Association of High Iodine Intake with the T1799A *BRAF* Mutation in Papillary Thyroid Cancer

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Context: Epidemiological studies have indicated that high iodine intake might be a risk factor for papillary thyroid cancer (PTC), which commonly harbors the oncogenic T1799A *BRAF* mutation.

Objective: The objective of the study was to investigate the relationship between *BRAF* mutation in PTC and iodine intake in patients.

Subjects and Methods: We analyzed and compared the prevalences of the T1799A *BRAF* mutation in classical PTC of 1032 patients from five regions in China that uniquely harbor different iodine contents in natural drinking water, ranging from normal (10–21 μg/liter) to high (104–287 μg/liter). The *BRAF* mutation was identified by direct DNA sequencing.

Results: The prevalence of *BRAF* mutation was significantly higher in any of the regions with high iodine content than any of the regions with normal iodine content. Overall, *BRAF* mutation was found in 387 of 559 PTC with high iodine content (69%) *vs.* 252 of 473 PTC with normal iodine content (53%), with an odds ratio of 1.97 (95% confidence interval 1.53–2.55) for the association of *BRAF* mutation with high iodine content ($P < 0.0001$). In addition, clinicopathological correlation analysis, the largest one of its type ever, showed that *BRAF* mutation was significantly associated with extrathyroidal invasion, lymph node metastasis, and advanced tumor stages of PTC.

Conclusions: High iodine intake seems to be a significant risk factor for the occurrence of *BRAF* mutation in thyroid gland and may therefore be a risk factor for the development of PTC. This large study also confirmed the association of *BRAF* mutation with poorer clinicopathological outcomes of PTC. **(*J Clin Endocrinol Metab* 94: 1612–1617, 2009)**

Follicular epithelial cell-derived thyroid cancer is the most common endocrine malignancy with a rapid rising incidence worldwide in recent decades (1–3). Histologically this cancer can be classified into papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), and anaplastic thyroid cancer, with PTC accounting for 80–85% of all thyroid malignancies. The increased worldwide use of screening and diagnostic testing is an important, but may not be the only, contributor to the currently rising incidence of thyroid cancer. Certain unknown environmental factors might also play a role in the occurrence of thyroid cancer.

Many epidemiological studies suggest that such environmental factors may include high intake of iodine (4–9), a normal nutrient that is uniquely accumulated and metabolized by thyroid cells for thyroid hormone synthesis. Many of these studies have revealed a lower incidence of thyroid cancer in naturally

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Abbreviations: FTC, Follicular thyroid cancer; HIC, high iodine content; NIC, normal iodine content; PTC, papillary thyroid cancer.

low-iodine-intake regions and a higher incidence in naturally high-iodine-intake regions, particularly in the case of PTC (4–9). Because iodine deficiency is associated with hypothyroidism that can cause serious developmental and growth abnormalities, implementation of iodine supplementation programs, such as salt iodination, have been widely used in the world (10–13). The paramount importance of this iodine prophylaxis, in terms of prevention of hypothyroidism and cretinism, has been unequivocally established. This iodine prophylaxis also seems to be associated with an increased incidence of thyroid cancer, particularly PTC, or a transition of the type of thyroid cancer to an increased predominance of PTC (8–13). A comprehensive meta analysis addressing this issue revealed a ratio of PTC to FTC to range from 3.4:1 to 6.5:1 in areas with high iodine intake *vs.* 1.6:1 to 3.7:1 in areas with moderate iodine intake and *vs.* 0.19:1 to 1.7:1 in iodine-deficient areas (13). These findings are interestingly consistent with the demonstration in animal studies that high dietary iodine intake favored chemically induced development of PTC, whereas insufficient iodine intake favored FTC (14). The molecular mechanism for this relationship of iodine with PTC is unclear.

Molecular studies in recent years have identified a T1799A transversion *BRAF* mutation that occurs in about 45% of PTC and 25% of apparently PTC-derived anaplastic thyroid cancer but not in any other type of thyroid tumors, such as FTC and benign thyroid tumors (15). This mutation causes a valine-to-glutamic amino acid change in codon 600 of the BRAF protein, resulting in constitutive activation of the BRAF kinase and the Ras → Raf → MAPK kinase → MAPK/ERK pathway. PTC tumorigenicity of this mutation was demonstrated in transgenic mouse studies showing that targeted thyroid overexpression of the *BRAF* mutant initiated specifically the development of PTC (16) and in metaanalysis studies on patients showing that this mutation promoted poorer clinicopathological outcomes of PTC (15).

