Published in final edited form as:

N Engl J Med. 2015 July 30; 373(5): 448–455. doi:10.1056/NEJMoa1502449.

Germline *HABP2* Mutation Causing Familial Nonmedullary Thyroid Cancer

Sudheer Kumar Gara, Ph.D., Li Jia, Ph.D., Maria J. Merino, M.D., Sunita K. Agarwal, Ph.D., Lisa Zhang, Ph.D., Maggie Cam, Ph.D., Dhaval Patel, M.D., and Electron Kebebew, M.D.

Endocrine Oncology Branch (S.K.G., L.Z., D.P., E.K.), Bioinformatics Core (L.J.) and Laboratory of Pathology (M.J.M.), Center for Cancer Research, and the Office of Science and Technology Resources (M.C.), National Cancer Institute, and the Metabolic Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases (S.K.A.) — both in Bethesda, MD

SUMMARY

Familial nonmedullary thyroid cancer accounts for 3 to 9% of all cases of thyroid cancer, but the susceptibility genes are not known. Here, we report a germline variant of *HABP2* in seven affected members of a kindred with familial nonmedullary thyroid cancer and in 4.7% of 423 patients with thyroid cancer. This variant was associated with increased *HABP2* protein expression in tumor samples from affected family members, as compared with normal adjacent thyroid tissue and samples from sporadic cancers. Functional studies showed that *HABP2* has a tumor-suppressive effect, whereas the G534E variant results in loss of function.

Thyroid cancer is common in the United States, with more than 62,000 cases projected in 2015. Thyroid cancers of follicular-cell origin account for more than 95% of all cases of thyroid cancer, with the remaining cancers originating from parafollicular cells (medullary thyroid cancer). Familial nonmedullary thyroid cancer, which accounts for 3 to 9% of all cases of thyroid cancer, has an autosomal dominant pattern of inheritance.^{1,2} It may be syndromic, occurring as a component of one of the familial cancer syndromes (familial adenomatous polyposis, Gardner's syndrome, Cowden's disease, Carney complex type 1, Werner's syndrome, and the DICER1 syndrome) for which the susceptibility genes are known, or it may occur as the only cancer (nonsyndromic).^{2,3} Nonsyndromic familial nonmedullary thyroid cancer accounts for more than 95% of all cases of familial nonmedullary thyroid cancer.² Most cases of familial nonmedullary thyroid cancer are papillary thyroid cancer, which is the most common type of thyroid cancer.

Nonsyndromic familial nonmedullary thyroid cancer is clinically defined by the presence of thyroid cancer and the absence of other predisposing hereditary or environmental factors in two or more first-degree relatives. Familial nonmedullary thyroid cancer is associated with a significantly increased rate of benign thyroid neoplasm among other family members, and different histologic subtypes of thyroid cancer of follicular-cell origin can occur within the

Address reprint requests to Dr. Kebebew at the Endocrine Oncology Branch, National Cancer Institute, National Institutes of Health, Bldg. 10, CRC, Rm. 4W-5952, 10 Center Dr., MSC-1201, Bethesda, MD 20892-1201, or at kebebewe@mail.nih.gov.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

same kindred.^{1,4,5} Several candidate chromosomal loci (1q21, 6q22, 8p23.1-p22, and 8q24) and susceptibility genes (*SRGAP1*, *NKX2-1*, and *FOXE1*) for nonsyndromic familial nonmedullary thyroid cancer have been reported, suggesting that it is a polygenic familial cancer syndrome.^{2,6-8}

In this study, we used next-generation sequencing to analyze the exome of a family with papillary thyroid cancer but without any known familial cancer syndrome. We identified a germline variant in *HABP2* (also known as *FSAP*, *PHBP*, and *HGFAL*) that segregated with all affected members of the kindred and was not present in unaffected spouses. The G534E variant of *HABP2* was associated with increased protein expression in thyroid neoplasms from affected members but was not expressed in normal thyroid tissue and was less prominent in tissue from sporadic cases of papillary thyroid cancer. Functional studies showed that the G534E variant resulted in increased colony and foci formation and cellular migration, which are the hallmarks of malignant transformation. Our findings suggest that *HABP2* is a susceptibility gene for thyroid cancer of follicular-cell origin.

