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ORIGINAL RESEARCH

Diagnostic potential of methylated *DAPK* in brushing samples of nasopharyngeal carcinoma

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Background: The death-associated protein kinase (*DAPK*) gene is an important member of the apoptotic pathway and is inactivated by abnormal methylation in numerous cancers, including nasopharyngeal carcinoma (NPC). However, the diagnostic value of *DAPK* methylation in brushing samples and tissue samples of NPC remains unclear.

Methods: We conducted a systematic meta-analysis based on 17 studies (including 386 tissue cases, 233 brushing cases, and 296 blood cases).

Results: Our results revealed an association between methylated DAPK and increased risk of NPC in blood, brushing, and tissue samples. In addition, the comparison of the pooled sensitivity, specificity, and area under the curve of methylated *DAPK* in brushing and tissue samples demonstrated the non-inferior effectiveness of methylated *DAPK* in brushing samples to monitor the development of NPC.

Keywords: death-associated protein kinase, methylation, nasopharyngeal carcinoma, diagnosis

Introduction

Nasopharyngeal carcinoma (NPC) is a cancer arising from the epithelial cells lining the nasopharynx. It has a wide geographic and racial distribution worldwide. The occurrence of NPC is rare in most parts of the world, but not in People's Republic of China. It is endemic in southern People's Republic of China, including Hong Kong, with a reported annual incidence of up to 50 cases per 100,000 people.[1] Approximately 60,000 new NPC cases and 34,100 deaths from NPC were projected to occur in 2015 in People's Republic of China.[2]

As in other major human cancers, the progression of NPC is a multistep process involving interactions between multiple factors, including Epstein-Barr virus (EBV) infection,[3,4] consumption of salted food,[5–7] cigarette smoking,[8,9] and alcohol consumption.[10,11] Among these, EBV infection is necessary for NPC progression. By adulthood, approximately 90% of individuals are EBV infected.[12,13] Although EBV is a ubiquitous pathogen, EBV-associated NPC develops in only a small fraction of infected individuals. Thus, it is believed that genetic factors may contribute significantly to the high risk of NPC in this population. To determine the differences present in the subset of EBV-positive individuals who develop cancer, multiple genome-wide studies have examined genetic and epigenetic abnormalities in specific oncogenes and tumor suppressor genes (TSGs).[14–16] In recent years, promoter methylation has been recognized as a common mechanism of inactivating TSGs in the tumorigenesis of NPC.[17,18] Because DNA hypermethylation is one of the earliest molecular alterations during malignant

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transformation in human epithelial cells and often occurs earlier than the morphological abnormalities of cancers,[19,20] the analysis of promoter methylation of TSGs may be a promising method for the detection of NPC.

Death-associated protein kinase (DAPK) is encoded by the *DAPK* gene, which is a novel serine/threonine kinase required for interferon gamma-induced apoptotic cell death.[21] Numerous cancer cell clones with highly aggressive metastatic behavior lack DAP kinase expression, whereas the clones with low metastatic capabilities express the protein.[22,23] Restoration of DAP kinase in highly metastatic cancer cells can suppress the metastatic ability of these cancer cells.[22] As a novel TSG, the expression of DAP kinase is repressed in several types of human cancers by hypermethylation in the promoter CpG region of the gene,[24,25] including in NPC.[26,27] However, the diagnostic power of *DAPK* methylation in NPC has not been investigated.

In the current study, we performed a meta-analysis of 17 studies to assess the association of *DAPK* methylation with the risk of NPC and implemented a diagnostic meta-analysis to evaluate the diagnostic potential of *DAPK* methylation for NPC.

Materials and methods

Literature search strategy

We performed a comprehensive literature search from a range of electronic databases, including PubMed, Embase, Google Scholar, and Web of Science (last search updated in January 2018) without language restrictions. The following search keywords were used: ("methylation" or "DNA methylation" or "promoter methylation" or "demethylation" or "hypermethylation") and ("nasopharyngeal cancer" or "nasopharyngeal neoplasm" or "nasopharyngeal carcinoma" or "NPC") and ("*DAPK*" or "death associated protein kinase").

Selection criteria

The following predefined criteria were used to evaluate the eligibility of included studies: 1) the study design must be a case–control study focused on the association between *DAPK* promoter methylation and NPC patients and 2) the study must provide sufficient information about *DAPK* promoter methylation to calculate odds ratio (OR) and 95% CI. The study was excluded if it did not meet the inclusion criteria. If the authors had published multiple studies using the same population, only the most recent or the largest-sample-size publication was used in our meta-analysis.

Data extraction

All the relevant data of the eligible studies were retrieved independently by all the authors of this study. The following information was extracted: the first author's name, the published year, the race distribution of the study subjects, the source of the samples, the number of participants, and the frequency of *DAPK* methylation.

Statistical analysis

The strength of the association between methylated *DAPK* and the risk of NPC is represented by the pooled overall OR across all the eligible studies. The heterogeneity of all eligible studies was quantified with the I^2 statistic and χ^2 test with the corresponding *P*-value.[28] A DerSimonian–Laird (D+L) model was applied to calculate pooled ORs when there existed heterogeneity in the meta-analysis ($I^2 > 50\%$, χ^2 test with $P < 0.05$). Otherwise, a Mantel–Haenszel (M-H) model was used.[28] A meta-regression was performed to identify the source of heterogeneity. Sensitivity analysis was performed to assess the stability of our results by omitting single studies in the meta-analysis iteration to determine the effect of the individual data on the overall pooled OR. The stability of our results was tested by switching between the D+L and M-H models. Publication bias was quantitatively estimated by Egger's linear regression test.

For the diagnostic meta-analysis, pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and their corresponding 95% CIs were calculated. The PLR is calculated as sensitivity/(1–specificity), and the NLR is calculated as (1–sensitivity)/specificity. The DOR is a measure that combines sensitivity and specificity and is calculated as PLR/NLR.[29] The Fagan plots, assessing the clinical utility of a tested indicator, were drawn based on the values of PLR and NLR. We evaluated pre-test probabilities of 25% and 50% versus corresponding post-test probabilities.[30] Summary receiver operation characteristic curves (SROCs) with the area under the receiver operating characteristic curve (AUC) were generated. All the data analyses were accomplished by STATA-12.0 software (Stata Corporation, College Station, TX, USA). All *P*-values were two sided, and a *P*-value less than 0.05 was deemed significant.

