Comparison Between Clinicopathological Characteristics, BRAF V600E and TERT Promoter Mutation of Familial Non-Medullary Thyroid Carcinomas, and Sporadic Case

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Background: It has been debated whether familial non-medullary thyroid carcinoma (FNMT) is more aggressive and has a worse prognosis than sporadic non-medullary thyroid carcinoma (SNMTC). Our aim was to compare the invasiveness and prognosis of FNMT and SNMTC by their biological behavior and molecular changes.

Method and Material: Our group mainly compared 106 patients with FNMT whom have complete clinicopathological data during 2011–2019 in West China Hospital, Sichuan University, and 212 randomly selected cases with SNMTC were included to compare their biological behavior, recurrence and mortality, and molecular expression of BRAF V600E and TERT promoter. At the same time, FNMT cases were divided into four subgroups, namely, two affected members group, three or more affected members, parent/offspring group, and sibling group, and they were compared with SNMTC separately to analyze the difference in their invasiveness and prognosis.

Results: We found that the mean tumor size of FNMT (0.96 ± 0.53 cm) was smaller than that of SNMTC (1.15 ± 0.72 cm) (p = 0.020), while no significant difference in the incidence of other clinicopathological factors, including bilateral growth, capsular invasion, with thyroid nodular goiter or not, multifocality, lymph node metastasis, extrathyroidal extension, iodine 131 treatments, T stage, and American Joint Committee on Cancer (AJCC) stage, was observed between FNMT and SNMTC (p > 0.05), between each FNMT subgroup (p > 0.05), and between each FNMT subgroup and SNMTC (p > 0.05). There was no significant difference in recurrence, mortality, and BRAF V600E and TERT promoter mutation between FNMT and SNMTC, among which 50/60 (83.33%) of FNMT patients had BRAF V600E mutation and 1/32 (3.13%) had TERT promoter mutation, while the mutation rates of SNMTC were 93/108 (86.11%) and 3/64 (4.69%) (p > 0.05).
**INTRODUCTION**

Due to the increased application of ultrasound and thyroid fine needle aspiration (FNA) biopsy, thyroid cancer (TC), a tumor originating from the thyroid follicular or parafollicular epithelium, has an increasing incidence and becomes the most common endocrine malignant tumor (1–3). Non-medullary thyroid carcinoma (NMTC) arises from thyroid follicular epithelial cells and includes papillary, follicular, and undifferentiated carcinomas, accounting for 95% of TC. NMTC, which mostly occurs in a sporadic fashion, usually has less aggressive behavior and a good prognosis (4).

Familial non-medullary thyroid carcinoma (FNMT), consisting of 5–10% of thyroid carcinoma, is defined as NMTC occurring in two or more first-degree relatives in the absence of other predisposing causes of thyroid cancer (3–6). There has been debate about the difference in aggressiveness between FNMT and sporadic non-medullary thyroid carcinoma (SNMTC). Several studies reported that FNMT tended to be more aggressive than SNMTC, and FNMT had increased risk of multifocal, bilateral growth, capsular and vascular invasion, and lymph node metastasis, with relatively short disease-free survival (5, 7–10). The patients with three or more cases in a family were more aggressive and had a poorer prognosis than those with only two cases in a family (7), and parent–offspring FNMT might have more aggressive biological behavior and a worse prognosis than sibling FNMT (11). However, there were other studies indicated that FNMT was no more aggressive than SNMTC (12–15).

v-Raf murine sarcoma viral oncogene homolog B1 (BRAF) V600E mutation is the most common genetic mutation in papillary thyroid carcinoma (PTC); it is associated with thyroid cancer by activating the mitogen-activated protein kinase pathway (16). Telomerase reverse transcriptase (TERT) is the catalytic subunit of telomerase. The two most common mutations in the TERT promoter region are C228T and C250T, which can enhance the transcriptional activity of the TERT promoter and are frequently found in TC (17). At present, some studies have performed combined analysis of BRAF V600E mutation and TERT promoter mutation in NMTC and found that these mutations were significantly related to the pathogenesis, development, and prognosis of NMTC (18, 19). Some studies compared the type of common gene mutations, including BRAF, Ras, between FNMT and SNMTC, and found no significant differences (13).

