RESEARCH ARTICLE

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Methylation of Tumor Suppressor Genes on Chromosome 9: Diagnostic Insights from a Nasopharyngeal Carcinoma in Vietnam

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Abstract

Background: Methylation represents the second hit in tumor development. In nasopharyngeal carcinoma (NPC), multiple tumor suppressor genes located on chromosome 9 under methylation. **Aims:** This first case-control study aims to explore the methylation characteristics of *DAPK*, $p16^{INK4\alpha}$, $p15^{INK4\alpha}$, and $p14^{ARF}$, individually and in combination, to evaluate their potential as promising biomarkers for nasopharyngeal carcinoma (NPC). **Materials and methods:** A total of 70 NPC biopsy samples and 60 non-cancerous swab samples were enrolled into the current study. Genomic DNA extraction, bisulfite modification, and methylation-specific PCR (MSP) were used to analyze methylation status. Statistical analyses, including odds ratio (OR) calculations and methylation index (MI) evaluation, were conducted to determine the association between methylation and nasopharyngeal carcinoma (NPC). **Results:** Promoter hypermethylation was observed in 75.71%, 78.57%, 52.86%, and 87.14% of samples for *DAPK*, $p16^{INK4\alpha}$, $p15^{INK4\alpha}$, and $p14^{ARF}$, respectively. A significant association between the methylation status of these candidate genes and nasopharyngeal carcinoma (NPC) was identified. The methylation of these genes significantly increased the risk of NPC compared to control samples (OR > 1). Based on the methylation index (MI), 100% of NPC samples showed methylation in at least one gene (MI ≥ 0.25). An MI ≥ 0.25 demonstrated a sensitivity of 100% and a specificity of 50%, highlighting its potential as a diagnostic marker. **Conclusion:** This study highlights the potential of *DAPK*, $p16^{INK4\alpha}$, $p15^{INK4\alpha}$, and $p14^{ARF}$ methylation as biomarkers for the diagnosis and early screening of nasopharyngeal carcinoma (NPC) in Vietnamese patients. The findings support the use of a composite methylation index as a sensitive biomarker for the early detection and screening of NPC.

Keywords: *DAPK- INK4α-* methylation- diagnosis- early screening- nasopharyngeal carcinoma

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Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor that originates from the epithelial cells of the nasopharynx and is notably characterized by its high geographical prevalence in East and Southeast Asia [1, 2]. According to the report of Globocan (2020), there were 133,354 newly diagnosed cases of NPC worldwide, with 80,008 reported deaths attributed to the disease [3]. Unfortunately, due to the absence of obvious symptoms in the early stages, most cases of nasopharyngeal carcinoma (NPC) are diagnosed at advanced stages, significantly reducing the chances of survival for NPC patients. [4-6]. A key challenge is developing effective strategies for the early identification and diagnosis of nasopharyngeal carcinoma (NPC), aiming to improve treatment outcomes and overall survival rates. This highlights the urgent need for reliable and effective biomarkers to facilitate the detection and screening of NPC in its early stages.

Precise etiological classification is crucial for a deeper understanding of the underlying causes of nasopharyngeal carcinoma (NPC) and for guiding the development of effective early diagnostic and screening methods [7, 8]. Recent efforts have highlighted the role of epigenetic alterations, particularly the methylation of tumor suppressor genes (TSGs), which silences their function and plays a critical role in the tumorigenesis of NPC [1, 2, 6-9]. These epigenetic changes hold significant potential as biomarkers for the early screening and detection of NPC.

Extensive research has highlighted that the inactivation of tumor suppressor genes (TSGs) through methylation typically occurs in specific regions of certain chromosomes [10-12]. Previous studies have proposed that the loss of chromosome 9, including the silencing of TSGs such as $p16^{INK4\alpha}$, $p15^{INK4\alpha}$, and $p14^{ARF}$ represents an early event in the transformation of normal nasopharyngeal epithelium [10, 13]. Among the chromosomal abnormalities, the silencing of TSGs located on chromosome 9, including *DAPK*, $p16^{INK4\alpha}$, $p15^{INK4\alpha}$, and $p14^{ARF}$, has been identified as a frequent event in nasopharyngeal tumorigenesis [2, 12, 14-17]. *DAPK* (Death-Associated Protein Kinase) and the INK4 family, including $p16^{INK4\alpha}$, $p15^{INK4\alpha}$, and

