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MTR, MTRR, and MTHFR Gene Polymorphisms and Susceptibility to nasopharyngeal cancer in Tunisian population

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Abstract

Background

Nasopharyngeal carcinoma is a multifactorial malignancy influenced by genetic, environmental, and viral factors, particularly Epstein-Barr Virus. Alterations in folate metabolism genes may impair DNA synthesis and methylation, promoting carcinogenesis. This study aimed to investigate the association between common polymorphisms in *MTHFR* (C677T and A1298C), *MTRR* (A66G), and *MTR* (A2756G) genes and nasopharyngeal carcinoma risk in a Tunisian cohort.

Methods

Between May 2020 and August 2023, 36 patients diagnosed with NPC and 41 healthy controls were enrolled. Genotyping was performed using TaqMan® Real-Time PCR assays. Allelic and genotypic distributions were compared using chi-square and Fisher's exact tests, and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated.

Results

A significant association was observed between the *MTHFR* A1298C polymorphism and NPC susceptibility. The C allele was associated with an increased risk of NPC (OR = 4.07; 95% CI: 1.47–11.24; p = 0.0068), and the CC genotype was detected exclusively among cases. No significant associations were found for *MTHFR* C677T, *MTRR* A66G, or *MTR* A2756G variants.

Conclusion

Our findings provide preliminary evidence that the *MTHFR A1298C* polymorphism may contribute to genetic susceptibility to NPC in the Tunisian population. Although confirmation in larger cohorts is required, these results support the growing evidence that alterations in folate metabolism could modulate NPC risk through interactions with environmental and viral factors.

Introduction

Nasopharyngeal cancer (NPC) is the most common cancer of the nasopharynx. Its incidence varies significantly by geographic location with higher rates in Southeast Asia, Southern region of China, Malaysia and Indonesia. Intermediate incidence rates were recorded in North Africa especially Tunisia, Morocco and Algeria, while in Western countries NPC is rare [1, 2, 3]. NPC remains a significant cancer to diagnose and treat early, as it can be aggressive and difficult to manage in its advanced stages.

Variations in the prevalence of NPC result from ethnic and environmental differences, and multifactorial etiologies involving environmental factors such as smoking, alcohol consumption salt-preserved food intake, smen and harr in Tunisia [2, 4, 5], infection with Epstein-Barr virus (EBV) and genetic predisposition [6,7].

Folate metabolizing genes including methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and methionine synthase reductase (MTRR) [8, 9, 10] play a central role in carcinogenesis. They are involved in the process of DNA repair and methylation [11,12]. Single nucleotide polymorphisms, *MTHFR* C677T, rs1801133; *MTHFR* A1298C, rs1801131; *MTR* A2756G, rs1805087; and *MTRR* A66G, rs1801394) reduce the serum folate level [11] and generates DNA damage [12].

The human gene *MTHFR* (OMIM number: 607093) is located in chromosome 1 (1p36.3) and consists of 11 exons that encode a protein of 656 amino acids; it is a key regulatory enzyme involved in the folate and homocysteine metabolic pathways. Converts 5, 10-methylenetetrahydrofolate into 5-methyltetrahydrofolate and this reaction provides a methyl for homocysteine into methionine in the catalyzed by MTR [13]. MTR requires vitamin B12 (cobalamin) as a coenzyme. Over time, the cobalamin (I) cofactor of MTR will be oxidized to cobalamin (II) which leads to inactivation of MTR. MTRR restores oxidized cobalamin (II) to CH3-cobalamin (III) in order to maintain the activity of MTR [9, 11, 13].

There are two common allelic variants of the *MTHFR* gene: C677T located at exon 4 and causes an amino acid substitution (Ala 222Val) and A1298C, located at exon 7 and results in a glutamate-to-valine substitution at codon 429. The C677T polymorphism is more strongly associated with decreased MTHFR enzyme activity [13].

The other crucial enzymes of the folic acid pathway are *MTRR* and *MTR*. *MTRR* is a housekeeping gene and located at chromosome 5p15.31 with 17 coding exons. The A66G polymorphism (rs1801394) is located in exon 3, resulting in an Ile22Met amino acid substitution in the protein [11, 14, 15].

MTR is located on chromosome 1 (1q43) and with 34 exons. *Methionine synthase* rs1805087 A/G variant is the most common mutation. It can lead to an aspartic acid to glycine transition at position 919 of the polypeptide chain [8, 16].

Several studies have shown associations between *MTR, MTRR*, and *MTHFR* polymorphisms and susceptibility to various cancers (colorectal, gastric, and head and neck cancers), including NPC, have been investigated in Asian populations, data remain limited and inconsistent [17]. Importantly, no study to date has specifically addressed this relationship in the Tunisian population, where genetic background and environmental exposures may differ significantly from other high-incidence regions. That's why we investigated, in this study, *MTHFR C677T, MTHFR A1298C, MTRR A66G*, and *MTR A2756G* as key factors in the occurrence of NPC in a Tunisian cohort.

This will be the first study to investigate these factors in this population, and may shed light on the role of folate metabolism genes in NPC etiology.