To clarify the clinicopathological characteristics of FNMT and compare the tumor aggressiveness between these two malignancies, we retrospectively analyzed the PTC patients who underwent thyroid FNA in West China Hospital.

**METHOD AND MATERIAL**

We reviewed 177 patients from 80 families who underwent thyroid FNA and were diagnosed with NMTC in West China Hospital, Sichuan University, during 2011–2019. Those patients including 175 PTC and 2 follicular carcinoma had no other predisposing causes of thyroid cancer and had been classified as FNMT according to criteria defined by Sturgeon (20).

However, only 106 patients with postoperative pathology diagnosis of PTC were included in this study due to the incomplete clinicopathological data caused by the fact that some patients did not receive surgery in our hospital (including 5 patients who did not receive surgery, and the rest were operated on in other hospitals), and some patients were lost to follow-up. A total of 7,663 patients were diagnosed with PTC in our hospital records system, and 212 cases were randomly selected to match 1:2 ratio for study purpose. All of those patients’ negative family history of thyroid carcinoma was confirmed during the telephone follow-up. The mean follow-up time for FNMT patients was 42 months (6–120 months) and that for SNMTC was 44.5 months (7–102 months).

T (the extent of the primary tumor), N (regional lymph-node metastases), and M (distant metastases) staging was determined according to the eighth edition of the American Joint Committee on Cancer (AJCC) thyroid cancer staging criteria.

DNA from paraffin-embedded tumor tissues (all samples came from preoperative FNA cell blocks or postoperative tumor tissue paraffin blocks) was extracted according to the instructions of QIAamp DNA FFPE Tissue Kit from QIAGEN, Germany. The iC-1100 ultrafine ultraviolet spectrophotometer produced by Hangzhou Suizhen Co., Ltd. was used to detect its purity and concentration and stored at −20°C for future use.

Polymerase chain reaction (PCR) was used for amplification. For BRAF V600E, PCR primers were used to amplify the exon 15 of BRAF gene containing mutation hotspots (16). For TERT promoter mutations, the previously established PCR primers 50-AGTGGATTCGCAGGCAAGTA-30 (sense) and 50-CACCGCTGCTGAAACTC-30 (antisense) were used to amplify the TERT promoters containing two mutation hotspots (C228T and C250T) (17). These materials were carried out with an initial denaturation step at 95°C for 3 min, followed by 10 cycles of 95°C denaturation for 30 s, 55°C annealing for 30s, and 68°C elongation for 1 min. Before

**Conclusion:** There was no significant difference in invasiveness and prognosis between FNMT and SNMTC by biological behavior, patient survival, and molecular level comparison.

**Keywords:** familial non-medullary thyroid carcinomas, clinicopathological characteristics, BRAF V600E, TERT promoter, papillary thyroid cancer
sequencing, 20 g/L agarose gel electrophoresis was used to detect the quality of PCR amplification products, and Sanger sequencing was performed on PCR amplification products with satisfactory quality. The sequencing results were compared with the BRAF and TERT gene sequences to confirm the mutation status.

**Statistical Method**
All data were analyzed by the Statistical Package for the Social Sciences 21.0 (SPSS Inc., Chicago, IL, USA). Chi-square test or Fisher’s exact test were used to compare differences in the intergroup count data, and t-test or Mann-Whitney U test was used to compare differences in the intergroup count data; \( p < 0.05 \) were considered statistical significant.

**Ethical Approval**
All procedures in this study were reviewed and approved by the Clinical Trial and Biomedical Ethics Committee of West China Hospital of Sichuan University. Informed consent in this retrospective study is excused.

**RESULTS**

**Clinicopathological Features of FNMTC Patients and SNMTC**
We collected 177 FNMTC patients from 80 families, and 106 of them were operated on in West China Hospital, Sichuan University, with a complete clinical and pathological data. The median age at diagnosis was 44.2 ± 12.1 years (range, 7–72 years) and 41.7 ± 11.65 years (range, 12–83 years), respectively, for FNMTC and SNMTC patients (\( p = 0.078 \)). There were 23 (21.70%) male patients and 83 (78.30%) female patients, with a M/F ratio of 1/3.6, while in the control group, 56 (26.42%) were male and 156 (73.58%) are female, with a M/F ratio of 1:2.8 (\( p = 0.359 \)) (Table 1). The mean tumor sizes of FNMTC was 0.96 ± 0.53 cm (range, 0.2–3.7 cm), smaller than that of SNMTC (1.15 ± 0.72 cm; range, 0.1–5.0 cm) (\( p = 0.020 \)).

