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Original Research

Early familial non-medullary thyroid cancer: differences with late-onset and sporadic forms

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Abstract

Objective **This study aimed to compare clinicopathological features of familial non-medullary thyroid cancer (FNMTC) and sporadic non-medullary thyroid cancer (sNMTC) and assess whether age at onset contributes to the different behavior of these 2 entities.**

Design **A retrospective study including 31 patients with FNMTC, and 31 age- and sex-matched patients with sNMTC.**

Methods **Histological variant, multifocality, tumor infiltration, angioinvasion, TNM stage, lymph node metastasis, BRAF and p53 expression, ATA risk stratification, and the presence of chronic lymphocytic thyroiditis (CLT) were compared between groups. Then, we identified a cutoff age to stratify patients by age of onset (≤35 vs >35 years).**

Results **FNMTC presented at a more advanced stage at diagnosis compared to sNMTC, with a higher proportion of medium-high TNM stages. Among FNMTC, early-onset (≤35 years) cases showed higher BRAF expression, more frequent lymph node metastases, a higher proportion of medium-high TNM stages, and intermediate-to-high ATA risk compared to late-onset cases (>35 years). Medium-high TNM stage, BRAF expression, and lymph node metastases were observed more frequently in early onset FNMTC than in age-matched sNMTC. Late-onset FNMTC patients exhibited a higher prevalence of CLT than early-onset FNMTC and late-onset sNMTC patients.**

Conclusions **These findings underscore the relevance of age at disease onset in shaping the clinical phenotypes of FNMTC. The distinct pathological features of early-onset vs late-onset FNMTC suggest different pathophysiological mechanisms, with the former likely driven by direct genetic/oncogenic alterations, and the latter influenced by autoimmune thyroiditis–related carcinogenesis, a hypothesis that warrants further investigation in larger prospective studies.**

Keywords thyroid cancer, familial thyroid cancer, early onset familial thyroid cancer, sporadic thyroid cancer, autoimmune thyroid disease

Significant

The clinical and pathological features of familial non-medullary thyroid cancer (FNMTC) remain debated. Leveraging a genetically distinct population and a patient stratification by age at disease onset, this study adds an important contribution to the current literature. We provide evidence that age at diagnosis might be a pivotal factor in defining distinct clinical phenotypes of FNMTC. Early-onset FNMTC presents with a more aggressive profile, characterized by advanced TNM stage, and higher rates of lymph node metastases and BRAF expression. In contrast, late-onset FNMTC seems to be associated with chronic lymphocytic thyroiditis, suggesting chronic inflammation as a potential oncogenic pathway. These findings underscore the need for a more nuanced approach to FNMTC classification and management, moving beyond a single, unified definition. Our data pave the way for future investigations into the specific pathogenic mechanisms behind these age-dependent subtypes.

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Introduction

Thyroid cancer is the most common malignancy of the endocrine system. Approximately 95% of thyroid cancers arise from follicular cells and are collectively referred to as differentiated thyroid carcinoma (DTC) or non-medullary thyroid carcinoma (NMTC).[1] It is estimated that up to 10% of NMTCs occur in a familial context (familial non-medullary thyroid carcinoma, FNMTC). Given the high overall prevalence of DTC, this proportion makes FNMTC a relatively common entity. However, many FNMTC cases are likely under-recognized due to challenges in accurately identifying familial aggregation.

A major challenge in the field is the lack of a universally accepted definition of FNMTC, which is generally defined as the occurrence of NMTC in at least 2 first-degree relatives.[2] However, due to the high prevalence of sporadic DTC, the presence of 2 affected family members may be coincidental. Indeed, probabilistic analyses indicate that kindreds with only 2 affected members have a 62%-69% chance of representing sporadic co-occurrence, whereas families with 3 or more affected first-degree relatives have a > 94%-96% probability of a true familial trait.[3] Accordingly, Capezzone et al. proposed that, in kindreds with only 2 affected members, an age at diagnosis <45 years in both relatives should be considered a key criterion to distinguish "true" familial NMTC from sporadic aggregations.[4] Based on these considerations, in the present study, FNMTC was defined as either: (1) families with at least 3 affected first-degree relatives, or (2) families with 2 affected first-degree relatives, both diagnosed before the age of 45 years. Moreover, FNMTC frequently exhibits genetic anticipation, characterized by an earlier age at diagnosis and greater severity, compared to sporadic cases.[5,6] This suggests that age at onset may be considered as an additional criterion to define familial disease, particularly in families with only 2 affected members, as an early diagnosis significantly reduces the likelihood of a random association.[7]

