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Original Article

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Active and Passive Smoking, *BRAF*^{V600E} Mutation Status, and the Risk of Papillary Thyroid Cancer: A Large-Scale Case-Control and Case-Only Study

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Purpose

The association between tobacco smoking and thyroid cancer remains uncertain. We evaluated the associations of active and passive smoking with the risk of papillary thyroid cancer (PTC), the most common type of thyroid cancer, and with the *BRAF*^{V600E} mutation, the most common oncogenic mutation in PTC related to poor prognosis.

Materials and Methods

We conducted this study with newly diagnosed PTC patients (n=2,142) and community controls (n=21,420) individually matched to cases for age and sex. Information on active and passive smoking and potential confounders were obtained from structured questionnaires, anthropometric measurements, and medical records. *BRAF*^{V600E} mutation status was assessed in PTC patients. We evaluated the associations of active and passive smoking with PTC and *BRAF*^{V600E} mutation risk using conditional and unconditional logistic regression models, respectively.

Results

We did not find associations between exposure indices of active and passive smoking and PTC risk in both men and women, except for the association between current smoking and lower PTC risk. Cumulative smoking ≥ 20 pack-years was associated with lower *BRAF*^{V600E} mutation risk in male PTC patients (odds ratio [OR], 0.55; 95% confidence interval [CI], 0.30 to 1.00). The CI for the association was wider in female PTC patients (OR, 0.23; 95% CI, 0.02 to 2.62), possibly owing to a smaller sample size in this stratum.

Conclusion

We did not find consistent associations between active and passive smoking and PTC risk. Cumulative smoking ≥ 20 pack-years was associated with lower *BRAF*^{V600E} mutation risk in male PTC patients.

Key words
Smoking, Passive smoking, Papillary thyroid cancer, *BRAF* mutation, Case-control studies, Case-only studies

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Introduction

The incidence of thyroid cancer has increased considerably in many regions of the world [1], including the Republic of Korea [2]. Previous studies reported that this increase cannot be explained solely by the over-diagnosis of patients due to the use of advanced diagnostic technologies (such as thyroid ultrasonography) [3,4] and that thyroid cancer incidence has truly increased [5].

Although tobacco smoking is an established risk factor for several types of cancer, the association between tobacco smoking and the risk of thyroid cancer remains unclear. Some studies reported no associations between smoking and thyroid cancer risk [6-9], while other studies demonstrated inverse [10-15] or positive associations [16].

BRAF^{V600E} mutation is the most common oncogenic mutation in papillary thyroid cancer (PTC), the most common type of thyroid cancer [17]. PTC patients with *BRAF*^{V600E} mutation have poor clinical outcomes and higher cancer-related mortality compared with those without mutation [18,19]. Despite the importance of *BRAF*^{V600E} mutation in oncogenesis and prognosis of PTC, to our knowledge, the association between tobacco smoking and *BRAF*^{V600E} mutation risk in PTC patients has not been investigated yet.

Most previous studies investigating the association between tobacco smoking and thyroid cancer did not consider passive smoking, resulting in the possibility of measurement error and knowledge gap. Therefore, in the present study, we investigated the associations of active and passive smoking with PTC risk using case-control design, and with *BRAF*^{V600E} mutation risk using case-only design. We hypothesized that active and passive smoking are associated with higher risk of PTC and *BRAF*^{V600E} mutation.

Materials and Methods

1. Study population

The cases were from the Thyroid Cancer Longitudinal Study (T-CALOS) [20]. We defined the cases as histologically confirmed incident PTC patients who underwent surgical procedures at the Seoul National University Hospital between April 2010 and April 2014. Among a total of 2,682 thyroid cancer patients originally included in the T-CALOS, 2,529 patients (94.2%) were diagnosed as PTC, 134 patients (5.0%) as follicular thyroid cancer, and 22 patients (0.8%) as medullary thyroid cancer. Of the 2,529 PTC patients, we excluded 387 patients (15.3%) due to inadequate responses in the questionnaire, leading to 2,142 cases in the final analyses.

