Biochimica et Biophysica Acta 1846 (2014) 468–476

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbacan

Review

Familial nonmedullary thyroid cancer: Screening, clinical, molecular and genetic findings

Diana Navas-Carrillo [a], Antonio Ríos [a], José Manuel Rodríguez [a], Pascual Parrilla [a], Esteban Orenes-Piñero [b,*]

[a] Department of Surgery, Hospital Universitario Virgen de la Arrixaca, University of Murcia, Murcia, Spain
[b] Department of Biochemistry and Molecular Biology-A, Biomedical Research Institute (IMIB), Campus of Lorca, University of Murcia, Spain

ARTICLE INFO

Article history:
Received 26 May 2014
Received in revised form 29 August 2014
Accepted 3 September 2014
Available online 11 September 2014

Keywords:
Familial nonmedullary thyroid cancer
Screening
Susceptibility loci
Telomerase
miRNA

ABSTRACT

Thyroid cancer, the commonest of endocrine malignancies, continues increasing in incidence being the 5th more prevalent cancer among women in the United States in 2012. Familial thyroid cancer has become a well-recognized, unique, clinical entity in patients with thyroid cancer originating from follicular cells, that is, nonmedullary thyroid carcinoma. Hereditary nonmedullary thyroid cancer may occur as a minor component of familial cancer syndromes (familial adenomatous polyposis, Gardner's syndrome, Cowden's disease, Carney's complex type 1, Werner's syndrome, and papillary renal neoplasia) or as a primary feature (familial nonmedullary thyroid cancer [FNMTC]).

Although there is some controversy, some epidemiologic and clinical kindred studies have shown that FNMTC is associated with more aggressive disease than sporadic cases, with higher rates of multicentric tumours, lymph node metastasis, extrathyroidal invasion, and shorter disease-free survival. This way, preventing screening will allow earlier detection, more timely intervention, and hopefully improved outcomes for patients and their families. On the other hand, in the last years, an important number of genetic studies on FNMTC have been published, trying to determine its genetic contribution. However, the genetic inheritance of FNMTC remains unclear; but it is believed to be autosomal dominant with incomplete penetrance and variable expressivity. This paper provides an extensive overview of FNMTC from several points of view. Firstly, the impact of early detection on prognosis, secondly, the management and follow-up of FNMTC patients, and finally, the role of susceptibility loci, microRNAs (miRNAs) and telomerases in recently identified isolated cases of FNMTC.

Contents

1. Introduction . . . 469
2. Search strategy . . . 470
3. Screening recommendations in FNMTC . . . 470
4. Clinical management and follow-up of FNMTC . . . 471
5. Genetic predisposition to FNMTC . . . 472
   5.1. FNMTC susceptibility loci . . . 472
      5.1.1. MNG1 14q31 locus . . . 472
      5.1.2. TCO 19p132 locus . . . 472
      5.1.3. fPTC/PRN 1q21 locus . . . 473
      5.1.4. NMTC1 2q21 locus . . . 473
      5.1.5. FTEN 8p23.1-p22 locus . . . 473
   5.2. miRNAs in FNMTC . . . 473
   5.3. Telomeres and telomerase in FNMTC . . . 474

Abbreviations: TC, Thyroid cancer; NMTC, Nonmedullary thyroid cancer; PTC, Papillary thyroid cancer; FTC, Follicular thyroid cancer; HTC, Hürthle cell thyroid cancer; ATC, Anaplastic thyroid cancer; FAP, Adenomatous polyposis of colon; FNAC, Fine-needle aspiration cytology; MNG, Multinodular goiter; TCO, Tumours with cell oxyphilia; PRN, Papillary renal neoplasm; FTEN, Familial thyroid epithelial neoplasia; SNP, Single-nucleotide polymorphism; FOXE1, Forkhead box protein E1; NKX2-1, NK2 homeobox 1; GWAS, Genome-wide association study; CDC6, Cell division control gene 6; PIP5K1C, Phosphatidylinositol-4-phosphate 5-kinase type-1 gamma gene; PXN, Paxillin; ZYX, Zyxin; RT-PCR, Retrotranscriptase-polymerase chain reaction; TERC, Telomerase RNA component; hTERT, Human telomerase reverse transcriptase; TA, Telomerase activity