CASE REPORT

A kindred with familial nonmedullary thyroid cancer was referred to our institution for evaluation of affected and unaffected members as part of a clinical protocol to study the clinical and genetic features of the disease. The proband (Patient II.2) was the youngest of seven children, with one affected brother (Patient II.4). Three members of this family (Patients II.2, II.4, and III.1) were affected at the time of the initial evaluation. Four other family members were found, on the basis of ultrasound screening, to have thyroid neoplasms (Fig. 1A and Table 1); papillary thyroid cancer was diagnosed in three of the four (Patients III.4, III.6, and III.7), and follicular adenoma was diagnosed in one (Patient III.8). Of eight family members in the fourth generation (age range, 7 to 24 years) who were screened, two (Patients IV.1 and IV.5) had small thyroid nodules (2 to 4 mm). None of the family members had a history of other primary cancers, but one member (Patient III.7) had had a dysplastic nevus removed 12 years before presentation. No other benign tumors were detected in the kindred.

METHODS

This study was approved by the institutional review board of the National Cancer Institute, National Institutes of Health. Patients gave written informed consent before undergoing evaluation and testing.

GENETIC STUDIES

We performed high-throughput sequencing of peripheral-blood DNA samples from the kindred after whole-exome capture (for details, see the Supplementary Appendix, available with the full text of this article at NEJM.org). Sanger sequencing was used to validate the variants identified by whole-exome sequencing in affected and unaffected family members.

SITE-DIRECTED MUTAGENESIS AND TRANSFECTIONS

We generated the recombinant G534E mutant by site-directed mutagenesis of wild-type complementary DNA and performed transfections to establish stable thyroid cancer and HEK293 cell lines (for details, see the Supplementary Appendix). We conducted knockdown experiments using small interfering RNA (siRNA) targeting *HABP2*; siRNA transfections were performed with the use of Lipofectamine RNAiMAX transfection reagent (Life Technologies), as described in the Supplementary Appendix.

FUNCTIONAL STUDIES

We performed clonogenic and cell-migration assays using established wild-type and G534E-expressing stable cell lines, with transient knockdown of wild-type *HABP2*. For cellular transformation assays, we used the mouse fibroblast cell line NIH-3T3. To determine the possible dominant-negative effect of the variant, we performed the foci assay with transient transfection of equal amounts of wild-type and mutant constructs.

RESULTS

IDENTIFICATION OF *HABP2* AS A CANDIDATE SUSCEPTIBILITY GENE

We performed whole-exome sequencing using peripheral-blood DNA from affected family members and unaffected spouses to identify candidate single-nucleotide variants and insertions and deletions that segregated with all affected members but were not present in unaffected spouses. Using filtering criteria (Table S1 in the Supplementary Appendix), we identified two single-nucleotide variants that were present only in affected family members, with no insertions or deletions. We validated these two non-synonymous single-nucleotide variants using Sanger sequencing. We selected *HABP2* as a candidate gene because the single-nucleotide variant in exon 13 segregated with all seven affected members, including the four in whom thyroid cancer was diagnosed on the basis of screening (Fig. 1A and Table 1). The *HABP2* variant results in a G→A substitution at base 1601, with a change in the amino acid from glycine to glutamate at position 534 (G534E). All affected family members were heterozygous for this variant in peripheral-blood DNA (Fig. 1A and 1B). The G534E variant is present within the serine protease trypsin domain of the *HABP2* protein and is a highly conserved site not only in *HABP2* but also in other serine protease domain-containing proteins, such as hepatocyte growth factor activator and factor XII, which may play a role in cancer (Fig. 1C).^{9,10}

To determine the possible effect of the *HABP2* G534E variant, we performed homologic modeling of the serine protease trypsin domain and found that the glycine-to-glutamate substitution resulted in a space constraint near the catalytic region, thereby disrupting the active site and surface accessibility of its substrates (Fig. 1E, 1F, and 1G).

We next investigated the expression of *HABP2* in thyroid-tissue samples from the familial and sporadic cases. Immunohistochemical analysis showed increased *HABP2* protein expression in papillary thyroid cancers and follicular adenoma tumors from affected members, but there was no staining in normal thyroid tissue from the same affected members (Fig. 1H, 1I, and 1J). In contrast, we found that only 3 of 12 sporadic papillary

thyroid cancers had faint HABP2 protein staining (Fig. 1K). We also analyzed messenger RNA expression of HABP2 and found that it was strongly expressed in the tumor tissue, as compared with adjacent normal thyroid tissue (Fig. 1D). HABP2 protein expression has been reported in 9 of 82 normal tissue types.¹¹ Thus, the overexpression of HABP2 in tumors from G534E variant carriers suggested a possible pathogenic role.