Materials and Methods

Study subjects

A total of 36 NPC patients (30 males and 6 females; mean age: 52.14 ± 12.90 years) were randomly recruited at the moment of diagnosis from the Department of medical oncology of Salah Azaiez institute, Tunis, Tunisia, between May 2020 and August 2023.

Patients were staged based on the 8th Edition of the UICC/AJCC Staging System for Nasopharyngeal Carcinoma [18] (Fig. 1). The exclusion criteria were as follows: (1) patients with associated hypertension, coronary heart disease, or other important organ diseases. All control group samples (N = 44) were collected at random, confirmed to be healthy by physical examinations and consisted of white, Tunisian blood donors of both sexes from the same geographic area as the study patients (mean age 50,82 ± 14,84 years). The characteristics of the case and control groups were comparable, including basal state of illness and age (all Ps > 0.05).

This study was approved by the Ethics Committee of Salah Azaiez Institute, Tunis, Tunisia **(approval No. ISA/2020/10).** All procedures performed in this study involving human participants were conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards [19]. Written informed consent was obtained from all patients and healthy donors prior to inclusion in the study.

TNM, Tumor–Node–Metastasis; T, primary tumor size and local extent; N, regional lymph node involvement; M, distant metastasis.

Stage IV A – *Locally advanced disease without distant metastasis.*

Stage IV B – *Advanced regional disease or with specific regional spread, still without distant metastasis*

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood collected in EDTA tubes of patients and controls using the salting out method [20]. DNA quantity and purity were assessed by a nano spectrophotometer and agarose gel electrophoresis, respectively. Genotype analyses for *MTHFR* C677T (rs1801133 C > T, 607093. 0003), *MTHFR* A1298C (rs1801131, 607093.0004), *MTR (*rs1805087 A > G), *MTRR (*rs1801394 A > G) were run on an ABI PRISM® 7900HT sequence-detection system (Applied Biosystems), using real-time polymerase chain reaction and TaqMan chemistry. Representative melting curves obtained during the real-time PCR analysis of the *MTHFR A1298C* polymorphism are shown in Fig. 2. Distinct melting

profiles enabled clear discrimination of the AA, AC, and CC genotypes, confirming the reliability of the genotyping assay.

Statistical analysis

Statistical analysis of the data was carried out using the STATA version 11.0 statistical software. The Chi-square test and Fisher's exact test were used to compare the frequencies of alleles and genotypes. p values less than 0.05 were considered statistically significant. Hardy–Weinberg equilibrium was evaluated by the $\chi 2$ test. The odds ratio (OR) and 95% confidence interval (CI) were calculated.

Results

1. Clinical and epidemiological characteristics of patients

The clinical and demographic characteristics of the 36 patients diagnosed with nasopharyngeal carcinoma (NPC) are summarized in Table 1. The patients had a mean age of 52.14 years **(± 12.90)** at diagnosis. The cohort was predominantly male 83.33% (6/36). Data completeness was limited, with several clinical variables available only for a smaller subset of cases. A history of smoking was documented in 50% (18/36) of cases, while alcohol consumption was less frequent (16.66%) (6/36). Concerning diet, 52.77% (19/36) reported dietary habits considered risk factors for NPC (e.g., high salt intake, preserved foods).

To ensure a comprehensive evaluation of known lifestyle risk factors for nasopharyngeal carcinoma, smoking and alcohol consumption data were gathered for the control group as well (Table 2). These variables were analyzed for their association with disease status, whereas clinical characteristics were collected only for patients.

Regarding lifestyle factors, smoking showed a strong association with nasopharyngeal carcinoma in our cohort. Smokers had a significantly higher risk of disease compared with non-smokers (OR = 8,53, 95% CI: 2,78 – 26,15, $P < 0.01$). By contrast, alcohol consumption was not associated with cancer status, with no significant difference observed between drinkers and non-drinkers (OR = 3,20, 95% CI: 0,73 – 13,96, $P = 0.11$) (Table 2).

In terms of disease staging, none of the patients were diagnosed at Stage I, indicating that the majority presented with advanced disease. The most frequent categories were T4NxM0 (19.44%) (7/36), TxN3M0 (13.88%) (5/36), and T1-3N2M0 (13.88%) (5/36). TxNxM1 category (11.11%) (4/36) as well was documented, which indicating the presence of distant metastases. Consequently, Stage III and IV (IVA, IVB) accounted for most cases, highlighting the typically late presentation of NPC.

The vast majority of patients exhibited head and neck (ENT) signs (77.77%) (28/36), including cervical lymphadenopathy (66.67%) (24/36), otologic findings (66.67%) (24/36), Rhinological symptoms (58.33%)

(21/36) and functional symptoms (69.44%). Neurological manifestations were present in 19.44% (7/36) of patients, suggesting a possible extension to the skull base.

Furthermore 50% (18/36) of tested patients, was seropositive for Epstein–Barr Virus (EBV), supporting its role in the NPC carcinogenesis. Radiological evaluation using MRI and CT scan (TDM) was performed in 66.66% (24/36) of cases. Distant metastases were observed, mainly involving the bones, detected in 11.11% (4/36) of patients, while no hepatic metastases were found. Clinical response to treatment was recorded in 25% (9/36) of documented cases, although radiological response was slightly less frequent (22.22%) (8/36).