Results

Study characteristics

First, 311 articles were collected by electronic searches in PubMed, Embase, Google Scholar, and Chinese National Knowledge Infrastructure. The selection process of eligible studies is illustrated in Figure 1. After carefully filtering all the potential papers according to the selection criteria, a total of 17 studies were included in the meta-analysis. Among

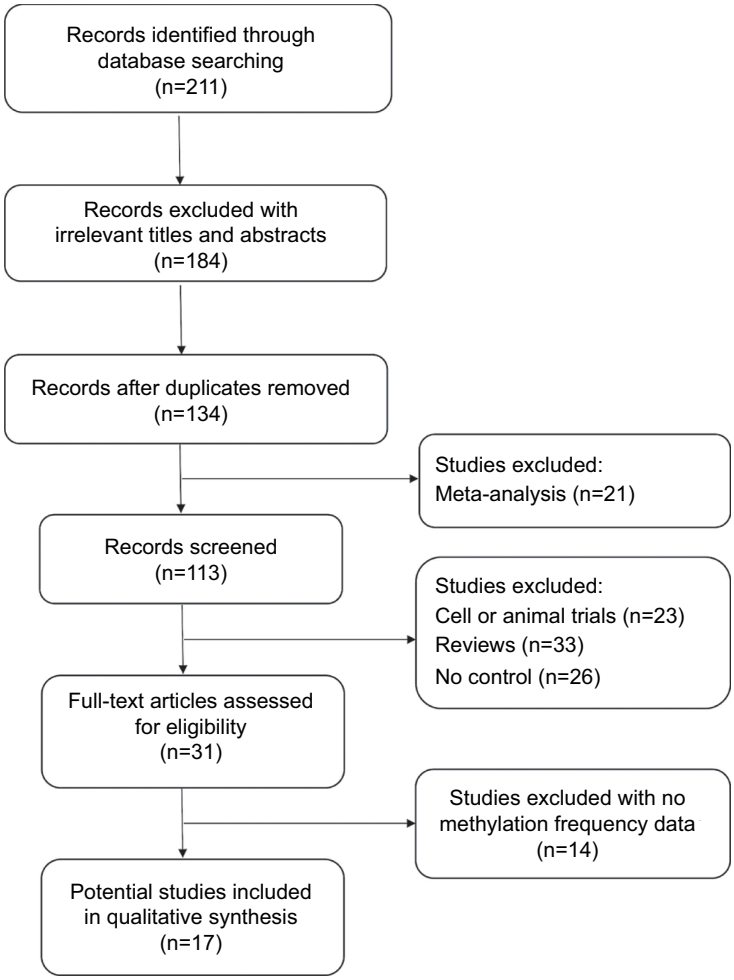

Figure 1 Flow diagram of the literature selection process.

them, three studies evaluated the association between *DAPK* methylation and the risk of NPC using blood samples,[31–33] and five studies used brushing samples.[33–36] Nine other studies assessed the correlation of *DAPK* methylation and the risk of NPC using NPC and non-tumorous tissue samples.[17,26,27,35–40] Table 1 shows the main characteristics of the included studies.

Association of DAPK methylation in NPC and controls

We assessed the difference in *DAPK* methylation between NPC and normal controls in the 17 studies, which included 915 NPC patients and 404 controls. As there was heterogeneity across studies ($I^2 = 54.7\%$, $P = 0.004$), we changed the fixed-effects model (M-H) and random-effects model (D+L) to affirm the reliability of our results ($OR_{(M-H)} = 13.82$, 95% CI: [9.13, 20.92]; $OR_{(D+L)} = 17.72$, 95% CI: [8.45, 37.14]). Besides, sensitivity analysis was also performed to support the robustness of our results (Table 2). The pooled ORs strongly suggested that *DAPK* methylation was associated with increased risk of NPC (Figure 2). Egger's test revealed no evidence of publication bias ($P = 0.58$; Figure 3).

Subgroup analysis of DAPK methylation in NPC and controls

To ascertain the source of heterogeneity, a meta-regression was performed. Our results attributed the heterogeneity to the three studies on Caucasians (Table 3). Therefore, subgroup analyses by ethnicity were conducted. As seen in Figure 4A, a significant decrease was observed in heterogeneity after exclusion of the three studies on Caucasians ($I^2 = 0.0\%$, $P = 0.58$, Figure 4A). Additionally, the pooled OR illustrated the association of *DAPK* methylation and elevated risk of NPC ($OR_{(M-H)} = 20.93$, 95% CI: [12.64, 34.64]).

In addition, we performed a subgroup analysis by sample type, and the results showed that the pooled OR