We selected controls from the Health Examinees (HEXA) cohort whose baseline survey was conducted between 2004 and 2013. Detailed information on the HEXA cohort has been presented elsewhere [21]. We used the HEXA cohort as a source of control because population characteristics, year of enrollment, contents of acquired information, and study protocols were comparable to T-CALOS. Among a total of 170,082 HEXA participants, we selected 156,844 participants (92.2%) who had never been diagnosed with cancer or thyroid disease by a doctor. Thereafter, we selected controls individually matched to each case for age and sex, using SAS macro GMATCH, which employs a greedy matching algorithm to find the smallest variations in matching factors (age, 50.6 years for cases vs. 50.2 years for controls; proportion of men, 19.5% for cases vs. 19.5% for controls). Cases and controls were matched in a 1:10 ratio, resulting in 21,420 controls in the final analyses.

2. Data collection

At the time of enrollment of the T-CALOS or HEXA cohort, participants underwent face-to-face interviews using a structured questionnaire to obtain information on sociodemographic characteristics, medical history, family history, lifestyles, diet, and reproductive factors. Anthropometric measurements were conducted, and body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). Clinical information such as histologic type of thyroid cancer was obtained from medical records by certified medical record officers and endocrine surgeons.

Information on active smoking status (never, past, or current), number of cigarettes smoked per day, duration of active smoking (years), and age at active smoking initiation (years) was obtained using a structured questionnaire. The cumulative smoking dose (pack-years) was calculated as the number of packs (20 cigarettes/pack) per day multiplied by the duration of active smoking (years). Information on current exposure to passive smoking at home or workplace (yes or no), passive smoking exposure time (minutes per day), duration of passive smoking exposure (years), and age at passive smoking exposure initiation (years) was also obtained using a structured questionnaire.

3. *BRAF*^{V600E} mutation analysis

Detailed information on *BRAF*^{V600E} mutation analysis has been described elsewhere [22]. In brief, paraffin-embedded primary tumor tissues obtained after thyroidectomy were used to assess *BRAF* mutation status. The somatic mutation *BRAF*^{V600E} (T1799A transversion) involving *BRAF* exon 15

was analyzed via DNA amplification by polymerase chain reaction (PCR), purification of PCR products with QIAquick PCR purification kit (Qiagen, Hilden, Germany), and direct bidirectional DNA sequencing with ABI 3130XL Genetic Analyzer BigDye Terminator (Applied Biosystems, Foster City, CA).

4. Statistical analysis

We performed chi-square tests to compare the baseline characteristics between cases and controls. Among the variables that differed between cases and controls ($p < 0.05$), we further selected variables which were also associated with active or passive smoking (yes or no) ($p < 0.05$): educational levels with high school graduation or more (no, yes, or unknown), marital status (single, married, or unknown), and history of chronic diseases (hypertension or dyslipidemia). We used these variables and age (year; unconditional logistic regression), sex (unconditional logistic regression), and BMI (< 25 kg/m^2, ≥ 25 kg/m^2, or unknown; conditional and unconditional logistic regression) as covariates in further analyses. Although the controls selected from HEXA cohort were individually matched to each case for age and sex, the above-mentioned covariates could also confound the results. Therefore, we included these covariates in all following analytical models to control potential confounding.

We used the following variables as exposure indices: active smoking status (never, past, or current), cumulative smoking dose (never, < 20 pack-years, ≥ 20 pack-years, or unknown), age at active smoking initiation (never, < 20 years of age, 20-24 years of age, ≥ 25 years of age, or unknown) as exposure indices of active smoking; current exposure to passive smoking (no or yes), exposure time to passive smoking (never, < 20 minutes/day, ≥ 20 minutes/day, or unknown), exposure duration of passive smoking (never, < 10 years, ≥ 10 years, or unknown), and age at passive smoking exposure initiation (never, < 31 years of age, ≥ 31 years of age, or unknown) as exposure indices of passive smoking.

To evaluate the associations between exposure indices of active and passive smoking and PTC risk, we employed a case-control study design with case and individually matched control samples, conducting conditional logistic regression adjusted for the above-mentioned covariates. Thereafter, we stratified the analyses for the associations between exposure indices of active smoking and PTC risk by the passive smoking status (no exposure to passive smoking, exposure to passive smoking with childhood exposure, or exposure to passive smoking without childhood exposure).