Table 1
Clinical and Epidemiological Characteristics of NPC Patients (n = 36)

Variable	Category	Frequency (n)	Percentage (%)
Age (mean ± SD)	—	—	**52.14 ± 12.90**
Sex	Male	30	83.33
	Female	6	16.67
Smoking history	Yes	18	50
	No	13	36.11
	Unknown	5	13.88
Alcohol consumption	Yes	6	16.66
	No	25	69.44
	Unknown	5	13.88
Dietary risk exposure	Yes	19	52.77
	No	11	30.55
	Unknown	6	16.66
TNM Classification	T2N0-1M0	4	11.11
	T3N0-2M0	3	8.33
	T1-3N2M0	5	13.88
	T4NxM0	7	19.44
	TxN3M0	5	13.88
	TxNxM1	4	11.11
	None	8	22.22
Tumor Stage	Stage I	0	0.00
	Stage II	4	11.11
	Stage III	8	22.22
	Stage IVA	12	33.33
	Stage IVB	4	11.11
	Unknown	8	22.22
Imaging (MRI)	Performed	24	66.66
	Not performed	5	13.88

Variable	Category	Frequency (n)	Percentage (%)
	Unknown	7	19.44
Imaging (CT)	Performed	24	66.66
	Not performed	5	13.88
	Unknown	7	19.44
Personal medical history	Yes	9	25
	No	20	55.55
	Unknown	7	19.44
Family history of cancer	Yes	8	22.22
	No	18	50
	Unknown	10	27.77
ENT signs	Yes	28	77.77
	No	1	2.77
	Unknown	7	19.44
Lymph node signs	Yes	24	66.67
	No	3	8.33
	Unknown	9	25
Rhinological signs	Yes	21	58.33
	No	7	19.44
	Unknown	8	22.22
Otological signs	Yes	24	66.67
	No	4	11.11
	Unknown	8	22.22
Functional symptoms	Yes	25	69.44
	No	3	8.33
	Unknown	8	22.22
Neurological signs	Yes	7	19.44
	No	19	52.77
	Unknown	10	27.77

Variable	Category	Frequency (n)	Percentage (%)
Liver metastasis	Yes	0	0.00
	No	26	72.22
	Unknown	10	27.77
Bone metastasis	Yes	4	11.11
	No	24	66.66
	Unknown	8	22.22
EBV serology	Positive	18	50
	Negative	2	5.55
	Unknown	16	44.44
Clinical response	Yes	9	25
	No	0	0.00
	Unknown	27	75
Radiologic response	Yes	8	22.22
	No	1	2.77
	Unknown	27	75

Table 2
Comparison of Tobacco and Alcohol Consumption between Patients and Controls

Risk factors	Statut	Patients	Controls	P-value	OR	CI	P-value
Tobacco	Non	41,93 (13)	86.05 (37)				
	Oui	58,06 (18)	13,95 (6)	$< 10^{-3}$ *	8,53	[2,78 – 26,15]	$< 10^{-3}$
Alcohol consumption	Non	80,64 (25)	93.02 (40)				
	Oui	19,35 (6)	6,98 (3)	0,153 ¶ (NS)	3,20	[0,73 – 13,96]	0,11

*Significant; (NS) Not significant; ¶ Fisher's exact test in cases where the chi-squared test is not valid.

2. Molecular Results and Genotypic Frequencies

Genotypic and allelic distributions of four polymorphisms in genes involved in folate metabolism among NPC cases and healthy controls were examined. All genotypes within the control group conformed to Hardy–Weinberg equilibrium ($p > 0.05$), indicating genetic stability.

The reliability of the genotyping assay was confirmed by the clear discrimination of melting profiles obtained during real-time PCR analysis (Fig. 2).

Among the different studied SNPs, only *MTHFR A1298C* (Glu429Ala) revealed a statistically significant association with NPC. The C allele was significantly more prevalent in patients than controls ($p = 0.00463$), conferring a 4.07-fold increased risk (OR = 4.07; 95% CI = 1.47–11.24; $p = 0.0068$). Moreover, The CC genotype was observed only among NPC patients in this cohort. However, the AA genotype was more frequent within controls ($p = 0.024$; OR = 3.51; CI = 1.27–9.71; $p = 0.015$) (Table 3).

No significant association was identified between NPC and the polymorphisms *MTHFR* C677T (Ala222Val), *MTRR* A66G (Ile22Met), or *MTR* A2756G (Asp919Gly) in either allelic or genotypic models (all $p > 0.20$) (Fig. 3).

These findings suggest that the *MTHFR A1298C* variant may contribute to genetic susceptibility to nasopharyngeal carcinoma in the Tunisian population, whereas the other investigated polymorphisms do not appear to play a major role. The significant associations observed at both the allelic and genotypic levels for *MTHFR A1298C* (Glu429Ala) were supported by the conformity of genotype frequencies in the control group with Hardy–Weinberg equilibrium, indicating the reliability of the genotyping data. Nevertheless, the relatively small sample size may have limited the statistical power to detect modest genetic effects and contributed to the wide confidence intervals observed. Therefore, larger studies integrating environmental, viral, and functional data are warranted to validate these findings and further clarify the role of folate metabolism genes in NPC susceptibility.