Given these epidemiological and molecular data on iodine and *BRAF* mutation in relation to PTC, in the present study, we asked whether high iodine intake was a risk factor for the occurrence of *BRAF* mutation in thyroid gland as a molecular basis for its association with PTC. To this end, we investigated the relationship of iodine intake in terms of iodine contents in drinking water with the prevalence of *BRAF* mutation in a large series of PTC patients from several unique regions in China that naturally have different iodine contents in drinking water.

Subjects and Methods

Study regions and tumor samples

Representative regions in China in which iodine contents in natural drinking water ranged from normal to high were chosen for the present study, including Shenyang, Shanghai, and Qingdao, where iodine contents in drinking water were documented to be normal (10–21 μg/liter), and Binzhou and Heze in the close vicinity of the Yellow River and Old Yellow River, where iodine contents in drinking water were high (104–287 μg/liter) (Fig. 1 and Table 1) (17–23). Urinary iodine levels in individuals living in these regions were documented to be correspondingly normal or high (17, 18, 20, 23, 24) (Table 1). None of these regions has

FIG. 1. Geographical distribution of the regions in China investigated in the present study. The three regions with normal iodine contents in drinking water are Shenyang, Shanghai, and Qingdao. Binzhou and Heze are close to the Yellow River and have very high iodine contents in drinking water.

a history of volcanic activities, radioactive nuclear pollution, or other extraordinary environment-contaminating events. Shenyang and Shanghai, where the iodine content in drinking water is normal, are industrialized regions, whereas Binzhou and Heze, where iodine content in drinking water is high, are relatively underdeveloped regions. Residents in these two regions had been traditionally drinking underground water until recent years. Qingdao is in between these regions in industrial level. With institutional review board approval, primary classical PTC samples (>1 cm) were randomly selected from 1032 adult patients in local hospitals, consisting of 240 from Shenyang, 76 from Shanghai, 157 from Qingdao, 170 from Binzhou, and 389 from Heze. Patients in the areas with normal iodine were operated between 1990 and 2006, and patients in the areas with high iodine were operated between 1991 and 2007. The histological diagnosis of tumors was made and agreed on by at least two local pathologists and confirmed by an experienced pathologist at the China Medical University (Y.Z.) based on World Health Organization criteria. Because the prevalence of *BRAF* mutation varied with different subtypes of PTC (15), we included only classical PTC for the present study. Patients from the five regions had comparable age and gender (Table 1).

DNA isolation

Paraffin-embedded PTC samples from patients were microdissected and DNA was isolated as previously described (25). Briefly, tissues dissected from paraffin-embedded specimen were treated for 8 h at room temperature with xylene, followed by digestion with 1% sodium dodecyl sulfate and 0.5 mg/ml proteinase K at 48 C for 48 h. To facilitate the digestion, a midinterval addition of a spiking aliquot of concentrated sodium dodecyl sulfate-proteinase K was added to the samples. DNA was subsequently isolated from the digested tissues by standard phenol-chloroform extraction and ethanol precipitation procedures.

Detection of *BRAF* mutation

Because the T1799A transversion mutation is virtually the only *BRAF* mutation that has been described in PTC with a high prevalence in previous studies (15), we sought this particular mutation in PTC in the present study. The *BRAF* mutation was analyzed using genomic DNA by direct sequencing. For direct DNA sequencing, exon 15 of the *BRAF* gene was amplified by PCR, followed by Big Dye terminator cycle sequencing reaction and sequence reading on an ABI PRISM 3730 genetic analyzer (Applied Biosystems, Foster City, CA). The PCR protocol and primers for exon 15 of the *BRAF* gene were as described previously (25).

TABLE 1. Association of the T1799A *BRAF* mutation in PTC with high iodine contents in drinking water

