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Brief Communication

BRIEF COMMUNICATION

HABP2 G534E Mutation in Familial Nonmedullary Thyroid Cancer

Tao Zhang, Mingzhao Xing

Affiliation of authors: Laboratory for Cellular and Molecular Thyroid Research, Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD (TZ, MX).

Correspondence to: Michael Mingzhao Xing, MD, PhD, Division of Endocrinology, Diabetes and Metabolism, The Johns Hopkins University School of Medicine, 1830 East Monument Street, Suite 333, Baltimore, MD 21287 (e-mail: mxing1@jhmi.edu).

Abstract

Papillary thyroid cancer (PTC) is a common endocrine malignancy, accounting for nearly 90% of all thyroid cancers. About 5% of PTC is hereditary familial nonmedullary thyroid cancer (FNMTC). No general susceptibility gene is known for FNMTC. An oncogenic *HABP2* G534E mutation has been recently reported in one FNMTC kindred, suggesting that *HABP2* is a susceptibility gene for FNMTC. Because of the limited kindred studied, how commonly this gene is responsible—and hence how important clinically it is—for FNMTC remains to be answered. By investigating a large number of FNMTC kindreds in the present study, we identified *HABP2* G534E in several independent kindreds of FNMTC. The overall prevalence of *HABP2* G534E was six per 43 (14.0%) PTC patients from the 29 kindreds and four per 29 (13.8%) kindreds. None of the subjects with benign thyroid neoplasm or the normal subjects from these kindreds had this mutation. These results are consistent with *HABP2* G534E being a susceptibility gene in a subgroup of FNMTC, providing important diagnostic implications for this hereditary thyroid cancer.

Follicular cell–derived thyroid cancer is a common endocrine malignancy with an estimated prevalence of 62 450 for 2015 in the United States (1). Among several histological types of thyroid cancer, papillary thyroid cancer (PTC) is the most common type, currently accounting for nearly 90% of all thyroid malignancies (1). The majority of thyroid cancers are sporadic and develop as a consequence of somatic genic alterations (2). About 5% of thyroid cancers are hereditary, and they are believed to be caused by germline oncogenic genetic events. This may occur in certain hereditary cancer syndromes, as exemplified by Cowden syndrome (3), which usually present with multiple types of cancers. There is also a rare familial para-follicular cell-derived medullary thyroid cancer, with *RET* being the well-known susceptibility gene (4). The most common type of hereditary thyroid cancer is familial nonmedullary thyroid cancer (FNMTC), which occurs in the form of PTC (5,6). Considerable effort has been devoted to identifying the susceptibility gene of FNMTC with limited success in the recent decades. Recently, exome sequencing revealed a 1601 G>A variant of *HABP2* (resulting in G534E) in a kindred with FNMTC, which was demonstrated to have oncogenicity in in vitro studies (7). This suggests that *HABP2*, located on chromosome 10q25-q26 in humans (8), is a susceptibility gene of FNMTC. Given the limited one kindred studied, it remains to be an important question how extensive the role this *HABP2* variant plays in general in FNMTC. We addressed this question by investigating the *HABP2* G534E in a large number of FNMTC kindreds.

With approval by our institutional review board and written patient consent, we investigated germline *HABP2* G534E in a cohort of 64 subjects from 29 kindreds with FNMTC (each with ≥ two first- or second-degree biologically related family members with PTC) that were available for genetic testing. All the 64 subjects were biologically related to their respective kindreds. These included 43 subjects with PTC, five subjects with benign thyroid neoplasm, and 16 normal subjects. There was no clinical evidence for other tumor syndromes in any of these kindreds. Each of the 29 kindreds had at least one individual subject with PTC who was available for genetic testing.

White blood cells were obtained from the subjects, and genomic DNA was isolated as previously described (9). *HABP2*

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G534E was detected by polymerase chain reaction (PCR) amplification of the G534E hot spot region of the *HABP2* gene and Sanger sequencing. Briefly, a 231-bp fragment comprising exon 13 of the *HABP2* gene containing the hot spot of genetic variant 1601 G>A (resulting in G534E) was generated by PCR using primers 5'- TGTCTCTGGTTCACGAGGATG -3' (sense) and 5'-TGAGGTCCAGAAGACAGTACC -3' (antisense) and HotStarTaq *Plus* DNA Polymerase (Qiagen, Valencia, CA). About 50 ng of white blood cell–derived DNA were used in the PCR reaction, which was run with an initial heat activation step at 95 °C for five minutes, followed by 35 cycles of 94°C denaturation for 30 seconds, 55°C annealing for 30 seconds, and 72°C extension for 20 seconds. The PCR was completed with a final elongation step at 72°C for 10 minutes. A single PCR product was produced and confirmed by electrophoresis on a 1.5% agarose gel, which was used as the template for the sequencing reaction using Big Dye reagents (Applied Biosystems, Foster City, CA). DNA sequencing was performed using an ABI PRISM 3730 automated genetic analyzer (Applied Biosystems). Positive mutation results were confirmed by independent PCR amplification/sequencing reactions both in forward and reverse directions.

