BRAF^{V600E} Mutation Analysis in Fine-Needle Aspiration Cytology Specimens for Evaluation of Thyroid Nodule: A Large Series in a *BRAF*^{V600E}-Prevalent Population

Sun Wook Kim,* Ji In Lee,* Jong-Won Kim, Chang-Seok Ki, Young Lyun Oh, Yoon-La Choi, Jung Hee Shin, Hee Kyung Kim, Hye Won Jang, and Jae Hoon Chung

Division of Endocrinology and Metabolism, Department of Medicine (S.W.K., J.I.L., H.K.K., H.W.J., J.H.C.), and Departments of Laboratory Medicine and Genetics (J.-W.K., C.-S.K.), Pathology (Y.L.O., Y.-L.C.), and Radiology (J.H.S.), Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 135-710, Korea

Background: The *BRAF*^{V600E} mutation is highly specific to papillary thyroid carcinoma. A test for this mutation may increase the diagnostic accuracy of fine-needle aspiration cytology (FNAC), especially in a *BRAF*^{V600E} mutation-prevalent population.

Methods: This prospective study enrolled 1074 patients with thyroid nodules who underwent both FNAC and *BRAF*^{V600E} mutation analysis by dual-priming oligonucleotide (DPO)-based multiplex PCR in FNA specimens.

Results: The ancillary test for *BRAF*^{V600E} significantly improved the sensitivity of FNA procedure, from 67.5% with FNAC alone to 89.6% with FNAC and the DPO-based multiplex PCR analysis combined. Diagnostic accuracy increased from 90.9 to 96.6%. Nine cases of papillary thyroid carcinoma were detected only by *BRAF*^{V600E} mutation analysis. Unexpectedly, the preoperative DPO-based multiplex PCR produced five false-positive results, which surgery showed to represent benign nodules.

Conclusions: Molecular testing for the *BRAF*^{V600E} mutation in FNA thyroid nodule specimens increases diagnostic value when applied in a *BRAF*^{V600E} mutation-prevalent population. However, when using this potentially powerful technique, we must consider both its strengths and its weaknesses. **(*J Clin Endocrinol Metab* 95: 3693–3700, 2010)**

Palpable thyroid nodules may be found in 4–7% of the general population, and this prevalence may approach 60% when high-resolution ultrasonography (USG) is used (1, 2). Fine-needle aspiration cytology (FNAC) has served for more than 30 yr as the initial diagnostic test in the evaluation of thyroid nodules (3–5). However, indeterminate results, including suspicious for malignancy and follicular neoplasm or lesion (6), variability in reporting systems (7), and inadequate specimens limit the utility of FNAC and may complicate the management of the thyroid nodules (8, 9).

Molecular tests for genetic alterations and different gene expressions in thyroid cancer may enhance the diagnostic value of FNAC (10). The thymine-to-adenine transversion at nucleotide position 1799 in exon 15 of the *BRAF* gene results in a valine-to-glutamate substitution at residue 600 (V600E), which leads to constitutive activation of MAPK signaling downstream (11, 12). The prevalence of *BRAF*^{V600E} in papillary thyroid carcinoma (PTC) ranges from 30% to more than 80%, depending on the geographic area and consumption of iodine (13, 14).

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2009-2795 Received December 30, 2009. Accepted April 26, 2010.
First Published Online May 25, 2010
* S.W.K. and J.I.L. contributed equally to this work.

Abbreviations: DPO, Dual-priming oligonucleotide; FN, false negative; FNA, fine-needle aspiration; FNAC, fine-needle aspiration cytology; FP, false positive; FTC, follicular thyroid carcinoma; MTC, medullary thyroid carcinoma; PPV, positive predictive value; PTC, papillary thyroid carcinoma; TN, true negative; TP, true positive; USG, ultrasonography.

BRAF^{V600E} is known to be highly specific to PTC and false-positive tests have been rarely reported (15, 16). In Korea, where more than 95% of newly diagnosed thyroid cancer is PTC and up to 83% of PTC cases harbor *BRAF*^{V600E}, a test for this mutation in FNAC specimens is likely to increase diagnostic accuracy (15, 17).

A mutation is best defined by direct sequencing, but as a test for *BRAF*^{V600E}, this method lacks the sensitivity to detect small but significant subpopulations of cells (*i.e.* less than 20% of the total) (18). Efforts to enhance sensitivity have produced methods that can detect *BRAF*^{V600E} in populations as small as 1–10% of total cells. These methods include a colorimetric mutation detection assay (19, 20); allele-specific amplification LightCycler PCR (Roche Molecular Biochemicals, Mannheim, Germany) with SYBR green 1 (21); LightCycler PCR with florescent resonance energy transfer probes (22, 23); and pyrosequencing (17). Recently a dual-priming oligonucleotide (DPO)-based multiplex PCR analysis was developed to detect the *BRAF*^{V600E} mutation and is now commercially available. This assay may detect the mutation in as few as 2% of cells in an FNA specimen of thyroid nodules (24).