Table 3
Frequencies of genes and genotypes (%) in the studied groups

Gene polymorphisms	Genotype & alleles	Patients	Controls	P-value	OR	CI	P-value
MTHFR: *677 C > T* (Ala222Val)	CC	38.24 (13)	48.57 (17)	0,142¶ (NS)	0,98	[0,48 – 1,98]	0.95
	CT	55.88 (19)	34.29 (12)				
	TT	5.88 (2)	17.14 (6)				
	C	66,18 (45)	65,71 (46)				
	T	33,82 (23)	34,29 (24)	0,95 (NS)	0,98	[0,48 – 1,98]	0,95
MTHFR: *1298 A > C* (Glu429Ala)	AA	53.57 (15)	80.65 (25)	0.024¶*	3,51	[1,27 – 9,71]	0.015
	AC	32.14 (9)	19.35 (6)				
	CC	14.29 (4)	0.00 (0)				
	A	69,64 (39)	90,32 (56)				
	C	30,36 (17)	09,68 (6)	0,00463*	4,07	[1,47 – 11,24]	0,0068*
MTRR: *66 A > G* (Ile22Met)	AA	35.29 (12)	45.95 (17)	0,503 (NS)	1,43	[0,77 – 2,67]	0,25
	AG	38.24 (13)	37.84 (14)				
	GG	26.47 (9)	16.22 (6)				
	A	54,41 (37)	64,86 (48)				
	G	45,59 (31)	35,14 (26)	0,20 (NS)	1,55	[0,79 – 3,04]	0,21
MTR: *2756A > G* (Asp919Gly)	AA	77.42 (24)	65.71 (23)	0,301¶ (NS)	0,82	[0,37 – 1,80]	0,61
	AC	12.90 (4)	28.57 (10)				
	CC	9.68 (3)	5.71 (2)				

Gene polymorphisms	Genotype & alleles	Patients	Controls	P-value	OR	CI	P-value
	A	83,87 (52)	80,00 (56)				
	C	16,13 (10)	20,00 (14)	0,56 (NS)	0,77	[0,31 – 1,88]	0,57

*Significant; (NS) Not significant; ¶ Fisher's exact test in cases where the chi-squared test is not valid.

Discussion

In this study, we first presented the clinical and sociodemographic features of NPC patients from our Tunisian cohort. The average age of the individuals in our cohort was circa 52 years, and male patients (83.3%) clearly outnumbered female patients. This sex predominance has been reported in virtually all other global and regional epidemiological studies, which present sex as a common feature of NPC susceptibility with possible connections with hormonal, occupational and/or behavioral characteristics including tobacco and alcohol use [21]. In fact, among individuals with available lifestyle information, smoking was reported by 50% and alcohol consumption by approximately 16.66%. Smoking emerged as a robust predictor of nasopharyngeal carcinoma risk, with smokers displaying a substantially higher likelihood of disease compared with non-smokers (OR = 8,53, 95% CI: 2,78 – 26,15, $P < 0.01$). These findings regarding tobacco and alcohol exposure are relevant in the context of NPC, where they are considered co-factors to modify the carcinogenic effects of other agents, especially Epstein - Barr virus (EBV). EBV seropositivity was detected in 50% of our cases which supports its primary role in the etiology of NPC and was reported in both endemic and non-endemic areas [21, 22, 23].

In the clinic, most of our patients with available data were seen with advanced disease. Using the TNM staging [18], about half of the patients had clinically T3 or T4 disease with most patients having nodal involvement (N2, N3). We also observed at least 66.7% were seen with advanced stages III, and IV at diagnosis, suggesting that there is a significant delay in diagnosis; this remains one of the most important factors affecting outcomes in NPC. During presentation, patients presented with ENT feature predominantly: cervical lymphadenopathy 66.67%), otologic signs (66.67%), rhinological complaints (58.33%) and general functional features (69.44%) and less than one-quarter with neurological signs (19.44%), which suggests potential skull base invasion in certain cases. Overall, the clinicopathological features observed in our cohort are in line with those reported in prior studies [23, 24, 25].

The role of imaging such as MRI and CT scan in the initial assessment was extensive in regard to the diagnostic process (each used in > 60% of cases) and highlights their central role in assessing tumor extension and for planning the treatment. Clinical response to treatment was seen in 25% patients followed over time, despite the severity of presentation in many cases, and although the data for clinical and radiologic response were less complete and reported in a smaller subset.

Personal and family history of cancer were noted in 25% and 22.22% of cases respectively suggestive of some genetic predisposition. Thus, these findings provide justification for searching for host genetic parameters, particularly those involved in the folate metabolism and DNA repair pathways that may modify the individual's susceptibility to NPC either alone or in conjunction with environmental exposures and viral exposures.

