Familial vs sporadic papillary thyroid carcinoma: a matched-case comparative study showing similar clinical/prognostic behaviour

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Abstract

Objective: Familial non-medullary thyroid cancer has been proposed as an aggressive clinical entity. Our aim in this study is to investigate potential distinguishing features as well as the biological and clinical aggressiveness of familial vs sporadic papillary thyroid carcinoma (PTC). We assessed clinicopathological characteristics, outcome measures and DNA ploidy.

Design: A matched-case comparative study.

Methods: A series of patients with familial PTC ($n = 107$) and two subgroups, one with three or more affected elements ($n = 32$) and another including index cases only ($n = 61$), were compared with patients with sporadic PTC ($n = 107$), matched by age, gender, pTNM disease extension and approximate follow-up duration. Histological variant, extrathyroidal extension, vascular invasion, tumour multifocality and bilateral growth were evaluated. Ploidy pattern was analysed in available samples by DNA flow cytometry. The probabilities of disease-free survival (DFS) and overall survival (OS) were estimated according to the Kaplan–Meier (K–M) method.

Results: No patient with familial PTC died of disease during follow-up (median, 72 months), contrarily to five patients (4.7%) ($P = 0.06$) with sporadic PTC (median, 90 months). There was a significantly higher tumour multifocality in familial PTC (index cases subgroup) vs sporadic PTC ($P = 0.035$), and a trend, in the familial PTC cohort with three or more affected elements, to show extrathyroidal extension ($P = 0.054$) more frequently. No difference was observed in DNA ploidy status. The K–M analyses showed no significant differences between both entities in relation to DFS or OS.

Conclusion: Apart from multifocality, familial PTC appears to have similar clinical/prognostic behaviour when compared with sporadic forms of the disease.

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Introduction

Familial non-medullary thyroid cancer (FNMTC) accounts for ~5% of all thyroid carcinomas of follicular cell origin (1, 2, 3, 4). A vast majority of patients with FNMTC present papillary thyroid carcinoma (PTC), although benign thyroid lesions, such as multinodular goitre, are also commonly found in members of these families (2, 4, 5).

FNMTC has been regarded as a specific clinical entity, with a possible inherited genetic susceptibility and distinct clinicopathological and outcome characteristics.

With regard to the genetic basis, three susceptibility genes have been identified: the *NKX2-1* gene (chromosome 14q13.3), which encodes the thyroid transcription

factor NKX2-1 (6), *DICER1* (chromosome 14q32), which encodes RNaseIII, an enzyme involved in microRNA processing (7) and *SRGAP1*, which regulates the small G-protein CDC42 (8). In addition, seven chromosomal loci (1p13.2-1q21, 1q21, 2q21, 6q22, 8p23.1-p22, 8q24 and 19p13.2) involved in FNMTC susceptibility have been mapped, but the causal genes remain to be identified (9, 10, 11, 12, 13, 14). Recently, we have found evidence of an association between common variants of the *FOXE1* gene and FNMTC susceptibility. This gene encodes the thyroid transcription factor FOXE1, which, together with NKX2-1, HHEX and PAX8, is required for thyroid differentiation and function (15).

Although some studies have reported a less favourable prognosis for FNMTC (3, 16, 17, 18, 19, 20, 21), others did not verify this (1, 22, 23, 24, 25), and therefore, the biological and clinical behaviour of this familial disease, as compared with the much more frequent sporadic forms, remains a matter of controversy. Furthermore, data on molecular biomarkers, e.g. DNA ploidy, for better characterisation of tumour biological aggressiveness, are missing. Aneuploidy is a molecular feature that identifies genetic instability, and potentially, higher metastatic capacity, being commonly associated with worse disease prognosis (26, 27). In two recent studies of familial PTC, although controversial (28), Capezzone *et al.* observed shorter telomere length in the leukocytes, other non-neoplastic tissues and tumours in the familial cases when compared with patients with sporadic PTC or healthy subjects (29, 30). The size reduction of these nucleoprotein complexes, which protect the ends of chromosomes from degradation and fusion, may induce chromosomal instability, and thus, aneuploidy in these familial tumours.

The purpose of this study was to evaluate clinicopathological characteristics, as well as tumour DNA ploidy and outcome measures (disease-free survival (DFS) and overall survival (OS)), in patients with familial vs sporadic PTC in order to determine potential distinguishing features and compare the biological and clinical aggressiveness of their disease.