We conducted this large prospective trial to test the added diagnostic value of *BRAF*^{V600E} detection in FNA specimens when combined with conventional FNAC in evaluation of thyroid nodules in a *BRAF*^{V600E}-prevalent population. We also report the cases of false-positive results caused by too sensitive molecular diagnostic methods.

Patients and Methods

Patients

For this study, we enrolled 1074 patients with thyroid nodules showing malignant or indeterminate features on USG at the Thyroid Nodule Clinic of Samsung Medical Center. Criteria for malignant features on USG included at least one of the following: 1) marked hypoechogenicity (decreased echogenicity compared with the anterior strap muscle), 2) presence of microcalcifications or macrocalcifications, 3) irregular or spiculated margins, and 4) a taller-than-wide shape (when the anteroposterior diameter of a nodule was longer than the transverse diameter) (25). Nodules with simple cystic appearance and typical spongiform (or honeycomb) appearance were classified as typical benign and excluded from the study. The sonographically indeterminate nodules were defined as having neither benign nor malignant features. The Institutional Review Board at Samsung Medical Center approved this study, and all patients gave informed consent to participate.

Fine-needle aspiration (FNA) procedure

All FNA was performed under USG guidance by three radiologists who specialize in thyroid USG and its interpretation. Ultrasound scanners (HDI 5000 or IU22; Philips Medical Systems, Bothell, WA) equipped with a commercially available 7- to 12-MHz linear-array transducer were used. The FNA specimens were obtained from the nodule in two or three passes with a 22- or 23-gauge needle attached to a 2-ml syringe. Mild negative pressure was applied during the procedure. The FNA aspirates were expressed onto frosted-end glass slides and immediately fixed in 95% alcohol for both Papanicolaou and May-Grunwald-Giemsa staining. The material remaining from the specimen after cytological preparation was collected for *BRAF*^{V600E} mutation analysis.

Criteria for reporting cytology

Two pathologists experienced in thyroid cytology reviewed all of the questionable specimens. The criterion for an adequate smear was the presence of six groups of cells with more than 10 cells per group. Cytological diagnoses consisted of four categories: malignant, indeterminate, benign, and inadequate. The indeterminate cytology results were subdivided into suspicious for malignancy and follicular neoplasm or lesion during the analysis.

DNA isolation

Gene analysis was performed with DNA extracted from FNA tissue remaining after cytological evaluation. Genomic DNA was extracted from the aspirated thyroid cells using the QIAamp DNA minikit (QIAGEN, Chatsworth, CA) according to the manufacturer's instructions and was stored at −70 C.

In thyroid nodules with benign histology after surgery, in which *BRAF*^{V600E} mutation was detected preoperatively by DPO-based multiplex PCR analysis for FNA specimen, hematoxylin and eosin slide was examined and marked by a pathologist for subsequent tumor dissection under the light microscope. Paraffin-embedded tissue was manually microdissected from one to five 5-μm sections, depending on the tumor size, by scraping the lesions. Genomic DNA was extracted using the QIAamp DNA minikit (QIAGEN).

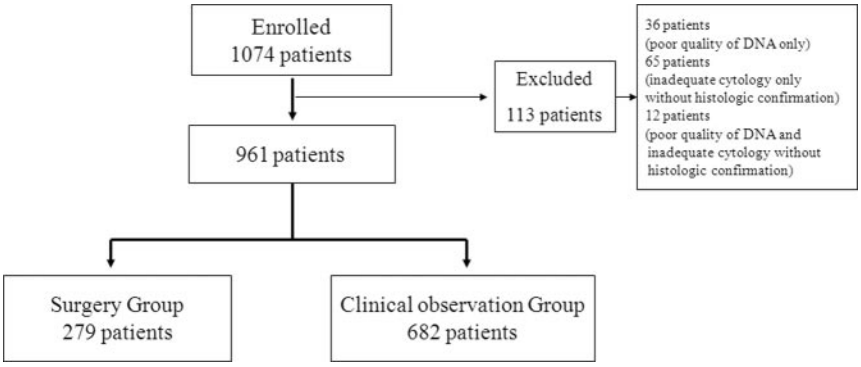

FIG. 1. Patient enrollment and exclusion.