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p14^{ARF}, are known to play critical roles in regulating cell cycle progression and apoptosis, thus functioning as tumor suppressor genes. Although numerous studies have identified the methylation of *DAPK, p16*$^{INK4\alpha}$, *p15*$^{INK4\alpha}$, and *p14*^{ARF} in NPC, determining which of these epigenetic alterations are directly involved in the initiation and progression of the disease remains challenging. This is due to the variations in genetic and environmental factors across different populations as well as the complexity of distinguishing causative alterations from incidental epigenetic changes [18, 19]. *DAPK*, located on chromosome 9q34.1, encodes a stress-regulated serine/threonine protein kinase involved in several cellular signaling pathways, including the p38MAPK/NF-κB pathway, which plays a role in apoptosis, autophagy, and immune response mechanisms [2, 15, 20, 21]. The INK4α family, consisting of *p16*$^{INK4\alpha}$, *p15* $^{INK4\alpha}$, and *p14*^{ARF}, is located on chromosome 9p21, and plays a vital role in the cellular growth-control network [22-24]. Together, these genes act as key regulators of cellular homeostasis, and their inactivation through genetic or epigenetic alterations is often implicated in tumorigenesis. Methylation-induced inactivation of these genes has been reported as a common molecular event in tumorigenesis.

The apoptosis-related and potential metastasis inhibitor gene *DAPK* has been reported to be inactivated in nasopharyngeal carcinoma (NPC). In our previous meta-analysis, the weighted frequencies of *DAPK* gene methylation in NPC and non-cancerous samples were 56.94% and 9.28%, respectively. Additionally, the pooled odds ratio of 13.13, derived from the random-effects model, highlighted the significantly higher likelihood of nasopharyngeal tumorigenesis in individuals with *DAPK* gene methylation [2]. The inactivation of *p16*$^{INK4\alpha}$, *p15*$^{INK4\alpha}$, and *p14*^{ARF} is also frequently observed in NPC. It has been previously reported that the loss of *p16*$^{INK4\alpha}$ due to promoter methylation, found in 52% of micro-dissected primary tumors, leads to dysregulated cell cycle control and promotes tumorigenesis in NPC [14]. Similarly, p15INK4α, and p14ARF are silenced by methylation in 21% and 18% of NPC primary tumors, respectively [14]. The coordinated silencing of p16INK4α, p15INK4α, and p14ARF through epigenetic modifications, particularly promoter methylation, disrupts cell cycle checkpoints, apoptosis, and tumor-suppressive signaling, collectively driving NPC progression. The frequent methylation of these target genes in NPC suggests their potential as biomarkers for early diagnosis and epigenetic therapy targets. Given the significant geographical prevalence of NPC in Vietnam, it is crucial to investigate the epigenetic mechanisms driving tumorigenesis in these candidate genes to identify promising biomarkers specifically relevant to Vietnamese NPC. This would facilitate the development of effective biomarkers for early diagnosis and screening, as well as the creation of culturally tailored strategies for managing the disease in the affected population.

Materials and Methods

Ethics Statement, and sample collection

The current study was approved by the Medical Ethics Committee of Cho Ray Hospital, Ho Chi Minh City, Vietnam (516/BVCR-HDDD). All participants agreed to the use of their samples for laboratory testing and analysis by signing consent forms.

The sample size calculation was based on previously reported methylation frequencies of D*APK, p16*$^{INK4\alpha}$, *p15*$^{INK4\alpha}$, and *p14*^{ARF}, which were 76.0%, 17.0%, 20.0%, and 22.5%, respectively [14, 25]. The formula used for sample size estimation is:

$$n \geq \frac{Z^2_{(1-\alpha)/2} pq}{d^2} \quad (1)$$

Where: $Z_{1-\alpha/2}{}^2 = 1.96$, $d = 0.1$, p is the methylation frequency, $q = 1 - p$

Using formula (1), the highest calculated sample size was 70, so the final sample size selected for the study was 70 samples A total of 70 biopsy samples from NPC patients, and 60 non-cancerous swab samples from healthy individuals were collected at Cho Ray Hospital, Ho Chi Minh City, Vietnam, for inclusion in the current study. For NPC confirmation, all samples were submitted to the histopathological department and subsequently confirmed through hematoxylin and eosin staining.

