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Investigation of Methylenetetrahydrofolate Reductase Gene Polymorphisms in Patients with Recurrent Aphthous Stomatitis

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Abstract

Background: This study examined whether the methylenetetrahydrofolate reductase (*MTHFR*) C677T variant was related to recurrent aphthous stomatitis (RAS) and assessed the accompanying hematological and biochemical findings.

Methods: The case–control sample comprised 90 individuals with RAS and 87 unaffected controls who reported no previous oral aphthous ulcers. Participant characteristics were documented, while pain and ulcer severity were quantified with validated assessment tools. Laboratory assessment covered serum iron, ferritin, folate, vitamin B12, and total iron-binding capacity. Genotyping of the C677T locus was performed by polymerase chain reaction followed by restriction fragment length polymorphism analysis.

Results: The proportion of smokers was lower among patients with RAS than among controls (15.6% vs. 41.4%, $P < .001$), whereas a family history of RAS was reported more often in the patient group (51.1% vs. 20.7%, $P < .001$). Neither genotype frequencies nor allele distributions showed a between-group difference. Low serum folate was observed in 17.8% of patients and 8.0% of controls ($P = .044$). Within the RAS cohort, the frequency of low folate varied across genotypes and was highest among TT carriers (44.4%, $P = .039$). In the adjusted model, gastrointestinal disease (odds ratio [OR] = 3.364, $P = .019$), a positive family history (OR = 4.516, $P < .001$), and low serum folate (OR = 5.996, $P = .011$) were independently related to RAS, while smoking was inversely associated with disease status (OR = 0.249, $P = .001$).

Conclusion: The results do not support a direct relationship between *MTHFR* C677T and RAS. In contrast, low folate remained independently associated with the disease, pointing to a possible contribution of altered folate metabolism.

Keywords: Folate deficiency, genetic polymorphism, *MTHFR* C677T polymorphism, recurrent aphthous stomatitis

What is already known on this topic?

- *Genetic predisposition and hematinic abnormalities have both been implicated in recurrent aphthous stomatitis (RAS), whereas evidence concerning methylenetetrahydrofolate reductase (MTHFR) C677T is still inconclusive.*
- *This study found no relationship between RAS and either MTHFR C677T genotype frequencies or allele distribution.*
- *Low serum folate levels, a positive family history of recurrent aphthous stomatitis, and gastrointestinal diseases were independently associated with the disease.*

What this study adds on this topic?

- *Low serum folate, rather than the MTHFR C677T polymorphism itself, was independently associated with recurrent aphthous stomatitis after adjustment for relevant clinical factors.*
- *The higher frequency of folate deficiency among TT carriers suggests that MTHFR C677T may influence metabolic susceptibility rather than directly determining the occurrence of recurrent apthous stomatitis.*

INTRODUCTION

Recurrent aphthous stomatitis (RAS) presents with repeated episodes of painful oral mucosal ulceration. Lesions are usually round or oval, often have an erythematous margin, and arise through a multifactorial process.[1,2] Recurrent aphthous stomatitis is clinically categorized as minor, major, or herpetiform, and the minor form accounts for

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most presentations.[3] Repeated painful episodes can interfere with eating, routine activities, and oral hygiene, thereby reducing quality of life.[4]

Proposed contributors to RAS include inherited susceptibility, altered immune regulation, hematinic deficiencies, hormonal influences, psychological stress, and local trauma, which may act separately or together.[5] Earlier onset and more frequent recurrences among individuals with affected relatives further support a hereditary contribution to RAS.[6]

Folate-dependent reactions support DNA synthesis, cellular replication, and methylation. Within this pathway, methylenetetrahydrofolate reductase (*MTHFR*) generates the folate form required for the remethylation of homocysteine to methionine.[7,8] Reduced *MTHFR* activity could influence mucosal renewal and inflammatory regulation; accordingly, *MTHFR* variants have been examined as possible modifiers of recurrent oral ulceration.[9,10]

The distribution of the *MTHFR* C677T variant was compared between participants with and without RAS, and its relationship with disease status and folate-related laboratory findings was examined. The findings obtained from this study are expected to contribute to the existing knowledge regarding the etiopathogenesis of the disease and to provide insight for the development of future genetically based approaches.