To assess the associations between exposure indices of active and passive smoking and *BRAF*^{V600E} mutation status in PTC patients, we employed a case-only study design with PTC patients with *BRAF*^{V600E} mutation and PTC patients with *BRAF*^{V600E} wild type, conducting unconditional logistic regression adjusted for the above-mentioned covariates. We performed this analysis because investigation of the associations between tobacco smoking and *BRAF*^{V600E} mutation status may provide insights on the underlying molecular mechanisms of epidemiological findings.

We restricted the analyses for the associations of exposure indices of passive smoking with PTC and *BRAF*^{V600E} mutation risk to never-smokers, due to the possibility of measurement error. We conducted all analyses stratified by sex, because active and passive smoking and prevalence of PTC may differ by sex and the results may be confounded by sex. We performed all statistical analyses with SAS ver. 9.4 (SAS Inc., Cary, NC).

5. Ethical statement

The present study was conducted in accordance with the principles of the Declaration of Helsinki. All study participants provided written informed consent, and the Institutional Review Board of Seoul National University Hospital reviewed and approved the study protocol (No. C-1001-067-307).

Results

Among a total of 2,142 cases (417 men and 1,725 women) and 21,420 controls (4,170 men and 17,250 women), cases were more likely to be unmarried (5.8% vs. 4.0%), have educational levels of high school graduation or more (83.9% vs. 75.1%), and have a previous history of hypertension (23.0% vs. 16.6%) and dyslipidemia (16.5% vs. 10.3%) compared with controls (Table 1).

We did not find any associations between exposure indices of active and passive smoking (cumulative smoking dose, age at active smoking initiation, current exposure to passive smoking, exposure time to passive smoking, exposure duration of passive smoking, and age at passive smoking exposure initiation) and PTC risk in both men and women, except for the association between current smoking and PTC risk (odds ratio [OR], 0.57; 95% confidence interval [CI], 0.43 to 0.76 for men; OR, 0.52; 95% CI, 0.35 to 0.78 for women) (Table 2).

When we stratified the analyses by the passive smoking status, exposure indices such as current smoking, cumulative smoking < 20 pack-years, and age at active smoking initiation ≥ 25 years of age were associated with lower PTC risk compared with never smoking in both men and women not exposed to passive smoking (S1 Table).

When we evaluated the associations between smoking and

Table 1. Baseline characteristics of papillary thyroid cancer patients and healthy controls by sex

Characteristic	Total[a] (2,142 cases/21,420 controls)			Men[a] (417 cases/4,170 controls)			Women[a] (1,725 cases/17,250 controls)		
	Case (n=2,142)	Control (n=21,420)	p-value[b]	Case (n=417)	Control (n=4,170)	p-value[b]	Case (n=1,725)	Control (n=17,250)	p-value[b]
Age (≥ 50 yr)	1,139 (53.2)	11,390 (53.2)	1.00	219 (52.5)	2,190 (52.5)	1.00	920 (53.3)	9,200 (53.3)	1.00
Body mass index (≥ 25 kg/m^2)	590 (27.6)	6,215 (29.0)	0.18	130 (31.2)	1,681 (40.4)	< 0.01	460 (26.8)	4,534 (26.3)	0.66
Ever drinkers	1,008 (47.1)	9,837 (45.9)	0.32	345 (82.7)	3,404 (81.6)	0.58	663 (38.4)	6,433 (37.3)	0.35
Educated (≥ high school graduation)	1,791 (83.9)	15,977 (75.1)	< 0.01	388 (93.3)	3,569 (86.1)	< 0.01	1,403 (81.6)	12,408 (72.4)	< 0.01
Married	2,012 (94.2)	20,534 (96.0)	< 0.01	390 (93.5)	3,948 (95.0)	0.21	1,622 (94.3)	16,586 (96.2)	< 0.01
History of diabetes mellitus	134 (6.3)	1,144 (5.3)	0.08	43 (10.3)	329 (7.9)	0.08	91 (5.3)	815 (4.7)	0.31
History of hypertension	492 (23.0)	3,544 (16.6)	< 0.01	125 (30.1)	877 (21.0)	< 0.01	367 (21.3)	2,667 (15.5)	< 0.01
History of dyslipidemia	351 (16.5)	2,204 (10.3)	< 0.01	82 (19.7)	484 (11.6)	< 0.01	269 (15.7)	1,720 (10.0)	< 0.01
Menopause							838 (50.2)	8,633 (50.1)	0.92

Values are presented as number (%). [a]Cases were individually matched to controls in a ratio of 1:10 for age and sex, [b]p-values were estimated by chi-square tests.