Detection of the *BRAF*^{V600E} mutation

DPO-based multiplex PCR analysis

The *BRAF*^{V600E} mutation was detected by DPO technology using the Seeplex BRAF ACE detection system (Seegene, Seoul, Korea). This DPO-based multiplex PCR analysis can reportedly detect the presence of *BRAF*^{V600E} in as few as 2% of cells in a wild-type population (24). Although allele-

specific PCR is more sensitive than direct sequencing for detecting small numbers of mutant cells, it is limited by low specificity in discriminating single-base point mutations. In DPO technology, five successive deoxyinosine linkers were used for 3′-end sensitization of the primer to enhance the specificity for single-base substitution. The shorter (~10 mer) 3′-portion is linked to the longer (~18 mer) 5′-portion by five successive deoxyinosine linkers. The binding energy of the shorter 3′-portion alone is sufficiently low to distinguish a single-base difference, which enhances the specificity of allele-specific PCR (24, 26). In accordance with the manufacturer's instructions, each 20-μl PCR assay contained 3 μl of DNA, 4 μl BRAF primer mixture, 3 μl 8-methoxypsoralen solution and 10 μl of Master mix (Seegene, Seoul, Korea). The PCR was carried out on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) with an initial denaturation step at 94 C for 15 min followed by 35 cycles (denaturation at 94 C for 30 sec; annealing at 62 C for 30 sec; and extension at 72 C for 60 sec) and a final extension cycle at 72 C for 10 min. Amplicon products from the DPO-based multiplex PCR were visualized and analyzed on the ScreenTape system (Lab901 Ltd., Edinburgh, UK) (see Fig. 2).

Direct sequencing analysis

Direct sequencing analysis for *BRAF*^{V600E} mutation was performed only for patients who underwent surgery, using the stored DNA samples. The DNA template was amplified in exon 15, which contains the T1799A mutation site, using the specific oligonucleotide primer pairs: 5′-TTCATGAAGACCTCACAGTAAAAA-3′ (forward) and 5′-CCACAAAATGGATCCAGACA-3′ (reverse). Cycling conditions were as follows: initial denaturation (94 C, 15 min) and then 35 cycles (denaturation 94 C for 30 sec; annealing 60 C for 30 sec; extension 72 C for 30 sec), followed by a final extension of 10 min at 72 C. All PCR products were visualized by electrophoresis on a 2% agarose gel and purified using a PCR product presequencing kit (United States Biochemical, Cleveland, OH). Direct sequencing was performed in an ABI PRISM 3100 sequencer using the BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems). DNA sequences were compared with those of the normal *BRAF* gene exon 15 in the GenBank database using sequence assembly software (Sequencher 4.8; Gene Codes Corp., Ann Arbor, MI).

Statistical analysis

To evaluate the diagnostic values of FNAC with ancillary detection of the *BRAF*^{V600E} mutation, we defined the true-positive (TP), true-negative (TN), false-positive (FP) and false-negative (FN) results as follows. First, in the FNAC analysis of thyroid nodules, TP denoted malignant cytology and malignant histology; TN, benign cytology and histology, or benign cytology and no evidence of malignancy during clinical monitoring; FN, benign cytology, but histologically confirmed malignancy; and FP, malignant cytology but benign histology. The indeterminate cytological diagnoses were considered benign cytology when calculating diagnostic values as described below. Second, in evaluation of the molecular test, TP denoted detection of the *BRAF*^{V600E} mutation in malignant nodules on the definitive pathology after surgery; TN, not finding *BRAF*^{V600E} on the benign histology; and FN, not finding the mutation in a malignant lesion; FP, detection of *BRAF*^{V600E} in the benign lesion on the permanent pathology.

Diagnostic values were calculated as follows: sensitivity = TP/(TP + FN) × 100; specificity = TN/(TN + FP) × 100; positive predictive value (PPV) = TP/(TP + FP) × 100; negative

TABLE 1. Results of FNAC- and DPO-based multiplex PCR analysis for *BRAF*^{V600E} mutation in the whole study population

Cytology/ histology	Malignancy		Indeterminate				Benign		Inadequate		Total
			Suspicious for malignancy		Follicular neoplasm						
	BRAF V600E (+)	*BRAF* V600E (−)	*BRAF* V600E (+)	*BRAF* V600E (−)	*BRAF* V600E (+)	*BRAF* V600E (−)	*BRAF* V600E (+)	*BRAF* V600E (−)	*BRAF* V600E (+)	*BRAF* V600E (−)	
Malignancy	162	19[a]	50	20	0	4[b]	2	0	7	4	268
Benign	0	0	0	2	1	3	3	683	1	0	693
Total	162	19	50	22	1	7	5	683	8	4	961

BRAF V600E (+), Thyroid cancer with *BRAF*^{V600E} mutation; *BRAF* V600E (−), thyroid cancer without *BRAF*^{V600E} mutation.