* Corresponding author at: Department of Biochemistry and Molecular Biology-A, Biomedical Research Institute (IMIB), Campus of Lorca, University of Murcia, Avda. de las Fuerzas Armadas, s/n, Lorca, Murcia, Spain . Tel.: +34 968 381027.
E-mail address: eorenes@um.es (E. Orenes-Piñero).

http://dx.doi.org/10.1016/j.bbcan.2014.09.002
0304-419X/

6. Conclusions . . . 474
Acknowledgements . . . 474
References . . . 474

1. Introduction

Thyroid cancer (TC) is the most common endocrine malignancy and its incidence has been increasing sharply since the mid-1990s, being the fastest-increasing cancers in both men and women in United States [1]. Increased medical surveillance, the effect of environmental factors and more sensitive diagnostic tests, such as ultrasound and confirmation via fine-needle aspiration, are thought to account for this increased incidence [2]. There is significant disparity in thyroid cancer incidence by gender. TC is more common in women (approximately 3:1 ratio) becoming the 5th more prevalent cancer among women [1]. Some investigators have suggested that the higher rate of papillary thyroid cancer in women may be due to reproductive, hormonal and dietary factors, but the molecular factors that account for gender disparity in thyroid cancer incidence are unknown [3].

TC is a general term that comprises two main groups of neoplasias, depending on the cell type affected by the malignant transformation. 1) Carcinomas originating from the follicular epithelium, referred to as nonmedullary thyroid cancer (NMTC) representing more than 95% of all TC; and 2) carcinomas originating from the parafollicular thyroid C cells, referred to as medullary thyroid cancer (MTC) accounting less than 5% of all TC. There are four histologic subtypes of NMTC: papillary (PTC) (85%), follicular (FTC) (11%), Hürthle cell (HTC) (3%) and the anaplastic histotype (ATC) (1%) [4].

Histologically, PTCs are composed of well differentiated epithelial cells and can be distinguished by distinctive nuclear alterations including pseudoinclusions, grooves, and chromatin clearing (Fig. 1A). PTC incidence is remarkably high in developed countries, it is typically slow growing, and when it spreads it usually metastasizes to local lymph nodes [5]. On the other hand, FTC lacks the morphological nuclear features of PTC (Fig. 1B), which tends to be more aggressive and produces distant metastasis rather than lymph node invasion [6]. Although some PTC and FTC behave aggressively, the vast majority can be managed effectively. An important histologic variant of FTC is the oncocytic (Hürthle cell, oxyphilic) follicular carcinoma composed of eosinophilic cells repleted with mitochondria [7]. ATCs, the most uncommon form of NMTCs, are characterized by undifferentiated cells with high mitosis rate, necrotic areas, spindle-like cell morphologies as well as giant and occasionally squamous cells. ATC behaves very aggressively, rapidly invades adjacent tissues and is considered one of the most lethal human cancers [5].

NMTC is prevalently sporadic, but evidence of a familial inheritance is accumulating over the last years with prevalence from 5–10% in different series [8]. The first description of familial nonmedullary thyroid cancer was reported in 1955 by Robinson and Orr in monozygotic twins [9], and since then, numerous cases of FNMTC were reported until FNMTC was recognized as a distinct clinical entity [10]. It is named as familial non-medullary thyroid carcinoma (FNMTC) and it is defined by the diagnosis of two or more first-degree relatives with thyroid cancer of follicular cell origin without another familial syndrome. Several large case–control studies have reported the heritability of FNMTC to be one of the highest of all cancers [11] (Fig. 2). FNMTC may occur as a minor component of familial cancer syndromes (Adenomatous polyposis of colon (FAP), Gardner's syndrome, Cowden's