We compared the clinicopathological features of FNMTC and SNMTC and found that the tumor size of FNMTC was significantly smaller than SNMTC (\( p = 0.026 \)), while no significant difference in the incidence of other clinicopathological factors was observed between the two groups, including bilateral growth, capsular invasion, with Hashimoto thyroiditis (HT) or thyroid nodular goiter or not, multifocality, lymph node metastasis, extrathyroidal extension, iodine 131 treatments, T stage, and AJCC stage (\( p > 0.05 \)) (Table 1).

**Recurrence and Mortality of FNMTC and SNMTC**
We collected 140 patients’ follow-up data, including 106 cases that we analyzed and one follicular carcinoma patient, from 177 FNMTC patients. There were four (2.86%) relapsed and one (0.71%) deceased from FNMTC, while nine (4.25%) relapsed but no deceased from SNMTC. There were no significant difference in recurrence rate and mortality between the two groups (\( p > 0.05 \)) (Table 2).

**Clinicopathological Features of FNMTC Subgroup Patients and SNMTC**
There were 92 (86.79%) patients of FNMTC who came from two affected members families and 14 (13.21%) from three or more affected families. Compared with the two affected members subgroup, SNMTC group has an increased incidence of Hashimoto thyroiditis (\( p = 0.046 \)). However, no significant difference was observed between the group of two affected FNMTC, three or more affected FNMTC, and SNMTC in other clinicopathological factors, including gender, age, tumor

| Parameter                  | FNMTC\(^a\) (n = 106) | SNMTC\(^b\) (n = 212) | \( p \) |
|----------------------------|------------------------|------------------------|--------|
| Gender                     |                        |                        |        |
| Male                       | 23 (21.70)             | 56 (26.42)             | 0.359  |
| Female                     | 83 (78.30)             | 156 (73.58)            |        |
| Age                        |                        |                        |        |
| \(<55\) years              | 87 (82.08)             | 186 (87.74)            | 0.172  |
| \(\geq 55\) years          | 19 (17.92)             | 26 (12.26)             |        |
| Tumor size (cm)            |                        |                        |        |
| \(<1.0\)                   | 76 (71.70)             | 125 (58.96)            | 0.026  |
| \(>1.0\)                   | 30 (28.30)             | 87 (41.04)             |        |
| Capsular invasion          |                        |                        |        |
| Yes                        | 74 (69.81)             | 155 (73.11)            | 0.536  |
| No                         | 32 (30.19)             | 57 (26.89)             |        |
| Multifocality              |                        |                        |        |
| Yes                        | 33 (31.13)             | 74 (34.91)             | 0.502  |
| No                         | 73 (68.87)             | 138 (65.09)            |        |
| Bilaterality               |                        |                        |        |
| Bilateral                  | 22 (20.75)             | 42 (22.64)             | 0.702  |
| Unilateral                 | 84 (79.25)             | 164 (77.36)            |        |
| With HT\(^c\)              |                        |                        |        |
| Yes                        | 14 (13.21)             | 46 (21.70)             | 0.068  |
| No                         | 92 (86.79)             | 166 (78.30)            |        |
| Thyroid nodular goiter     |                        |                        |        |
| Yes                        | 63 (59.43)             | 114 (53.77)            | 0.338  |
| No                         | 43 (40.57)             | 98 (46.23)             |        |
| Lymph node metastasis      |                        |                        |        |
| Yes                        | 66 (62.26)             | 136 (64.15)            | 0.511  |
| No                         | 40 (37.74)             | 70 (33.02)             |        |
| Treatment of iodine-131    |                        |                        |        |
| Treated                    | 30 (28.30)             | 78 (36.79)             | 0.057  |
| Untreated                  | 54 (50.94)             | 83 (39.15)             |        |
| Missing data               | 22 (20.76)             | 51 (24.06)             |        |
| T stage                    |                        |                        |        |
| T1 + T2                    | 95 (89.62)             | 186 (87.74)            | 0.621  |
| T3 + T4                    | 11 (10.38)             | 26 (12.26)             |        |
| AJCC stage                 |                        |                        |        |
| I + II                     | 104 (98.11)            | 208 (98.11)            | NS\(^d\) |
| III + IV                   | 2 (1.89)               | 4 (1.89)               |        |

\(^a\)FNMTC, familial non-medullary thyroid carcinoma.