Table 1 General characteristics of studies included in the current meta-analysis

Author	Year	Country	Ethnicity	Sample type	Case, N (M, %)	Control, N (M, %)
Kwong et al[17]	2002	People's Republic of China	Asian	Tissue	33 (72.72%)	6 (0.00%)
Liang et al[39]	2015	People's Republic of China	Asian	Tissue	48 (75.00%)	26 (0.00%)
Wonget al[40]	2002	People's Republic of China	Asian	Tissue	32 (75.00%)	5 (0.00%)
Zhang et al[36]	2012	People's Republic of China	Asian	Tissue	49 (67.35%)	20 (0.00%)
Nawaz et al[27]	2015	Morocco	Caucasian	Tissue	44 (25.00%)	18 (27.78%)
Kong et al[38]	2006	People's Republic of China	Asian	Tissue	46 (76.00%)	6 (0.00%)
Fendri et al[26]	2009	Tunisia	Caucasian	Tissue	68 (88.24%)	9 (0.00%)
Challouf et al[37]	2012	Tunisia	Caucasian	Tissue	36 (47.22%)	19 (15.79%)
Chang et al[35]	2003	People's Republic of China	Asian	Tissue	30 (76.67%)	6 (0.00%)
Tong et al[34]	2002	People's Republic of China	Asian	Brushing	28 (50.00%)	12 (0.00%)
Yang et al[33]	2015	People's Republic of China	Asian	Brushing	96 (68.75%)	43 (18.60%)
Chang et al[35]	2003	People's Republic of China	Asian	Brushing	30 (50.00%)	43 (2.33%)
Zhang et al[36]	2012	People's Republic of China	Asian	Brushing	49 (55.10%)	20 (0.00%)
Chang et al[35]	2003	People's Republic of China	Asian	Brushing	30 (63.33%)	37 (0.00%)
Yang et al[33]	2015	People's Republic of China	Asian	Blood	220 (27.27%)	50 (4.00%)
Wong et al[32]	2004	People's Republic of China	Asian	Blood	41 (19.51%)	43 (0.00%)
Tian et al[31]	2013	People's Republic of China	Asian	Blood	35 (51.43%)	41 (9.76%)

Abbreviation: M, Methylation.

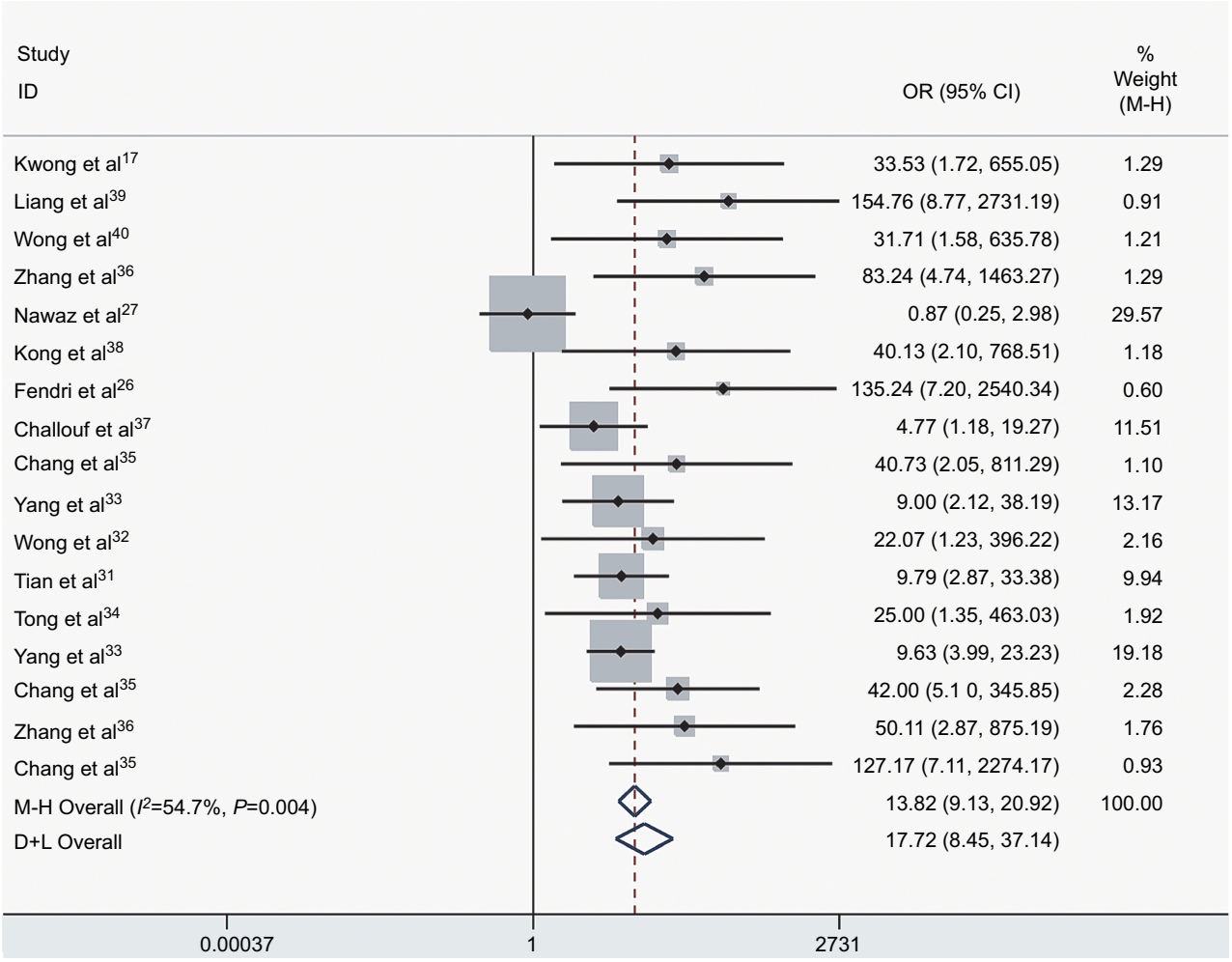

Figure 2 The forest plot for the association between *DAPK* methylation and the risk of NPC by the fixed-effects model (M-H) and random-effects model (D+L) in NPC vs controls.

Abbreviations: NPC, nasopharyngeal carcinoma; M-H, Mantel–Haenszel; D+L, DerSimonian–Laird; OR, odds ratio; CI, confidence interval.

Table 2 Sensitivity analysis of pooled OR for *DAPK* methylation between NPC and controls

Study omitted	Year	Estimated OR (95% CI)
Kwong et al[17]	2002	17.44 (8.10, 37.54)
Liang et al[39]	2015	15.65 (7.52, 32.57)
Wong et al[40]	2002	17.50 (8.12, 37.69)
Zhang et al[36]	2012	16.39 (7.74, 34.71)
Nawaz et al[27]	2015	17.21 (10.20, 29.06)
Kong et al[38]	2006	17.25 (8.03, 37.08)
Fendri et al[26]	2009	15.94 (7.59, 33.45)
Challouf et al[37]	2012	20.49 (9.27, 45.297)
Chang et al[35]	2003	17.25 (8.03, 37.07)
Yang et al[33]	2015	19.64 (8.73, 44.18)
Wong et al[32]	2004	17.82 (8.25, 38.53)
Tian et al[31]	2013	19.75 (8.68, 44.92)
Tong et al[34]	2002	17.71 (8.19, 38.23)
Yang et al[33]	2015	20.40 (8.71, 47.79)
Chang et al[35]	2003	16.82 (7.79, 36.32)
Zhang et al[36]	2012	16.96 (7.93, 36.287)
Chang et al[35]	2003	15.92 (7.59, 33.36)

Abbreviations: OR, odds ratio; NPC, nasopharyngeal carcinoma.