Our molecular examination focused on four polymorphisms in folate metabolism genes: *MTHFR C677T; MTHFR A1298C; MTRR A66G; and MTR A2756G.T*hese genes contribute to the regulation of one-carbon metabolism, DNA methylation, and nucleotide synthesis processes, all of which are pivotal in cancer. These pathways are especially relevant to tissues that undergo chronic processes (e.g., viral infections, environmental exposures, or stress).

Among the four studied SNPs, it was only the *MTHFR A1298C* (Glu429Ala) variant that statistically correlated with NPC in all allelic and genotypic genetic models. The C allele was significantly more common in patients (30.4%) versus controls (9.7%), with an over four-fold increase in risk of disease (OR = 4.07, 95% CI: 1.47–11.24, p = 0.0068). While the CC genotype was only seen in NPC cases, the AC heterozygous genotype was also significantly more common in patients (32.1% v 19.4%). The significance of the genotype distributions was further confirmed (p = 0.024); importantly, a model with covariates remained statistically significant (OR = 3.51, CI: 1.27–9.71, p = 0.015). This result is consistent with the findings reported by Cao et al. (2010) [26] in a population from southern China.

Genotype frequencies in the control group were consistent with Hardy–Weinberg equilibrium, reducing the likelihood of findings due to population structure or selection effects.

In all, no statistically significant associations were observed between NPC and the other three SNPs (*MTHFR C677T, MTRR A66G, and MTR A2756G)*. For *MTHFR C677T*, there was an equal distribution of T allele between the patients and controls (~ 34%), and the distribution of genotypic frequencies did not demonstrate significant differences (p = .142). This indicates that, in our cohort, this variant does not significantly contribute to NPC susceptibility. These results are in agreement with several studies from East Asia that reported no significant association between the *MTHFR C677T* polymorphism and susceptibility to head and neck cancer [25] These differences may relate to ethnicity or variations in dietary consumption of folate that may, in part, interact with viral status.

The *MTRR A66G* and *MTR A2756G* variants also did not demonstrate significant differences in allele or genotype frequencies between cases and controls (p = 0.20 and p = 0.56 respectively). Although the frequency of the GG genotype of *MTRR A66G* variant was higher in patients, this variation was not statistically relevant. Similar inconsistencies have been observed in other diseases [21, 28], but no data are yet available for nasopharyngeal **carcinoma**. Our result supports that it is possible these variants are relatively minor contributors to NPC risk, or that their effect could depend on the genetic context or operate through particular gene-gene or gene-environment interactions.

Overall, the present findings suggest *MTHFR A1298C* is a potential biomarker of genetic susceptibility to NPC in the Tunisian population. Given that the A1298C substitution may act biologically similar to the C677T variant, it is plausible that the association may affect MTHFR activity differently, which could produce altered levels of folate available for DNA synthesis and methylation. These precipitating events may provoke genomic instability and oncogenesis, particularly in chronic EBV infection, which is the main feature of NPC in our cohort.

Importantly, the association seen for A1298C is not driven by evolutionary forces, as this SNP was found to be in Hardy-Weinberg equilibrium in the control group. This strengthens the case for A1298C acting as a true disease-associated variant rather than just a byproduct of demographic admixture or selection.

Along with the clinical data, the molecular results indicate the need for expanded studies within North African populations to confirm the associations, identify potential mechanisms, and to acknowledge that genetic screening should be included in future studies for NPC.

Conclusion

Overall, this study provides the first evidence for an association between the *MTHFR A1298C* (Glu429Ala) polymorphism and NPC risk in the Tunisian population. The implications of our findings support the notion that host genetic variation in folate metabolism may play a role in individual susceptibility to NPC, especially in populations that have been exposed to EBV and other environmental risk factors. Although no associations were observed for the *MTHFR C677T, MTRR A66G, or MTR A2756G* variants, the A1298C SNP emerged as a potential biomarker for risk stratification. Overall, this study underlines the utility of combining molecular and epidemiological evidence as a strategy to improve our understanding of NPC disease mechanisms; this would give way to future studies completed in large cohorts, with a more robust characterization of viral and epigenetic factors, and biological validation of the SNPs through experimental studies. In this regard, integrated approaches will ultimately lead to optimally tailored, personalized risk mitigation strategies for preventing and detecting disease early in high-risk individuals.

Abbreviations

EBV
Epstein-Barr virus
MTHFR
Methylenetetrahydrofolate Reductase
MTR
Methionine Synthase
MTRR
Methionine synthase reductase
NPC
Nasopharyngeal cancer

Declarations

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Salah Azaiez Institute, Tunis, Tunisia (approval No. ISA/2020/10). All procedures performed in this study involving human participants were conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent was obtained from all patients and healthy donors prior to inclusion in the study.

Consent for Publication

Not applicable.

Competing Interests

The authors declare no competing interests.

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Conflict of Interest statement

The authors declare no conflict of interest

Data Availability (NA)

The datasets generated and/or analyzed during the current study are not publicly available due to privacy and ethical restrictions involving human participant data, but are available from the corresponding author on reasonable request, subject to compliance with the relevant ethical requirements.