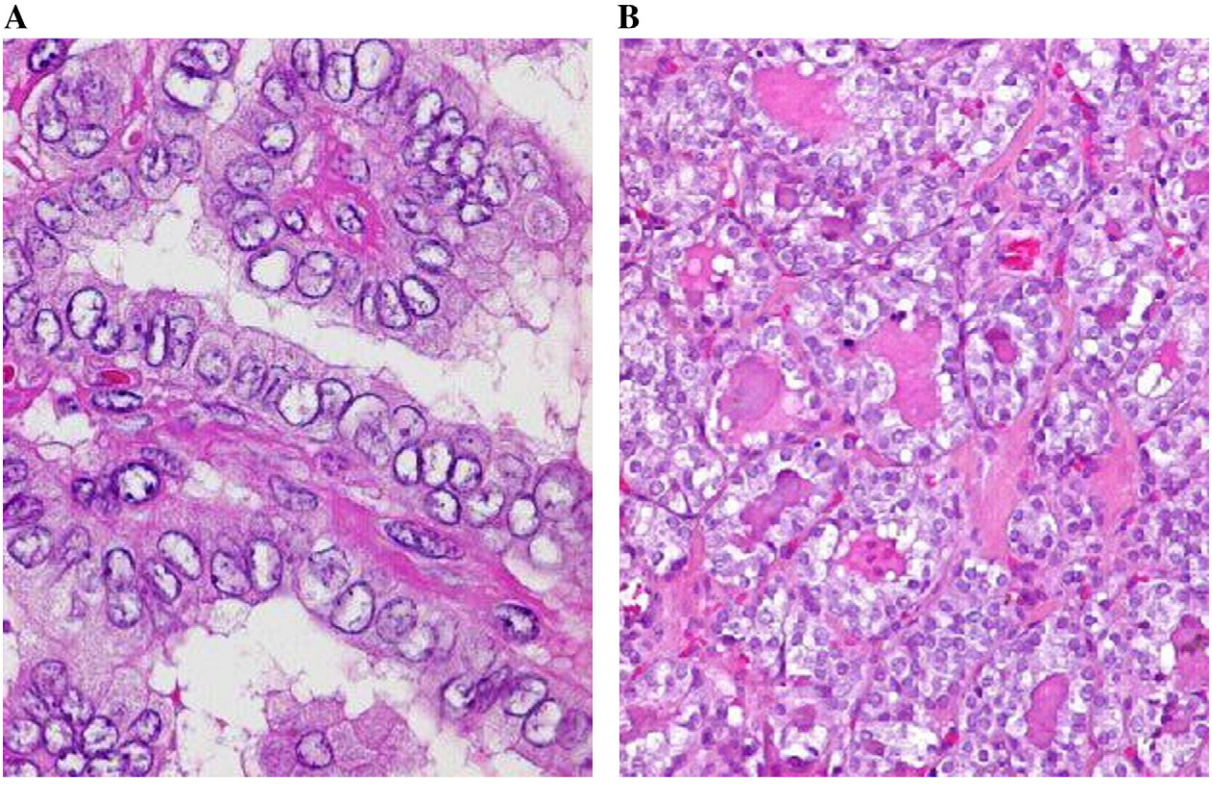

Fig. 1. Histopathology of the two differentiated subtypes of non-medullary thyroid neoplasias: A. Papillary thyroid cancer (PTC) characterized by distinctive nuclear alterations; B. Follicular thyroid cancer (FTC) without the morphological nuclear features of PTC.