[a] One MTC is included.

[b] All are FTC.

predictive value = TN/(TN + FN) × 100; accuracy = (TP + TN)/(TP + TN + FP + FN) × 100.

Categorical data were summarized using frequencies and percent values. Group comparisons of categorical variables were performed using the χ^2 test or, for small cell values, Fisher's exact test. $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

We prospectively enrolled 1074 patients with thyroid nodules, from whom we could obtain cytology results and FNA specimens for *BRAF*^{V600E} mutation analysis. We excluded 113 patients with inadequate cytology or inadequate DNA amplification for mutation analysis. Among the patients excluded, 65 (of 1074, 6.1%) showed inadequate cytology but adequate DNA amplification; 36 (of 1074, 3.4%) showed inadequate DNA amplification but adequate cytology; and 12 (of 1074, 1.1%) had both inadequate cytology and inadequate DNA amplification.

Twelve patients with inadequate cytology but whose surgical pathology reports were available were not excluded and were counted in the final analysis. The final analysis thus included a total of 961 patients (Fig. 1). The mean age of the patients was 50.6 ± 10.8 yr (range 19–83 yr). Of the 961 patients, 773 (80.4%) were female and 188 (19.6%) were male. The mean size of the nodule at the longest diameter was 1.29 ± 0.96 cm (range 0.2–6.3 cm). The proportion of nodules with maximum diameter less than 1 cm was 457 (47.8%).

Among 961 patients included in the analysis of diagnostic values, 279 underwent surgery (the surgery group). The remaining 682 patients were observed by either repeated FNAC or follow-up with USG after 6 months (the clinical observation group).

FNAC

Of 961 FNAC analyses performed, 12 (1.2%) were reported as inadequate. Malignant cytology was reported in 181 patients (18.8%); 80 patients (8.3%) showed indeterminate cytology (72, suspicious for malignancy and eight, follicular neoplasm or lesion); 688 patients (71.6%) showed benign cytology (Table 1).

The FNAC results for 279 thyroid nodules whose final histology were available after surgery are listed in Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org.

DPO-based multiplex PCR analysis for the *BRAF*^{V600E} mutation

Among 268 nodules histologically confirmed as malignant [including four follicular thyroid carcinoma (FTC) and one medullary thyroid carcinoma (MTC)], 221 (82.5%) showed positive results for the *BRAF*^{V600E} mutation by DPO-based multiplex PCR analysis. The positive rate of *BRAF*^{V600E} mutation in PTC alone was 84.0% (221 of 263). The results of *BRAF*^{V600E} mutation analysis in relation to FNAC are shown in Table 1 and Supplemental Table 1.

Diagnostic value of FNAC and DPO-based multiplex PCR analysis for *BRAF*^{V600E}

The analysis of diagnostic value included 961 patients in the surgical and clinical observation groups combined. Of these, 279 patients (of 961, 29.0%) underwent surgery and 268 were found to have malignancy at the nodule. For these surgeries, we had results of both preoperative FNAC and *BRAF*^{V600E} mutation analysis. Among the 268 thyroid malignancies were 263 PTCs, four FTC, and one MTC. Of the 11 surgically proven benign nodules, eight were nodular hyperplasias and three were follicular adenomas. The remaining 682 patients had thyroid nodules that were benign by FNAC analysis and negative for the *BRAF*^{V600E} mutation. We observed these patients for at least 6 months by either repeat ultrasound-guided FNAC or USG follow-up and found no changes to alter the benign status of the nodules. We therefore included these 682 patients as true negatives in the evaluation of diagnostic values. The sensitivity of FNAC alone was 67.5% and the specificity was 100%. Adding the molecular test to FNAC significantly improved sensitivity, from 67.5 to 89.6%,

TABLE 2. Diagnostic values for FNAC and *BRAF*^{V600E} analysis in FNA specimens of thyroid nodule in a *BRAF*^{V600E}-prevalent population

Diagnostic modality	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
FNAC					
Malignancy	67.5	100	100	88.8	90.9
Malignancy + indeterminate	95.1	99.1	97.7	98.1	98.0
BRAF^{V600E} alone	82.5	99.3	97.8	93.6	94.6
BRAF^{V600E} + FNAC (malignancy)	89.6	99.3	98.0	96.1	96.6
BRAF^{V600E} + FNAC (malignancy + indeterminate)	98.5	98.6	96.4	99.4	98.5

BRAF^{V600E}, Mutation analysis based on the DPO-based multiplex PCR analysis; PPV, positive predictive value; NPV, negative predictive value.

and accuracy from 90.9 to 96.6% (Table 2). Thirteen patients presented nodules with benign or inadequate FNAC but positive results for *BRAF*^{V600E} by DPO-based multiplex PCR analysis. These patients underwent thyroidectomy and nine showed PTC in the final histology (see Supplemental Table 2 for clinicopathologic features).