\(^b\)SNMTC, sporadic non-medullary thyroid carcinoma.

\(^c\)HT, Hashimoto thyroiditis.

\(^d\)ETE, extrathyroidal extension.

\(^\text{NS}\), not significant.

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Yang et al. Comparison of Invasiveness Between FNMTC and SNMTC
The 106 patients were divided into the parent–offspring group and the sibling group according to their family identity. Among them, 53 (50.00%) patients belonged to the parent–offspring group, 49 (46.23%) patients to the sibling group, and 4 (3.77%) patients belonged to both two group. However, all observed clinicopathological factors between parent–offspring group, sibling group, and SNMTC did not have statistically significant difference ($p > 0.05$) (Table 3).

The 106 patients were divided into the parent–offspring group and the sibling group according to their family identity. Among them, 53 (50.00%) patients belonged to the parent–offspring group, 49 (46.23%) patients to the sibling group, and 4 (3.77%) patients belonged to both two group. However, all observed clinicopathological factors between parent–offspring group, sibling group, and SNMTC did not have statistically significant difference ($p > 0.05$) (Table 4).

### TABLE 2 | Recurrence and mortality of FNMTC versus SNMTC, n (%).

| Parameter         | FNMTC* (n = 140) | SNMTC* (n = 212) | $p$ |
|-------------------|------------------|------------------|-----|
| Recurrence        |                  |                  |     |
| Yes               | 4 (2.86)         | 9 (4.25)         | 0.447 |
| No                | 136 (97.14)      | 193 (95.76)      |     |
| Death from disease|                  |                  |     |
| Yes               | 1 (0.71)         | 0 (0)            | NS  |
| No                | 139 (99.29)      | 212 (100)        |     |

*FNMTC, familial non-medullary thyroid carcinoma.

**TABLE 3 | Clinicopathological characteristics and prognostic factors of two affected members group and three or more affected members group vs. SNMTC, n (%).**