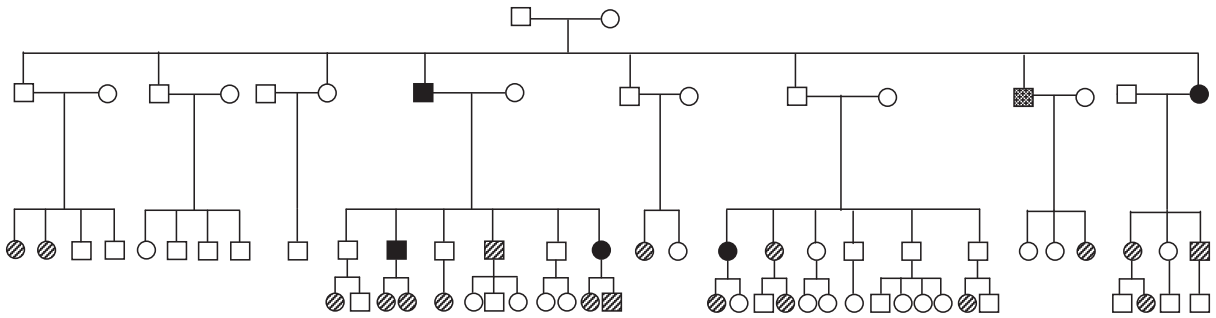

Fig. 2. Example of a complete pedigree affected by familiar non-medullary thyroid cancer (FNMTC) where □ and ○ represent non-affected relatives; ■ and ● represent relatives affected by FNMTC; ▨ and ◍ represent relatives affected by benign thyroid disease; and ▩ represents an individual with thyroid metastasis from bladder cancer.

disease, Carney complex type-1, Werner's syndrome, McCune–Albright syndrome) or as the predominate feature [12]. The genetic inheritance of FNMTC remains unknown, but it is believed to be autosomal with incomplete penetrance and variable expressivity as observed on reports of families with three or more affected members, horizontal transmission in siblings and increased percentage of male patients with FNMTC compared to those with sporadic NMTC [13].

FNMTC cannot be differentiated from the sporadic form of NMTC; and no specific clinical or histological features have been identified. However, it may have a trabecular struma with oxyphilia, and when associated with familial adenomatous polyposis it has a cribiform pattern [14]. Of note, there is some controversy about the higher aggressiveness of FNMTC compared with its sporadic counterpart. While some authors have reported this difference [15–17], some others did not find it [10,18]. However, incidence of tumour multifocality, local invasion, lymph node metastasis, and local or regional recurrences has been also observed. In addition, FNMTC occurs at an earlier age, with some evidence to suggest genetic anticipation in successive generations [17]. All these features have led many medical centres to recommend more vigilant screening and management in affected families. Thus, the aim of this paper is to extensively review FNMTC from several points of view. Firstly, we determine the impact of early detection on prognosis; secondly, we study the management and follow-up of FNMTC patients and finally, we analyze the role of susceptibility loci, microRNAs (miRNAs) and telomerases in recently identified isolated cases of FNMTC.

2. Search strategy

Published data for this review were identified by search and selection in MEDLINE database and reference lists from relevant articles and reviews. A two-step approach was used. Firstly, a search with the keywords "familial thyroid cancer", "familial nonmedullary thyroid cancer" and "familial papillary thyroid cancer" was carried out. Second, familial nonmedullary thyroid cancer was used as a keyword with the addition of the following keywords "screening", "genetics", "aggressiveness", "miRNA" and "telomerase". Bibliographies of all selected articles and review articles about familial nonmedullary thyroid cancer were reviewed for other relevant articles.

3. Screening recommendations in FNMTC

As heritability of FNMTC is one of the highest of all cancers, all first-degree relatives of affected families, even if asymptomatic, should be assessed. Some authors go beyond and strongly recommend the screening not only to first-degree relatives but also to second-degree [19]. In this study, a comprehensive screening examination was carried out in a huge cohort of 128 relatives of 21 FNMTC patients from 10 different families. The most outstanding observation of this analysis was that nearly 50% of second-degree relatives were diagnosed of thyroid pathology [19]. This percentage was very similar to the one obtained in first degree relatives (54%) suggesting that an exhaustive screening should be performed in both, first and second degree relatives, when possible [19]. Moreover, the authors did not find differences in multicentricity or lymphatic metastases when comparing first vs second-degree relatives showing similar aggressiveness of the tumour in both groups [19].

The gene(s) responsible for FNMTC have not yet been identified, and only some susceptibility genes of this familial disorder have been analyzed in selected kindred, but usually have not been validated by other subsequent studies. Thus, genetic testing for FNMTC is not available and it is mandatory that these individuals undergo a careful history and comprehensive physical examination. This should be followed by ultrasonography of the thyroid gland and cervical lymph nodes, fine needle aspiration biopsy when thyroid nodules or suspicious nodules are identified, and appropriate surgical treatment. Regarding patient and family history, the presence of locoregional symptoms should be questioned in all patients with thyroid abnormalities [20] because an augmenting incidence of tumour multifocality, local invasion, lymph node metastasis, local or regional recurrences and intraglandular metastasis in patients with FNMTC compared to sporadic counterparts has been reported [15–18]. The increased incidence of benign thyroid lesions that can progress from normal thyroid tissue to benign neoplasms and subsequent malignant transformation, together with familial clustering of multinodular goiters should be annotated because a personal or family history of benign thyroid conditions has been observed in about 45% of patients with FNMTC [10,21].