Several studies have investigated the clinical and histopathological characteristics of FNMTC compared to its sporadic counterpart, but the results have been inconsistent. Some reports describe a more aggressive profile for FNMTC, with higher rates of multifocality, bilaterality, extrathyroidal extension, and lateral lymph node metastases,[8-11] whereas others have found no major differences.[12-14] In some cohorts, tumor node metastasis (TNM) staging and tumor size were even lower in familial than in sporadic ones, with unclear differences in prognosis.[15,16] Despite this heterogeneity, clinical guidelines generally recommend a more cautious approach in the management of FNMTC; for instance, total thyroidectomy is often preferred over hemithyroidectomy due to the increased risk of multifocality.[17] One of the main reasons for the lack of consensus is the heterogeneity in the criteria used to define familial cases. Additionally, although FNMTC is widely recognized to encompass multiple subtypes with different clinical presentations and pathogenic mechanisms, only a few studies have considered age at onset as a key factor for subclassifying FNMTC. Another underexplored aspect in the literature is the potential association between autoimmune thyroiditis and FNMTC. While the association between papillary thyroid carcinoma (PTC) and chronic lymphocytic thyroiditis (CLT) has been extensively described in sporadic DTC, the potential link between CLT and FNMTC has been poorly investigated.[18,19]

Starting from these considerations, this single-center study aimed to systematically compare the clinical and histopathological features of FNMTC and sporadic NMTC (sNMTC). Exploiting the distinct genetic background of the Sardinian population, we sought to identify potential differences between FNMTC and sNMTC and to determine whether age at onset and concomitant pathological features contribute to distinct biological behaviors of these 2 entities.

Material and methods

Study design

This was a retrospective, single-center, non-profit study to evaluate patients diagnosed with FNMTC, comparing them with patients affected with sNMTC. Clinical and laboratory records along with histopathological data were reviewed and analyzed to identify potential differences between the 2 groups. To better characterize tumor behavior and aggressiveness, patients were further stratified by age at diagnosis (≤35 vs >35 years) for subgroup analyses, using a 35-year cutoff that was empirically identified from tumor behavior and ROC analysis, as described below.

Study setting and ethical considerations

This study was conducted at the Endocrinology Unit of the University Hospital of Cagliari and was approved by the local Institutional Review Board (ethical approval code: PG/2017/3273-3.42/2022). All procedures complied with the principles of the Declaration of Helsinki, good clinical practice guidelines, and the General Data Protection Regulation (GDPR) for data privacy. Written informed consent was obtained from all patients before inclusion, after they had received comprehensive information about the nature, objectives, and potential implications of the research.

Patients

We retrospectively analyzed the medical records of patients who underwent total thyroidectomy at the University Hospital of Cagliari between 2005 and 2020. The study included 2 groups: 31 patients with a diagnosis of FNMTC, and 31 age- and sex-matched patients with sNMTC. Patients were considered eligible for the familial group (FNMTC) if they met the following criteria: (1) histological diagnosis of PTC, belonging to a family with at least 3 members, first-degree relatives with PTC, or more rarely, 2 members with PTC and 1 with follicular thyroid carcinoma (FTC); alternatively, (2) only 2 members with PTC, but with an early-onset diagnosis before the age of 45; (3) age between 12 and 80 years at the time of diagnosis; and (4) total thyroidectomy performed at our institution, with histopathological evaluation conducted by the same pathologist (MLL) to ensure consistency in diagnosis.

The control group (sNMTC) included patients without a familial history of thyroid carcinoma, consecutively selected with individual pairwise matching to match the familial cases by sex and age at diagnosis. Familial history was thoroughly assessed for each patient to exclude the presence of relatives affected with thyroid carcinoma.

Exclusion criteria for both groups were hereditary genetic cancer syndromes, exposure to ionizing radiation, and any condition impairing the ability to provide informed consent.

Outcomes

The primary objective of this study was to compare the histopathological features of FNMTCs and sNMTCs to identify potential differences in tumor behavior. The secondary objective was to determine whether age at disease onset influences the aggressiveness of FNMTCs. To this end, we explored the relationship between age at diagnosis and tumor aggressiveness using simple logistic regression analyses. To identify the optimal age cutoff, a Receiver Operating Characteristic (ROC) analysis was performed with advanced TNM stage as the outcome variable. We identified a cutoff of 35 years as the age threshold maximizing the Youden index. Therefore, we stratified patients into subgroups based on age at diagnosis: early-onset cases (≤35 years) and late-onset cases (>35 years), and their histopathological features were compared accordingly.

Procedures

For each patient, detailed histopathological data were collected, including tumor size, histological variant, presence of multifocality, tumor infiltration, angioinvasion, TNM stage (according to VIII AJCC/TNM edition), lymph node metastasis, BRAF and p53 expression patterns, American Thyroid Association (ATA) 2015 risk classification, and the presence of CLT.