We identified six subjects from four kindreds that were positive for *HABP2* G534E, which was heterozygous in all the cases (Figure 1A and Table 1). All of these six subjects had PTC. The overall prevalence of *HABP2* G534E in this study was six per 43 (14.0%) PTC patients and four per 29 (13.8%) kindreds. Neither the subjects with benign thyroid neoplasm nor the

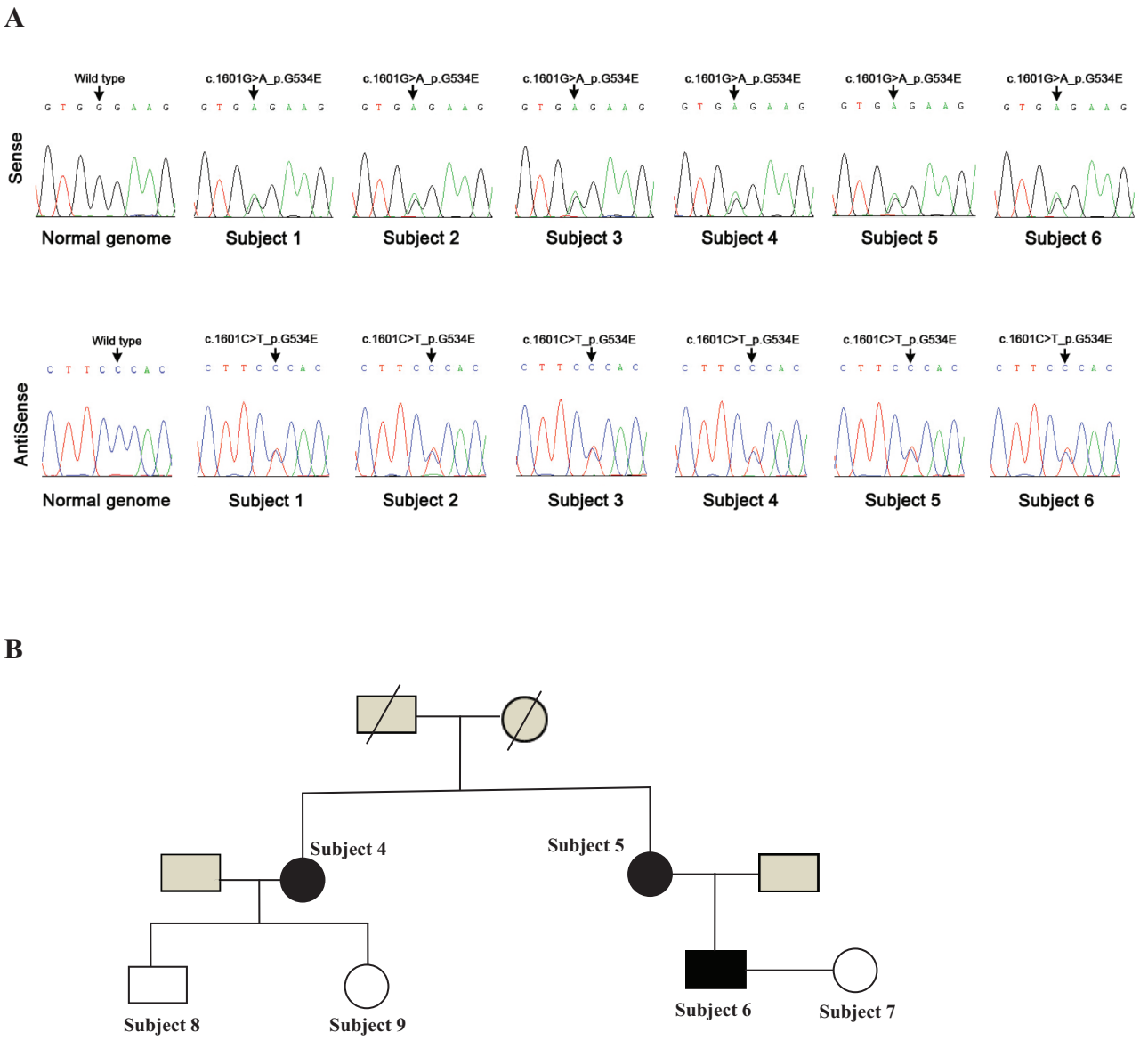

Figure 1. *HABP2* G534E mutation in familial nonmedullary thyroid cancer (FNMTC). **A)** Sequencing electropherogram presentation of six mutation-positive subjects from four kindreds (all heterozygous) corresponding to the six thyroid cancer subjects with FNMTC in Table 1. The **upper panel** represents the sequencing results using the sense primer, and the **lower panel** represents the sequencing results using the antisense primer. **B)** Pedigree of a kindred of FNMTC (kindred #4 in Table 1). All the three subjects of both sexes with papillary thyroid cancer involving two generations were positive for heterozygous germline *HABP2* G534E mutation (**black-filled symbols**). All the three normal subjects without thyroid tumor, with two being biologically and one being through marriage related to the family, carried the germline wild-type *HABP2* (**empty symbols**). The genotype and phenotype status of the remaining family members were unknown (**gray-filled symbols**). The subject and kindred numbers are as defined in Table 1.

Table 1. Germline *HABP2* mutation status in the individual members from four kindreds with familial nonmedullary thyroid cancer

Subject number	Kindred number	Genotype of *HABP2*	Sex	Age at diagnosis*, y	Diagnosis	Comments
1	1	G534E	Male	34	PTC	PTC in father
2	2	G534E	Female	32	PTC	PTC in a sister and a cousin
3	3	G534E	Female	27	PTC	PTC in a cousin
4	4	G534E	Female	69	PTC	PTC in a sister (subject #5) and a nephew (subject #6)
5	4	G534E	Female	60	PTC	PTC in a sister (subject #4) and son (subject #6)
6	4	G534E	Male	40	PTC	PTC in mother (subject #5) and aunt (subject #4)
7†	4	Wild-type	Female	44	Normal subject	Wife of subject #6
8	4	Wild-type	Male	38	Normal subject	PTC in mother (subject #4), and aunt (subject #5) and a nephew (subject #6)
9	4	Wild-type	Female	44	Normal subject	PTC in mother (subject #4), and aunt (subject #5) and a nephew (subject #6)

* For patient subjects, the age refers to the age at the diagnosis of thyroid cancer; for normal subjects, the age refers to the age at the time of blood sample collection for genetic testing. PTC=papillary thyroid cancer.