MATERIALS AND METHODS

Study Design

A case–control design was used to compare *MTHFR* C677T status between participants with RAS and unaffected controls. Recruitment and data collection took place from May 2007 to September 2023. The same eligibility criteria and clinical, biochemical, and genetic procedures were maintained throughout the study; participants with RAS formed the case group, and individuals with no history of oral aphthous ulceration served as controls.

Ethics and Consent

The Local Ethics Committee of İstanbul University approved the Decision/Protocol No.: 2007/404, Date: March 21, 2007). Before enrollment, eligible individuals received an explanation of the study and signed the informed consent form. Participant information was entered on a standardized follow-up form, and all procedures conformed to the Declaration of Helsinki.

Participants and Sample

Patients with RAS were identified through medical history and intraoral clinical examination during the study period. The RAS group included 90 adults aged 18–72 years, of whom 51 were women and 39 were men. Controls were recruited from accompanying persons, individuals attending for tooth extraction, and volunteer staff members. This group comprised 87 adults aged 19–71 years, including 48 women and 39 men. Control eligibility required no previous oral aphthous ulceration, as confirmed during history taking.

Inclusion and Exclusion Criteria

For inclusion in the RAS group, participants were required to have a clinical diagnosis of recurrent aphthous stomatitis. To standardize the recurrent nature of the disease, participants were additionally required to have experienced at least 3 episodes of aphthous ulceration within the preceding 6 months and to present with at least 1 active aphthous ulcer in the oral cavity at the time of clinical examination. Eligibility also required an age of at least 18 years, voluntary participation, and written consent. Exclusion criteria were defined to encompass systemic diseases known to be associated with aphthous ulceration as well as clinical conditions that could potentially influence the study outcomes. Accordingly, individuals with a diagnosis of Behçet's disease, celiac disease, HIV infection, cyclic neutropenia, Crohn's disease, Sweet's syndrome, or MAGIC (Mouth and Genital Ulcers with Inflamed Cartilage) syndrome were excluded. In addition, individuals who had used iron, vitamin B12, or folic acid supplements within the previous 6 months, those with a diagnosed psychological or psychiatric disorder, and pregnant individuals were excluded from the study. These criteria were applied to minimize potential confounding effects on hematological parameters and folate metabolism–related indicators.

Clinical Data Collection and Clinical Assessment

A structured form captured age, sex, occupation, education, comorbidities, allergies, tobacco and alcohol use, disease duration, medications, family history, and clinical RAS subtype. Information from history taking and intraoral examination was considered together when establishing the RAS diagnosis. Oral hygiene was judged clinically from overall cleanliness, visible plaque, and gingival appearance. Participants were subsequently categorized as having good, fair, or poor oral hygiene, and this 3-level classification described baseline oral health. Pain intensity was rated on a 10-cm Visual Analog Scale anchored at 0 for no pain and 10 for the worst imaginable pain. The prespecified categories were 0–3 cm, 3–7 cm, and ≥7 cm for no, mild, and severe pain, respectively. Disease severity was also quantified with the Ulcer Severity Score (USS). The USS incorporates ulcer number, duration, ulcer-free interval, location, and pain. Each component receives 0–3 points, producing a total score between 0 and 15. Higher total scores indicate greater clinical severity, with 0 and 15 representing the lowest and highest possible values, respectively.[11]

Hematological and Biochemical Analyses

Fasting venous samples were collected from both groups between 08:30 and 11:00 to reduce potential diurnal variability. Complete blood counts were obtained with a Coulter LH 750; serum iron and total iron-binding capacity (TIBC)

were measured on a Roche/Hitachi MODULAR DPP platform, and folate, ferritin, and vitamin B12 were measured with a MODULAR E-170 electrochemiluminescence assay. The laboratory cutoffs were 12 g/dL for hemoglobin, 37% for hematocrit, and 50 µg/dL for serum iron. Total iron-binding capacity values above 410 µg/dL or below 250 µg/dL were categorized as high or low, respectively. Low ferritin was defined as below 30 ng/mL in men and below 13 ng/mL in women, whereas folate and vitamin B12 deficiency were defined as below 3 ng/mL and below 191 pg/mL, respectively.

Genetic Analyses: Methylenetetrahydrofolate Reductase C677T Polymorphism

For genetic testing, venous blood was collected after completion of the hematological assessment. Specimens were placed in plain and EDTA (ethylenediaminetetraacetic) tubes and transferred to the laboratory for genetic analysis. Peripheral-blood DNA was extracted by an ammonium acetate salting-out procedure. The extracted DNA was washed with 70% ethanol, resuspended in TE (Tris-EDTA) buffer, and kept at 4°C until use.