Regarding tumor size evaluation, tumors were categorized as microcarcinomas (<10 mm) and larger tumors (≥10 mm). Histological variants were classified as low grade (including classic, solid, and follicular variant of PTC) and medium-high grade (including tall cell and oncocytic, variants of PTC and FTC). Based on TNM staging, patients were categorized into low risk (up to T3 N0 M0) and medium-high risk (T4 or any T with N1 or M1), according to current staging guidelines.[20] The ATA risk stratification (low vs intermediate-high risk score) was based on the 2015 guidelines recommendations.[17] The presence of CLT was defined by the histopathological identification of diffuse lymphocytic infiltration throughout the entire thyroid parenchyma.[21] All histological specimens underwent qualitative immunohistochemical analysis for BRAF and p53 expression performed by the same dedicated pathologist (MLL) to ensure consistency and minimize inter-observer variability. Specifically, BRAF expression was assessed by immunohistochemistry using the VENTANA anti-BRAF V600E monoclonal antibody, and staining was considered positive when a diffuse (approximately 100%) cytoplasmic signal was observed in tumor cells. Staining intensity was not taken into account, and immunostaining was thus scored qualitatively (positive/negative). Likewise, p53 was evaluated qualitatively and classified as positive when tumor nuclei showed diffuse staining according to our institutional practice.

Statistical analysis

The assumption of normality was assessed using the Shapiro–Wilk test. Continuous variables are expressed as mean ± standard deviation (SD), while categorical variables are presented as absolute frequencies and percentages. Age cutoff was identified by applying simple logistic regression and receiver operating characteristic (ROC) analysis. Pairwise comparisons between groups were performed by chi-square test or Fisher's exact, as appropriate. For continuous variables with normal distribution, comparisons between groups were conducted using the independent Student's *t*-test. A *P*-value of <.05 was considered statistically significant. All statistical analyses and graphical representations were performed using GraphPad Prism software (version 10.1.1). A post hoc power analysis for 2 proportions (α=.05) was performed using ClinCalc (©2025—ClinCalc LLC) to evaluate the power of the main comparisons between familial and sporadic cases and of the subgroup analyses.

Table 1 Gender and age distribution between FNMTC and sNMTC.

Population ($n = 62$)	FNMTC ($n = 31$)	sNMTC ($n = 31$)	*P*-value
Gender			
N (%)			
F	23 (74.2)	22 (70.9)	1
M	8 (25.8)	9 (29.1)	1
Age at diagnosis (yr)	38.4 (±16)	38.7 (±15)	.93

FNMTC, familial non medullary thyroid carcinoma; sNMTC, sporadic non medullary thyroid carcinoma; F, female; M, male; yr, years.

Results

Patients' characteristics and age cutoff identification

A total of 62 patients were included in the study and divided into 2 groups: (1) 31 FNMTC patients and (2) 31 age and sex matched sNMTC patients. Matching was conducted sequentially: for each patient with FNMTC who underwent thyroidectomy, we identified the next consecutive patient with sporadic NMTC showing the closest possible match in age and sex. Among the FNMTC cases, a total of 10 families were identified: 8 with at least 3 first-degree relatives affected (including one family with 5 affected relatives, two with 4, and five with 3 relatives) while 2 families had 2 first-degree relatives, both diagnosed at an early age (enrolled using the 45 years threshold, but it is worth mentioning that both members were actually under 35 years old).

As shown in Tables 1 and 2, the mean age at diagnosis was 38.4 ± 16 years (range: 13-72 years) in the FNMTC group and 38.7 ± 15 years (range: 17-67 years) in the sNMTC group.

During the initial exploratory analysis, younger age at diagnosis appeared to be associated with more aggressive histopathological features. This observation was supported by simple logistic regression analysis, which demonstrated a significant inverse association between age and markers of tumor aggressiveness, indicating that a younger age of onset was associated with a higher likelihood of aggressive disease.

Specifically, the inverse correlation between age and advanced TNM stage was statistically significant when considering

Table 2 Gender and age distribution between the subgroups.

	FNMTC ≤35 ($n = 16$)	FNMTC >35 ($n = 15$)	sNMTC ≤35 ($n = 14$)	sNMTC >35 ($n = 17$)	*P*-value
Gender					
N (%)					
F	13 (81.3)	10 (66.6)	10 (71.5)	12 (70.5)	.82
M	3 (18.7)	5 (33.3)	4 (28.5)	5 (29.5)	.82
Age at diagnosis (yr)	25.1 (±7.4)	52.6 (±9.2)	24.2 (±6.1)	50.7 (±7.5)	<.0001

FNMTC, familial non medullary thyroid carcinoma; sNMTC, sporadic non medullary thyroid carcinoma; F, female; M, male; yr: years.

Table 3 Histopathological features of FNMTC and sNMTC.