We sequenced the coding regions (exons 1 through 13) of *HABP2* in peripheral-blood DNA from all affected members of the kindred and in tumor DNA samples from four affected members. We found that the variant was heterozygous in both the germline and tumor DNA (Fig. 1B). Sanger sequencing identified no other abnormalities within the *HABP2* coding region that would suggest an alteration of the other *HABP2* allele in the tumor tissue. Furthermore, copy-number analysis in the tumor tissue showed no copy-number variation, as compared with adjacent normal tissue (Fig. S3 in the Supplementary Appendix). We also found no somatic mutations or known rearrangements in *BRAF*, *KRAS*, *NRAS*, or *RET/PTC1* and *RET/PTC3* in the tumor samples (Table 1). Analysis of the Cancer Genome Atlas (TCGA) database for *HABP2* somatic mutation status showed mutation rates of up to 6% (Fig. S4 in the Supplementary Appendix).

ROLE OF *HABP2* IN THYROID CANCER

Next, we tested the hypothesis that *HABP2* functions as a tumor-suppressor gene or oncogene. Experimental knockdown of wild-type *HABP2* increased colony formation and cellular migration, suggesting a tumor-suppressive function (Fig. 2A). Stable overexpression of wild-type *HABP2* in cell lines reduced colony formation and cellular migration (Fig. 2B). In contrast, overexpression of the G534E variant increased colony formation, as compared with the wild type and the empty-vector control. To test whether the G534E variant could result in cellular transformation, we transiently overexpressed wild-type *HABP2* and the G534E variant in the mouse fibroblast NIH-3T3 cell line and performed a foci assay. We observed that the G534E variant induced a significantly higher number of foci and increased cellular migration, as compared with the wild type (Fig. 2C). Since we did not find alterations in the other *HABP2* allele in the tumor tissue and our functional studies suggested that *HABP2* has a tumor-suppressive effect, we cotransfected equal amounts of the wild-type and G534E mutant constructs into NIH-3T3 cells. We found greater foci formation and cellular migration than in wild-type *HABP2* overexpression, suggesting that the G534E variant has a dominant-negative tumor-suppressive effect (Fig. 2C). Finally, analysis of TCGA data in 423 patients with papillary thyroid cancer showed that 4.7% carried the *HABP2* G534E variant, as compared with 0.7% of persons with unknown disease status in multiethnic population databases ($P<0.001$). This suggests that the *HABP2* G534E germline variant may be a susceptibility gene for additional cases of familial nonmedullary thyroid cancer.

DISCUSSION

We identified *HABP2* as a susceptibility gene for cancer in a large kindred with familial papillary thyroid cancer. We provided several lines of evidence to support our finding that the *HABP2* G534E variant confers susceptibility to familial papillary thyroid cancer. First,

we found complete segregation of this variant in affected members, and carriers of this variant in the youngest generation had thyroid nodules, on the basis of ultrasound screening. Second, we found *HABP2* overexpression in thyroid neoplasms (both malignant and benign) from variant carriers but not in normal thyroid tissue. Third, the protein structure modeling and functional studies of *HABP2* suggest that the variant is pathogenic and has a dominant-negative tumor-suppressive function.

HABP2 was initially discovered as a plasma protein that binds to hyaluronan and cleaves the alpha and beta chains of fibrinogen and, more recently, was found to degrade the extracellular matrix and maintain vascular integrity.^{12–14} The G534E variant has been associated with an increased risk of venous thrombosis and carotid-artery stenosis in some, but not all, studies.^{15–20} *HABP2* was also reported to have a protective role in liver fibrosis.²¹ Although the ligands of *HABP2*, such as hyaluronan, have been implicated in the development of cancer,²² most studies have focused on its function in thrombosis and fibrosis. Here, we report a novel role of *HABP2* as a tumor-suppressor gene. Although tumor-suppressor genes classically follow the two-hit hypothesis, with biallelic inactivating mutations of a single gene or mutations in another gene of an affected tissue site, several tumor-suppressor genes, such as *TP53* and *ATM*, have been shown to have a dominant-negative effect in cancer.^{23–25} We propose that the *HABP2* G534E variant is a dominant-negative tumor suppressor because we found no mutations or copy-number changes in the other allele of *HABP2* and detected none of the somatic oncogenes that are commonly mutated in thyroid cancer. Additional support for the idea that the G534E variant has a dominant-negative effect is provided by the results of our cell-transformation assays, the autosomal pattern of inheritance in the kindred described, and the finding that almost all the family members carrying the variant had thyroid neoplasms; the exceptions were the members in the youngest generation.