Genomic DNA isolation, bisulfite modification, and Nested methylation-specific polymerase chain

Total genomic DNA from collected samples were isolated by the method of phenol/chloroform. Cell were lysed in a lysis buffer consisted of 10 mM Tris-HCl pH = 8, 10 mM EDTA, 150 mM NaCl, 2% SDS, and 0.1 mg/ml Proteinase. Finally, the ethanol precipitation was applied to purify the extracted DNA. Bisulfite modification was performed by using 2 µg genomic DNA according to the guideline of EpiJet Bisulfite Conversion Kit (Thermo Scientific, #1461).

For evaluating the methylation status of candidate genes, including *DAPK, p16*$^{INK4\alpha}$, *p15*$^{INK4\alpha}$, and *p14*^{ARF}, were performed by MSP assays. Table 1 presented the sequences of primers used for amplification. Each PCR reaction was conducted in a total volume of 15 µl, consisting of 200 ng bisulfite-modified template DNA, 7.5 µl MyTaqTM Mix (Bioline, #25041), and 10 mM primers. The thermal cycling was set as following steps: 95°C for 5 minutes, then following 40 cycles of denaturation at 95°C for 30 seconds, annealing at XoC for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. (XoC: each methylation or unmethylated primer annealing temperature were listed in Table 1). Electrophoresis was applied to visualize the products of MSP.

Statistical analysis

MedCalc® software (Version 12.7.0.0) was employed to statistically analyze the associations between the methylation status of individual and combined candidate

Table 1. Sequences of Primers for Nested-PCR assay

Gene	Kí hiệu	Trình tự mồi (5'-3')	X°C	L
DAPK [25]	DAPK_S1-F*	GTTTGTAGGGTTTTTATTGGT	50	220
	DAPK_S1-R*	AAAACACAACTAAAAAATAAAT		
	DAPK-M-F	GATAGTCGGATCGAGTTAACGTC	50	102
	DAPK-M-R	CAAATCCCTCCCAAACGCCGA		
	DAPK-U-F	GGAGGATAGTTGGATTGAGTTAATGTT	50	108
	DAPK-U-R	CACAAATCCCTCCCAAACACCAA		
$p14^{ARF}$ [14]	P14-S1_F*	GGTGCGTGGGTTTTAGTTTGTA	52	256
	P14-S1_R*	AAC ATC AAC ACG AAA ACC ACA A		
	P14-MF	GTGTTAAAGGGCGGCGTAGC	59	122
	P14-MR	AAAACCCTCACTCGCGACGA		
	P14-UF	TTTTTGGTGTTAAAGGGTGGTGTAGT	59	132
	P14-UR	CACAAAAACCCTCACTCACAACAA		
$p15^{INK4\alpha}$ [14]	P15_S1-F*	GGTTTAGTTGAAAACGGAATTT	50	236
	P15_S1-R*	TCTCTCCTTCCTAAAAAACCTAAA		
	P15-MF	GCGTTCGTATTTTGCGGTT	55	148
	P15-MR	CGTACAATAACCGAACGACCGA		
	P15-UF	TGTGATGTGTTTGTATTTTGTGGTT	55	154
	P15-UR	CCATACAATAACCAAACAACCAA		
$p16^{INK4\alpha}$ [32]	P16_S1-F	GGAGGAAGAAAGAGGAG	50	277
	P16_S1-R	CTACAAACCCTCTACCCA		
	P16-M-F	TTATTAGAGGGTGGGGCGGATCGC	55	150
	P16-M-R	GACCCCGAACCGCGACCGTAA		
	P16-U-F	TTATTAGAGGGTGGGGTGGATTGT	58	151
	P16-U-R	CAACCCCAAACCACAACCATA		

M, Methyl; U, Unmethyl; F, Forward primer; R, Reverse primer, X°C, annealing temperature

genes and nasopharyngeal carcinoma (NPC), as well as their correlations with clinicopathological variables, using the Chi-square (χ^2) test. Statistically significant differences in aberrant methylation frequencies among groups were determined based on a significance threshold of $p \leq 0.05$. The strength of association between promoter methylation of the candidate genes and NPC risk was evaluated by calculating odds ratios (ORs) with 95% confidence intervals (95% CI).