Artificial Intelligence (AI) Disclosure Statement

The authors used ChatGPT (OpenAI) solely to assist with language editing, grammar correction, and improvement of the manuscript's readability. All scientific content, study design, data analysis, interpretation of results, and conclusions were developed and verified by the authors. The authors take full responsibility for the content of this manuscript.

Authors' contributions

Abir Gmidène conceptualized the study, designed the experiments, analyzed the data, drafted the initial version of the manuscript, and submitted it for publication. Imen Sdiri analyzed the data, prepared the tables. Chaima Bessadi performed the molecular analyses. Khaoula Ghrairi contributed to the collection

of samples. Yosr Zenzri, Hajer ben Mansour, Azza Gabsi, Henda Raies and Amel Mezlini contributed to the clinical design of the study, patient recruitment and collection of clinical data., Nizar Ben Halim contributed to the statistical analysis and prepared the tables. Ghassen Hammami performed the experiments and contributed the qPCR data analysis. Ameur supervised the literature search and providing administrative and logistical support. All authors have contributed to and approved the final version of the manuscript.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.CA Cancer J Clin. 2021;71(3): 209–249.
2. Yosra M, Sameh S, Randa G, Yassmine R, Sallouha G, Nadia B, Noureddine B, Abdelfattah Z, Elham H. Functional Polymorphisms and Gene Expression of TLR9 Gene as Protective Factors for Nasopharyngeal Carcinoma Severity and Progression. J Immunol Res. 2019; https://doi: 10.1155/2019/2826563.
3. Tang LL, Chen WQ, Xue WQ, He YQ, Zheng RS, Zeng YX, Jia WH. Global trends in incidence and mortality of nasopharyngeal carcinoma. Cancer Lett. 2016; *374*(1): 22-30.
4. Feng BJ, Khyatti M, Ben-Ayoub W, Dahmoul S, Ayad M, Maachi F, Bedadra W, Abdoun M, Mesli S, Bakkali H , Jalbout M ,Hamdi-Cherif M, Boualga k, Bouaouina N, Chouchane L, Benider UN, Ben-Ayed F, Goldgar DE, Corbex M. Cannabis, tobacco and domestic fumes intake are associated with nasopharyngeal carcinoma in North Africa. Br J Cancer.2009;101(7): 1207–1212.
5. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics.GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68(6):394-424
6. Tsao SW, Tsang CM, Lo KW (2016) Epstein–Barr virus infection and nasopharyngeal carcinoma. Philosophical Transactions of the Royal Society B: Philos Trans R Soc Lond B Biol .2018; https://doi: 10.1098/rstb.2016.0270.
7. Taflin H, Wettergren Y, Odin E, Carlsson G, Derwinger K. Folate Levels and Polymorphisms in the Genes MTHFR, MTR, and TS in Colorectal Cancer. Clin Med Insights Onco.2014;l8: 15-20.
8. Perpétua Peres N, Ana Lívia Silva Galbiatti-Dias ALS, Castanhole-Nunes MMU, da Silva RF, Pavarino EC, Goloni-Bertollo EM, Ruiz-Cintra MT.Polymorphisms of folate metabolism genes in patients with cirrhosis and hepatocellular carcinoma. World J Hepatol. 2016; 8(29):1234–1243.
9. Leclerc D, Wilson A, Dumas R, Gafuik C, Song D, Watkins D, Heng HH, Rommens JM, Scherer SW, Rosenblatt DS, Gravel RA. Cloning and mapping of a cDNA for methionine synthase reductase, a

flavoprotein defective in patients with homocystinuria. Proc. Natl. Acad. Sci. USA. 1998;95: 3059–3064.