BRAF^{V600E} mutation in PTC patients, cumulative smoking ≥ 20 pack-years was associated with lower *BRAF*^{V600E} mutation risk compared with never smoking in male PTC patients (OR, 0.55; 95% CI, 0.30 to 1.00). In female PTC patients, point estimate for the association between cumulative smoking ≥ 20 pack-years and *BRAF*^{V600E} mutation was also less than 1; however, the CI was wider (OR, 0.23; 95% CI, 0.02 to 2.62), possibly due to a smaller sample size in this stratum (n=3) (Table 3).

Discussion

We did not find the associations between exposure indices of active and passive smoking and PTC risk, except for the association between current smoking and PTC. When we evaluated the associations between smoking and *BRAF*^{V600E} mutation, higher cumulative smoking was associated with lower *BRAF*^{V600E} mutation in male PTC patients.

Current smoking was found to be associated with lower PTC risk, although other exposure indices of active and passive smoking were not associated with PTC risk in the present study. Based on our findings, we believe that active smoking might be a more direct and important exposure index associated with PTC risk, than past, cumulative, or passive smoking. However, this observed association should be interpreted cautiously due to the possibility of reverse causality, because we could not determine the time patients had quit smoking and categorize those who had quitting smoking just before the diagnosis or surgery as a separate group, due to the lack of relevant information. Information on active smoking status was obtained upon enrollment. PTC patients might have been more likely to quit smoking than the control participants. In addition, the possibility that the observed association might be due to random chance cannot be ruled out, because we performed the analyses using various exposure indices.

Although some previous studies reported inverse [10-15] or positive associations between active smoking and thyroid cancer risk [16], other studies demonstrated no associations [6-9]. This inconsistency may be explained by differences in accuracy of exposure measurement and outcome ascertainment, heterogeneity of study population in terms of genetic, social, and lifestyle factors, analytical models and adjusted covariate sets, and random error.

Passive smoking is one of the potential sources of measurement error of exposure, which might explain inconsistent results of previous studies at least in part. However, there have been limited number of studies exploring the association between passive smoking and thyroid cancer risk. In a

Table 2. Associations[a] between active and passive smoking and the risk of papillary thyroid cancer assessed using case-control study design

	Men		Women	
	Cases/Controls	OR (95% CI)	Cases/Controls	OR (95% CI)
Active smoking (total)	417/4,170		1,725/17,250	
Active smoking status				
Never	122/1,115	1.00	1,664/16,515	1.00
Past	202/1,598	1.15 (0.91-1.47)	36/250	1.27 (0.88-1.82)
Current	93/1,457	0.57 (0.43-0.76)	25/475	0.52 (0.35-0.78)
Cumulative smoking dose (pack-year)				
Never	122/1,115	1.00	1,664/16,515	1.00
< 20	150/1,720	0.80 (0.61-1.02)	43/662	0.64 (0.47-0.88)
≥ 20	125/1,307	0.87 (0.66-1.14)	11/63	1.73 (0.91-3.29)
Age at active smoking initiation (yr)				
Never	122/1,115	1.00	1,664/16,515	1.00
≥ 25	32/440	0.64 (0.44-0.97)	25/468	0.53 (0.35-0.80)
< 25	259/2,611	0.91 (0.71-1.17)	32/265	1.17 (0.72-1.90)
Passive smoking (total)[b]	122/1,115		1,664/16,515	
Current exposure to passive smoking				
No	99/915	1.00	1,343/13,536	1.00
Yes	23/200	1.06 (0.64-1.75)	321/2,979	1.09 (0.95-1.24)
Exposure time to passive smoking (min/day)				
No	99/915	1.00	1,343/13,536	1.00
< 20	16/130	1.15 (0.65-2.01)	220/1,938	1.15 (0.97-1.35)
≥ 20	7/70	0.93 (0.38-2.09)	101/1,041	0.98 (0.79-1.21)
Exposure duration of passive smoking (yr)				
No	99/915	1.00	1,343/13,536	1.00
1-9	5/53	0.88 (0.34-2.24)	70/710	0.99 (0.76-1.31)
≥ 10	9/108	0.78 (0.38-1.58)	168/1,868	0.91 (0.77-1.08)
Age at passive smoking exposure initiation (yr)				
No	99/915	1.00	1,343/13,536	1.00
≥ 31	10/104	0.90 (0.45-1.77)	117/1,135	1.05 (0.88-1.32)
< 31	4/57	0.67 (0.19-2.40)	121/1,443	0.85 (0.68-1.05)