Population ($n = 62$)	FNMTC ($n = 31$)	sNMTC ($n = 31$)	*P*-value
CLT			
N (%)	17 (54.8)	10 (32.3)	.12
Histological variants grade			
N (%)			
Low	18 (58.1)	22 (71)	
Medium-high	13 (41.9)	9 (29)	.42
Multifocality			
N (%)	18 (58)	11 (35.4)	.12
Infiltration			
N (%)	11 (35.4)	9 (29)	.78
Angioinvasion			
N (%)	6 (19.3)	6 (19.3)	1
Tumor size			
N (%)			
<10 mm	11 (35.4)	9 (29)	
≥10 mm	20 (64.5)	22 (70.9)	.78
Lymph node metastases			
N (%)	10 (34.4)	3 (10)	.05
TNM			
N (%)			
1/2	16 (51.6)	27 (87)	
3/4	15 (48.4)	4 (12.9)	.005
BRAF +			
N (%)	8 (27.5)	2 (6.6)	.08
P53 +			
N (%)	3 (9.7%)	0	.23
ATA risk			
N (%)			
Low	16 (51.6%)	18 (58%)	
Intermediate-to-high	15 (48.4%)	13 (41.9%)	.79

FNMTC, familial non medullary thyroid carcinoma; sNMTC, sporadic non medullary thyroid carcinoma; CLT, chronic lymphocytic thyroiditis; TNM, tumor node metastasis; ATA, American Thyroid Association.

the entire study population (OR 4.5, $P = .0012$) and even stronger when the analysis was restricted to the FNMTC group alone (OR 18.7, $P = .003$).

To further characterize this relationship, we performed an ROC curve analysis using advanced TNM stage as the outcome. The age threshold of 35 years provided the best trade-off between sensitivity (67.4%) and specificity (68.4%), with an AUC of 0.73 ($P = .0048$), indicating good discriminative ability. Notably, 35 years also corresponded to the median age of the FNMTC cohort.

Based on these findings, patients were stratified into early-onset (≤35 years) and late-onset (>35 years) subgroups. This cutoff yielded well-balanced subgroups, comprising in the FNMTC group 16 early-onset and 15 late-onset patients, and in the sNMTC group 14 early-onset and 17 late-onset patients.

Regarding gender distribution, female predominance was observed in both groups, consistent with the well-documented higher incidence of DTC in women. Indeed, in the FNMTC group, 74.2% were female and 25.8% were male; in the sporadic group, the female-to-male ratio was similar, with 70.9% females and 29.1% males. Gender distribution within the age-stratified subgroups confirmed equal distribution. Statistical analysis showed no significant difference between the 2 groups and the age-matched subgroups (Tables 1 and 2).

It is important to note that the 35-year threshold, identified through the analysis of our cohort, was used to better characterize the clinicopathological behavior of tumor within the already defined FNMTC population, whereas the 45-year threshold, as proposed in previous literature, was applied to define familial disease in families with only 2 affected members. These 2 age cutoffs therefore serve different non-overlapping purposes (definition versus phenotypic stratification) and are not in conflict with one another.

FNMTC shows a more advanced stage at diagnosis compared to sNMTC

We compared all histopathological features between FNMTC and sNMTC (Table 3). Regarding tumor histotype, the FNMTC group included 13 classic PTC, 3 follicular variants of PTC, 2 solid variants, 7 tall cell variants, 2 FTC, and 4 oncocytic carcinomas. In the sNMTC group, we identified 11 classic PTC, 8 follicular variants, 2 solid variants, 1 cystic variant of PTC, 4 tall cell variants, 1 diffuse sclerosing variant, 2 FTC, and 2 oncocytic carcinomas. As detailed in the methods section, for the statistical analysis, histotypes were categorized into low and medium-high grade variants. Distribution of low-grade and intermediate–high-grade histologic variants in the main groups (Table 3) and in the age-based subgroups (Table 4) did not differ significantly ($P = .42$ and $P = .43$, respectively), suggesting that histologic heterogeneity was comparable across groups and is unlikely to have substantially confounded the study findings. Overall, regardless of age, the only histological finding significantly associated with FNMTC was a more advanced TNM stage at diagnosis compared with sNMTC. Indeed, a higher proportion of patients in the FNMTC group had medium-high TNM stage than in the sNMTC (15/31 FNMTC vs 4/31; OR 6.328, 95% CI

Table 4 Histopathological characteristics of the age-stratified subgroups.