On the basis of our findings, several questions come to mind that could be addressed in future studies. Should members of affected families who are carriers of the G534E variant but have only small thyroid nodules or no clinical evidence of disease undergo thyroidectomy to prevent the development of thyroid cancer? Should carriers of the G534E variant in the general population undergo screening for thyroid neoplasms? Does the G534E variant account for cases of familial nonmedullary thyroid cancer in other families or kindreds and confer a predisposition to other cancers?

In conclusion, our results suggest that the *HABP2* G534E variant is a susceptibility gene for familial nonmedullary thyroid cancer. It functions as a dominant-negative tumor-suppressor gene. Its potential role as a susceptibility gene in other cancers needs to be explored, as well as its precise role in cancer initiation and progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institutes of Health.

We thank the patients and their family members for participating in our study, the health care providers who referred them to us for evaluation through our clinical protocol, and our colleagues Drs. Stephen J. Marx and Karel Pacak for reviewing an earlier version of the manuscript and providing helpful suggestions.

References

1. Moses W, Weng J, Kebebew E. Prevalence, clinicopathologic features, and somatic genetic mutation profile in familial versus sporadic nonmedullary thyroid cancer. *Thyroid*. 2011; 21:367–71. [PubMed: 21190444]
2. Vriens MR, Suh I, Moses W, Kebebew E. Clinical features and genetic predisposition to hereditary nonmedullary thyroid cancer. *Thyroid*. 2009; 19:1343–9. [PubMed: 20001717]
3. Slade I, Bacchelli C, Davies H, et al. DICER1 syndrome: clarifying the diagnosis, clinical features and management implications of a pleiotropic tumour predisposition syndrome. *J Med Genet*. 2011; 48:273–8. [PubMed: 21266384]
4. Uchino S, Noguchi S, Kawamoto H, et al. Familial nonmedullary thyroid carcinoma characterized by multifocality and a high recurrence rate in a large study population. *World J Surg*. 2002; 26:897–902. [PubMed: 11965446]
5. Iacobone M, Jansson S, Barczy ski M, Goretzki P. Multifocal papillary thyroid carcinoma — a consensus report of the European Society of Endocrine Surgeons (ESES). *Langenbecks Arch Surg*. 2014; 399:141–54. [PubMed: 24263684]
6. He H, Bronisz A, Liyanarachchi S, et al. SRGAP1 is a candidate gene for papillary thyroid carcinoma susceptibility. *J Clin Endocrinol Metab*. 2013; 98(5):E973–E980. [PubMed: 23539728]
7. Pereira JS, da Silva JG, Tomaz RA, et al. Identification of a novel germline FOXE1 variant in patients with familial non-medullary thyroid carcinoma (FNMTTC). *Endocrine*. 2015; 49:204–14. [PubMed: 25381600]
8. Ngan ES, Lang BH, Liu T, et al. A germline mutation (A339V) in thyroid transcription factor-1 (TTF-1/NKX2. 1) in patients with multinodular goiter and papillary thyroid carcinoma. *J Natl Cancer Inst*. 2009; 101:162–75. [PubMed: 19176457]
9. Parr C, Watkins G, Mansel RE, Jiang WG. The hepatocyte growth factor regulatory factors in human breast cancer. *Clin Cancer Res*. 2004; 10:202–11. [PubMed: 14734471]
10. Schmeidler-Sapiro KT, Ratnoff OD, Gordon EM. Mitogenic effects of coagulation factor XII and factor XIIa on HepG2 cells. *Proc Natl Acad Sci U S A*. 1991; 88:4382–5. [PubMed: 1852005]
11. Uhlén M, Fagerberg L, Hallström BM, et al. Proteomics: tissue-based map of the human proteome. *Science*. 2015; 347:1260419. [PubMed: 25613900]
12. Choi-Miura NH, Tobe T, Sumiya J, et al. Purification and characterization of a novel hyaluronan-binding protein (PHBP) from human plasma: it has three EGF, a kringle and a serine protease domain, similar to hepatocyte growth factor activator. *J Biochem*. 1996; 119:1157–65. [PubMed: 8827452]
13. Römisch J, Vermöhlen S, Feussner A, Stöhr H. The FVII activating protease cleaves single-chain plasminogen activators. *Haemostasis*. 1999; 29:292–9. [PubMed: 10754382]
14. Choi-Miura NH, Yoda M, Saito K, Takahashi K, Tomita M. Identification of the substrates for plasma hyaluronan binding protein. *Biol Pharm Bull*. 2001; 24:140–3. [PubMed: 11217080]
15. Weisbach V, Ruppel R, Eckstein R. The Marburg I polymorphism of factor VII-activating protease and the risk of venous thromboembolism. *Thromb Haemost*. 2007; 97:870–2. [PubMed: 17479202]
16. Franchi F, Martinelli I, Biguzzi E, Bucciarelli P, Mannucci PM. Marburg I polymorphism of factor VII-activating protease and risk of venous thromboembolism. *Blood*. 2006; 107:1731. [PubMed: 16461761]