False positive findings with DPO-based multiplex PCR analysis

Unexpectedly, five patients who underwent surgery because of a positive result for *BRAF*^{V600E} by DPO-based multiplex PCR had benign nodules on final histology (Table 3). In these patients, FNAC revealed one follicular neoplasm, three benign, and one inadequate result. The sequencing analysis for *BRAF*^{V600E} mutation, which was performed on both the stored preoperative FNA specimens and DNA extracted from the same area as the preoperative FNA by microdissection on histology slides after surgery, showed negative results in all five thyroid nodules (Fig. 2). We considered these results to be FP outcomes of DPO-based multiplex PCR analysis.

Discussion

Here we report the enhanced diagnostic value of *BRAF*^{V600E} mutation analysis with conventional FNAC, over FNAC alone, in evaluation of thyroid nodules for cancer. In this relatively large prospective study conducted in a *BRAF*^{V600E}-prevalent population, the molecular test increased the sensitivity of the FNA procedure from 67.5 to 89.6%. Diagnostic accuracy also increased from 90.9 to 96.6%.

The greatest increase in sensitivity came from the detection of PTC in the patients with indeterminate FNAC results. In particular, the PPV of the *BRAF*^{V600E} mutation in the suspicious for PTC subgroup of those with indeterminate cytology was 100%. Thus, preoperative *BRAF*^{V600E} testing clarified the plan for surgery in this group of patients.

These findings support in part those of Nikiforov *et al.* (16), who reported gains in sensitivity (from 44 to 80%) and accuracy (from 93.3 to 97.4%) by introducing multiple molecular tests in combination for genetic alterations such as the *BRAF*^{V600E} and K601E mutations, *RAS* (N-, H-, and K-) mutations, *RET/PTC* rearrangements and *PAX8/PPARγ* rearrangement in FNA specimens. However, we used only the test for the *BRAF*^{V600E} mutation and obtained higher sensitivity and accuracy than in that previous report. The prevalence of all genetic alterations combined in the thyroid cancers in the study by Nikiforov *et al.* was 63.8%, whereas the prevalence of *BRAF*^{V600E} alone in our thyroid cancer patients was 82.5%. These

TABLE 3. Characteristics of patients who showed FP results for thyroid cancer by DPO-based multiplex PCR analysis for *BRAF*^{V600E} mutation in FNAB specimens

Age (yr)	Sex	USG tumor size (cm)	USG finding	FNAB diagnosis	*BRAF*^{V600E} mutation		Histology
					FNAB specimens DPO-based multiplex PCR	Tumor tissue Direct sequencing	
48	F	0.7	Suspicious for malignancy	Follicular proliferative lesion	(+)	(−)	FA
55	F	3.0	Benign	Nodular hyperplasia	(+)	(−)	NH
51	F	2.3	Benign	Nodular hyperplasia	(+)	(−)	NH
60	F	0.4	Indeterminate	Nodular hyperplasia	(+)	(−)	NH
40	F	0.7	Indeterminate	Nodular hyperplasia	(+)	(−)	NH

FA, Follicular adenoma; NH, nodular hyperplasia.