	FNMTC ≤35 ($n = 16$)	FNMTC >35 ($n = 15$)	sNMTC ≤35 ($n = 14$)	sNMTC >35 ($n = 17$)	χ^2 (df), *P*-value
CLT					
N (%)	6 (37.5%)	11 (73.3%)	6 (42.9%)	4 (23.5%)	8.425 (3), .03
Histological variants grade					
Medium-high	8 (50%)	5 (33.3%)	3 (21.4%)	6 (35.3%)	2.711 (3), .43
Low	8 (50%)	10 (66.6%)	11 (78.6%)	11 (64.7%)	
Multifocality					
N (%)	12 (75%)	6 (40%)	5 (35.7%)	6 (35.3%)	6.984 (3), .72
Tumor infiltration					
N (%)	8 (50%)	3 (20%)	2 (14.3%)	7 (41.2%)	6.024 (3), .11
Angioinvasion					
N (%)	4 (25%)	2 (13.3%)	5 (35.7%)	1 (5.9%)	5.052 (3), .16
Tumor size					
<10 mm	4 (25%)	7 (46.7%)	1 (7.1%)	8 (47%)	7.556 (3), .05
≥10 mm	12 (75%)	8 (53.3%)	13 (92.9%)	9 (53%)	
Lymph node metastases					11.38 (3), .009
N (%)	8 (57%)	2 (13.3%)	2 (15.3%)	1 (6.2%)	
TNM					
I/II	5 (31.25%)	11 (73.3%)	11 (78.6%)	16 (94.1%)	16.51 (3), .0009
III/IV	11 (68.75%)	4 (26.7%)	3 (21.4%)	1 (5.9%)	
BRAF +					
N (%)	7 (50%)	1 (6.7%)	1 (7.7%)	1 (6.2%)	10.29 (3), .01
P53 +					
N (%)	2 (12.5%)	1 (6.7%)	0	0	3.725 (3), .29
ATA risk					
Low	6 (37.5%)	10 (66.6%)	9 (64.3%)	9 (53%)	3.319 (3), .34
Medium-high	10 (62.5%)	5 (33.3%)	5 (35.7%)	8 (47%)	

FNMTC, familial non medullary thyroid carcinoma; sNMTC, sporadic non medullary thyroid carcinoma; CLT, chronic lymphocytic thyroiditis; TNM, tumor node metastasis; ATA, American Thyroid Association.

1.837-19.34, $P = .0051$) (Table 3). All other histopathological features did not differ significantly between the 2 groups.

Early onset FNMTC shows a more aggressive profile compared to either late-onset FNMTC and sNMTC

Next, we compared histopathological features between the age-stratified subgroups (Table 4). The proportion of lymph node metastases was significantly higher in patients with early-onset FNMTC than in both late-onset FNMTC and sNMTC, irrespective of age at diagnosis (≤35 or >35 years). Moreover, early-onset FNMTC cases more frequently showed advanced TNM stage and a higher prevalence of BRAF positivity on immunohistochemistry (Table 4). Notably, late-onset FNMTC patients exhibited the highest prevalence of CLT compared with all other groups (Table 4).

FNMTC: a distinctly more aggressive tumor profile in younger patients

To better define any difference between early and late-onset FNMTC, we analyzed these 2 groups separately. Early-onset FNMTC showed a significantly more aggressive histopathological profile than late-onset FNMTC, with a higher TNM stage (11/16 vs 4/15, OR 6.050, 95% CI 1.140-24.71, $P = .0320$) (Figure 1, panel A), an increased rate of lymph node metastases (8/14 vs 2/15, OR 8.667, 95% CI 1.493-46.15, $P = .0209$) (Figure 1, panel B) a higher ATA risk score (9/16 vs 2/15, OR 8.357, 95% CI 1.577-43.57, $P = .0233$) (Figure 1, panel C) and a greater prevalence of BRAF positivity (7/14 vs 1/15, OR 14, 95% CI 1.547-166.5, $P = .0142$) (Figure 1, panel D). It is worth noting that, in the comparison of lymph node metastases and BRAF expression, patients with FTC were excluded, as these tumors are known not to give rise to lymph node metastases and typically do not express BRAF.

Early-onset FNMTC is associated with greater tumor aggressiveness compared to early-onset sNMTC

Subsequently, to further investigate the role of age at disease onset in both familial and sporadic forms, we isolated patients with either FNMTC or sNMTC diagnosed before the age of 35 and analyzed them separately. When comparing