10. Saeedi S, Owji AA, Ansari M, Ghafarpour M, Ebrahimi A, Fallah MS. MTHFR gene polymorphisms and susceptibility to migraine attacks.Arch. med. lab. Sci. 2015;1(2): 61–6.
11. Li WX, Dai SX, Zheng JJ, Liu GQ, Huang JF. Homocysteine Metabolism Gene Polymorphisms (MTHFR C677T, MTHFR A1298C, MTR A2756G and MTRR A66G) Jointly Elevate the Risk of Folate Deficiency. Nutrients. 2015;7(8): 6670–6687.
12. Duthie SJ. Folate & cancer: how DNA damage, repair and methylation impact on colon carcinogenesis . J. Inherit Metab. Dis. 2011; (1): 101–9.
13. Niu YM, Deng MH, Chen W, Zeng XT, Luo J. MTHFR C677T Gene Polymorphism and Head and Neck Cancer Risk: A Meta-Analysis Based on 23 Publications. Dis Mar.2015; https:// doi: 10.1155/2015/681313.
14. Shi TL, Wu Y, Li Y, Chen ZF, Ma YN, Zhang ZT, Zhang YH, Zhang. The relevance of MTHFR C677T, A1298C, and MTRR A66G polymorphisms with response to male infertility in Asians: A meta-analysis. Medicine (Baltimore).2019; https://doi: 10.1097/MD.0000000000014283.
15. Li D, Zhao Q, Zhang C, Huang X, Godfrey O, Zhang W.Associations of MTRR A66G polymorphism and promoter methylation with ischemic stroke in patients with hyperhomocysteinemia. J Gene Med. 2020;https://doi.org/10.1002/jgm.3170.
16. Leclerc D, Odièvre M, Wu Q, Wilson A, Huizenga JJ, Rozen R, Scherer SW, Gravel RA/Molecular cloning, expression and physical mapping of the human methionine synthase reductase gene. Gene 1999;240:75–88.
17. Wang YW, Huang Zy, xue Jin C, Shen XH, He XE, Chang-qing Yang CQ. Unveiling the link: Evaluating MTHFR gene polymorphisms and colorectal cancer risk through meta-analysis. PLoS One. 2025; https:// doi:10.1371/journal.pone.0305517.
18. Tang LL, Chen YP, Mao YP, Wang ZX, Guo R, Chen L, Chen L,Tian L, Lin AH, Li L, Sun Y, Ma, J .Validation of the 8th edition of the UICC/AJCC staging system for nasopharyngeal carcinoma from endemic areas in the intensity-modulated radiotherapy era. J Natl Compr Canc Netw. 2017; 15(7): 913-9. https:// doi:10.6004/jnccn.2017.0121.
19. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA.2013;310(20): 2191–2194. doi:10.1001/jama.2013.281053.
20. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research. 1989;16(3): 1215.
21. Xu FH, Xiong D, Xu YF, Cao SM, Xue WQ, Qin HD, Liu WS, Cao JY, Zhang Y, Feng QS, Chen LZ, Li MZ, Liu ZW, Liu Q, Hong MH, Shugart YY, Zeng YX, Zeng MS, Jia WH. An epidemiological and molecular study of the relationship between smoking, risk of nasopharyngeal carcinoma, and Epstein-Barr virus activation. J NatL Cancer Inst. 2012;104(18): 1396-1410.

22. Stan DJ, Niculet E, Lungu M, Onisor C, Rebegea L, Vesa D, Bezman L, Bujoreanu FC , Sarbu MI, Mihailov R , Fotea S, Tatu AL. Nasopharyngeal carcinoma: A new synthesis of literature data (Review).Exp Ther Med.2022; https://doi: 10.3892/etm.2021.11059.
23. Song Y, Cheng W, Li H, Liu X .The global, regional, national burden of nasopharyngeal cancer and its attributable risk factors (1990–2019) and predictions to 2035. Cancer Med. 2022;11(22): 4310–4320.
24. Ly A, Hoyt L, Crowell J, In Kim Y (2012) Folate and DNA methylation. Antioxid Redox Signal. 2012; 17(2):302–326.
25. Liu Z, Fang F, Chang ET, Ye W. Cancer risk in the relatives of patients with nasopharyngeal carcinoma—a register-based cohort study in Sweden. Br J Cancer.2015; 112(11): 1827–1831.
26. Cao Y, Miao XP, Huang MY, Deng L, Liang XM, Lin DX, Zeng YX, Shao JY. Polymorphisms of methylenetetrahydrofolate reductase are associated with a high risk of nasopharyngeal carcinoma in a smoking population from Southern China. Mol Carcinog.2010; 49(11):928-934.
27. Yang B, Fan S, Zhi X, Wang D, Li Y, Wang Y, Wang Y, Wei J, Zheng Q, Sun G.Associations of *MTHFR* C677T and MTRR A66G Gene Polymorphisms with Metabolic Syndrome: A Case-Control Study in Northern China. Int J Mol Sci.2014; 15(12): 21687-21702.
28. Chung-Wen Jen, Yu-Chen Tsai, Jia-Shing Wu, Pei-Lin Chen, Jia-Hua Yen, Wei-Kai Chuang, Skye Hung-Chun Cheng. Prognostic classification for patients with nasopharyngeal carcinoma based on American Joint Committee on cancer staging system T and N categories. Ther Radiol Oncol. 2020:4:21.

Figures

A A, T and N groupings, eighth edition

		T1	T2	T3	T4
M0	N0	I	II	III	IVA
	N1	II	II	III	IVA
	N2	III	III	III	IVA
	N3	IVA	IVA	IVA	IVA
M1	Any N	IVB			

Figure 1

TNM stage grouping for NPC according to the 8th edition of the AJCC/UICC staging system [18].

TNM, Tumor–Node–Metastasis; T, primary tumor size and local extent; N, regional lymph node involvement; M, distant metastasis.

Stage IV A – *Locally advanced disease without distant metastasis.*

Stage IV B – *Advanced regional disease or with specific regional spread, still without distant metastasis*