17. Hoppe B, Tolou F, Radtke H, Kiesewetter H, Dörner T, Salama A. Marburg I polymorphism of factor VII-activating protease is associated with idiopathic venous thromboembolism. *Blood*. 2005; 105:1549–51. [PubMed: 15486068]
18. Sidelmann JJ, Vitzthum F, Funding E, Münster AM, Gram J, Jespersen J. Factor VII-activating protease in patients with acute deep venous thrombosis. *Thromb Res*. 2008; 122:848–53. [PubMed: 18394684]
19. Ahmad-Nejad P, Dempfle CE, Weiss C, Bugert P, Borggreffe M, Neumaier M. The G534E-polymorphism of the gene encoding the factor VII-activating protease is a risk factor for venous thrombosis and recurrent events. *Thromb Res*. 2012; 130:441–4. [PubMed: 22421107]
20. van Minkelen R, de Visser MC, Vos HL, Bertina RM, Rosendaal FR. The Marburg I polymorphism of factor VII-activating protease is not associated with venous thrombosis. *Blood*. 2005; 105:4898–9. [PubMed: 15933067]
21. Martinez-Palacian A, Kanse SM, Weiskirchen R. Factor VII activating protease (FSAP): a novel protective factor in liver fibrosis. *Proteomics Clin Appl*. 2014; 8:438–46. [PubMed: 24497464]
22. Karbownik MS, Nowak JZ. Hyaluronan: towards novel anti-cancer therapeutics. *Pharmacol Rep*. 2013; 65:1056–74. [PubMed: 24399703]
23. Willis A, Jung EJ, Wakefield T, Chen X. Mutant p53 exerts a dominant negative effect by preventing wild-type p53 from binding to the promoter of its target genes. *Oncogene*. 2004; 23:2330–8. [PubMed: 14743206]
24. de Vries A, Flores ER, Miranda B, et al. Targeted point mutations of p53 lead to dominant-negative inhibition of wild-type p53 function. *Proc Natl Acad Sci U S A*. 2002; 99:2948–53. [PubMed: 11867759]
25. Chenevix-Trench G, Spurdle AB, Gatei M, et al. Dominant negative ATM mutations in breast cancer families. *J Natl Cancer Inst*. 2002; 94:205–15. Erratum, *J Natl Cancer Inst* 2002;94:952. [PubMed: 11830610]

Figure 1. Identification and Expression of the G534E Variant of HABP2
Panel A shows the family pedigree, status with respect to nonmedullary thyroid cancer, and the *HABP2* genotype for each heterozygous mutation (G534E). Squares denote male family members, circles female members, shaded symbols affected members, and slashes deceased members; the arrow points to the proband (Patient II.2). Panel B shows a sequence chromatogram of the normal and mutant genome. Panel C shows the protein domain architecture of HABP2 and conservation of the G534 position across species. EGF denotes epidermal growth factor, HAF Hageman factor (factor XII), and HGFA hepatocyte growth factor activator. Panel D shows quantitative messenger RNA (mRNA) expression of HABP2

from the tumors and adjacent normal tissue in two affected patients in the kindred. The control serves as the reference. T bars indicate standard deviation. Structural superimposition of the wild-type (pink) and G534E mutant (blue–green) trypsin domains in Panel E shows the disorientation of the loop as a result of the polar side chain of glutamate, which is depicted with the use of ball-and-stick modeling. Panels F and G show surface accessibility of the trypsin domain catalytic site in the wild-type and G534E mutant domains, respectively, with the G534E position (orange) encircled. Panels H through K show representative immunohistochemical staining for HABP2 in a papillary thyroid cancer sample from an affected family member with the G534E variant of *HABP2* (Panel H), normal thyroid tissue from the same family member (Panel I), a sample of follicular adenoma from a family member (Panel J), and a sample of sporadic papillary thyroid cancer (Panel K). Staining is positive (brown) in the cytoplasm and nucleus of the samples of papillary thyroid cancer.