Deoxyribonucleic acid quality was evaluated spectrophotometrically. After 1 : 100 dilution in TE buffer, absorbance was recorded at 260 and 280 nm. An A260/A280 ratio of 1.7-1.8 was accepted as indicating adequate purity. DNA concentration was derived from the 260-nm reading, assuming 50 µg/mL of double-stranded DNA at an absorbance of 1.0 and correcting for the dilution factor. Polymerase chain reaction (PCR) targeted exon 4 of *MTHFR*, which contains the C677T locus. The primer sequences used for amplification were 5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ (forward) and 5′-AGGACGGTGCGGTGAGAGTG-3′ (reverse). Each PCR was prepared in a final volume of 25 µL using a master mix to reduce pipetting variation. All preparation steps were performed under cold-chain conditions on ice. The thermal cycling conditions consisted of an initial denaturation at 94°C for 2 minutes, followed by cycles of denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 1 minute, with a final extension step at 72°C for 5 minutes. Polymerase chain reaction products were analyzed by electrophoresis on a 2% agarose gel, and the presence of a 198-bp amplicon was confirmed under ultraviolet illumination. Restriction fragment length polymorphism (RFLP) analysis was performed to determine the presence of the C677T polymorphism. Polymerase chain reaction products were digested with the restriction enzyme HinfI at 37°C for 2 hours. The digestion products were subsequently separated by electrophoresis on a 2% agarose gel. Samples without the C677T mutation were expected to show a single band of 198 bp. Homozygous mutant samples were expected to yield 2 bands of 175 bp and 23 bp following enzyme digestion, whereas heterozygous samples were expected to display 3 bands of 198 bp, 175 bp, and 23 bp. A previously confirmed homozygous C677T mutant sample was used as a positive control for the restriction enzyme digestion.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Chicago, IL, USA). Continuous variables were assessed for normality using the Kolmogorov–Smirnov test. Descriptive data are expressed as mean ± SD for normally distributed variables and as number (percentage) for categorical variables. Comparisons between the recurrent aphthous stomatitis and control groups were conducted using the independent samples *t*-test for normally distributed continuous variables and the chi-square test for categorical variables. Laboratory parameters were analyzed both as continuous variables and as categorical variables based on predefined thresholds. When appropriate, Fisher's exact test was applied. Comparisons of clinical and laboratory parameters according to *MTHFR* genotypes and allele carrier status were performed using the 1-way analysis of variance (ANOVA) or chi-square test, as applicable. To identify factors independently associated with recurrent aphthous stomatitis, a multivariate logistic regression analysis was performed. Variables that were considered clinically relevant or showed significance in univariate analyses were included in the regression model. Results were presented as odds ratios (ORs) with corresponding 95% CIs. The discriminative performance of the multivariate model was evaluated using receiver operating characteristic (ROC) curve analysis, and the area under the curve (AUC) was calculated to assess the overall predictive accuracy of the model. A *P* value of less than .05 was considered statistically significant for all analyses.

Use of Generative Artificial Intelligence and/or LLM Tools

This study did not use artificial intelligence (AI) and/or LLM tools during the preparation of this manuscript.

RESULTS

Smoking was significantly less common in the RAS group than in the control group, being observed in 14 patients corresponding to 15.6% in the RAS group and in 36 individuals corresponding to 41.4% in the control group ($P < .001$). Toothbrushing frequency differed significantly between groups ($P < .001$), with no individuals in the control group reporting lack of daily toothbrushing, whereas this was reported by 10 patients corresponding to 11.1% in the RAS group. A positive family history of recurrent aphthous stomatitis was significantly more frequent in the RAS group, observed in 46 patients corresponding to 51.1%, compared with 18 individuals corresponding to 20.7% in the control group ($P < .001$) (Table 1).