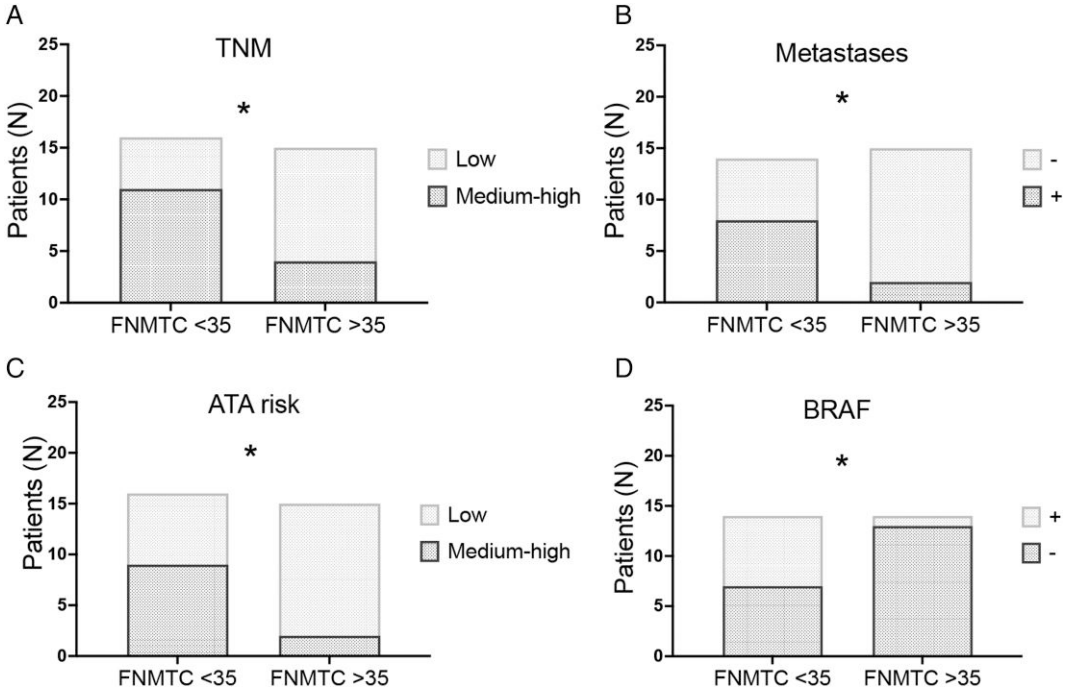

Figure 1 Comparison between early- and late-onset familial non-medullary thyroid cancer (FNMTC). Distribution of: A) tumor node metastasis (TNM) stage (low vs medium-high risk) in early-onset FNMTC (≤35 years; $n = 16$) vs late-onset FNMTC (>35 years; $n = 15$); B) Presence (+) or absence (–) of lymph node metastases in early-onset (≤35 years; $n = 16$) vs late-onset (>35 years; $n = 15$) FNMTC; C) American Thyroid Association (ATA) risk categories (low vs intermediate-high) in early-onset (≤35 years; $n = 16$) vs late-onset (>35 years; $n = 15$) FNMTC; D) BRAF immunohistochemistry status (positive vs negative) in early-onset (≤35 years; $n = 16$) vs late-onset (>35 years; $n = 15$) FNMTC. Bars represent the absolute number of patients in each category. Comparisons between groups have been performed by Fisher exact test (*P-value < .05).

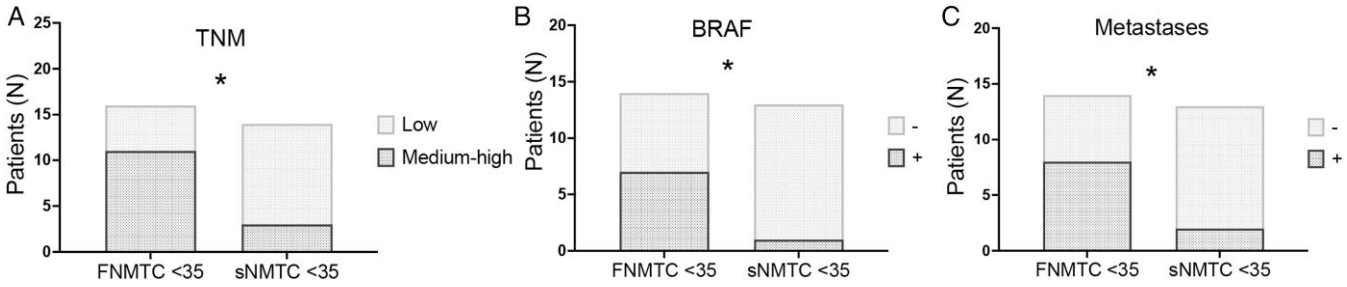

Figure 2 Comparison between early-onset familial non-medullary thyroid cancer (FNMTC) and early-onset sporadic non-medullary thyroid cancer (sNMTC). Distribution of: A) tumor node metastasis (TNM) stage (low vs medium-high risk) in early-onset FNMTC (≤35 years; $n = 16$) vs early-onset sNMTC (≤35 years; $n = 14$); B) BRAF immunohistochemistry status (positive vs negative) in early-onset FNMTC vs early-onset sNMTC; C) Presence (+) or absence (–) of lymph node metastases in early-onset FNMTC vs early-onset sNMTC. Bars represent the absolute number of patients in each category. Comparisons between groups have been performed by Fisher exact test (*P-value < .05).

early-onset patients with familial vs sporadic form, younger FNMTC exhibited a significantly more aggressive profile. Patients in this subgroup presented more advanced TNM (11/16 vs 3/14, OR 8.067, 95% CI 1.642-33.82 $P = .0136$) (Figure 2, panel A) along with higher BRAF expression levels (7/14 vs 1/13, OR 12, 95% CI 1.285-144.2 $P = .0329$) (Figure 3, panel B), and increased rate of lymph node metastases (8/14 vs 2/13, OR 7.333, 95% CI 1.217-39.65, $P = .0461$) (Figure 2, panel C).