Results

Characteristics of the participants

The current study enrolled a total of 70 NPC biopsy samples, which were collected from local NPC patients. They included 55 male subjects (counting for 78.57%), and 15 female subjects (accounting for 21.43%). The age of the patients ranged from 20 to 77 (mean = 52.50 ± 12.86). The age group with the highest frequency was 41-60 years old, accounting for 50.0%, followed by those aged 61 to ≤ 80 years (24, 34.29%), and fewer individuals aged 21 to ≤ 40 years (10, 14.29%) or ≤ 20 years (1, 1.43%). The sample was predominantly composed of Type 3 individuals (48, 68.57%), with smaller groups classified as Type 2 (19, 27.14%) or Type 1 (3, 4.29%). In terms of stage distribution, no cases were observed at Stage I, whereas the majority were in Stage IV (33, 47.14%), followed by Stage II (27, 38.57%) and Stage III (10, 14.29%).

Methylation status in NPC biopsy samples and non-cancerous nasopharyngeal swab samples

Nested-Methylation specific PCR (Nested-PCR) were conducted to determine the methylation status of the enrolled samples. As illustrated in Figure 1, the MSP products for the candidate genes in both NPC biopsy samples and non-cancerous control samples were detected. The methylated bands were observed at 102 bp for *DAPK*, 150 bp for $p16^{INK4\alpha}$, 148 bp for $p15^{INK4\alpha}$, and 122 bp for $p14^{ARF}$. Correspondingly, the unmethylated bands appeared at 108 bp for *DAPK*, 151 bp for $p16^{INK4\alpha}$, 154 bp for $p15^{INK4\alpha}$, and 132 bp for $p14^{ARF}$. The status of candidate genes' methylation was calculated and compared between NPC biopsy samples and normal swab samples, revealing the significant difference (Table 2). The incidences of promoter methylation in NPC were 75.71%, 78.57%, 52.86%, and 87.14% for *DAPK*, $p16^{INK4\alpha}$, $p15^{INK4\alpha}$, and $p14^{ARF}$, respectively. Meanwhile, the lower frequencies of methylation of candidate gene in healthy samples were observed, particularly no methylation detected in the $p15^{INK4\alpha}$ gene. Statistically, as presented in Table 2, a p-value of < 0.0001 demonstrated a highly significant association between the methylation status of individual genes and the presence of NPC. However, there was no significant associations between the methylation status of individual genes and specific clinical features.