Genotype and allele frequencies were comparable between groups. Hemoglobin, hematocrit, serum iron, TIBC, ferritin, and vitamin B12 showed no between-group differences,

Table 1. Comparison of Demographic and Clinical Characteristics Between Control and RAS Groups

		Control	RAS	*P*
Age (years)		41.1 ± 12.5	38.8 ± 12.1	.217
Sex	Male (%)	48 (55.2)	51 (56.7)	.841
	Female (%)	39 (44.8)	39 (43.3)	
Education (years)		8.4 ± 4.5	7.8 ± 4.6	.578
Working status (%)		54 (62.1)	56 (62.2)	.983
Gastrointestinal diseases (%)		17 (19.5)	11 (12.2)	.182
Systemic disease (%)		34 (39.1)	27 (30.0)	.204
Alcohol consumption (%)		7 (8.0)	11 (12.2)	.358
Smoking status (%)		36 (41.4)	14 (15.6)	**<.001**
Toothbrushing frequency (day)	No (%)	0 (0.0)	10 (11.1)	**<.001**
	Once (%)	29 (33.3)	50 (55.6)	
	Twice (%)	37 (42.5)	23 (25.6)	
	3 times (%)	21 (24.1)	7 (7.8)	
Oral health status	Poor (%)	29 (33.4)	60 (66.7)	**.001**
	Fair (%)	36 (41.4)	23 (25.6)	
	Good (%)	22 (25.2)	7 (7.7)	
Family history of RAS (%)		18 (20.7)	46 (51.1)	**<.001**

Values are presented as mean ± SD or n (%).
RAS, recurrent aphthous stomatitis.

whereas low folate was more frequent in RAS (17.8% vs. 8.0%, *P* = .044; Table 2).

Clinical characteristics, disease duration, healing duration, pain intensity, ulcer severity score, and most hematological parameters did not differ significantly among patients with different *MTHFR* C677T genotypes. Low serum folate levels, however, showed a significant difference according to *MTHFR* genotype distribution, being observed in 9 patients corresponding to 19.6% in the CC (homozygous wild-type) genotype, 3 patients corresponding to 8.6% in the CT (heterozygous) genotype, and 4 patients corresponding to 44.4% in the TT (homozygous variant) genotype (*P* = .039) (Table 3).

Table 2. Comparison of *MTHFR* Allele and Genotype Distributions and Hematological and Biochemical Parameters Between Control and RAS Groups

Parameter	Category	Control	RAS	*P*
C allele carrier	C−	8 (9.2)	9 (10.0)	.856
	C+	79 (90.8)	81 (90.0)	
T allele carrier	T−	49 (56.3)	46 (51.1)	.487
	T+	38 (43.7)	44 (48.9)	
Genotype	CC	49 (56.3)	46 (51.1)	.784
	CT	30 (34.5)	35 (38.9)	
	TT	8 (9.2)	9 (10.0)	
Anemia		5 (5.7)	11 (12.2)	.133
Low serum iron (µg/dL)		10 (11.5)	14 (15.6)	.430
Low TIBC (µg/dL)		1 (1.1)	2 (2.2)	.580
Low sFerritin (ng/mL)		7 (8.0)	8 (8.9)	.840
Low sFolate (ng/mL)		7 (8.0)	16 (17.8)	**.044**
Low vitamin B12 (pg/mL)		17 (19.5)	14 (15.6)	.486

Values are presented as n (%).
MTHFR, methylenetetrahydrofolate reductase; RAS, recurrent aphthous stomatitis; TIBC, total iron-binding capacity.

When clinical and laboratory characteristics were compared according to allele carrier status, disease duration was significantly longer in patients without the C allele compared with C allele carriers, with mean durations of 11.6 ± 9.5 years and 7.4 ± 6.5 years, respectively (*P* = .009). In addition, low serum folate levels were significantly more frequent among patients without the C allele, observed in 4 patients corresponding to 44.4%, compared with 12 patients corresponding to 14.8% among C allele carriers (*P* = .027). Low total iron-binding capacity was also more frequently observed in patients without the C allele, occurring in 1 patient corresponding to 11.1%, compared with 1 patient corresponding to 1.2% among C allele carriers (*P* = .047). No significant differences were observed between C and T allele carrier groups with respect to healing duration, pain intensity, ulcer severity score, anemia, serum iron, ferritin, folate levels according to T allele status, or vitamin B12 levels (Table 4).