Subjects and methods

Clinicopathological data

All the patients involved in this study were diagnosed and treated for PTC between 1979 and 2011 at the Portuguese Oncology Institute (IPO). They encompassed two series: one with familial PTC ($n=107$), including 88 patients

from IPO Lisbon Center and 19 from IPO-Porto Research Center and the other with sporadic PTC ($n=107$), retrieved from a prospective thyroid surgery patient database, which includes DNA ploidy status information (25). The series were matched by number of patients, age, gender, disease extension (including tumour size, regional lymph node involvement and distant metastasis) and approximate follow-up time duration (Table 1). From the whole series of familial PTC ($n=107$), two patients' subgroups, one belonging to families with three or more affected elements ($n=32$) and another including index cases only ($n=61$), were selected.

FNMTC cases were defined when two or more first-degree family members were affected with NMTC, without another familial syndrome. Information about the family history of thyroid cancer was obtained from the patients and/or from hospital records. Sixty-six families, the majority previously reported (15), were included in this study. NMTC frequency in these families was as follows: one family had five members affected, five had four members affected, 11 had three members affected and the remaining 49 families had two affected members.

Table 1 Sporadic PTC vs familial PTC matched series.

Variables	Sporadic PTC, <i>n</i> (%)	Familial PTC, <i>n</i> (%)
Age (years)		
Mean (range)	46.2 (5–76)	46.1 (8–72)
Median	47	47
Follow-up time (months)		
Mean (range)	89.3 (20–144)	92.7 (8–402)
Median	90	72
Gender		
Female	82 (76.6)	82 (76.6)
Male	25 (23.4)	25 (23.4)
Histological variants		
Classic	71 (66.3)	71 (66.4)
Follicular	29 (27.1)	24 (22.4)
Mixed	5 (4.7)	9 (8.4)
Tall cell	2 (1.9)	3 (2.8)
Tumour size		
pT1	42 (39.3)	49 (46.2)
pT2	25 (23.4)	12 (11.3)
pT3	35 (32.7)	39 (36.8)
pT4	5 (4.7)	6 (5.7)
Regional lymph nodes		
pN0	72 (67.3)	72 (67.9)
pN1	35 (32.7)	34 (32.1)
Distant metastasis		
M0	106 (99.1)	105 (99.1)
M1	1 (0.9)	1 (0.9)

The PTC histological subtype, defined by common features or the most specific component of the tumour area, included the four variants found in the familial PTC group: i) classic, ii) follicular, iii) mixed and iv) tall cell (Table 1). The TNM classification was estimated according to the AJCC criteria from 2010 (31).

Total thyroidectomy was carried out in 100 of 107 patients (93.5%) with familial PTC and in 101 of 107 patients (94.4%) with sporadic PTC, while 30 (28.0%) and 39 (36.4%) patients, respectively, were submitted to lymph node dissection. When the procedure was not undergone, the nodal status was clinically assessed. In addition, 86 patients (80.4%) with familial PTC and 80 (74.8%) with sporadic PTC received adjuvant ablative iodine radiotherapy.

This study was performed with the approval of the Institutional Research Ethics Board. Follow-up data information was obtained by review of the patients' clinical records and consultation of an Oncological Registry Service (ROR-SUL). Each patient's clinical outcome was assessed by two parameters, the DFS and the OS, defined as the time elapsed from diagnosis to the first disease recurrence and death from the disease respectively. Patients not experiencing the relevant end point were censored at last clinical observation.

DNA flow cytometry

Flow cytometric analysis was performed in all sporadic PTCs and in 44 familial PTC cases, in which tumour samples were available. The sporadic series and 15 familial PTCs were analysed on representative fresh/frozen samples of tumour specimens obtained at surgical resection, according to a technique described previously (32), which uses propidium iodide (Sigma) as fluorochrome for DNA staining, and RNase (Sigma) and non-ionic detergent Nonidet P40 (Sigma) for double-stranded RNA digestion and cell membrane lysis respectively. The remaining 29 familial PTC cases were assessed on formalin-fixed paraffin-embedded material following the technique of Hedley *et al.* (33), with slight modifications, such as the thickness of block cut section (50 µm) and the incubation period for DNA staining (overnight). Fluorescent microbeads were used for daily calibration of the flow cytometer. The Multicycle Software program (Phoenix Flow Systems, San Diego, CA, USA) was used for cell-cycle analysis and further classification of tumours as diploid vs aneuploid according to DNA ploidy pattern.