† Subject #7 was not biologically related to kindred #4 and was therefore not included in the subject/kindred data calculation and summary described in the text.

normal subjects from the 29 kindreds had this mutation. In kindred #4, there were three family members in two generations that had PTC who were all positive for *HABP2* G534E and there were three normal family members available for genetic testing who were all negative for the mutation (Figure 1B and Table 1). Among the three normal subjects in kindred #4, one (subject #7) was a non–biologically related family member, who was not included in the above pooled analyses of the subjects. The remaining three kindreds (kindreds #1–3 in Table 1), positive for the *HABP2* G534E mutation, each had two or more family members with known PTC, and one such PTC patient from each kindred was available for genetic testing (Table 1). The affected mutation-positive subjects included both men and women. Although all of the mutation-positive subjects had PTC, none of the subjects with benign thyroid neoplasm or clinically normal subjects had the mutation. These results were consistent with a high hereditary disease penetrance of *HABP2* G534E in an autosomally dominant manner. A limitation of this study is that some kindreds only had one cancer subject available for genetic testing.

This study demonstrates occurrence of *HABP2* G534E in several independent kindreds of FNMTC, providing strong evidence that *HABP2* is a susceptibility gene that is responsible for a subgroup of FNMTC—14.0% of clinically defined FNMTC patients/kindreds. This study also showed that many clinically defined FNMTC patients lacked *HABP2* G534E, suggesting two possibilities: 1) many cases of PTC that currently meet the clinical criteria for FNMTC are likely sporadic; and 2) additional susceptibility genes may also exist for FNMTC. By demonstrating a relatively general role of *HABP2* G534E in FNMTC, this study consolidates the clinical significance of the recent finding of this genetic variant in FNMTC. As such, testing for germline *HABP2* G534E mutation will likely become a useful measure in helping identify family members at risk for FNMTC although further studies are needed to determine how this genetic testing can be most appropriately used clinically.

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MX conceived and designed the study; TZ and MX conducted the study and analyzed the data. MX wrote the paper. TZ and MX revised and approved the final version of the paper.

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