Table 3 Meta-regression analysis of *DAPK* methylation in NPC vs controls

Characteristics	Coefficient	*P*	95% CI
Year	–0.053	0.57	(–0.26, 0.15)
Ethnicity			
Caucasian	–2.37	0.043	(–4.79, 0.058)
Sample type			
Blood	–0.67	0.51	(–2.83, 1.48)
Tissue	0.39	0.69	(–1.75, 2.52)
Method			
MS-HRM	–0.73	0.74	(–5.55, 4.09)
MSP	–0.03	0.99	(–4.27, 4.22)

Abbreviations: MS-HRM, methylation-sensitive high resolution melting; MSP, methylation specific PCR; NPC, nasopharyngeal carcinoma.

was 22.27 for tissue samples, 10.43 for blood samples, and 20.50 for brushing samples (tissue: $OR_{(D+L)}$ = 22.27, 95% CI [5.09, 97.55]; blood: $OR_{(M-H)}$ = 10.43, 95% CI [4.15, 26.20]; brushing: $OR_{(M-H)}$ = 20.50, 95% CI [10.15, 41.41]; Figure 4B).

Diagnostic value of DAPK methylation for NPC and controls

As shown in Figure 4B, the pooled OR in tissue samples was congruent with that in brushing samples, implying that the *DAPK* methylation in brushing samples may serve as a useful and noninvasive biomarker for NPC. However, the comparison of the diagnostic capability of *DAPK* methylation for NPC in tissue samples and in brushing sample has not been investigated. Therefore, we performed a diagnostic meta-analysis of 9 studies on NPC tissue samples and a separate one of 5 studies on NPC brushing samples. The summary specificity and sensitivity of methylated *DAPK* for distinguishing NPC from control tissue samples were 0.99 and 0.69 (0.55–0.80), respectively (tissue: specificity = 0.99, 95% CI [0.85, 1.00]; sensitivity = 0.69, 95% CI [0.55, 0.80]; Figure 5A). The summary specificity and sensitivity of methylated *DAPK* for identification of NPC from control brushing samples were 0.98 and 0.58, respectively (brushing: specificity = 0. 98, 95% CI [0.85, 1.00]; sensitivity = 0. 58, 95% CI [0.50, 0.67]; Figure 5B).

The SROCs based on specificity and sensitivity are shown in Figure 6. The AUC was 0.92 for tissue and 0.71 for brushing samples (tissue: AUC = 0. 92, 95% CI [0.90–0.94], Figure 6A; brushing: AUC = 0.71, 95% CI [0.67, 0.75], Figure 6B). In addition, the summary DOR, another diagnostic-strength parameter that indicates better diagnostic strength with higher values, was 184 for tissue and 87 for brushing (tissue: DOR = 184, 95% CI [9, 3725]; brushing: DOR = 87, 95% CI [9, 865]). There was no publication bias in this diagnostic meta-analysis (Figure 7).

The abovementioned results confirm that the detection of *DAPK* methylation could serve as an auxiliary technology for the diagnosis of NPC. Therefore, it is necessary to evaluate the clinical value of *DAPK* methylation during clinical practice. The PLR and NLR are effective indicators of clinical utility, as is the Fagan plot. The PLR and NLR for tissue were 58.8 and 0.32, respectively (tissue: PLR = 58.8, 95% CI [3.7, 930.0]; NLR = 0.32, 95% CI [0.21, 0.47]), while the PLR and NLR for brushing samples were 36.7 and 0.42, respectively (brushing: PLR = 36.7, 95% CI [3.7, 367.3]; NLR = 0.42, 95% CI [0.35, 0.51]). As indicated by PLR in tissue, NPC patients had a nearly a 59 times higher chance of positive detection of *DAPK* methylation than in control tissue samples. The NLR indicated that normal control tissue samples had a threefold greater chance (the reciprocal of the value of NLR) of having unmethylated *DAPK* than NPC patients. The PLR and NLR for brushing samples indicated a nearly 37 times higher chance of positive detection of *DAPK* methylation in NPC brushing samples than in controls and twofold greater chance of having unmethylated *DAPK* in control brushing samples than in NPC patients.

The Fagan plot was generated for the visual presentation of the diagnostic performance of the detection of methylated *DAPK*. As shown in Figure 8A and B, when the prior probability was taken as 25% and 50%, the Fagan plot illustrated that the probability of an individual being diagnosed with

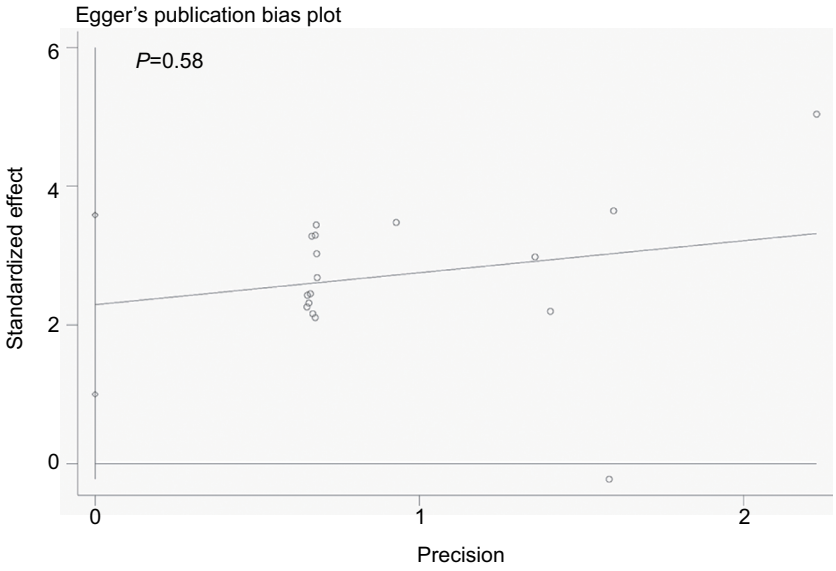

Figure 3 Egger's plot of publication bias for *DAPK* methylation in the current study.