| Parameter          | Two affected members (n = 92) | Three or more affected members (n = 14) | $p$  | SNMTC* (n = 212) | $pa^b$ | $pb^b$ |
|--------------------|-------------------------------|----------------------------------------|------|------------------|--------|--------|
| Gender             |                               |                                        |      |                  |        |        |
| Male               | 22 (23.91)                    | 1 (7.14)                               | 0.294| 56 (26.42)       | 0.646  | 0.199  |
| Female             | 70 (76.09)                    | 13 (92.86)                             | 156 (73.58) |                  |        |        |
| Age                |                               |                                        |      |                  |        |        |
| <55 years          | 74 (80.43)                    | 13 (92.86)                             | 0.456| 186 (87.74)      | 0.096  | NS     |
| ≥55 years          | 18 (19.57)                    | 1 (7.14)                               | 26 (12.26) |                  |        |        |
| Tumor size (cm)    |                               |                                        |      |                  |        |        |
| ≤1.0               | 65 (70.65)                    | 11 (78.57)                             | 0.456| 125 (58.96)      | 0.053  | 0.170  |
| >1.0               | 27 (29.35)                    | 3 (21.43)                              | 87 (41.04) |                  |        |        |
| Capsular invasion  |                               |                                        |      |                  |        |        |
| Yes                | 65 (70.65)                    | 9 (64.29)                              | 0.629| 155 (73.11)      | 0.659  | 0.538  |
| No                 | 27 (29.35)                    | 5 (35.71)                              | 57 (26.89) |                  |        |        |
| Multifocality      |                               |                                        |      |                  |        |        |
| Yes                | 27 (29.35)                    | 6 (42.86)                              | 0.309| 74 (34.91)       | 0.345  | 0.547  |
| No                 | 65 (70.65)                    | 8 (57.14)                              | 138 (65.09) |                  |        |        |
| Bilaterality       |                               |                                        |      |                  |        |        |
| Bilateral          | 18 (19.57)                    | 4 (28.57)                              | 0.439| 48 (22.64)       | 0.550  | 0.743  |
| Unilateral         | 74 (80.43)                    | 10 (71.43)                             | 164 (77.36) |                  |        |        |
| With HT            |                               |                                        |      |                  |        |        |
| Yes                | 11 (11.96)                    | 3 (21.43)                              | 0.329| 46 (21.70)       | 0.046  | NS     |
| No                 | 81 (88.04)                    | 11 (78.57)                             | 166 (78.30) |                  |        |        |
| Thyroid nodular goiter |                           |                                        |      |                  |        |        |
| Yes                | 55 (59.78)                    | 8 (57.14)                              | 0.851| 114 (53.77)      | 0.333  | 0.806  |
| No                 | 37 (40.22)                    | 6 (42.86)                              | 96 (46.23) |                  |        |        |
| Lymph node metastasis |                             |                                        |      |                  |        |        |
| Yes                | 55 (59.78)                    | 9 (64.29)                              | 0.898| 138 (64.15)      | 0.562  | 0.895  |
| No                 | 33 (35.87)                    | 5 (35.71)                              | 70 (33.02) |                  |        |        |
| ETE^e              |                               |                                        |      |                  |        |        |
| Yes                | 15 (16.30)                    | 2 (14.29)                              | 0.797| 48 (22.64)       | 0.279  | 0.740  |
| No                 | 73 (79.55)                    | 12 (85.71)                             | 164 (77.36) |                  |        |        |
| Treatment of iodine-131 |                         |                                        |      |                  |        |        |
| Treated            | 28 (30.43)                    | 2 (14.28)                              | 0.705| 78 (36.79)       | 0.088  | 0.283  |
| Untreated          | 48 (52.18)                    | 6 (42.86)                              | 83 (39.15) |                  |        |        |
| Missing data       | 16 (17.39)                    | 6 (42.86)                              | 51 (24.06) |                  |        |        |
| T stage            |                               |                                        |      |                  |        |        |
| T1+T2              | 82 (89.13)                    | 13 (92.86)                             | NS  | 186 (87.74)      | 0.730  | NS     |
| T3+T4              | 10 (10.87)                    | 1 (7.14)                               | 26 (12.26) |                  |        |        |
| AJCC stage         |                               |                                        |      |                  |        |        |
| I+II               | 91 (98.91)                    | 13 (92.86)                             | 0.248| 208 (98.11)      | NS     | 0.276  |
| III+IV             | 1 (1.09)                      | 1 (7.14)                               | 4 (1.89) |                  |        |        |

*SNMTC, sporadic non-medullary thyroid carcinoma.

^pa, two affected members group vs. SNMTC.

^pb, three or more affected members group vs. SNMTC.

^HT, Hashimoto thyroiditis.

^ETE, extrathyroidal extension.

^NS, not significant.
BRAF V600E Mutation and TERT Promoter Mutation of FNMTC and SNMTC

Only 49 of the 106 included FNMTC patients were tested for BRAF V600E mutations, and 28 were also tested for TERT promoter mutations (Figure 1). Of the 212 cases in the SNMTC group, 108 patients were tested for BRAF V600E mutations, and 64 were also tested for TERT promoter (Figure 2).

Only one (3.57%) of the FNMTC patients tested for TERT promoter mutation was positive (C228T), and BRAF V600E mutation was positive in 39 (79.59%). Compared with SNMTC patients, there was no significance difference in BRAF V600E mutation and TERT promoter mutation between FNMTC and SNMTC (p > 0.05) (Tables 5, 6).

We also analyzed BRAF V600E mutation and clinicopathological characteristics in FNMTC and SNMTC and found that BRAF mutation in this study was not associated with gender, age, tumor size, inclusion, bilateral growth, thyroid nodular goiter, multifocal, lymph node metastasis, thyroid extradiffusion, iodine 131 treatment, T stage, and AJCC stage (p > 0.05) (Tables 7, 8).