Table 3. Clinical and Laboratory Characteristics of Patients With Recurrent Aphthous Stomatitis According to *MTHFR* Genotypes

Parameter	Category	CC	CT	TT	*P*
Disease duration (years)		7.2 ± 6.4	7.5 ± 6.6	11.6 ± 9.5	.476
Healing duration (days)	3-7 days	20 (43.5%)	14 (40.0%)	3 (33.3%)	.710
	8-14 days	17 (37.0%)	17 (48.6%)	5 (55.6%)	
	>14 days	9 (19.6%)	4 (11.4%)	1 (11.1%)	
Pain intensity (VAS score)		2.6 ± 2.1	2.7 ± 2.1	2.8 ± 2.2	.910
Ulcer severity score		9.0 ± 2.9	8.8 ± 2.6	8.9 ± 3.8	.899
Anemia		6 (13.0%)	3 (8.6%)	2 (22.2%)	.521
Low serum iron (µg/dL)		5 (10.9%)	7 (20.0%)	2 (22.2%)	.449
Low TIBC (µg/dL)		1 (2.2%)	0 (0.0%)	1 (11.1%)	.131
Low sFerritin (ng/mL)		4 (8.7%)	4 (11.4%)	0 (0.0%)	.560
Low sFolate (ng/mL)		9 (19.6%)	3 (8.6%)	4 (44.4%)	**.039**
Low vitamin B12 (pg/mL)		7 (15.2%)	4 (11.4%)	3 (33.3%)	.269

Values are presented as mean ± SD or n (%).
MTHFR, methylenetetrahydrofolate reductase; TIBC, total iron-binding capacity; VAS, Visual Analog Scale.

Table 4. Comparison of Clinical and Laboratory Characteristics According to C and T Allele Carrier Status in Patients with Recurrent Aphthous Stomatitis

Parameter	Category	C−	C+	*P*	T−	T+	*P*
Disease duration		11.6 ± 9.5	7.4 ± 6.5	**.009**	7.2 ± 6.4	8.3 ± 7.3	.427
Healing duration (days)	3-7 days	3 (33.3%)	34 (42.0%)	.734	20 (43.5%)	17 (38.6%)	.371
	8-14 days	5 (55.6%)	34 (42.0%)		17 (37.0%)	22 (50.0%)	
	>14 days	1 (11.1%)	13 (16.0%)		9 (19.6%)	5 (11.4%)	
Pain intensity (VAS score)		2.8 ± 2.2	2.7 ± 2.1	.860	2.6 ± 2.1	2.7 ± 2.1	.788
Ulcer severity score		8.9 ± 3.8	9.0 ± 2.8	.935	9.0 ± 2.9	8.8 ± 2.9	.649
Anemia		2 (22.2%)	9 (11.1%)	.334	6 (13.0%)	5 (11.4%)	.808
Low serum iron (µg/dL)		2 (22.2%)	12 (14.8%)	.561	5 (10.9%)	9 (20.5%)	.210
Low TIBC (µg/dL)		1 (11.1%)	1 (1.2%)	.047	1 (2.2%)	1 (2.3%)	.975
Low sFerritin (ng/mL)		0 (0.0%)	8 (9.9%)	.323	4 (8.7%)	4 (9.1%)	.947
Low sFolate (ng/mL)		4 (44.4%)	12 (14.8%)	**.027**	9 (19.6%)	7 (15.9%)	.650
Low vitamin B12 (pg/mL)		3 (33.3%)	11 (13.6%)	.121	7 (15.2%)	7 (15.9%)	.928

Values are presented as mean ± SD or n (%).
TIBC, total iron-binding capacity; VAS, Visual Analog Scale.

After adjustment, gastrointestinal disease was associated with higher odds of RAS (odds ratio [OR] = 3.364, *P* = .019). Smoking was associated with lower odds of disease (OR = 0.249, *P* = .001). A positive family history of RAS was the strongest predictor of disease presence, associated with an approximately 4.5-fold increased risk (OR = 4.516, *P* < .001). In addition, lower serum folate levels were independently associated with RAS, conferring an almost 6-fold increased risk (OR = 5.996, *P* = .011). Age, sex, *MTHFR* carrier status, ferritin, and vitamin B12 were not independent correlates of RAS (Table 5).

The receiver operating characteristic curve (ROC) illustrates that the multivariate logistic regression model demonstrated good discriminatory performance in predicting recurrent aphthous stomatitis (Figure 1).

Receiver operating characteristic analysis demonstrated that the multivariate logistic regression model showed good discriminative ability for predicting recurrent aphthous stomatitis, with an area under the curve of 0.775 and a statistically significant performance (*P* < .001), indicating acceptable diagnostic accuracy (Table 6).