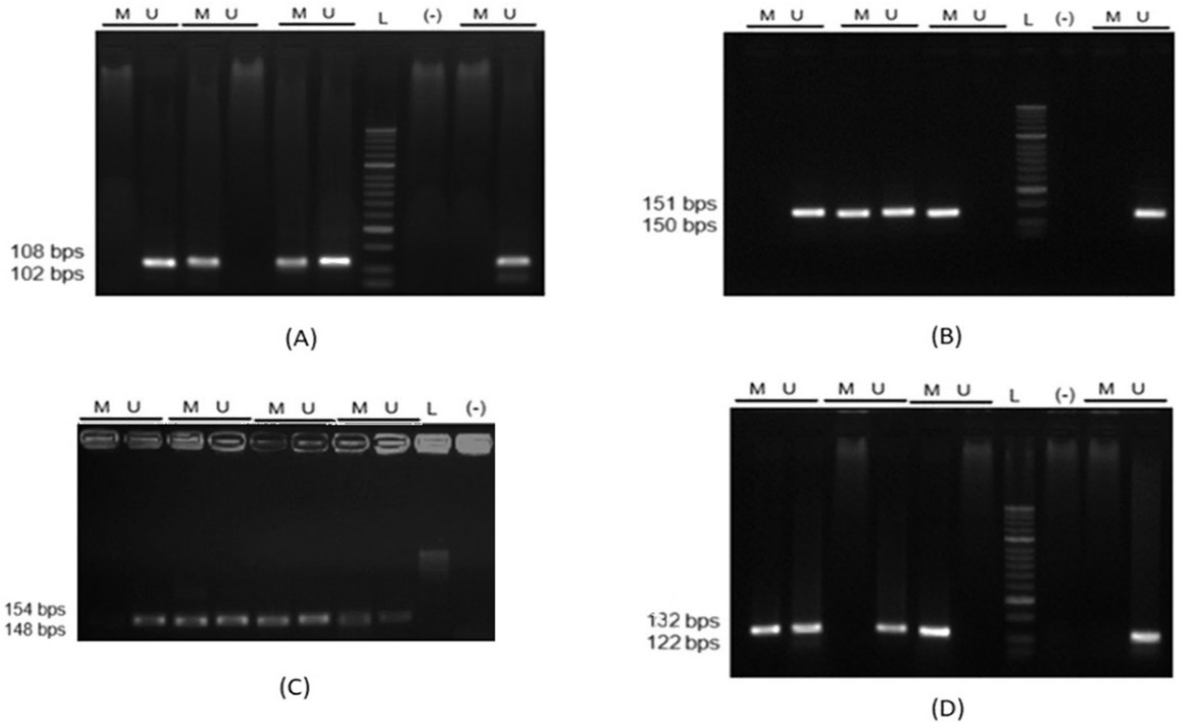

Figure 1. Electrophoresis of (A) *DAPK;* (B) $p16^{INK4\alpha}$, (C) $p15^{INK4\alpha}$, and (D) $p14^{INK4\alpha}$. Note: L: ladder 50-bps (A, B, D), 100-bps (C), M: methylated; U: unmethylated; (-) negative control

Table 2. Methylation of Multiple Genes in NPC and Normal Nasopharyngeal Swab Samples

		DAPK	$p14^{INK4\alpha}$	$p15^{INK4\alpha}$	$p16^{INK4\alpha}$
NPC biopsy samples (n = 70)	M (n, %)	53 (75.71)	61 (87.14)	37 (52.86)	55 (78.57)
	U (n, %)	17 (24.29)	9(12.86)	33 (47.14)	15 (21.43)
Normal swab samples (n = 60)	M (n, %)	15 (25.00)	15 (25.00)	0 (0.00)	13 (21.67)
	U (n, %)	45 (75.00)	45 (75.00)	60 (100.00)	47 (78.33)
p		< 0.0001	< 0.0001	< 0.0001	< 0.0001

M, Hypermethylation; U, Unmethylation

Additionally, Odds ratio (OR), sensitivity (Se), specificity (Sp), and Cohen's kappa (κ) were calculated. The odds ratios (OR) provide robust evidence of the strong associations between gene methylation and NPC. Specifically, the OR values were as follows: *DAPK* (OR = 9.35, 95% CI: 4.20–20.81), p16INK4α (OR = 13.26, 95% CI: 5.73–30.66), $p15^{INK4\alpha}$ (OR = 135.45, 95% CI: 8.06–2276.99), and $p14^{ARF}$ (OR = 20.33, 95% CI: 8.17–50.60). Among the analyzed genes, $p14^{ARF}$ demonstrated the highest sensitivity at 87.14%, followed by $p16^{INK4\alpha}$ with 78.57%, *DAPK* with 75.71%, and $p15^{INK4\alpha}$, which showed the lowest sensitivity at 52.86%. The specificity were 100.00%, and 78.33% for $p15^{INK4\alpha}$, $p16^{INK4\alpha}$, respectively. *DAPK*, and $p14^{ARF}$ all exhibited specificity values of 75.00%. Regarding to Cohen's kappa, among these candidate genes, $p14^{ARF}$ had the highest κ value at 0.626, $p16^{INK4\alpha}$ followed with 0.568, and *DAPK* and $p15^{INK4\alpha}$ had similar κ values of 0.506 and 0.509, respectively.