The use of cervical ultrasound for screening of asymptomatic patients is increasing because ultrasound permits earlier detection of occult non-palpable thyroid cancers in asymptomatic family members of patients with FNMTC [22]. It is important to remark that thyroid cancer detection rate with neck palpation alone is low, reportedly only 0.19% in a huge screening study of more than 18,000 women, whereas the incidence of ultrasonographically detecting thyroid nodules in healthy adults was reported to be about 20% [23]. Moreover, a study with 149 patients who were symptom-free relatives of patients from 53 FNMTC families, reported a 52% prevalence of thyroid nodules detected by screening neck ultrasounds [22]. In addition, 10% of this cohort was subsequently diagnosed with thyroid cancer, with tumours averaging less than 1 cm in size. Despite the relatively tumour small size, 47% showed multifocal disease and 43% had lymph node metastases [22]. Several studies have shown excellent specificity (95.7–100%) and reasonable sensitivity (83.3–92.6%) of neck ultrasonography for identifying cervical lymph node metastases in the lateral neck [24,25]. Furthermore, preoperative ultrasonography results may alter the surgical approach in as many as 34% of cases, including those with palpable lymphadenopathy on preoperative exam [24]. The only drawback of thyroid ultrasonography is that regarding the high frequency of multifocal disease, it can identify thyroid nodules that are benign and of little risk to these individuals. However, when balancing the advantages vs disadvantages of neck ultrasonography, the use of ultrasound is strongly recommended as an important part of the evaluation of any patient at risk for FNMTC. In the last years, thyroid elastography is gaining more

importance for thyroid tumour screening. It is a new technique based on the principle that when body tissues are compressed, softer parts deform more easily than harder ones. The amount of displacement is determined from ultrasound signals reflected by tissues when they are compressed. Elastography is a more accurate and sensitive technology that allows the detection and differential diagnosis of thyroid cancers. In a recent study, the reproducibility of real-time freehand elastography in thyroid nodules was determined with statistically significant agreement between two radiologists, making elastography a reproducible and suitable technique that could be used in daily clinical practice [26].

Fine needle aspiration cytology (FNAC) is a cost-effective and reliable diagnostic test for thyroid nodules being the gold standard for the differential diagnosis between benign and malignant thyroid nodules. However, as FNMTC is associated with an increased rate of multifocality, bilaterality, and multiple benign nodules, the results of FNAC may be indeterminate and inconclusive in nearly 5–15% of these lesions [27]. This rate of inconclusive results led us to recommend total thyroidectomy in any patient with a thyroid nodule and a strong family history, regardless of the FNAC result [20].

To sum up, we believe that a complete screening should be developed in all, first and second-degree relatives, even if asymptomatic. Regarding the special features of FNMTC, a complete personal and familiar history and an exhaustive physical examination should be carried out. This should be followed by ultrasonography of the thyroid gland and cervical lymph nodes, fine needle aspiration biopsy when thyroid nodules or suspicious nodules are identified, and appropriate surgical treatment. This preventing screening will allow earlier detection, more timely intervention, and hopefully improved outcomes for patients and their families.