OR, odds ratio; CI, confidence interval. [a]Conditional logistic regression models (matching variables: age and sex) adjusted for body mass index, education, marital status, and history of chronic diseases were used to estimate the association between active and passive smoking and papillary thyroid cancer risk, [b]The analyses for the associations between passive smoking and papillary thyroid cancer were conducted among never-smokers.

case-control study with 467 cases and 255 controls in the United States, passive smoking was not associated with thyroid cancer risk [23]. However, a case-control study with 151 cases and 139 controls in the United States reported that mothers of controls were more likely to be smokers than mothers of cases [24].

In addition, in the present study, exposure indices such as current smoking, cumulative smoking dose < 20 pack-years, and age at active smoking initiation ≥ 25 years of age were associated with lower PTC risk in participants not exposed to passive smoking; however, these associations were not observed in participants exposed to passive smoking. To the best of our knowledge, this is the first study reporting different associations between active smoking and thyroid cancer by passive smoking exposure. However, because information on active and passive smoking was obtained using a structured questionnaire and, therefore, may not be precise, further thorough investigations on the potential effects of passive smoking on thyroid cancer are warranted to confirm the results.

Genetic difference between study populations might also explain inconsistent results of previous studies at least in part. In the present study, higher cumulative smoking was associated with lower *BRAF*^{V600E} mutation in male PTC pati-

Table 3. Associations[a] between active and passive smoking and the risk of *BRAF*^{V600E} mutation in papillary thyroid cancer patients

	Men			Women		
	BRAF^{V600E}	*BRAF*^{WT}	OR (95% CI)	*BRAF*^{V600E}	*BRAF*^{WT}	OR (95% CI)
Active smoking (total)	306	90		1,131	532	
Active smoking status						
Never	90	24	1.00	1,094	512	1.00
Past	146	50	0.78 (0.45-1.35)	24	9	1.25 (0.58-2.70)
Current	70	16	1.17 (0.55-2.33)	13	11	0.55 (0.25-1.24)
Cumulative smoking dose (pack-year)						
Never	90	24	1.00	1,094	512	1.00
< 20	126	25	1.34 (0.71-2.52)	29	15	0.90 (0.44-1.79)
≥ 20	76	37	0.55 (0.30-1.00)	1	2	0.23 (0.02-2.61)
Age at active smoking initiation (yr)						
Never	90	24	1.00	1,094	512	1.00
≥ 25	72	10	1.92 (0.84-4.29)	22	8	1.28 (0.55-2.93)
< 25	136	52	0.71 (0.37-1.35)	12	11	0.51 (0.22-1.19)
Passive smoking (total)[b]	90	24		1,094	512	
Current exposure to passive smoking						
No	65	18	1.00	878	396	1.00
Yes	25	6	1.15 (0.41-3.25)	255	116	1.00 (0.79-1.30)
Exposure time to passive smoking (min/day)						
No	65	18	1.00	878	396	1.00
< 20	8	2	1.11 (0.20-5.70)	130	55	1.07 (0.74-1.53)
≥ 20	9	3	0.83 (0.20-3.39)	125	61	0.92 (0.65-1.31)
Exposure duration of passive smoking (yr)						
No	65	18	1.00	878	396	1.00
1-9	9	3	0.83 (0.15-3.59)	25	69	1.08 (0.65-1.79)
≥ 10	5	2	0.69 (0.12-3.87)	85	185	0.96 (0.72-1.30)
Age at passive smoking exposure initiation (yr)						
No	65	18	1.00	878	396	1.00
≥ 31	8	1	2.22 (0.26-18.90)	65	20	1.46 (0.88-2.45)
< 31	6	4	0.42 (0.08-2.22)	86	53	0.87 (0.59-1.26)