A panel of markers established to identify abnormalities associated with NPC

The value of the methylation index (MI), indicating that at least one of these genes (MI ≥ 0.25) had abnormal methylation, was computed in all 70 NPC biopsy samples. The methylation status of candidate genes within the MI ≥ 0.25 in the NPC biopsy samples and non-cancerous samples were 100.00%, and 50.00%, respectively. The p-value < 0.0001 statistically indicated the strong association between the MI value of ≥ 0.25 and disease. Moreover, the OR for the MI ≥ 0.25 in the cases compared to controls was 141.00 (95% CI: 8.35–2381.17), significantly indicating a strong association between them. The values of Se, Sp and κ were 100.00%, 50.00%, and 0.52, respectively. The correlation between the methylation of at least one gene and clinical parameters, including: sex, age, histopathology, as well as stage, were indicated in Table 3. As shown in Table 3, these findings suggest that MI is closely linked to sex, age, histopathology, and stage, reinforcing its potential as a clinical biomarker.

Discussion

In studies of DNA methylation patterns, it is essential to account for potential differences between various sample types, such as tumor biopsies and control subject

Table 3. Correlation between the Methylation Status (MI ≥ 0.25) and Clinical Parameters

	MI ≥ 0.25	
	P (%)	N (%)
Sex		
Male	55 (78.57)	0 (0.00)
Female	15 (21.43)	0 (0.00)
p	< 0.0001	
Age		
≤ 20	1 (1.43)	0 (0.00)
From 21 to ≤ 40	10 (14.29)	0 (0.00)
From 41 to ≤ 60	35 (50.00)	0 (0.00)
From 61 to ≤ 80	24 (34.29)	0 (0.00)
p	< 0.0001	
Histopathology		
Type 1	3 (4.29)	0 (0.00)
Type 2	19 (27.14)	0 (0.00)
Type 3	48 (68.57)	0 (0.00)
p	< 0.0001	
Stage		
I	0 (0.00)	0 (0.00)
II	27 (38.57)	0 (0.00)
III	10 (14.28)	0 (0.00)
IV	33 (47.14)	0 (0.00)
p	0.0022	

swabs, as these may influence the study's conclusions. Several investigations have explored the variability in methylation profiles between tissue and swab samples. Recent findings suggest that the methylation profiles of tumor suppressor genes (TSGs) in nasopharyngeal carcinoma (NPC) are largely consistent between tumor biopsies and non-cancerous swab samples. For example, studies on the methylation of SEPT9, H4C6, and RASSF1A have demonstrated comparable profiles in both tumor tissues and swab-derived DNA, supporting their potential use as minimally invasive diagnostic tools. Moreover, the sensitivity and specificity of detecting methylation in NPC are often similar when using tissue samples or more invasive specimens, such as plasma, brushing, or swabs [19].

This pioneering study is the first case-control investigation of the methylation profile of TSGs: *DAPK, p16$^{INK4\alpha}$, p15$^{INK4\alpha}$*, and *p14^{ARF}* located on Chromosome 9 in Vietnamese NPC populations. It provides critical insights into the epigenetic alterations of candidate genes driving nasopharyngeal carcinoma (NPC) and examines whether these genes could be identified as valuable potential biomarkers for the prognosis and screening of NPC in the Vietnamese region. The methylation of Chromosome 9 has been identified as a critical epigenetic mechanism in NPC tumorigenesis, as evidenced by the aberrant methylation of *DAPK, p16$^{INK4\alpha}$, p15$^{INK4\alpha}$*, and *p14^{ARF}*, highlighted in both previous studies [2, 14, 15, 17, 19, 22]. In this study, we investigated the methylation changes of individual candidate genes, which were found to be significantly higher in NPC biopsy samples compared to non-cancerous nasopharyngeal swab samples (Table 2). The observed methylation of these candidate genes, located at regions 9p34 (*DAPK*) and 9p21 (*INK4α* family), highlights the pivotal role of Chromosome 9 methylation in nasopharyngeal tumorigenesis, suggesting their potential as ideal biomarkers for early detection and risk stratification. The current findings provide compelling evidence that methylation profiling of these genes in nasopharyngeal tumor biopsies could serve as potential biomarkers for early detection and screening of NPC. Based on the methylation pattern profile, further studies and clinical application should focus on the development and establishment of highly sensitive and specific non-invasive diagnostic and prognostic tools, such as liquid biopsy-based methylation assays using circulating tumor DNA, or plasma cell-free DNA, for NPC. Recently, the exploration of DNA methyltransferase inhibitors, such as 5-Aza-4'-thio-2'-deoxycytidine, 5-azacytidine and decitabine, has highlighted their emerging role as promising epigenetic therapeutics for the treatment of solid tumors and hematologic cancers [26, 27]. NPC, associated with the methylation of TSG genes', such as *DAPK, p16$^{INK4\alpha}$, p15$^{INK4\alpha}$,* and *p14^{ARF}*, represents a promising target for DNA methyltransferase inhibitors-based therapy.