DISCUSSION

Recurrent aphthous stomatitis is a multifactorial disorder in which genetic susceptibility, systemic conditions, mucosal immune responses, and nutritional factors may interact in a complex manner. Rather than supporting a direct effect of *MTHFR* C677T polymorphism alone, the findings suggest that disease susceptibility may be more strongly influenced by familial predisposition and folate-related metabolic factors. In this context, the present results should be interpreted within the broader framework of RAS pathogenesis, where inherited tendency, environmental exposures, and systemic comorbidities are likely to contribute simultaneously to disease development and recurrence.

Recurrent aphthous stomatitis is a multifactorial disorder in which genetic susceptibility, immune dysregulation, and hematinic deficiencies interact to determine disease onset

Table 5. Multivariate Logistic Regression Analysis for Factors Associated with Recurrent Aphthous Stomatitis

Variable	B	S.E.	OR (Exp[B])	*P*	95% CI for OR
Age (years)	−0.014	0.015	0.986	.332	0.958-1.015
Sex (male)	0.070	0.361	1.072	.846	0.528-2.177
Gastrointestinal diseases	1.213	0.518	3.364	**.019**	1.218-9.294
Smoking status	−1.389	0.416	0.249	**.001**	0.110-0.563
Family history of RAS	1.508	0.386	4.516	**<.001**	2.119-9.626
C allele carrier	−0.177	0.631	0.838	.779	0.243-2.884
T allele carrier	−0.387	0.380	0.679	.308	0.322-1.430
Serum ferritin (ng/mL)	0.354	0.603	1.425	.557	0.437-4.645
Serum folate (ng/mL)	1.791	0.705	5.996	**.011**	1.505-23.900
Vitamin B12 (pg/mL)	−0.843	0.551	0.430	.126	0.146-1.267
Constant	−0.474	0.860	0.623	.582	—

Values are presented as regression coefficients (B), standard errors (S.E.), odds ratios (OR), and 95% CI.
OR, odds ratio; RAS, recurrent aphthous stomatitis.

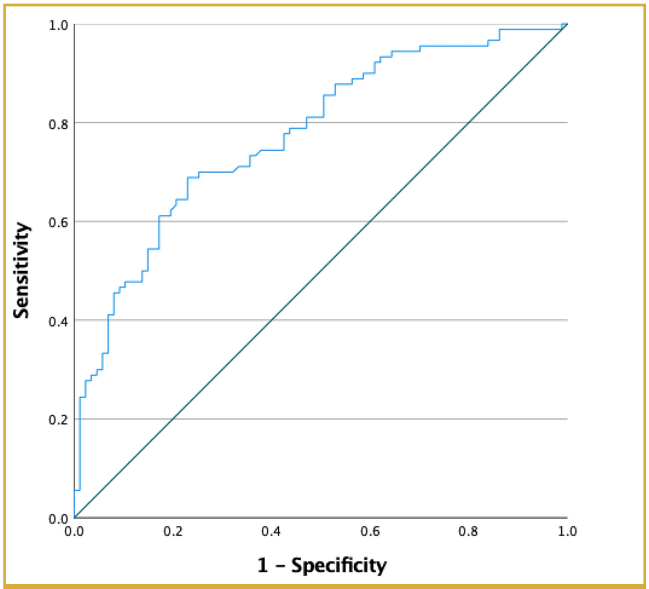


Figure 1. Receiver operating characteristic curve of the multivariate logistic regression model for predicting recurrent aphthous stomatitis.

and clinical severity. Comprehensive reviews emphasize that although RAS is highly prevalent, its etiopathogenesis remains incompletely understood, and no single factor can fully explain disease development across different populations.[12] In this study, the simultaneous presence of familial aggregation, gastrointestinal comorbidities, and hematological alterations supports this multifactorial model and suggests that both systemic and inherited factors contribute to disease susceptibility.

Hematinic deficiencies have long been proposed as important contributors to RAS. Early observational studies demonstrated that deficiencies of iron, folic acid, and vitamin B12 are more common in patients with recurrent oral ulcers than in healthy controls and that correction of these deficiencies often leads to clinical improvement.[13,14] Consistent with this concept, the findings revealed a significantly higher prevalence of low serum folate levels in the RAS group compared with controls, reinforcing the relevance of folate metabolism in the pathophysiology of RAS.

More recent studies further support the association between folate status and RAS. Large cohort analyses have shown that RAS patients exhibit higher frequencies of folate deficiency and related hematological abnormalities, including anemia and iron deficiency, compared with matched healthy individuals.[15] In parallel with these observations, serum folate levels in the cohort were significantly lower in RAS patients, and folate deficiency emerged as an independent risk factor in multivariate analysis, highlighting its potential role as a key metabolic determinant rather than a coincidental finding.