4. Clinical management and follow-up of FNMTC

In the past years, there has been a controversy about the existence of the familiar form of NMTC, however, nowadays, the existence of this FNMTC is well documented and has little discussion [7–13,15–17,20,22,27]. Although NMTC is mostly sporadic, evidence for a familial form, not associated with other Mendelian cancer syndromes is unequivocal and is thought to cause more aggressive disease [17]. For that reason, the search for genetic susceptibility loci for FNMTC started two decades ago. The clinical characteristics of FNMTC are being clarified, not only by family studies, but also by large epidemiologic revisions, differing from sporadic NMTC [18]. FNMTC has been associated with early age of onset [10,17], an increased incidence of benign thyroid nodules [15], multifocality [8,15], bilateral tumour occurrence [8,18], higher frequency of lymph node metastasis at diagnosis [18], recurrence [17] and worse outcome [17] when compared with the sporadic NMTC. All these differences between the familiar and the sporadic form of NMTC explain that patients with FNMTC should have a more aggressive surgical treatment and should be followed closely. On the other hand, it is not clear if higher incidence in thyroid pathology might be due to a more exhaustive screening in kindred of patients, or it is a real higher incidence. It is a difficult question to answer due to the lack of bibliography, but in our point of view and according to unpublished results of our group [19], the incidence of thyroid pathology (including benign diseases) is higher in the familiar than in the sporadic form.

Although being a controversial topic, most studies, including large cohort studies, suggest that FNMTC is more aggressive than sporadic NMTC. As commented above, FNMTC has been associated with early age of onset, an increased incidence of multiple benign thyroid nodules, multifocality, bilaterality, nodal involvement, intraglandular dissemination, extrathyroidal invasion, lymph node metastasis, shorter disease-free survival period and recurrence [15,17,19,21,22,28–31]. In a study conducted in Japan containing records of 8422 patients treated by thyroidectomy between 1946 and 2000, 258 patients from 154 families were identified as having FNMTC [15]. Among them, intraglandular dissemination of the tumour was more frequent in FNMTC patients than in sporadic thyroid carcinoma patients (40.7% vs 28.5%; $p < 0.0001$). In addition, multiple benign nodules were found more frequently in the FNMTC patients than in the sporadic thyroid carcinoma patients (41.5% vs 29.8%; $p < 0.0001$) and tumour recurrence was also seen more frequently in the FNMTC patients (16.3% vs 9.6%; $p = 0.0005$). Recurrence at ipsilateral and contralateral lymph nodes and the contralateral thyroid lobe were significantly more frequent in FNMTC patients than in sporadic disease patients. Regarding surgical procedure, total thyroidectomy, subtotal thyroidectomy, and lymph node dissection were more frequent in the FNMTC patients than in the sporadic thyroid carcinoma patients [15]. Furthermore, the disease-free survival period was also significantly shorter for the FNMTC patients, although survival curves did not differ statistically between groups. It is important to remark that it is very difficult to perform a survival analysis in a relatively rare disease with a low death rate. Hence, no study will probably ever be able to prove a difference in overall survival [20]. Thus, disease-free survival is a much more appropriate endpoint for this disease, and there are, apart from this, several well-conducted studies that support the conclusion that FNMTC has a shorter disease-free survival.

A descriptive study of 119 patients with papillary thyroid microcarcinoma showed that FNMTC may be more aggressive than the sporadic disease [31]. Despite the fact that only seven of these patients had FNMTC, the differences with the sporadic cohort were remarkable. Although FNMTC patients showed an average tumour size of only 5.9 mm, rates of tumour multicentricity (71% vs 19%), bilateral disease (43% vs 8%), lymph node metastasis (57% vs 28%) and vascular invasion (43% vs 5%) were all significantly higher than in sporadic cases.

A retrospective analysis with 698 confirmed cases of nonmedullary well-differentiated thyroid cancer demonstrated that the presence of distant metastases was statistically significantly higher in the familial cases than in the sporadic cases ($p = 0.003$). Interestingly, distant metastases were seen particularly in families with three or more affected members [28]. The familial group had statistically significant increased rates of recurrent/persistent disease as judged by the requirement for reoperation ($p = 0.05$), the requirement of additional radioactive iodine therapy administered at least two years after the initial therapy ($p = 0.03$), or a combination of these two therapeutic modalities ($p = 0.03$). Furthermore, there was a statistically significant trend for greater mortality ($p = 0.01$) in the FNMTC cohort.

A population-based case–control study including 332 cases with papillary or follicular carcinoma diagnosed between 1993–1999 and 412 controls, matched by sex and 5-year age-group was carried out in New Caledonia, where high incidence rates of thyroid cancer have been observed, particularly in Melanesian women [29]. In this study, a 3.2-fold increased odds ratio of non-medullary thyroid cancer in individuals with a family history of thyroid cancer among first-degree relatives was observed. This study also found an odds ratio of 3.6 for thyroid cancer in individuals with a family history of multinodular goiter in first-degree relatives [29].