Increased prevalence of CLT in late-onset familial NMTC compared to sporadic and early-onset cases

A notable finding in our cohort was the higher prevalence of CLT in patients with late-onset FNMTC (>35 years). Compared to late-onset sNMTC, the presence of CLT was significantly increased (11/15 vs 4/17, OR 9.938, 95% CI 1.619-42.45,

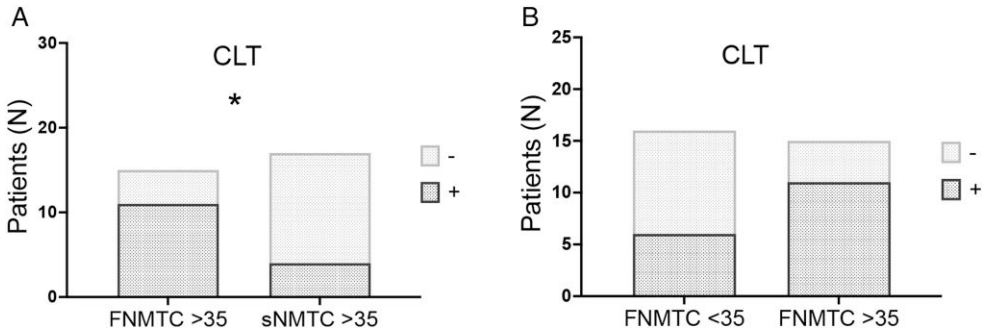

Figure 3 Comparison of chronic lymphocytic thyroiditis (CLT) prevalence between a) late-onset (>35 years old) familial non-medullary thyroid cancer (FNMTC, $n = 15$) and late-onset sporadic non-medullary thyroid cancer (sNMTC, $n = 17$), and B) early-onset (≤35 years old, $n = 16$) FNMTC and late onset (>35 years old, $n = 15$) FNMTC. Bars represent the absolute number of patients in each category. Comparisons between groups have been performed by Fisher exact test (*P-value < .05).

$P = .0118$) (Figure 3, panel A). A trend toward significance was also observed when comparing late-onset FNMTC to early-onset FNMTC (11/15 vs 6/16, OR 4.583, 95% CI 0.890-17.32 P=.0731) (Figure 3, panel B).

Discussion

Several studies have compared the clinical and histopathological features of FNMTC and sNMTC, with most suggesting greater tumor aggressiveness in FNMTC,[22] although overall evidence remains inconclusive, largely because of heterogeneous definitions of familial disease.[23] To minimize the risk of random co-occurrence rather than true familial clustering, we applied stringent criteria, primarily including families with either at least 3 affected first-degree relatives and, more rarely, kindreds with 2 affected relatives both diagnosed before 45 years.[4] This approach increased the likelihood of capturing genuine familial disease and strengthened the internal validity of our FNMTC cohort. Within this rigorously defined series, we found a significantly more advanced TNM stage in familial cases compared with sporadic NMTC, irrespective of age at diagnosis. However, we observed an inverse correlation between age at diagnosis and tumor aggressiveness across all histopathological features. For this reason, we sought to identify an age cutoff to stratify the study population into subgroups and better highlight the impact of age at onset on pathological characteristics. ROC analysis identified 35 years as the optimal age threshold, and stratifying patients into early-onset (≤35 years) and late-onset (>35 years) groups enabled the key differences in tumor aggressiveness that underpin our main findings to become evident. In the subanalysis, early-onset FNMTC patients showed significantly higher BRAF expression, more frequent lymph node metastases, more advanced TNM stage and higher ATA risk compared to late-onset familial cases. Notably, medium-high TNM stage, BRAF expression and lymph node metastases were also more frequent in early-onset familial cases than in age-matched sNMTC patients. Moreover, the most aggressive cases clustered at younger ages at onset, without significant differences between patients belonging to the first or second generation of affected families. These findings suggest that tumor aggressiveness in FNMTC is influenced by age at onset itself and that an age cutoff may better distinguish clinically distinct FNMTC subtypes.

Interestingly, BRAF V600E mutations were significantly more prevalent in early-onset FNMTC than in both late-onset FNMTC and early-onset sNMTC, while P53 expression was rare, being detected in only 3 familial cases. Literature data on BRAF mutation prevalence in FNMTC versus sNMTC remain inconsistent, with studies reporting lower,[24] higher,[25] or similar[26,27] rates in familial cases. Taken together, the early age at onset and the high prevalence of BRAF V600E mutations in our cohort support the hypothesis that, in this subset of FNMTC, genetic/oncogenic mechanisms may drive early and accelerated thyroid carcinogenesis.