Table 6. Receiver Operating Characteristic (ROC) Analysis of the Multivariate Logistic Regression Model for Predicting Recurrent Aphthous Stomatitis

			Asymptotic 95% CI	
AUC	Std. Error	*P*	Lower bound	Upper bound
0.775	0.035	**<.001**	0.707	0.843

Values are presented as area under the curve (AUC) with standard error and 95% CI.
AUC, area under the curve; ROC, receiver operating characteristic.

Dietary intake studies have suggested that reduced folate and vitamin B12 intake may be present in some patients with RAS.[16] However, the interpretation of lower serum folate levels remains complex, since hematinic status may be influenced not only by dietary intake, but also by overall nutritional status, anthropometric characteristics, gastrointestinal absorption, systemic conditions, and disease-related factors such as ulcer severity and recurrence frequency. Because these variables were not evaluated in the present study, the observed association between folate deficiency and RAS should be interpreted cautiously and should not be considered evidence of a direct causal relationship. Further prospective studies incorporating detailed nutritional and metabolic assessments are needed to clarify this relationship.

Vitamin B12 deficiency has been variably associated with RAS in previous studies. While some investigations reported significantly lower serum vitamin B12 levels in RAS patients, others found no consistent difference compared with controls.[15,17] In the cohort, vitamin B12 deficiency was not significantly more frequent in the RAS group, suggesting that the contribution of vitamin B12 may be population-dependent or secondary to other metabolic disturbances, such as folate deficiency.

Genetic predisposition represents another important component of RAS pathogenesis. Familial clustering and early disease onset among affected relatives have been consistently reported, indicating a heritable susceptibility to mucosal ulceration.[18] In agreement with these reports, family history of RAS was significantly more common in the patient group and constituted 1 of the strongest independent risk factors in logistic regression analysis, underscoring the importance of inherited factors in disease development.

The *MTHFR* C677T polymorphism has been proposed as a candidate genetic factor linking folate metabolism to RAS. A Turkish case–control study reported an association between the *MTHFR* C677T mutation and RAS severity, particularly in relation to ulcer number.[19] However, subsequent genetic analyses and meta-analyses have highlighted substantial heterogeneity across populations and failed to confirm a consistent association between *MTHFR* polymorphism and RAS susceptibility.[20] In line with these data, the study did not identify significant differences in *MTHFR* allele or genotype distributions between RAS patients and controls, suggesting that *MTHFR* C677T alone may not be a major determinant of disease risk.

Importantly, despite the lack of a direct association between *MTHFR* polymorphism and RAS in the cohort, folate

deficiency remained independently associated with disease presence. This finding supports the notion that functional folate status may have a greater clinical impact than genetic variation in folate-metabolizing enzymes.[16] Taken together, the results indicate that while genetic predisposition contributes to RAS susceptibility, metabolic factors—particularly folate deficiency—may play a more prominent and potentially modifiable role in disease development.

This study has certain limitations that should be considered when interpreting the results. The case–control design does not allow causal inferences between recurrent aphthous stomatitis and the identified risk factors. The study was conducted at a single center with a relatively limited sample size, which may have reduced the power to detect weak genetic associations, particularly regarding the *MTHFR* C677T polymorphism. Only 1 polymorphism related to folate metabolism was analyzed, and serum homocysteine levels were not measured, limiting functional interpretation of folate-related pathways. Dietary intake of folate and vitamin B12 was not evaluated, and gastrointestinal diseases were recorded based on medical history. In addition, lifestyle-related variables such as smoking were self-reported. Despite these limitations, the findings provide meaningful insights into the metabolic and clinical factors associated with RAS.

In conclusion, this study showed that *MTHFR* C677T polymorphism was not directly associated with recurrent aphthous stomatitis, whereas low serum folate levels, positive family history, and gastrointestinal diseases were significantly associated with the disease. These findings suggest that metabolic and familial factors may contribute more prominently to RAS susceptibility than this single genetic polymorphism. Although the historical nature of the dataset and the limited sample size should be considered when interpreting the results, the study provides additional evidence supporting the relevance of folate-related mechanisms in the pathogenesis of RAS.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Artificial Intelligence Usage Statement: The authors declared that no artificial intelligence tool was used in the preparation of the manuscript.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of İstanbul University (Approval No.: 2007/404, Date: March 21, 2007).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

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