DISCUSSION

With up to 10% of NMTC (3–6), FNMTC was generally considered as a separate malignancy in thyroid cancer with...
controversial clinical behavior and prognosis. At present, several studies have shown that FNMTC was more aggressive and had a worse prognosis than SNMTC (5, 7–11), but some studies have shown that there was no significant difference in biological behavior or prognosis between the two diseases (12–14). Since lots of medical records did not demonstrate whether the patient had a family history, according to the data we collected, FNMTC cases accounted for nearly 2% in our research, lower than those of former studies. We decided to randomly select 212 patients without family history to match 1:2 ratio. However, due to limited sample data, age, gender, and tumor stage were not matched.

Cao et al. (10) and Ito et al. (15) found that FNMTC had higher multiple foci incidence, and Cao’s study also showed that it has a higher bilateral incidence. Zhang et al. found a higher incidence of lymph nodes metastases of FNMTC (5). Meanwhile, compared with SNMTC, FNMTC had a higher recurrence rate and mortality (7, 10, 21, 22). However, Moses et al. (13) found that there were no significant differences between FNMTC and SNMTC in multiple foci, bilateral incidence, and the rate of lateral lymph node metastasis; Ito et al. and Zhang et al. also proved that there was no significant difference in recurrence mortality between the two types.

Recently, with the extensive use of thyroid ultrasound technology and FNA in China, TC was found and treated at an early stage. In our study, we compared some of the invasion-related risk factors, including gender, age, tumor size, capsular invasion, bilateral growth, with thyroid nodular goiter or not, multifocality, lymph node metastasis, extrathyroidal extension, iodine 131 treatment, T stage, and AJCC stage, for FNMTC and SNMTC. We found that the tumor size of FNMTC is significantly smaller than SNMTC \( (p = 0.020) \), while there was no significant difference in the incidence of other factors \( (p > 0.05) \).

| TABLE 5 | BRAF V600E mutations in FNMTC and SNMTC, n (%) |
|-----------------------------------------------|
| **Parameter** | **FNMTCA** | **SNMTCB** | **p** |
| | \((n = 49)\) | \((n = 108)\) |
| BRAF V600E | 10 (20.41) | 15 (13.89) | 0.301 |
| Wild type | 39 (79.59) | 93 (86.11) |
| Mutation | |
| | |

\( ^a \)FNMTC, familial non-medullary thyroid carcinoma.
\( ^b \)SNMTC, sporadic non-medullary thyroid carcinoma.

| TABLE 6 | TRET promoter mutations in FNMTC and SNMTC, n (%) |
|-----------------------------------------------|
| **Parameter** | **FNMTCA** | **SNMTCB** | **p** |
| | \((n = 28)\) | \((n = 64)\) |
| TRET promoter | 27 (96.43) | 61 (95.31) | NS\(^c\) |
| Wild type | 1 (3.57) | 3 (4.69) |
| Mutation | |

\( ^a \)FNMTC, familial non-medullary thyroid carcinoma.
\( ^b \)SNMTC, sporadic non-medullary thyroid carcinoma.
\( ^c \)NS, not significant.
compared the recurrence rate and mortality between FNMTC and SNMTC; same with Moses et al. and Ito et al, there was no significant difference between these two types \((p > 0.05)\). One hundred four FNMTC patients (98.11%) and 208 (98.11%) SNMTC patients belong to AJCC I and II stage. These results suggested that the aggressiveness of FNMTC was not significantly different from that of SNMTC, while the average tumor size of our data (0.96 and 1.15 cm) was smaller than that of Park et al. (11) (1.2 and 1.4 cm), Robenshtok et al. (14) (1.78 and 2.02 cm), and Uchino et al. (22) (1.98 and 2.05 cm). These might mean that NMTC was detected early with thyroid ultrasound technology and FNA, and FNMTC was detected earlier than SNMTC.