Another study, with two different cohorts from Italy and Greece, compared 34 FNMTC with 235 sporadic NMTC patients [17]. These authors obtained that tumours of FNMTC patients were more frequently multifocal ($p = 0.001$), tended to have higher recurrence rate ($p = 0.05$), and had worse outcome ($p = 0.001$) when compared with sporadic PTC patients [17]. In this analysis, the phenomenon of "genetic anticipation" was observed. It has gained new attention in the last decades and has been fully demonstrated for other inherited benign and malignant disorders, such as Huntington's disease [32] or dyskeratosis congenital [33]. The authors found that mean age of tumour at presentation and at diagnosis was significantly younger in the second generation ($p < 0.0001$). In addition, patients of the second generation had a higher rate of local or distant metastases ($p = 0.02$), tumour multicentricity ($p = 0.003$), and bilaterality ($p = 0.01$) at the time of diagnosis and a worse outcome ($p = 0.04$) at final follow-up when compared with their parents [17].

A higher aggressiveness of the tumour in FNMTC patients compared with sporadic counterparts was also reported in a case–control study

with 28 FNMTC and 85 sporadic NMTC [30]. The presence of adenopathies ($p = 0.02$), thyroid nodules ($p = 0.01$) and multicentric disease ($p = 0.007$) was higher in the FNMTC patients. However, this study failed in finding significant differences in the presence of lymph node metastases, recurrence or mortality.

On the other hand, a retrospective analysis of 67 patients with FNMTC who were compared with 375 control subjects with sporadic NMTC did not find a higher frequency of multicentric disease and lymph node metastases at diagnosis. In addition, the authors showed FNMTC to have a similar long-term outcome when compared with sporadic disease [34]. Similarly, a meta-analysis performed with 178 patients concluded that FNMTC was not more aggressive than the sporadic form of the disease [10].

In view of the results thus far reviewed, we believe that there is convincing evidence supporting the fact that FNMTC is more aggressive than the sporadic form of the disease, thus suggesting that earlier treatment is beneficial. We therefore recommend total thyroidectomy with a prophylactic central neck lymph node dissection if the patient has a malignant thyroid nodule and a thorough family history of thyroid cancer. If lymph node involvement is present in the lateral neck compartment by preoperative imaging or by palpation, a therapeutic lymph node dissection should also be performed. Moreover, all patients treated with a total thyroidectomy should be considered for postoperative radioactive iodine therapy, regardless of tumour size, as there is a high incidence of local recurrence and nodal involvement. Upon completion of radioactive iodine therapy, patients should be placed on lifelong thyroid suppression. The optimal follow-up for patients with a diagnosis of FNMTC is not known. Because all patients with FNMTC have thyroid cancer, their follow-up should be, at a minimum, the standard of care for their stage of disease [20]. However, due to aggressiveness of FNMTC and the high risk of recurrence, a more aggressive postoperative treatment and more rigorous follow-up should be considered.

5. Genetic predisposition to FNMTC

On a molecular level, the genetic basis of FNMTC as a distinct syndrome remains poorly understood (Table 1). Unlike in the case of MTC syndrome, caused by germline point mutations in the RET proto-oncogene [35]; the causative genes predisposing to FNMTC have not been yet identified. The variable expression of FNMTC suggests that the responsible gene(s) may lead to predisposition or susceptibility to thyroid cancer. With the advent of new techniques in molecular genetics, a number of potential loci for FNMTC genes have been identified. In addition, the role of different miRNAs and the effect of telomeres and telomerases in the genetic predisposition to FNMTC have been investigated.

5.1. FNMTC susceptibility loci

Studies employing genome wide linkage analysis using microsatellite markers and informative large pedigrees with multiple affected members have revealed potential loci and also excluded some important genes that were thought to be good candidates in susceptibility to FNMTC [7].