	Regions with NIC in drinking water			Regions with HIC in drinking water		
	Shenyang	**Shanghai**	**Qingdao**	**Binzhou**	**Heze**	***P* value**[a]
Iodine in drinking water (μg/liter, means ± SD) (number of water samples tested)	21 ± 14 (18) (n = 30)[b]	17 (19) (n = 62)[c]	10 (20) (n = 200)[d]	104 ± 136 (21) (n = 128)	287 ± 151 (22) (n = 24)	
Urinary iodine excretion in population (μg/liter, median) (number of urine samples tested)	188 (18) (n = 200)	198 (24) (n = 437)	82.77 (20) (n = 338)[d]	>900 (17)[e]		
PTC to non-PTC ratio in hospitals that provided samples during the sampling period	304/70 (4.34)	148/40 (3.70)	564/126 (4.48)	228/29 (7.86)	577/71 (8.13)	<0.0001
Number of PTC cases	240	76	157	170	389	
Age of patients at diagnosis (yr, means ± SD)	43.66 ± 12.41	43.88 ± 15.21	44.25 ± 13.71	43.63 ± 10.49	42.52 ± 14.01	0.63
Gender of patients (female/male)	210/30	64/12	130/27	141/29	322/67	0.57
T1799A *BRAF* mutation [mutation/total cases (%)]	128/240 (53.33)	38/76 (50.00)	86/157 (54.78)	115/170 (67.65)	272/389 (69.92)	<0.0001
	Overall for NIC: 252/473 (53.28)			Overall for HIC: 387/559 (69.23)		<0.0001

[a] Statistical analysis is as described in *Subjects and Methods*. *P* = 0.66 and 0.26 on comparison of patient age and gender between NIC and HIC groups, respectively.

[b] These data on normal drinking water iodine were obtained but not reported in this study (18), which reported the data only on urinary iodine (also normal) instead.

[c] The data were reported only as mean.

[d] These data represent medians. These testings were performed in 1995 before the universal salt iodinization in 1996 in China, and a later testing in 2003 in Qingdao showed iodine in water to be 19.07± 6.81 mg/liter (n = 3) and median urinary iodine 164.15 mg/liter (n = 36) (23). All the iodine testings shown for other regions were performed from 1998 to 2005.

[e] Sampling size was not reported in the reference.

Statistical analysis

Categorical data were summarized using frequencies and percentiles. Age at the diagnosis was normally distributed and was summarized as means ± SD. Comparison of two groups of categorical variables was performed using the χ^2 test. Comparison of multigroups of categorical variables was performed using the m × n χ^2 test. Comparison of patient ages between two groups and among multiple groups were performed using *t* test and ANOVA test, respectively. All reported *P* values were two sided. $P < 0.05$ was considered to be statistically significant. Analysis was performed using the SPSS software (versions 11.5, Chicago, IL).

Results

The overall prevalence of the T1799A *BRAF* mutation in this series of PTC was 62% (639 of 1032). The prevalences of *BRAF* mutation were similar among regions with comparable iodine contents in drinking water (*i.e.* Shenyan *vs.* Shanghai *vs.* Qingdao, 53 *vs.* 50 *vs.* 55%, $P = 0.79$; Binzhou *vs.* Heze, 68 *vs.* 70%, $P = 0.59$) (Table 1 and Fig. 2, *left panel*). In contrast, the prevalences were significantly different on multigroup analysis for the five regions ($P < 0.0001$) and in pairwise comparisons for normal-iodine-content (NIC) *vs.* high-iodine-content (HIC) regions [*i.e.* Shenyang *vs.* Binzhou ($P = 0.004$), Shenyang *vs.* Heze ($P < 0.001$), Shanghai *vs.* Binzhou ($P = 0.008$), Shanghai *vs.* Heze ($P < 0.001$), Qingdao *vs.* Binzhou ($P = 0.017$), and Qingdao *vs.* Heze ($P < 0.001$)], showing a clear association of *BRAF* mutation with HIC in drinking water (Table 1). The ratio of PTC/non-PTC during the sampling period in the hospitals in which the thyroid tumors were collected for this study was higher in HIC regions than NIC regions (Table 1), consistent with the widely reported association of a higher ratio of PTC to non-PTC with higher iodine intake (13). To further analyze the relationship between iodine intake and *BRAF* mutation in PTC, we divided the data into two groups according to the levels of iodine contents in drinking water: one group consisted of Shenyang, Shanghai, and Qingdao with NIC and the other consisted of Binzhou and Heze with HIC. The prevalences of the *BRAF* mutation were 53% (252 of 473) in the NIC group and 69% (387 of 559) in the HIC group, with the latter being significantly higher than the former (Table 1 and Fig. 2, *right panel*) and carrying an increased risk of harboring *BRAF* mutation (odds ratio 1.97, 95% confidence interval 1.53–2.55, $P < 0.0001$).