Another study by Zhang et al. (7) showed that the subgroup with three or more members in the family affected with the disease was more invasive than the subgroup with only two members infected. Park et al. (11) also believed that the parent-child group was more aggressive than the sibling group, while Cao et al. (10) came to a different conclusion. Therefore, we also compared the invasive differences between different subgroups and SNMTC patients. Similar to Cao et al. (10), SNMTC only showed more significant opportunity with Hashimoto thyroiditis than two affected members group \((p = 0.046)\). This could be explained by the research conclusions of Zeng et al. and Azizi et al.; they suggested that Hashimoto’s thyroiditis played a protective factor in NMTC (23, 24). Our results showed that there were no significant statistical differences in other factors between each subgroup and between each subgroup and SNMTC \((p > 0.05)\), i.e., the aggressiveness of each subgroup was no more

### TABLE 7 | Relationship of BRAF V600E with clinicopathological factors of FNMTC, n (%).

| Parameter | BRAF V600E | p   |
|-----------|------------|-----|
| Gender    |            |     |
| Male      | 10 (25.64) | 0 (0.00) | 0.097 |
| Female    | 29 (74.36) | 10 (100.00) |
| Age       | 30 (76.92) | 8 (80.00) | NS |
| ≥55 years | 9 (23.07)  | 2 (20.00) |
| Tumor size (cm) | 21 (53.85) | 7 (70.00) | 0.482 |
| ≤1.0      | 18 (46.15) | 3 (30.00) |
| Capsular invasion | Yes | 26 (66.67) | 8 (80.00) | 0.702 |
| No        | 13 (33.33) | 2 (20.00) |
| Multilocality | Yes | 14 (35.90) | 1 (10.00) | 0.145 |
| No        | 25 (64.10) | 9 (90.00) |
| Bilaterality | Bilateral | 8 (20.51) | 1 (10.00) | 0.663 |
| Unilateral | 31 (79.49) | 9 (90.00) |
| With HT† | 3 (7.69)  | 2 (20.00) | 0.267 |
| No        | 36 (92.31) | 8 (80.00) |
| Thyroid nodular goiter | Yes | 26 (66.67) | 6 (60.00) | 0.721 |
| No        | 13 (33.33) | 4 (40.00) |
| Lymph node metastasis | Yes | 24 (61.54) | 7 (70.00) | 0.726 |
| No        | 15 (38.46) | 3 (30.00) |
| ETE‡ | Yes | 9 (23.08) | 1 (10.00) | 0.663 |
| No        | 30 (76.92) | 9 (90.00) |
| Treatment status of iodine-131 | Treated | 11 (28.21) | 6 (60.00) | 0.27 |
| Untreated | 20 (51.28) | 4 (40.00) |
| Missing data | 8 (20.51) | 0 (0.00) |
| T stage | T1 + T2 | 33 (84.62) | 9 (90.00) | NS |
| T3 + T4 | 6 (15.38) | 1 (10.00) |
| AJCC stage | I + II | 38 (97.44) | 10 (100) | NS |
| III + IV | 1 (2.56)  | 0 (0.00) |

†HT, Hashimoto thyroiditis.
‡ETE, extrathyroidal extension.
NS, not significant.

### TABLE 8 | Relationship of BRAF V600E with clinicopathological factors of SNMTC, n (%).

| Parameter | BRAF V600E | p   |
|-----------|------------|-----|
| Gender    |            |     |
| Male      | 27 (29.03) | 6 (40.00) | 0.392 |
| Female    | 66 (70.96) | 9 (60.00) |
| Age       | 88 (94.62) | 15 (100.00) | NS |
| ≥55 years | 5 (5.37)  | 0 (0.00) |
| Tumor size (cm) | 55 (59.13) | 8 (53.33) | 0.672 |
| ≤1.0      | 38 (40.86) | 7 (46.66) |
| Capsular invasion | Yes | 64 (68.81) | 11 (73.33) | NS |
| No        | 29 (31.18) | 4 (26.66) |
| Multilocality | Yes | 32 (34.40) | 5 (33.33) | 0.935 |
| No        | 61 (65.60) | 10 (66.66) |
| Bilaterality | Bilateral | 22 (23.65) | 3 (20.00) | NS |
| Unilateral | 71 (76.34) | 12 (80.00) |
| With HT† | Yes | 21 (22.58) | 2 (13.33) | 0.417 |
| No        | 72 (77.42) | 13 (86.66) |
| Thyroid nodular goiter | Yes | 54 (58.06) | 8 (53.33) | 0.731 |
| No        | 39 (41.94) | 7 (46.66) |
| Lymph node metastasis | Yes | 63 (67.74) | 9 (60.00) | 0.555 |
| No        | 30 (32.25) | 6 (40.00) |
| ETE‡ | Yes | 18 (19.35) | 4 (26.66) | 0.502 |
| No        | 75 (80.64) | 11 (73.33) |
| Treatment status of iodine-131 | Treated | 33 (35.48) | 7 (46.66) | 0.725 |
| Untreated | 35 (37.63) | 6 (40) |
| Missing data | 25 (26.88) | 2 (13.33) |
| T stage | T1 + T2 | 82 (88.17) | 11 (73.33) | 0.218 |
| T3 + T4 | 11 (11.82) | 4 (26.66) |
| AJCC stage | I + II | 93 (100.00) | 15 (100.00) | NS |
| III + IV | 0 (0.00)  | 0 (0.00) |