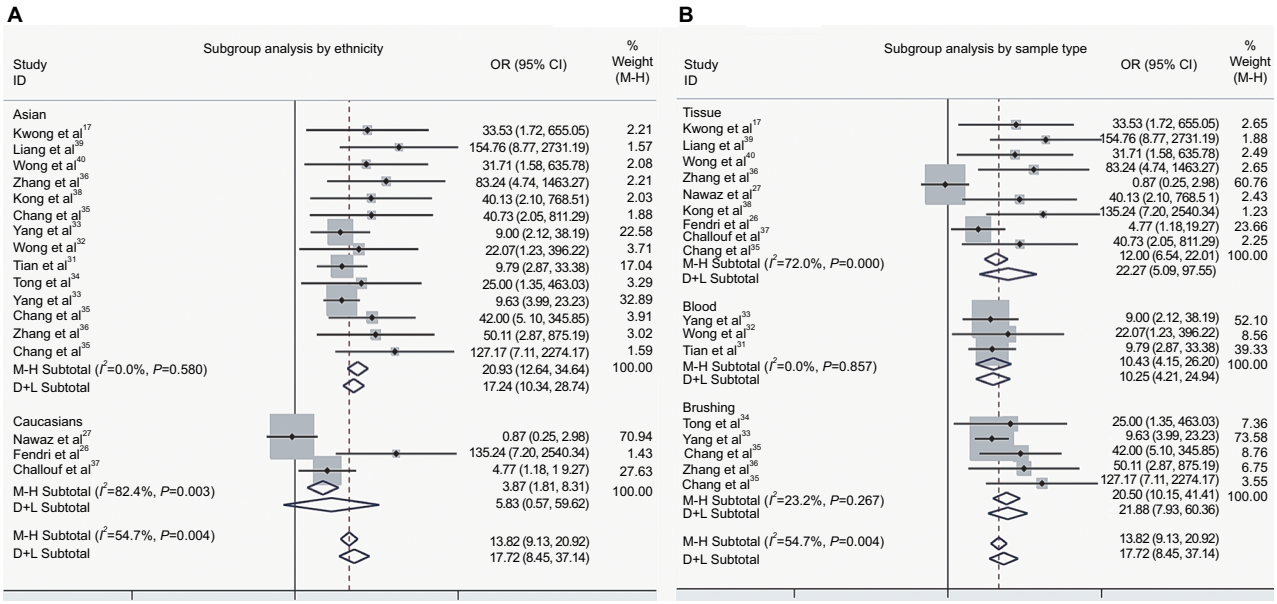

Figure 4 The forest plots for the association of *DAPK* methylation and the risk of NPC by subgroup analyses.
Notes: (**A**) Subgroup analysis by ethnicity; (**B**) Subgroup analysis by sample type.
Abbreviations: NPC, nasopharyngeal carcinoma; OR, odds ratio; CI, confidence interval; M-H, Mantel-Haenszel ; D+L, DerSimonian–Laird.

NPC was 95% and 98%, respectively, following a methylated *DAPK* in tissue samples. However, the probability of an exclusion diagnosis of NPC was 16% and 26% following a non-methylated *DAPK* in tissue samples. As illustrated in Figure 8C and D, with prior probabilities of 25% and 50%, the Fagan plot illustrated that the probability of an individual being diagnosed with NPC was 93% and 97%, respectively, following a methylated *DAPK* in brushing samples. However, the probability of an exclusion diagnosis of NPC was 13% and 20% following a non-methylated *DAPK* in brushing samples. The results of our diagnostic meta-analysis imply that the detection of methylated *DAPK* in brushing samples could serve as an effective biomarker for diagnosis of NPC.

Discussion

The incidence of NPC is rare in western populations, with rates below 2 per 100,000 person-years.[41] NPC is much more common in People's Republic of China and in the