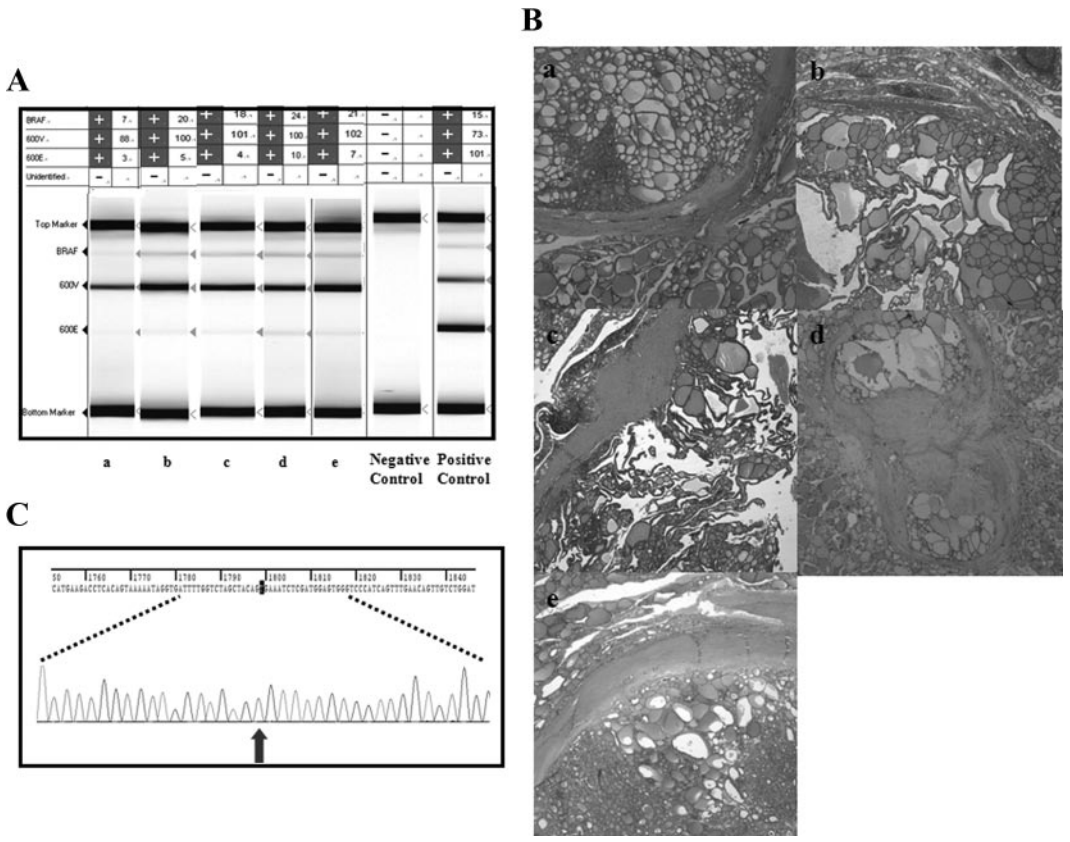

FIG. 2. FP results for PTC by DPO-based multiplex PCR analysis for the *BRAF*^{V600E} mutation. A, DPO-based multiplex PCR analysis on preoperative FNA specimens and controls. In examination of the density of the mutation band in the DPO-based multiplex PCR analysis, the band was scarcely visible, and the density was very low. B, Histology of surgical specimens: a, follicular adenoma; b–e, nodular hyperplasia (hematoxylin-eosin stain, ×50). C, Direct sequencing analysis for *BRAF*^{V600E} mutation performed on microdissected surgical specimens showed negative results.

differences originate from the variable prevalence of genetic alterations in thyroid malignancy according to geographic area, ethnicity, and iodine consumption (27, 28). In Korea, where iodine consumption is very high, 95% of thyroid cancers are PTCs and 80% or more of PTCs harbor the *BRAF*^{V600E} mutation (15, 17). In Western countries, the prevalence of PTC in thyroid cancer (80–90%) and the prevalence of *BRAF*^{V600E} mutation in PTC (30–50%) are much lower than in Korea (13, 14). For optimal diagnostic value and cost-effectiveness, the use of an ancillary test with FNAC for evaluation of thyroid nodule must be adapted to the characteristics of the population at risk.

The high PPV (100%) of the molecular test for PTC in patients with indeterminate FNAC may be of particular benefit in planning the extent of surgery for this group. Guidelines of the American Thyroid Association recommend thyroid lobectomy or total thyroidectomy for patients with indeterminate FNAC to assure safety when malignancy is uncertain (4). This imposes a dilemma concerning the extent of surgery. A surgeon may decide to order a frozen biopsy during the operation, which provides a rapid result but prolongs time in surgery and may prove inconclusive. If the permanent histology proves the frozen biopsy incorrect, a completion thyroidectomy may be required. On the other hand, total thyroidectomy carries an unavoidable complication rate of 1–3% for permanent hypoparathyroidism and vocal cord injury. An accurate preoperative assessment is therefore highly desirable.