†HT, Hashimoto thyroiditis.
‡ETE, extrathyroidal extension.
NS, not significant.
than that of SNMTC. However, the number of patients from three or more person families was relatively small, and further studies are needed to compare. These results might be caused by the early detection and intervention of FNMT when the tumor size of FNMT remains small. We speculated that due to the extensive application of ultrasound and FNA in China and the active monitoring of asymptomatic first-degree relatives of patients with FNMT, it can be detected early.

Studies have shown that BRAF V600E mutation was associated with large tumor size, thyroid capsule invasion, extraglandular invasion, lymph node metastasis, and high AJCC stage in PTC patients (25, 26). The TERT promoter mutation was related to the age of PTC patients, the maximum tumor diameter, the status of thyroid capsule invasion, and AJCC stage, according to Jin et al. (27) and Alzahrani et al. (28). Unfortunately, similar results were not obtained in our current study, which may be related to the small sample size of our genetic testing, and TERT was not analyzed due to the small number of mutations. Moreover, in one of our previous studies (29), we found that BRAF V600E mutations were mainly associated with recurrence and metastasis, which also confirmed that BRAF could be used as an indicator to evaluate the aggressiveness of NMTC. Therefore, BRAF V600E and TERT promoter mutations were also used as the evaluation indicators of FNMT aggressiveness and was compared with SNMTC; the difference between them was not statistically significant (p > 0.05). This also indicated that there was no difference in clinicopathological invasiveness between FNMT and SNMTC from a genetic perspective, while researchers generally believed that BRAF V600E can drive the growth of PTC through the mitogen-activated protein kinase (MAPK) pathway, and TERT promoter mutation might have a similar effect (25–30).

There were some limitations in our study. First, only 106 patients with FNMT were finally included in the comparison of clinicopathological features. In the subgroup analysis, the case number of some subgroups was small, so the comparison could not be made in the statistical analysis, and the comparison results were meaningless. Furthermore, the biological behavior of NMTC patients was less aggressive, and the prognosis was better than that of other malignant tumors, so long-term follow-up is needed to explore the recurrence and metastasis. The average follow-up time of this study was 3–4 years, and some patients still need to be followed up to evaluate the recurrence and metastasis. All the patients in this study received surgical treatment shortly after the diagnosis of NMTC by FNA, so it was impossible to compare the biological behavior and prognosis differences between early and late intervention of FNMT in this study, which need further exploration and study by follow-up researchers.

CONCLUSION
In summary, by comparing the biological behavior, prognosis, and molecular level of FNMT and SNMTC, we draw conclusion that the biological behavior and prognosis of FNMT were no more aggressive and worse than SNMTC, and BRAF V600E and TERT also provided a genetic explanation for this conclusion. We speculated that it might be the early detection of FNMT with increasing emphasis on the family history that led to the result of a significantly smaller mean tumor size of FNMT than that of SNMTC.

DATA AVAILABILITY STATEMENT
The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT
All procedures in this study were reviewed and approved by the Clinical Trial and Biomedical Ethics Committee of West China Hospital of Sichuan University.

AUTHOR CONTRIBUTIONS
TY was responsible for the experimental design, experimental data collection, analysis and drafting of papers. LH assisted in data collection and analysis. CC was mainly responsible for genetic testing of samples. HL was responsible for experimental guidance and assisted in data collection. YJ was responsible for the overall experimental design, experimental guidance, and important modification of papers. All authors contributed to the article and approved the submitted version.

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