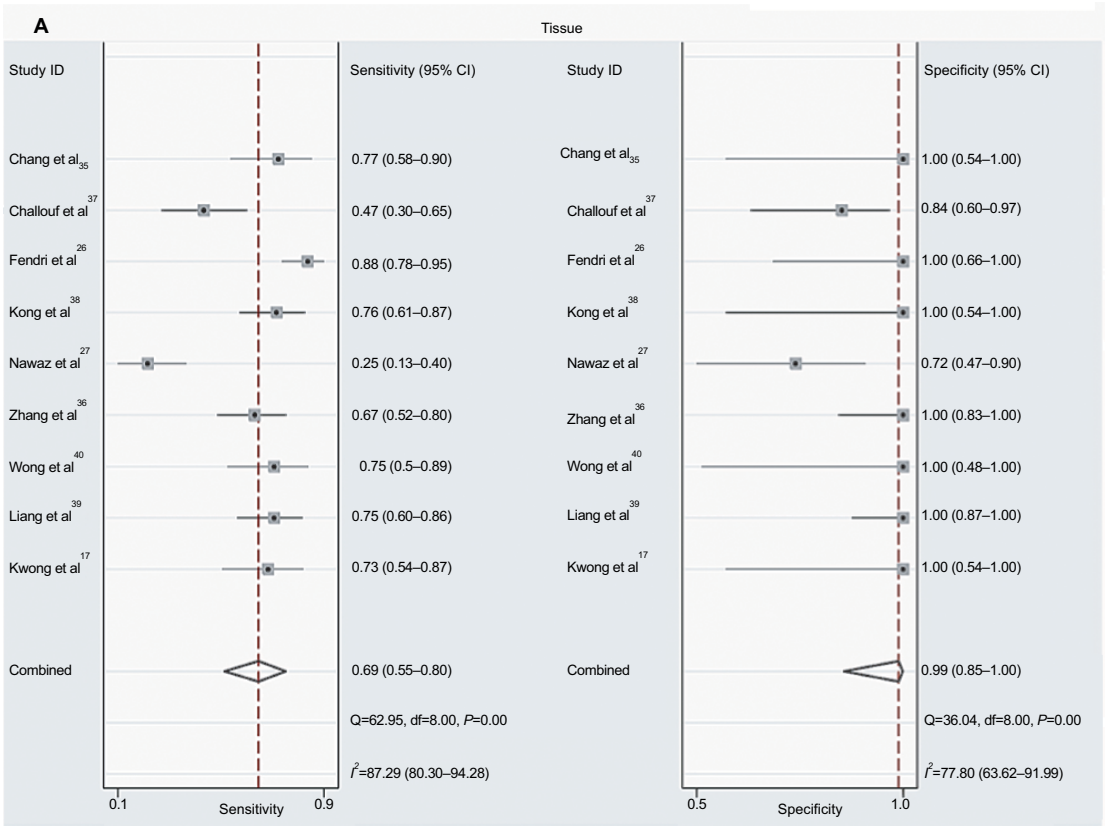

Figure 5: Forest sensitivity and specificity of methylated *DAPK* for diagnosis of NPC.
Notes: (**A**) Forest sensitivity and specificity of methylated *DAPK* for diagnosis of NPC using tissue sample; (**B**) Forest sensitivity and specificity of methylated *DAPK* for diagnosis of NPC using brushing sample.
Abbreviation: NPC, nasopharyngeal carcinoma; OR, odds ration; CI, confidence interval.

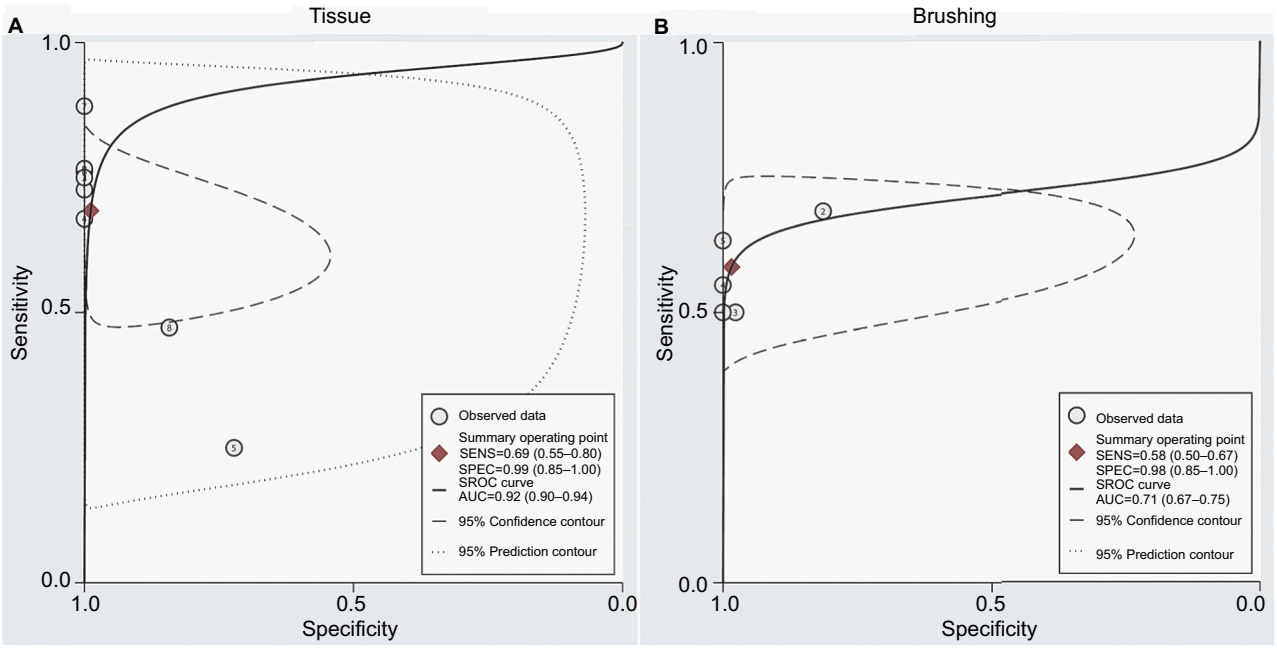

Figure 6 SROC plots with best fitting asymmetric curve of methylated *DAPK* for NPC by sample type.
Notes: (**A**) SROC plots with best fitting asymmetric curve of methylated *DAPK* for NPC by tissue sample; (**B**) SROC plots with best fitting asymmetric curve of methylated *DAPK* for NPC by brushing sample.
Abbreviations: SENS, sensitivity; SPEC, specificity; SROC, summary of receiver-operator characteristic; NPC, nasopharyngeal carcinoma; AUC, area under the receiver operating characteristic curve.