Statistical analysis

Comparisons between familial and sporadic PTC groups were evaluated, for categorical parameters, by means of χ^2 test with Yates' modification and Fisher's exact test when appropriate (small samples); for continuous variables, Student's *t*-test and Wilcoxon' rank-sum test were used to compare mean and median values respectively. The probabilities of DFS and OS were estimated according to the Kaplan–Meier (K–M) method, and the differences between survival curves were analysed by the log-rank test. *P* values <0.05 were considered statistically significant.

Results

In the familial PTC subgroup with three or more affected elements (*n*=32), the mean and median values for age at time of diagnosis were 41.1 and 40 years (range, 22–72 years), revealing a trend to be lower as compared with sporadic (46.2 and 47 years; *P*=0.070 and *P*=0.084 respectively). The same figures for patients in the familial PTC subgroup with index cases only (*n*=61) were 47.6 and 47 years (range, 22–72 years) respectively.

Table 2 shows the tables of contingency and respective tests of independence of the clinicopathological and outcome characteristics and DNA ploidy status of familial vs sporadic PTC. With regard to pathological features, there was a statistically significant difference related to tumour multifocality (*P*=0.035) between sporadic PTC and the subgroup of familial PTC with index cases. Moreover, as compared with sporadic PTC, there was a trend in familial PTC with three or more affected members to show more frequently extrathyroidal extension (*P*=0.054). When the latter subgroup (*n*=32) was compared with familial PTC with only two affected elements (*n*=75), no differences were verified in relation to clinicopathological features, ploidy or survival parameters (data not shown). Overall, a higher percentage of vascular invasion, tumour multifocality and bilateral growth were found in familial PTC (whole series), but without reaching statistical significance.

Regarding clinical outcome, we observed that no patient in the familial PTC cohort died from the disease during follow-up, as compared with five patients (4.7%) in the sporadic PTC group, although the difference was not statistically significant (*P*=0.060). No differences were found in relation to disease recurrence either.

No difference between both entities was observed for DNA ploidy status, in which two of 44 (4.5%) cases (classic type) of familial PTC, and five of 107 (4.7%) cases

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Table 2 Clinicopathological and outcome characteristics and DNA ploidy status of sporadic PTC vs familial PTC.

Variables	Sporadic PTC, n (%)	Familial PTC (whole series), n (%)	P value	Familial PTC (three or more affected members), n (%)	P value	Familial PTC (index cases), n (%)	P value
Extrathyroidal spread			NS		0.054		NS
Negative	71 (66.4)	60 (57.1)		14 (45.2)		31 (51.7)	
Positive	36 (33.6)	45 (42.9)		17 (54.8)		29 (48.3)	
Vascular invasion			NS		NS		NS
Negative	98 (91.6)	91 (88.4)		26 (83.9)		49 (84.5)	
Positive	9 (8.4)	12 (11.6)		5 (16.1)		9 (15.5)	
Multifocality			NS		NS		0.035
Negative	65 (60.7)	54 (50.9)		19 (61.3)		26 (42.6)	
Positive	42 (39.3)	52 (49.1)		12 (38.7)		35 (57.4)	
Bilateral growth			NS		NS		NS
Negative	78 (72.9)	74 (69.8)		22 (71.0)		42 (68.9)	
Positive	29 (27.1)	32 (30.2)		9 (29.0)		19 (31.1)	
DNA ploidy			NS		NS		NS
Diploid	102 (95.3)	42 (95.5)		14 (93.3)		20 (95.2)	
Aneuploid	5 (4.7)	2 (4.5)		1 (6.7)		1 (4.8)	
Recurrence of disease			NS		NS		NS
Negative	91 (85.0)	88 (83.0)		25 (79.1)		51 (85.0)	
Positive	16 (15.0)	18 (17.0)		7 (21.9)		9 (15.0)	
Death of disease			0.060		NS		NS
Negative	102 (95.3)	106 (100)		32 (100)		60 (100)	
Positive	5 (4.7)	0 (0)		0 (0)		0 (0)	

(one classic, three follicular and one mixed) of sporadic PTC were found to be aneuploid.

The K–M survival estimates analyses revealed that there were no statistically significant differences in DFS (Fig. 1) and OS (disease-specific; Fig. 2) between familial PTC (whole series and the two subgroups) and sporadic PTC.

