10030035. PubMed PMID: 35892729; PubMed Central PMCID: PMC9326573.

3 Gou YK, Zhou J, Liu P, Wang MY. Research progress on monocyte/macrophage in the development of gastric cancer.

Future Oncol. 2025;21:1939-49. doi: 10.1080/14796694.2025.2504334. PubMed PMID: 40351251; PubMed Central PMCID: PMC12153212.
4 Guo J, Yang Y, Buettner R, Rosen ST. Targeting the methionine-methionine adenosyl transferase 2A- S -adenosyl methionine axis for cancer therapy. Curr Opin Oncol. 2022;34:546-51. doi: 10.1097/CCO.0000000000000870. PubMed PMID: 35788128; PubMed Central PMCID: PMC9365249.
5 Yang C, Ou Y, Zhou Q, Liang Y, Li W, Chen Y, et al. Methionine orchestrates the metabolism vulnerability in cisplatin resistant bladder cancer microenvironment. Cell Death Dis. 2023;14:525. doi: 10.1038/s41419-023-06050-1. PubMed PMID: 37582769; PubMed Central PMCID: PMC10427658.
6 Secker KA, Bloechl B, Keppeler H, Duerr-Stoerzer S, Schmid H, Schneidawind D, et al. MAT2A as Key Regulator and Therapeutic Target in MLLr Leukemogenesis. Cancers (Basel). 2020;12. doi: 10.3390/cancers12051342. PubMed PMID: 32456310; PubMed Central PMCID: PMC7281730.
7 Lou Z, Zeng X, Zhao F, Zhang L, Weinshilboum RM, Tao K. Targeting MTAP creates a therapeutic vulnerability to PARP inhibition in brain metastatic TNBC by disrupting MAT2A mediated methionine metabolism. Research Square. 2023. doi: 10.21203/rs.3.rs-3579438/v1.
8 Sanderson SM, Gao X, Dai Z, Locasale JW. Methionine metabolism in health and cancer: a nexus of diet and precision medicine. Nat Rev Cancer. 2019;19:625-37. doi: 10.1038/s41568-019-0187-8. PubMed PMID: 31515518.
9 Murin R, Vidomanova E, Kowtharapu BS, Hatok J, Dobrota D. Role of S-adenosylmethionine cycle in carcinogenesis. Gen Physiol Biophys. 2017;36:513-20. doi: 10.4149/gpb_2017031. PubMed PMID: 29372684.
10 Ma M, Kong P, Huang Y, Wang J, Liu X, Hu Y, et al. Activation of MAT2A-ACSL3 pathway protects cells from ferroptosis in gastric cancer. Free Radic Biol Med. 2022;181:288-99. doi: 10.1016/j.freeradbiomed.2022.02.015. PubMed PMID: 35182729.
11 Di Francesco AM, Verrecchia E, Manna S, Urbani A, Manna R. The chitinases as biomarkers in immune-mediate diseases. Clin Chem Lab Med. 2023;61:1363-81. doi: 10.1515/cclm-2022-0767. PubMed PMID: 36473071.
12 Zhang S, Chen Q, Guo ZB, Wan R. Expression and methionine adenosyltransferase 2A in gastric cancer and its clinical significance. Chin J Gen Surg. 2014;23:1711-3.
13 Hollak CE, van Weely S, van Oers MH, Aerts JM. Marked elevation of plasma chitotriosidase activity. A novel hallmark of Gaucher disease. J Clin Invest. 1994;93:1288-92. doi: 10.1172/JCI117084. PubMed PMID: 8132768; PubMed Central PMCID: PMC294082.
14 Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi B, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia. 2017;19:649-58. doi: 10.1016/j.neo.2017.05.002. PubMed PMID: 28732212; PubMed Central PMCID: PMC5516091.
15 Yang PW, Jiao JY, Chen Z, Zhu XY, Cheng CS. Keep a watchful eye on methionine adenosyltransferases, novel therapeutic opportunities for hepatobiliary and pancreatic tumours. Biochim Biophys Acta Rev Cancer. 2022;1877:188793. doi: 10.1016/j.bbcan.2022.188793. PubMed PMID: 36089205.
16 Tellai AD, Haghnejad V, Antoine J, Khemiri Merouani B, Bronowicki JP, Dreumont N. The complex post-transcriptional regulation of genes coding for methionine adenosyl transferase: New insights for liver cancer. Biochimie. 2025;238:19-28. doi: 10.1016/j.biochi.2025.05.001. PubMed PMID: 40348354.
17 Pulous FE, Steurer B, Pun FW, Zhang M, Ren F, Zhavoronkov A. MAT2A inhibition combats metabolic and transcriptional reprogramming in cancer. Drug Discov Today. 2024;29:104189. doi: 10.1016/j.drudis.2024.104189. PubMed PMID: 39306235.
18 Cacciatore A, Shinde D, Musumeci C, Sandrini G, Guarrera L, Albino D, et al. Epigenome-wide impact of MAT2A sustains the androgen-indifferent state and confers synthetic vulnerability in ERG fusion-positive prostate cancer. Nat Commun. 2024;15:6672. doi: 10.1038/s41467-024-50908-7. PubMed PMID: 39107274; PubMed Central PMCID: PMC11303763.
19 Zhao C, Bu X. Promoter methylation of tumor-related genes in gastric carcinogenesis. Histol Histopathol. 2012;27:1271-82. doi: 10.14670/hh-27.1271. PubMed PMID: 22936446.
20 Lee SY, Lee CM, Ma B, Kamle S, Elias JA, Zhou Y, et al. Targeting Chitinase 1 and Chitinase 3-Like 1 as Novel Therapeutic Strategy of Pulmonary Fibrosis.

Front Pharmacol. 2022;13:826471. doi: 10.3389/fphar.2022.826471. PubMed PMID: 35370755; PubMed Central PMCID: PMC8969576.

21 Nechita VI, Hajjar NA, Drugan C, Catana CS, Mois E, Nechita MA, et al. Chitotriosidase and Neopterin as Two Novel Potential Biomarkers for Advanced Stage and Survival Prediction in Gastric Cancer-A Pilot Study. Diagnostics (Basel). 2023;13. doi: 10.3390/diagnostics13071362. PubMed PMID: 37046579; PubMed Central PMCID: PMC10093230.

22 Dong Z, Kumar R, Yang X, Fidler IJ. Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma. Cell. 1997;88:801-10. doi: 10.1016/s0092-8674(00)81926-1. PubMed PMID: 9118223.

23 Xia L, Oyang L, Lin J, Tan S, Han Y, Wu N, et al. The cancer metabolic reprogramming and immune response. Mol Cancer. 2021;20:28. doi: 10.1186/s12943-021-01316-8. PubMed PMID: 33546704; PubMed Central PMCID: PMC7863491.

24 Hobson-Gutierrez SA, Carmona-Fontaine C. The metabolic axis of macrophage and immune cell polarization. Dis Model Mech. 2018;11. doi: 10.1242/dmm.034462. PubMed PMID: 29991530; PubMed Central PMCID: PMC6124558.

25 Yu W, Wang Z, Zhang K, Chi Z, Xu T, Jiang D, et al. One-Carbon Metabolism Supports S-Adenosylmethionine and Histone Methylation to Drive Inflammatory Macrophages. Mol Cell. 2019;75:1147-60. doi: 10.1016/j.molcel.2019.06.039. PubMed PMID: 31420217.