Figure 2. Functional Characterization of HABP2

Panel A shows colony formation and cell migration with transient knockdown of wild-type *HABP2* in three different cell lines (FTC133, TPC1, and HEK293); siRNA denotes small interfering RNA, and scrambled denotes an antisense RNA sequence that has the same molar content of each base as the *HABP2* siRNA but in a random sequence that is not complementary to authentic *HABP2*. Panel B shows colony formation and cell migration in three cell lines with stable wild-type (WT) and G534E *HABP2* overexpression. Panel C shows foci formation and cell migration of NIH-3T3 mouse fibroblasts with transient overexpression of wild-type and G534E *HABP2*. One asterisk denotes $P < 0.05$, two asterisks denote $P < 0.01$, and three asterisks denote $P < 0.001$; T bars indicate standard deviation.

Table 1

Clinical Characteristics, Pathological Findings, and Treatment in Family Members with Nonmedullary Thyroid Cancer.

Variable	Patient II.2	Patient II.4	Patient III.1	Patient III.4	Patient III.6	Patient III.7	Patient III.8
Age at diagnosis (yr)	53	62	31	42	39	34	37
Finding at presentation	Thyroid nodule on ultrasound screening	Thyroid mass	Thyroid mass	Thyroid nodule on ultrasound screening	Thyroid mass	Thyroid nodule on ultrasound screening	Thyroid nodule on ultrasound screening
Histologic type of cancer	Papillary thyroid cancer	Papillary thyroid cancer	Papillary thyroid cancer	Papillary thyroid cancer	Papillary thyroid cancer	Papillary thyroid cancer	Follicular adenoma
Stage of cancer (TNM)*	III (T4N1M0)	III (T3N1M0)	I (T1N0M0)	I (T1N0M0)	I (T3N1M0)	I (T1N1M0)	NA
Treatment							
Surgical resection	Total thyroidectomy and lymph node dissection	Total thyroidectomy and lymph node dissection	Total thyroidectomy	Total thyroidectomy	Total thyroidectomy and lymph node dissection	Total thyroidectomy and lymph node dissection	Total thyroidectomy
Radioiodine ablation	Yes	Yes	Yes	Yes	Yes	Yes	No
Thyroid hormone for thyrotropin suppression (thyrotropin level) [†]	Yes (<0.1 mU/ml)	Yes (<0.1 mU/ml)	Yes (<0.5 mU/ml)	Yes (<0.5 mU/ml)	Yes (<0.1 mU/ml)	Yes (<0.1 mU/ml)	No
External beam radiation	Yes	No	No	No	No	No	No
Disease status at last follow-up or death [‡]	No evidence of disease	Evidence of disease	No evidence of disease	No evidence of disease	No evidence of disease	No evidence of disease	No evidence of disease
Vital status	Alive	Dead	Alive	Alive	Alive	Alive	Alive
Germline gene mutations tested for in <i>APC</i> , <i>PTEN</i> , <i>WRN</i> , <i>DICER1</i> , <i>SRGAP1</i> , <i>NKX2-1</i> , and <i>FOXE1</i>	No germline mutation	Not tested	No germline mutation	No germline mutation	No germline mutation	No germline mutation	No germline mutation
Somatic mutations tested for in <i>BRAF</i> , <i>RAS</i> , and <i>RET/PTC1</i> and <i>RET/PTC3</i>	No somatic mutation	Not tested	Not tested	No somatic mutation	Not tested	No somatic mutation	Not tested

* Staging was based on the tumor–node–metastasis (TNM) classification of the American Joint Committee on Cancer. NA denotes not applicable.

[†]Thyrotropin suppression was defined as a level below 0.5 mU per milliliter.

[‡]Disease status was assessed on the basis of follow-up cervical ultrasonography, radioiodine scanning, and the stimulated serum thyroglobulin level.