Unexpectedly, the preoperative DPO-based multiplex PCR analysis diagnosed five thyroid nodules as malignant that surgery revealed as benign. The final histological findings were benign, and the sequencing analyses for *BRAF*^{V600E}, which were performed on both the DNA stored after preoperative FNA and DNA extracted from the corresponding microdissected area on the surgical specimen, showed negative results for all five nodules. To the best of our knowledge, no nodules reported as benign tested positive for the *BRAF*^{V600E} mutation. In examination of the scanned image and the density of the mutation bands in the DPO-based multiplex PCR analysis, the band was scarcely visible and the density was very low. We therefore conclude that the test produced FP results for the five nodules as the result of setting the positive cutoffs as low as possible. The occurrence of five FP results decreased the specificity and PPV of combining FNAC and the DPO-based multiplex PCR technique, which we thought the

trade-off of increasing sensitivity by ancillary molecular diagnostic testing. We think that this trade-off applies to not only DPO-based multiplex PCR analysis but also other complex molecular techniques used to enhance diagnostic sensitivity (with the exception of direct sequencing analysis). For each molecular technique used in conjunction with FNAC, the strengths and weaknesses of the test must be weighed against the patient's risk for cancer, the USG finding and the FNAC.

This study is limited by the relatively short period of follow-up to confirm that patients in the clinical observation group were TN as assumed in the analysis of diagnostic values. The rate of FN with FNAC generally averages less than 5% (7), and this rate has decreased with the use of USG guidance during FNA procedures (29). In this study, experienced radiologists performed all FNA procedures under USG guidance and pathologists experienced in thyroid cytology read all the cytological preparations. Furthermore, the FNAC analysis missed nine malignancies (showing two as benign and seven as inadequate) that were detected only by molecular testing of FNA specimens. In the era of conventional FNAC, these would have been FN cases that are now detected by the ancillary molecular test. We therefore believe that very few FN cases remain in the clinical observation group and that detection of these would not affect the main outcomes of this study.

In conclusion, testing for the $BRAF^{V600E}$ mutation in thyroid nodules increases the diagnostic value of conventional FNAC in a $BRAF^{V600E}$ mutation-prevalent population. However, when we apply these modern and sensitive molecular techniques in routine clinical practices, we must be familiar with the strength and the weakness of each molecular testing.

Acknowledgments

Address all correspondence and requests for reprints to: Jae Hoon Chung, M.D., Ph.D., Professor, Division of Endocrinology and Metabolism, Samsung Medical Center, Seoul 135-710, Republic of Korea. E-mail: thyroid@skku.edu.

This work was supported by IN-SUNG Foundation for Medical Research Grant CA98131 and Samsung Biomedical Research Institute Grant SBRI C-B0-233-1.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Hegedüs L** 2004 Clinical practice. The thyroid nodule. N Engl J Med 351:1764–1771
2. **Gharib H, Papini E** 2007 Thyroid nodules: clinical importance, assessment, and treatment. Endocrinol Metab Clin North Am 36:707–735, vi
3. **Gershengorn MC, McClung MR, Chu EW, Hanson TA, Weintraub BD, Robbins J** 1977 Fine-needle aspiration cytology in the preoperative diagnosis of thyroid nodules. Ann Intern Med 87:265–269
4. **Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, Tuttle RM** 2009 Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 19:1167–1214
5. **Schmitt FC** 2006 Thyroid cytology: FNA is still the best diagnostic approach. Cytopathology 17:211–212
6. **Kelman AS, Rathan A, Leibowitz J, Burstein DE, Haber RS** 2001 Thyroid cytology and the risk of malignancy in thyroid nodules: importance of nuclear atypia in indeterminate specimens. Thyroid 11:271–277
7. **Lewis CM, Chang KP, Pitman M, Faquin WC, Randolph GW** 2009 Thyroid fine-needle aspiration biopsy: variability in reporting. Thyroid 19:717–723
8. **Borget I, Vielh P, Leboulleux S, Allyn M, Iacobelli S, Schlumberger M, de Pouvourville G** 2008 Assessment of the cost of fine-needle aspiration cytology as a diagnostic tool in patients with thyroid nodules. Am J Clin Pathol 129:763–771
9. **Oertel YC** 2009 Thyroid fine-needle aspiration. Am J Clin Pathol 132:308
10. **Schmitt FC, Longatto-Filho A, Valent A, Vielh P** 2008 Molecular techniques in cytopathology practice. J Clin Pathol 61:258–267
11. **Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE, Fagin JA** 2003 High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. Cancer Res 63:1454–1457
12. **Melillo RM, Castellone MD, Guarino V, De Falco V, Cirafici AM, Salvatore G, Caiazzo F, Basolo F, Giannini R, Kruhoffer M, Orntoft T, Fusco A, Santoro M** 2005 The RET/PTC-RAS-BRAF linear signaling cascade mediates the motile and mitogenic phenotype of thyroid cancer cells. J Clin Invest 115:1068–1081
13. **Xing M** 2007 BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. Endocr Rev 28:742–762
14. **Guan H, Ji M, Bao R, Yu H, Wang Y, Hou P, Zhang Y, Shan Z, Teng W, Xing M** 2009 Association of high iodine intake with the T1799A BRAF mutation in papillary thyroid cancer. J Clin Endocrinol Metab 94:1612–1617
15. **Chung KW, Yang SK, Lee GK, Kim EY, Kwon S, Lee SH, Park do J, Lee HS, Cho BY, Lee ES, Kim SW** 2006 Detection of BRAFV600E mutation on fine needle aspiration specimens of thyroid nodule refines cyto-pathology diagnosis, especially in BRAF600E mutation-prevalent area. Clin Endocrinol (Oxf) 65:660–666
16. **Nikiforov YE, Steward DL, Robinson-Smith TM, Haugen BR, Klopper JP, Zhu Z, Fagin JA, Falciglia M, Weber K, Nikiforova MN** 2009 Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. J Clin Endocrinol Metab 94:2092–2098
17. **Kim SK, Kim DL, Han HS, Kim WS, Kim SJ, Moon WJ, Oh SY, Hwang TS** 2008 Pyrosequencing analysis for detection of a BRAFV600E mutation in an FNAB specimen of thyroid nodules. Diagn Mol Pathol 17:118–125
18. **Jin L, Sebo TJ, Nakamura N, Qian X, Oliveira A, Majerus JA, Johnson MR, Lloyd RV** 2006 BRAF mutation analysis in fine needle aspiration (FNA) cytology of the thyroid. Diagn Mol Pathol 15:136–143
19. **Xing M, Tufano RP, Tufaro AP, Basaria S, Ewertz M, Rosenbaum E, Byrne PJ, Wang J, Sidransky D, Ladenson PW** 2004 Detection of BRAF mutation on fine needle aspiration biopsy specimens: a new diagnostic tool for papillary thyroid cancer. J Clin Endocrinol Metab 89:2867–2872
20. **Xing M, Clark D, Guan H, Ji M, Dackiw A, Carson KA, Kim M, Tufaro A, Ladenson P, Zeiger M, Tufano R** 2009 BRAF mutation testing of thyroid fine-needle aspiration biopsy specimens for pre-