OR, odds ratio; CI, confidence interval; WT, wild type. [a]Unconditional logistic regression models adjusted for age, sex, body mass index, education, marital status, and history of chronic diseases were used to estimate the association between active and passive smoking and papillary thyroid cancer risk, [b]The analyses for the associations between passive smoking and *BRAF*^{V600E} mutation were conducted among never-smokers.

ents. Among patients with non-small-cell lung cancer, *BRAF*^{V600E} mutation was found to be more common in never-smokers than current or ever-smokers, while *BRAF*$^{Non\text{-}V600E}$ mutation was only found in current or ever-smokers [25]. However, among patients with colon cancer, active smoking was associated with *BRAF*^{V600E} mutation risk [26]. Although tobacco smoking can reduce thyroid stimulating hormone levels, which may lead to lower iodine uptake and subsequent lower *BRAF*^{V600E} mutation risk [10,27-29], the biological mechanisms underlying these results are still unclear. To our knowledge, this is the first study reporting the association between active smoking and lower *BRAF*^{V600E} mutation risk in PTC patients. As such, and since the biological mechanisms and implications of these results are still unclear, further studies are warranted.

The case-only design employed in the analyses regarding *BRAF*^{V600E} mutation can be used to estimate gene-environment interactions on a multiplicative scale in case genetic and environmental factors are independent in the general population [26,30]. *BRAF*^{V600E} mutation is an oncogenic mutation and, to the best of our knowledge, there have been no studies directly investigating whether tobacco smoking would be associated with *BRAF*^{V600E} mutation in the general population. If we assume that active and passive smoking are not

associated with *BRAF*^{V600E} mutation status in the general population, the results of the present study suggest that interactions between smoking and *BRAF*^{V600E} mutation status on PTC risk exist, and that the association between smoking and PTC risk may differ by *BRAF*^{V600E} mutation status, which might explain the inconsistent results of previous studies. Because there is little evidence available, investigations using general population samples are warranted to obtain further insight on the potential gene-environment interactions.

There are some limitations to the present study. First, we evaluated active and passive smoking at study enrollment using structured questionnaires, instead of using biomarkers such as urinary cotinine levels. However, urinary cotinine levels may not be adequate to evaluate cumulative or past smoking, because the average half-life of cotinine is reported to be < 20 hours [31]. In addition, in the present study, trained interviewers obtained information from participants using a structured questionnaire following a standardized protocol to minimize the possibility of exposure misclassification. Second, thyroid cancer patients were recruited from one hospital, which may lower the generalizability of the results. Third, we could not confirm that controls did not have PTC with diagnostic tools such as thyroid ultrasonography. Therefore, it is possible that a few subclinical PTC patients were assigned as controls, which may result in underestimation of the true associations. Fourth, although we used large-scale data, some strata such as female PTC patients with cumulative smoking ≥ 20 pack-years still had small sample size, lowering the power to test hypotheses. Fifth, because we used various exposure indices, some observed associations might have occurred by random chance. However, we presented all results analyzed under a pre-established hypothesis, and therefore, did not adjust the analyses for multiple comparison. Sixth, the information on the time of quitting smoking was not available, leading to a potential information bias and reverse causality regarding the association between current smoking and lower PTC risk.

However, the present study has several strengths. First, this is the first study to report the association between active smoking and lower *BRAF*^{V600E} mutation risk in PTC patients. Second, we obtained detailed information on active smoking behaviors, passive smoking exposure, and various potential confounders. Third, we used large-scale data (2,142 cases and 21,420 controls) with a sufficient number of male PTC patients (n=417).

In conclusion, we did not find consistent associations between active and passive smoking and PTC risk. Higher cumulative smoking was associated with lower *BRAF*^{V600E} mutation in male PTC patients. Because tobacco smoking can increase the risk of various diseases including other types of cancer, a comprehensive evaluation is needed before deriving clinical and public health implications from these results.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (https://www.e-crt.org).

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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