Conversely, an unexpected and notable finding of our study was the higher prevalence of CLT in late-onset FNMTC. Compared with both early-onset FNMTC patients and late-onset sporadic patients, late-onset FNMTC had a greater association with CLT along with a lower prevalence of BRAF V600E mutations, suggesting a key role of chronic inflammation as an oncogenic mechanism in this subgroup. To our knowledge, our study is the first to suggest an age-dependent association between FNMTC and CLT using a 35-year threshold. We hypothesize that chronic inflammation and prolonged exposure to a pro-inflammatory environment may contribute to neoplastic transformation in late-onset FNMTC patients. This observation aligns with our previous findings that thyroid autoimmunity and increased serum TSH levels are independent risk factors for malignancy in patients with thyroid nodules.[28,29] In contrast, the early-onset FNMTC subgroup showed higher BRAF expression and a lower prevalence of CLT than late-onset FNMTC, in line with a recent meta-analysis suggesting that BRAF-mutated PTC is more common in patients without CLT rather than those with autoimmune thyroiditis.[19]

Although larger studies are required to confirm our findings, a pathogenic interpretation can be proposed. Given the complex, multifactorial, and polygenic nature of FNMTC, it is plausible that this entity encompasses distinct subtypes with different pathogenic mechanisms and inheritance patterns. In particular, early-onset FNMTCs may be primarily driven by specific genetic variants that promote a more direct oncogenic process, relatively independent from other local causes, such as inflammatory background. Conversely, the higher prevalence of CLT in late-onset FNMTCs suggests that chronic inflammation and prolonged exposure to inflammatory mediators may contribute to tumor initiation or progression, thereby acting as a tumorigenic

factor in these patients. An intriguing framework that may account for the different tumor presentations across age groups is the "double-edged sword" role of CLT. A recent meta-analysis indicates that autoimmune thyroiditis can increase the risk of thyroid cancer while at the same time being associated with more indolent tumor features.[30,31] Hence, carcinogenesis in patients with late-onset FNMTC may be driven by long-standing thyroiditis through a slower process, leading to later clinical onset and a less aggressive tumor phenotype. Consistently, experimental data from a transgenic mouse model combining BRAF V600E-driven PTC with thyroiditis susceptibility (NOD.H2h4 background) show that when thyroiditis precedes tumor development, PTC occurs less frequently and with less aggressive histopathological features than when thyroiditis is concomitant or absent.[32] Finally, telomere shortening, which promotes genomic instability, has emerged as a potential unifying mechanism in FNMTC pathogenesis.[33] A chronic inflammatory milieu such as CLT may further accelerate telomere shortening, thereby increasing the likelihood of malignant transformation over time.[34]

We acknowledge that the main limitation of our study is the relatively small sample size, particularly for subgroup analyses. Post hoc power analysis indicated that the primary comparisons between familial and sporadic cases were supported by high statistical power (>80%), whereas several subgroup comparisons (eg, early- vs late-onset strata) were only moderately powered (approximately 65%-75%). This implies a non-negligible risk of type II error in some subgroup analyses and warrants caution when interpreting these findings. Larger, preferably multicenter studies are needed to confirm and refine the observed age-related and familial–sporadic differences in tumor behavior. Another limitation is the absence of longitudinal data, which would have been valuable for assessing prognosis and long-term outcomes. Furthermore, this was a single-center study, which may limit the generalizability of our findings to other populations. However, the single-center design also represents a strength, given the relative insularity and genetic homogeneity of our region. Lastly, potential shared environmental co-exposures within families, which could have influenced tumor characteristics at diagnosis, were systematically investigated through detailed anamnestic evaluation, and no relevant common exposures were identified. However, subtle or unrecognized shared exposures cannot be completely ruled out and represent a limitation inherent to retrospective family-based studies.

Our findings underscore the importance of age at disease onset in defining clinical phenotypes in FNMTC and highlight the need for further investigations on its pathophysiology, including genetic, molecular, and immunological mechanisms, as well as the potential relationship with autoimmune thyroid disorders. A deeper understanding of these processes could pave the way for refined risk stratification and personalized management strategies for patients with familial thyroid cancer.

Authors' contributions

Chiara Mura (Data curation, Resources, Software, Formal analysis, Writing—original draft [lead], Investigation, Methodology [equal]), Silvia Corrias (Data curation, Investigation, Methodology [equal], Validation, Visualization [equal]), Mara Lecca (Data curation, Investigation), Maria L. Lai (Methodology, Validation, Visualization [equal]), Gian Luigi Canu (Investigation, Methodology, Validation, Visualization [equal]), Fabio Medas (Investigation, Methodology, Validation, Visualization [equal]), Stefano Mariotti (Conceptualization, Validation, Visualization, Writing—review & editing [equal]), Pietro G. Calò (Validation, Visualization [equal]), Paola Caria (Validation, Visualization [equal]), Giulia Lanzolla (Conceptualization, Data curation, Formal analysis, Resources, Software, Investigation, Supervision [equal], Writing—review & editing [lead]), and Francesco Boi (Conceptualization, Funding acquisition, Project administration, Writing—review & editing [equal], Supervision [lead])

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Data availability

The datasets generated and analyzed in this study are available from the corresponding author on reasonable request.

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