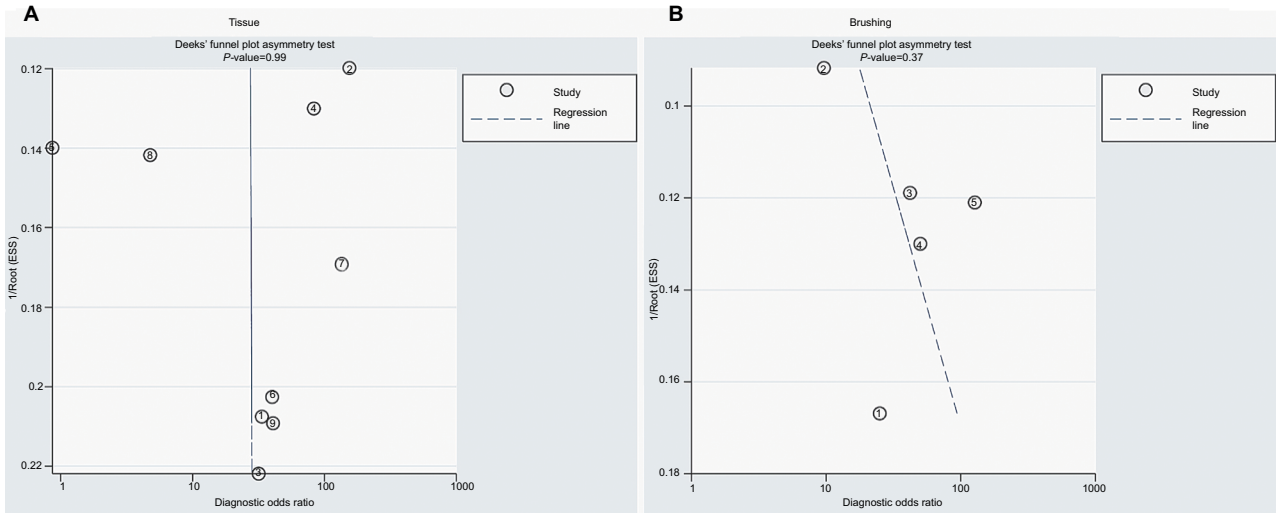

Figure 7 The publication bias of methylated *DAPK* for diagnosis of NPC by sample type.
Notes: (**A**) The publication bias of methylated *DAPK* for diagnosis of NPC by tissue sample. (**B**) The publication bias of methylated *DAPK* for diagnosis of NPC by brushing sample.
Abbreviations: ESS, effective sample size; NPC, nasopharyngeal carcinoma.

Arctic region, with incidence rates up to 30 per 100,000 persons.[41] However, even within Asian populations, dramatic differences in NPC incidence are observed across regions. Higher incidences of NPC are found in urban areas than in rural areas.[42]

Although multiple specific environmental factors, including early exposure to salted food and latent EBV infection, have been suggested to be risk factors in the endemic regions, the predisposition to NPC among southern Chinese population strongly suggests the involvement of both genetic and epigenetic susceptibility and environmental factors.[43,44] Genome-wide linkage analyses of high-risk Chinese NPC identified several candidate NPC susceptibility loci, including chromosome 3p21.[44,45] Many TSGs, such as *RASSF1A* and *MLH1*, have been isolated from this region.[46,47] Inactivation of multiple TSGs attributed to high frequencies of deletion of this region has been associated with the progression of NPC.[14,48] Additionally, aberrant methylation of the 5′ CpG