operative risk stratification in papillary thyroid cancer. J Clin Oncol 27:2977–2982
21. **Jarry A, Masson D, Cassagnau E, Parois S, Laboisse C, Denis MG** 2004 Real-time allele-specific amplification for sensitive detection of the BRAF mutation V600E. Mol Cell Probes 18:349–352
22. **Nikiforova MN, Kimura ET, Gandhi M, Biddinger PW, Knauf JA, Basolo F, Zhu Z, Giannini R, Salvatore G, Fusco A, Santoro M, Fagin JA, Nikiforov YE** 2003 BRAF mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. J Clin Endocrinol Metab 88:5399–5404
23. **Rowe LR, Bentz BG, Bentz JS** 2007 Detection of BRAF V600E activating mutation in papillary thyroid carcinoma using PCR with allele-specific fluorescent probe melting curve analysis. J Clin Pathol 60:1211–1215
24. **Kwak JY, Kim EK, Kim JK, Han JH, Hong SW, Park TS, Choi JR** 2010 Dual priming oligonucleotide-based multiplex PCR analysis for detection of BRAF(V600E) mutation in FNAB samples of thyroid nodules in BRAF(V600E) mutation-prevalent area. Head Neck 32:490–498
25. **Moon WJ, Jung SL, Lee JH, Na DG, Baek JH, Lee YH, Kim J, Kim HS, Byun JS, Lee DH** 2008 Benign and malignant thyroid nodules: US differentiation—multicenter retrospective study. Radiology 247: 762–770
26. **Chun JY, Kim KJ, Hwang IT, Kim YJ, Lee DH, Lee IK, Kim JK** 2007 Dual priming oligonucleotide system for the multiplex detection of respiratory viruses and SNP genotyping of CYP2C19 gene. Nucleic Acids Res 35:e40
27. **Xing M** 2008 Recent advances in molecular biology of thyroid cancer and their clinical implications. Otolaryngol Clin North Am 41: 1135–1146, ix
28. **Fagin JA, Mitsiades N** 2008 Molecular pathology of thyroid cancer: diagnostic and clinical implications. Best Pract Res Clin Endocrinol Metab 22:955–969
29. **Gharib H, Papini E, Valcavi R, Baskin HJ, Crescenzi A, Dottorini ME, Duick DS, Guglielmi R, Hamilton Jr CR, Zeiger MA, Zini M** 2006 American Association of Clinical Endocrinologists and Associazione Medici Endocrinologi medical guidelines for clinical practice for the diagnosis and management of thyroid nodules. Endocr Pract 12:63–102