The statistically significant associations, indicated by a p-value < 0.0001 and the odds ratio (OR), reinforce the importance of these events in driving nasopharyngeal tumorigenesis. By focusing on these tumor suppressor genes (TSGs), the current research highlights the unique contribution of Chromosome 9 methylation-specifically *DAPK, p16$^{INK4\alpha}$, p15I$^{NK4\alpha}$*, and *p14^{ARF}* -in NPC pathogenesis within the Vietnamese population, where genetic and environmental influences may differ significantly from other regions. These candidate genes are postulated to play vital roles in maintaining cellular homeostasis and preventing tumor development [14, 15, 21, 24]. Thus, their inactivation through epigenetic alterations is often implicated as the second hit in cancer development, as described in Knudson's two-hit model [28, 29].

Also, to establish the powerful epigenetics-biomarker, the computation of the methylation index (MI) strengthens the argument for a panel-based approach to NPC diagnosis. An MI value of ≥ 0.25, indicating the presence of abnormal methylation in at least one candidate gene, demonstrated a perfect sensitivity of 100.00% and a significant association with NPC (OR = 141.00, p < 0.0001). The sensitivity of 100.00% pointed out the ability of the MI ≥ 0.25 to correctly identify a true positive outcome among individuals who actually have the epigenetic alterations. Also, the odds of methylation of at least one gene were 141.00 times higher (95% CI: 8.35–2381.17) among NPC patients compared to non-cancerous samples. The odds ratios (ORs) greater than 1 indicate that promoter methylation is strongly associated with an increased risk of NPC. This OR value was higher than that observed for individual genes, suggesting that the combined methylation index (MI ≥ 0.25) is more strongly associated with NPC. As shown in Table 3, the combination of four candidate genes revealed a

significant association between epigenetic alterations and NPC in Vietnamese NPC patients. Therefore, the use of a composite biomarker (MI ≥ 0.25) outperforms single-gene analyses in understanding and diagnosing NPC. This highlights the potential of multi-gene methylation analysis in improving diagnostic accuracy and risk stratification for NPC.

The identification of methylated genes, including at least one methylated gene, raises the prospect of using a variety of medications, such as 5-azacytidine and 5'-aza-2'deoxycytidine, which specifically target the phenomenon of DNA aberrant methylation [30, 31]. Therefore, our finding provides molecular evidence that encourages future research to explore the epigenetic landscape of chromosome 9, which could reveal additional biomarkers and therapeutic targets for NPC.

In conclusion, a first case – control study was performed to determine the methylation profile of tumor suppressor genes located on chromosome 9, including DAPK, and INK4α family, in Vietnamese NPC population. The present study observed a high prevalence of promoter methylation in the candidate genes among nasopharyngeal tumor cases. A significant correlation was identified between the methylation status of individual genes and the incidence of nasopharyngeal carcinoma (NPC). Also, in the case where at least one gene was methylated, diagnostic accuracy significantly improved, as evidenced by the higher sensitivity and odds ratio (OR) observed. This finding suggests that the current panel of candidate genes is sufficient for a strong diagnostic association, supporting the utility of composite methylation indices in clinical settings.

Author Contribution Statement

Conceptualization, data curation, formal analysis, methodology: Hue TH, Thuy LHA, Thuan LD; Writing-original draft: Thuan LD; Writing-review and editing: Thuy LHA, Thuan LD.

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Institutional Review Board Statement

Ethical permission were approved by the permission of Medical Ethics Committee of Cho Ray Hospital, Ho Chi Minh City, Vietnam (516/BVCR-HDDD).

Conflicts of Interest

The authors declared no conflict of interest.

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