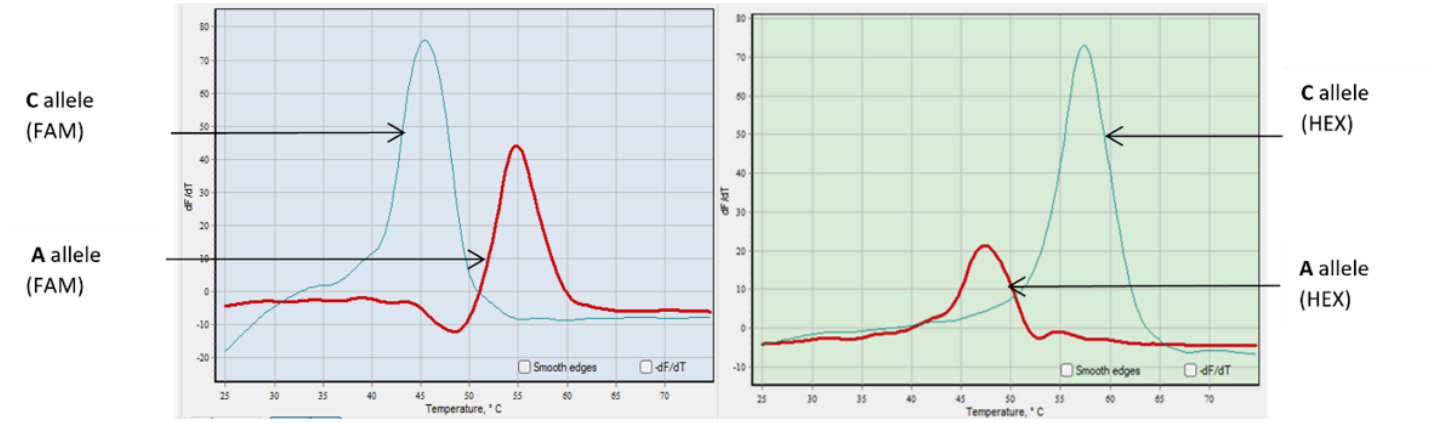


Figure 2

Representative melting curves obtained for *MTHFR A1298C* genotyping by TaqMan real-time PCR. Distinct melting profiles allowed discrimination of the three genotypes (AA, AC, and CC), demonstrating the specificity and reproducibility of the assay.

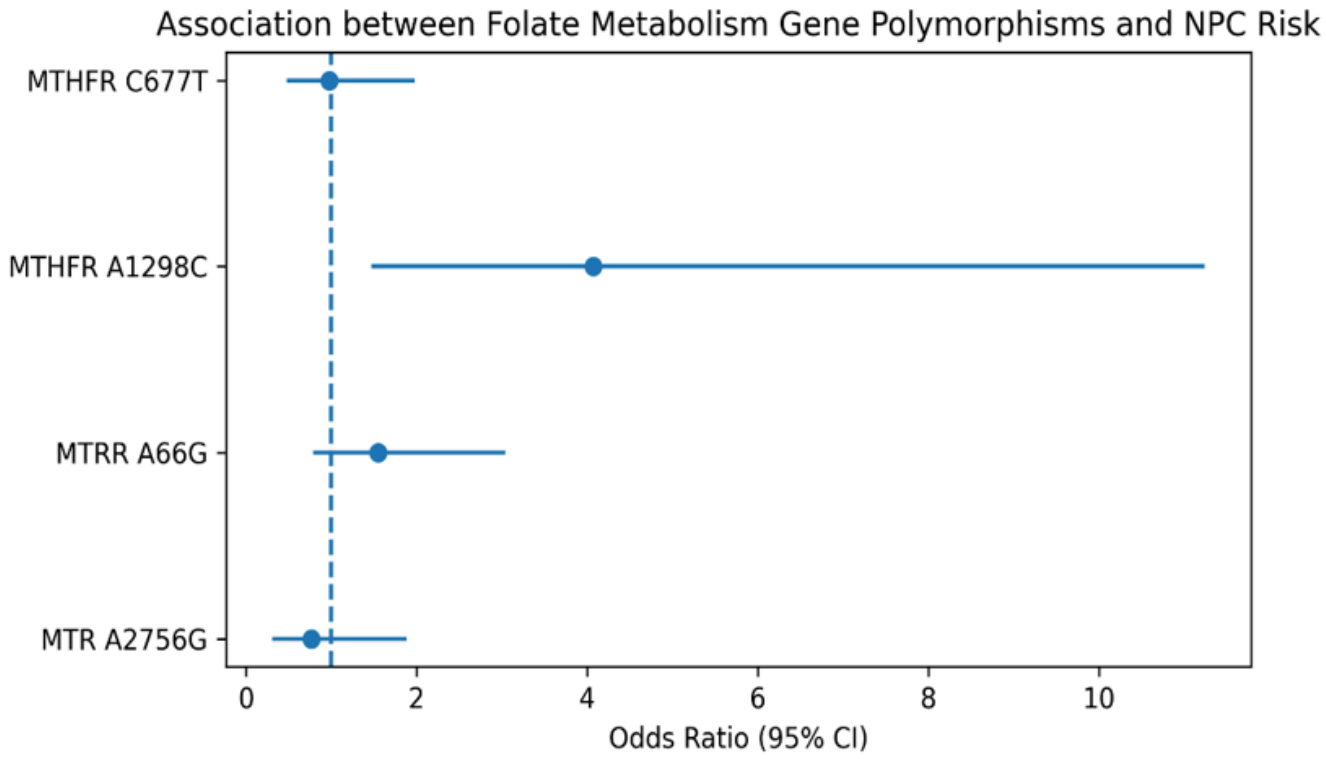


Figure 3

Forest plot showing the association between folate metabolism gene polymorphisms and NPC a risk