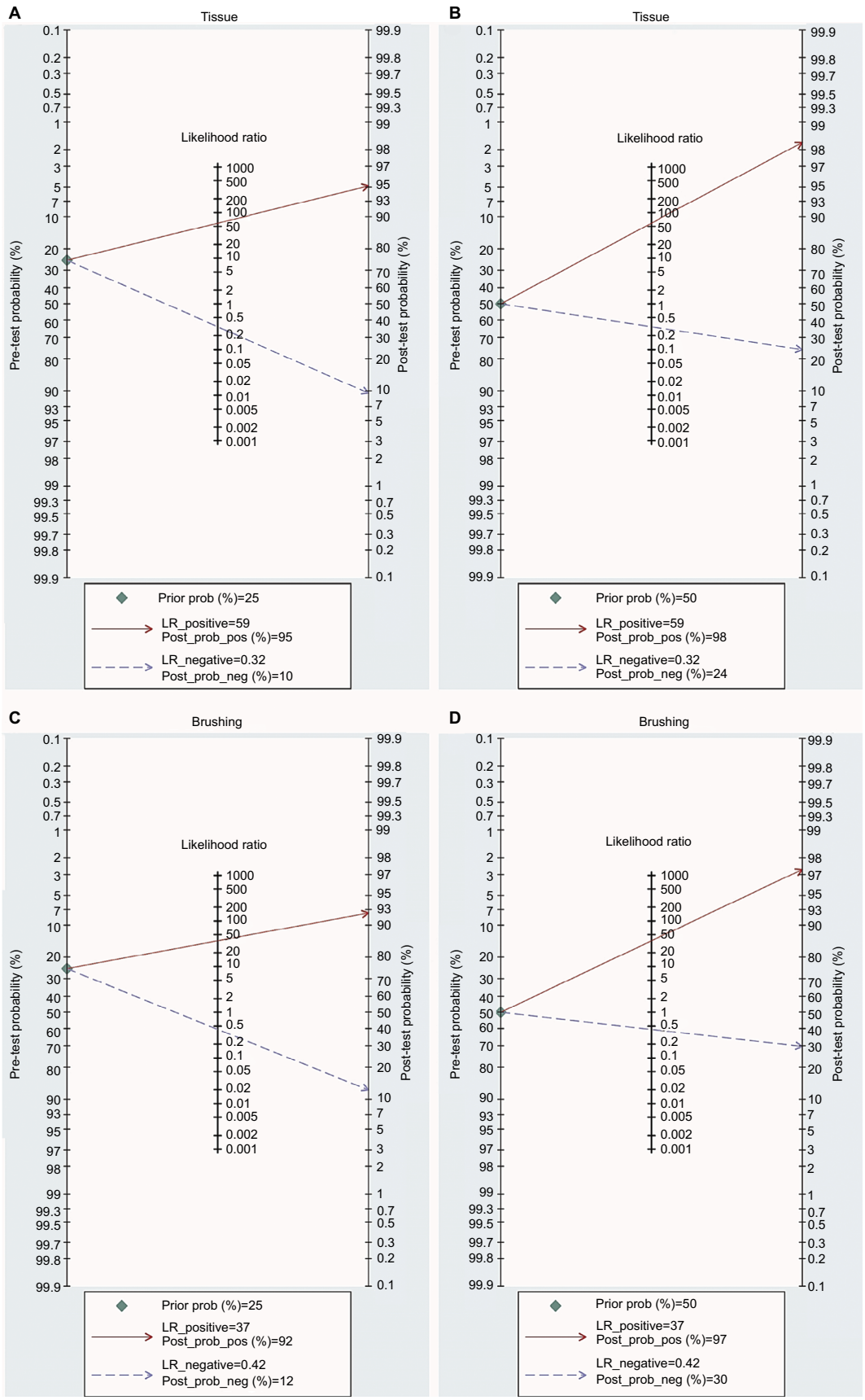

Figure 8 Fagan plots analysis to illustrate the clinical utility of methylated DAPK for identification of NPC by sample type.
Notes: (**A**) The post-test probability of NPC was 95% at a pretest probability of 25% by tissue samples. (**B**) The post-test probability of NPC was 98% at a pretest probability of 50% by tissue samples. (**C**) The post-test probability of NPC was 92% at a pretest probability of 25% by brushing sample. (**D**) The post-test probability of NPC was 97% at a pretest probability of 50% by brushing sample.

island is also a major mechanism for the silencing of these genes.[49–51]

DNA methylation is the process of formation of methylcytosine in DNA by the addition of a methyl group to a cytosine.[52] Neoplastic cells simultaneously harbor widespread genomic hypomethylation and more regional areas of hypermethylation. Each component of methylation imbalance may contribute to tumor progression.[53,54] Hypermethylation of gene promoter regions is associated with gene repression and can be considered an alternative modification to coding mutations that induces the inactivation of TSGs (such as *P16INK4a*, *MGMT*, *GSTP1*, and *APC*) in numerous human cancers.[55–58] The dysregulation of TSGs resulting in the imbalance of biological process and uncontrolled cell proliferation is contributed to the transformation of neoplastic cell.[59] Among these abnormal methylated genes in human cancers, the hypermethylation of *CDKN2A* is well characterized that is involved in tumorigenesis by inducing the loss of negative regulator of cell proliferation.[60] Besides, the identities of the hypermethylated regions can vary between cancer types.[61] The use of the hypermethylation events of *MGMT* in glioma and *GSTP1* in prostate cancer[62] is effective for diagnosis of cancer, indicating that alterations of epigenetic markers could be used as cancer biomarkers.

DAPK, encoded by the *DAPK* gene, belongs to the DAPK family. The DAPK family contains three closely related serine/threonine kinases, named DAPK, ZIPK, and DRP-1.[21] Several lines of evidence indicate that the most studied member of the DAPK family, *DAPK*, has tumor and metastasis suppressor properties.[26,63] DAPK downregulation or inactivation through epigenetic modification, especially DNA methylation, has been observed in a number of metastatic cancers.[64,65] The imbalance of proliferation and apoptosis, partly induced by the inactivation of the apoptotic pathway, has been considered as one of the hallmarks of cancer, specifically the initiation and progression of human cancers, including NPC.[66] The dysfunction of apoptosis-related genes could decrease apoptosis induced by chemotherapy. Thus, the inactivation of the apoptotic pathway by aberrant methylation has been associated with chemoresistance.[67,68] As an important member of the apoptotic pathway, the role of abnormal hypermethylation of *DAPK* in NPC has been investigated by many researchers.[31–36] The detection of *DAPK* methylation in different NPC patients may be one reason for the inconsistent conclusions about the association between *DAPK* methylation and the risk of NPC.[33,35] To solve this problem, we performed a meta-analysis and conducted a subgroup analysis by sample source, and our results support the correlation between hypermethylated *DAPK* and increased risk of NPC, which was also confirmed by subgroup analysis of sample type. Moreover, the pooled OR in brushing samples was close to that in tissue samples, indicating that the detection of methylated *DAPK* in brushing samples could serve as an important alternative non-invasion measurement for diagnosis of NPC. Traditional diagnosis of NPC is made by biopsy of the nasopharyngeal mass. Fused positron emission tomography/computed tomography is a valuable imaging tool in patients for staging diagnosis of NPC. However, NPC is commonly diagnosed late due to its deep location and vague symptoms.[69] Thus, by measuring the nuclear DNA content, DNA diploidy was found to occur earlier in the progression from premalignant to malignant head and neck squamous cell carcinomas (including NPC). However, the diagnostic strength of methylated *DAPK* has not been investigated in NPC.

The current study aims to demonstrate that methylation of *DAPK* was readily applicable for routine diagnostic work. Therefore, diagnostic meta-analyses were performed in brushing samples and tissue samples separately to assess the power of methylated *DAPK* in distinguishing NPC from control tissue. Since the minimum number of included studies for a diagnostic meta-analysis is four, the diagnostic strength of methylated *DAPK* in blood samples was unable to be evaluated. The summary specificity and sensitivity of methylated *DAPK* for tissue samples were 0.99 and 0.69, respectively, and for brushing samples, they were 0.98 and 0.58, respectively, which shows the non-inferior effect for early monitoring of NPC in brushing samples. Fagan plots were drawn based on the values of PLR and NLR to assess the clinical utility of methylated *DAPK*. The Fagan plot is calculated based on Bayes' rule, which is used to formalize how the pre-test probability of the risk of NPC is changed by the detection of methylated *DAPK* to yield the post-test probability of the risk of NPC.[30] From our results, in both diagnostic meta-analyses, the post-test probability of NPC risk increased to more than 90% when an individual with 25% of pre-test probability of NPC had a positive result of *DAPK* methylation. For the exclusion diagnosis, the post-test probability of NPC risk decreased to less than 30% when an individual with the 50% pre-test probability of NPC had a negative result of *DAPK* methylation. According to the results of this diagnostic meta-analysis, the detection of methylated *DAPK* in brushing samples for distinguishing NPC from non-tumor samples could serve as an alternative non-invasive biomarker.

In summary, this integrated analysis demonstrated the correlation of methylated DAPK and increased risk of NPC.

In addition, the detection of methylated *DAPK* in brushing samples of NPC could serve as a promising alternative measurement for monitoring the initiation of NPC. Well-designed prospective studies with larger sample sizes will be indispensable to confirm our results.

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Disclosure

The authors report no conflicts of interest in this work.

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