Discussion

The issue concerning the putative clinical aggressiveness of FNMTc as compared with sporadic forms of the disease is not consensual. The low prevalence of FNMTc, together with small sampling series and short follow-up studies, has limited consistent information on patient survival as well. Some studies have reported distinct clinical behaviours (3, 16, 17, 18, 19, 20, 21), but others have claimed that there are no differences between the two entities (1, 22, 23, 24, 25). Our present data seem to support the latter view, as we did not observe, overall, statistical differences in clinicopathological features, DNA ploidy status and outcome measures between familial PTC (whole series) and sporadic PTC. However, two findings may suggest the opposite in our study: the significantly higher presence of tumour multifocality in the subgroup of familial PTC with index cases and the trend to higher occurrence of extrathyroidal extension in the subgroup of familial PTC with three or more affected elements, where accurate

diagnosis is more easily demonstrated. It is well known about the association of these adverse pathological characteristics with disease aggressiveness, the former often being documented in familial PTC (16, 19, 20). The same holds true to the higher incidence of vascular invasion and bilateral tumour growth found in this group, as compared with sporadic PTC, although without reaching statistical significance.

There were no deaths among the familial PTC series during follow-up (100% absolute survival), in contrast to sporadic PTC which showed a higher, although not significant, incidence of disease mortality (4.7%). In agreement with some authors (24), but contrarily to

Figure 1

The probability of disease-free survival between familial PTC (whole series) and sporadic PTC ($P=0.693$).

Figure 2

The probability of overall survival between familial PTC (whole series) and sporadic PTC ($P=0.062$).

others (18, 19), no significant differences were observed in relation to disease recurrence and corresponding DFS between both entities. These relatively surprising findings might be explained by the fact that we have matched both familial and sporadic PTC series for pTNM parameters. Our FNMTC cohort is, contrarily to most similar comparative studies in the literature, histologically homogeneous, not including other much less frequent but more aggressive histopathological forms, such as the follicular, Hürthle cell, poorly differentiated or anaplastic carcinomas. Furthermore, the awareness of thyroid pathology and early diagnosis in affected families could lead to a more careful patient management, improving the clinical outcome of familial PTC. In a retrospective matched-case control study of FNMTC, the majority being PTCs, Maxwell *et al.* (24) reported that familial cases tend to have, but not significantly, improved DFS and disease-specific survival.

In a classical study (34), based on probability calculation analysis, Charkes pointed out that up to 60% of familial PTCs diagnosed in families with only two affected members might, actually, represent a fortuitous aggregation of sporadic cases, suggesting that the disease may be more accurately diagnosed in families with three or more affected members (17, 21). This fact could, therefore, also contribute to misestimate the aggressiveness of familial PTC. In our study, despite earlier disease onset and the trend above mentioned for extrathyroidal extension, we did not find significant differences in the clinicopathological and outcome characteristics between familial PTC with three or more affected members and sporadic cases, which indicates that even this particular familial subgroup does not present a more aggressive behaviour.

In a recently published paper (26) we found that, despite there being an uncommon molecular feature (<10%) in PTC, DNA aneuploidy can identify (mainly in disease recurrence) most aggressive tumours and patients with unfavourable clinical course. Therefore, in this work, we studied ploidy essentially for three reasons: i) to test its value as a biomarker of tumour aggressiveness; ii) to gather ploidy information in familial PTC (no data on this issue has been published so far); and iii) to investigate a potential link between shorter telomere length, chromosomal instability and aneuploidy. In thyroid cancer, patients displaying aneuploid tumours are in general associated with decreased survival (26, 27). However, as mentioned earlier, the low incidence of aneuploidy is a common finding in sporadic PTC (26), consistent with its mostly indolent course and favourable prognosis. In this sense, we found an overall low percentage of aneuploid cases, and no differences in DNA ploidy status between familial (whole series and the PTC subgroups) and sporadic PTC were depicted. These findings suggest that chromosomal instability, which could result from the reported telomere length shortening (29, 30), is not a common feature in these familial tumours.

In conclusion, our comparative analysis between familial and sporadic PTC appears to show a similar clinical behaviour, although higher tumour multifocality in index cases and a borderline tendency to higher extrathyroidal spread have been observed in the former. In particular, the worse prognosis of familial PTC in relation to sporadic forms of the disease was not proved. These findings do not impair, however, the careful patient's genetic screening and counselling, as well as the continuing search for elucidating the causative underlying mutations involved in FNMTC disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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