We did not see an age or gender preference of the *BRAF* mutation in the current cohort of patients, in the NIC, HIC, or overall group (Table 1). Given the sometimes controversies from different studies in recent years on the role of *BRAF* mutation in the aggressiveness of PTC (15), we took the advantage of this large series of cases to also analyze the relationship of *BRAF* mutation with clinicopathological characteristics of PTC. In the 799 cases of PTC from Shenyang, Binzhou, and Heze for which information on pathological characteristics of tumors was available, the analysis showed a significant association of *BRAF* mutation with extrathyroidal invasion, lymph nodes metastasis, and advanced tumor stages (III/IV) in these regions, particularly the HIC regions (Table 2). This was the largest single analysis of its type and may help reconcile the inconsistent findings in some previous smaller studies on the relationship between *BRAF* mutation and clinicopathological outcomes of PTC (15).

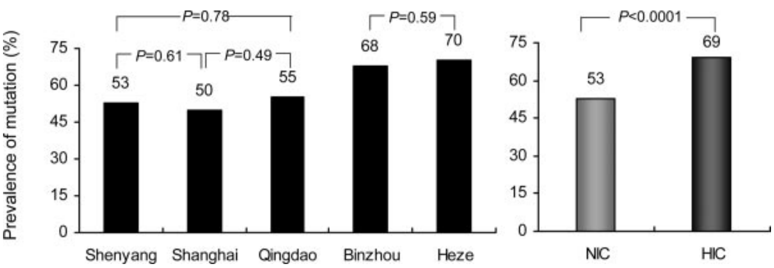

FIG. 2. Comparison of prevalences of the T1799A *BRAF* mutation in PTC from regions with NIC or HIC in drinking water. The prevalences of T1799A *BRAF* mutation in PTC were similar in different regions within the NIC (Shenyang, Shanghai, and Qingdao) or HIC (Binzhou and Heze) groups (*left panel*), whereas it is significantly higher in the HIC than NIC group ($\chi^2 = 27.66$; $P <$ 0.0001) (*right panel*). The values shown at the *top of the bars* represent the prevalences of *BRAF* mutation.

Discussion

Using this large series of PTC patients from several unique regions in China with different iodine contents in natural drinking water, we demonstrated a significant association of the T1799A *BRAF* mutation in PTC with high iodine intake. The T1799A *BRAF* mutation is the most common oncogenic genetic alteration in PTC and, through aberrant activation of the MAPK pathway, can initiate the development and promote the progression of PTC (15, 16). Therefore, the present results suggest that iodine, when excessively taken, could be a risk factor for the occurrence of *BRAF* mutation and hence development of PTC. Thyroid cells have a unique physiological function to take up, concentrate, and metabolize iodide and can therefore be specifically affected by high iodine. This, together with the relationship between *BRAF* mutation in PTC and high iodine intake shown in the present study, might provide an explanation why, compared with cancers originated from other tissues, PTC harbors *BRAF* mutation with an unusually high prevalence (15).

There is no known environmental factor that can cause *BRAF* mutation. Frasca *et al.* (26) recently examined the relationship of *BRAF* mutation in PTC with iodine intake in some regions in Italy. The authors found *BRAF* mutation in 107 of 270 cases in an iodine-sufficient region (40%) *vs.* 18 of 53 cases of PTC in an iodine-deficient region (34%) ($P = 0.44$). Although the difference in *BRAF* mutation between the two regions was not statistically significant, the trend of a higher prevalence of *BRAF* mutation in the higher-iodine (normal iodine) region than the lower-iodine (iodine deficient) region is in line with our finding of the significant association of *BRAF* mutation with high iodine intake in the present study. It is possible that very high iodine intake, seen in the present study, had a stronger impact on the occurrence of *BRAF* mutation. A recent study in an Irish population demonstrated a higher prevalence of *BRAF* mutation in PTC from the recent decades than that in earlier times and the authors suspected that the cause might be an environmental factor (27). It would be interesting to see whether this environmental factor could be increased iodine intake. The molecular mechanisms in which high iodine might promote the development of *BRAF* mutation in thyroid cells remain to be elucidated. Speculatively, it could involve generation of harmful molecular species from the normal oxidation process of iodide in the thyroid cell that may lead to DNA damage or impairment of DNA repair with consequent formation of *BRAF* mutation.

The demonstration of high iodine intake as a risk factor for the occurrence of *BRAF* mutation in thyroid cells seems to provide a molecular explanation for the association of increased iodine intake with PTC or a shift of the type of thyroid cancer to PTC observed in many epidemiological studies (4–13). In this context, increased iodine intake, which may occur through iodine supplementation programs, might have contributed, to some extent, to the overall rising incidence of PTC worldwide in recent decades. This impact of iodine intake on the rising incidence of PTC in the general population, however, is likely smaller than that of increased use of screening and diagnostic testings, as suggested by the rising incidence of PTC accompanied by an actually somewhat decreased iodine intake in the United States in recent years (28). In this case, some decrease in PTC incidence that presumably could occur with decreased iodine intake might have been over-offset by an increase in the incidence of PTC caused by the increased use of screening and diagnostic testings for thyroid cancer. It also appears that the amount of iodine intake from normal sea fish consumption may not be a significant risk for the occurrence of thyroid cancer as a metaanalysis study did not show an overall increase in the incidence of thyroid cancer (mostly PTC) in association with fish consumption (29). In this analysis, however, a relatively small positive effect of sea fish iodine on the incidence of PTC could be masked by the effect of the increased use of screening and diagnostic testings on PTC incidence that could more profoundly and equally affect all the subjects regardless of their fish-eating backgrounds.

A high incidence of goiter is seen in high-iodine regions investigated in the present study (17). A high incidence of autoimmune thyroiditis and hypothyroidism was reported in other similar high-iodine regions in China (7). Demonstration of high iodine intake as a risk factor for the occurrence of *BRAF* mutation in thyroid cells, and hence the development of PTC, provides further evidence that excessive iodine intake can have a negative impact on human health as iodine deficiency does. Therefore, it is imperative that iodine intake be maintained at an appropriate level. It should be emphasized that it is the very high iodine intake that was shown in the present study to be associated with *BRAF* mutation in PTC. This high level of iodine intake is not reachable with the normal iodine prophylaxis widely administered on iodine-deficient populations in different parts of the world. Such iodine prophylaxis is therefore likely safe. However, the present study does support the notion that an iodine supplementation program needs to be tailored to a particular region in such a way that it appropriately suits with the level of the natural iodine content of the region and avoids excessive iodine intake to optimize the balance between benefit and potential harm of iodine supplementation.

Given the association of *BRAF* mutation with aggressiveness of PTC and high iodine intake, one might expect the regions with high iodine intake to have higher levels of aggressive disease of

TABLE 2. Association of the T1799A *BRAF* mutation with clinicopathological characteristics in PTC

	Extrathyroidal invasion				Lymph nodes metastasis				Disease stages III/IV			
	Overall	***BRAF+***	***BRAF−***	***P* value**	**Overall**	***BRAF+***	***BRAF−***	***P* value**	**Overall**	***BRAF+***	***BRAF−***	***P* value**
Overall	12% (99/799)	15% (77/515)	8% (22/284)	0.003	35% (279/799)	38% (198/515)	29% (81/284)	0.005	25% (196/799)	29% (151/515)	16% (45/284)	< 0.001
SY^{NIC}	11% (27/240)	14% (18/128)	8% (9/112)	0.20	31% (75/240)	35% (45/128)	27% (30/112)	0.16	25% (61/240)	31% (40/128)	19% (21/112)	0.026
BZ	12% (20/170)	14% (16/115)	7% (4/55)	0.32	38% (64/170)	42% (48/115)	29% (16/55)	0.11	27% (46/170)	32% (37/115)	16% (9/55)	0.047
HZ	13% (52/389)	16% (43/272)	8% (9/117)	0.038	36% (140/389)	39% (105/272)	30% (35/117)	0.102	23% (89/389)	27% (74/272)	13% (15/117)	0.002
$BZ+HZ^{HIC}$	13% (72/559)	15% (59/387)	8% (13/172)	0.012	36% (204/559)	40% (153/387)	30% (51/172)	0.025	24% (135/559)	29% (111/387)	14% (24/172)	<0.001
P value (HIC *vs.* NIC)	0.52	0.74	0.93		0.15	0.38	0.60		0.70	0.58	0.28	

PTC. Indeed, our data on extrathyroidal invasion and lymph node metastasis appear to be consistent with this possibility (Table 2), although no statistical difference was achieved, probably due to insufficient sample size.

There are a few limitations in this study, including, for example, the lack of information on the incidence of PTC in different regions investigated and other unknown environmental factors that might affect the occurrence of *BRAF* mutation and development of thyroid cancer. We also cannot be certain whether different industrial levels of the regions investigated could account for the difference in the *BRAF* mutation rate. This is unlikely, however, because industrialization usually tends to be associated with increased cancer risk, whereas we observed a higher *BRAF* mutation rate in the less industrialized regions in the present study. Nevertheless, we believe that this is an important study as it has for the first time linked high iodine intake with the occurrence of the T1799A *BRAF* mutation, the major oncogenic genetic alteration in PTC.

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