5.1.1. MNG1 14q31 locus

It was the first locus identified to be potentially implicated in FNMTC [36]. A Canadian pedigree with 18 cases of multinodular goiter (MNG) and 2 cases of NMTC were studied [36]. After genotyping 34 individuals, a potential susceptibility locus at 14q31 was identified. Haplotype analysis concurred with the linkage data and showed an autosomal dominant mode of inheritance. To confirm these initial findings, linkage analysis was repeated on several pedigrees. The locus was confirmed in other families with MNG [37], but no evidence of linkage was found in additional FNMTC pedigrees suggesting that this locus may not be involved in FNMTC, or that it may account for only a minority of FNMTC cases with MNG. Alternatively, it is possible that the MNG1 locus may harbour a gene for MNG alone but not for FNMTC.

5.1.2. TCO 19p132 locus

The thyroid tumours with cell oxyphilia (TCO) locus were mapped for the first time on chromosome 19p132 in a French family consisting of six cases of MNG and three of NMTC by the French NMTC Consortium [38]. The TCO locus extends up to ~2 Mb and has been further refined to a 1.6 Mb interval by adding more markers and more families with affected individuals showing oxyphilic tumours. Initially, it was speculated that the TCO locus is associated only with this unique form of FNMTC

Table 1
Genetic alterations in FNMTC.

Susceptibility gene	Location	Author	Year	Findings	References
MNG1	14q31	Bignell et al.	1997	No evidence of linkage was found in additional FNMTC pedigrees	[36,37]
TCO	19p132	Canzian et al.	1998	Confirmed in independent studies. Evidence for the genetic interaction between the TCO and NMTC1.	[16,38,39]
fPTC/PRN	1q21	Malchoff et al.	2000	Not yet confirmed in any other independent study and no further families with a PTC and PRN association have been reported.	[40]
NMTC1	2q21	McKay et al.	2001	Interaction between the TCO and NMTC1 loci may increase the risk of FNMTC in patients who inherit both susceptibility loci. Alterations at TCO and NMTC1 could be important in a fraction of cases with FNMTC.	[39,41–43]
FTEN	8p23.1-p22	Cavaco et al.	2008	Potential tumour suppressor gene. Not confirmed in further analysis.	[44]
FOXE1	9q22.33	Gudmundsson et al.	2009	GWAS conducted in a huge cohort of patients and controls. This gene encodes for a thyroid transcription factor.	[45]
NKX2-1	14q13.3	Gudmundsson et al.	2009	GWAS conducted in a huge cohort of patients and controls. This gene encodes for a thyroid transcription factor.	[45]
Not yet identified	1q21	Suh et al.	2009	SNP array-genotype analysis in 38 families. Several genes of the neuroblastoma breakpoint family (NBPF) reside on this chromosomal region.	[46]
Not yet identified	6q22	Suh et al.	2009	SNP array-genotype analysis in 38 families. Same chromosomal region as the PRN1 locus.	[46]
miR-886-3p	5q31.2	Xiong et al.	2011	3-Fold downregulated in FNMTC as compared to NMTC. Regulates DNA replication (CDC6) and focal adhesion (PIP5K1C, PXN, ZYX) genes.	[51,52,55]
miR-20a	13q31.3	Xiong et al.	2011	4-fold downregulated in FNMTC as compared to NMTC. Promotes cellular proliferation and invasion, and higher expression levels have been associated with tumour dedifferentiation.	[51,53–55]
TERC-hTERT complex	3q26 and 5p15.33	Capezzone et al.	2008	Short telomeres, increased hTERT gene copy number and higher telomerase activity contribute to genomic instability and immortalization. Differences found between FNMTC and NMTC patients.	[56–65]

Abbreviations: MNG1: Multinodular goiter-1; FNMTC: Familiar non-medullary thyroid cancer; TCO: Tumours with cell oxyphilia; NMTC1: Non-medullary thyroid cancer-1; fPTC: Familiar papillary thyroid cancer; PRN: Papillary renal neoplasm; FTEN: Familial thyroid epithelial neoplasia; FOXE1: Forkhead box protein E1; NKX2-1: NK2 homeobox 1; GWAS: Genome-wide association study; NBPF: neuroblastoma breakpoint family; SNP: Single nucleotide polymorphism; CDC6: Cell division control gene 6; PIP5K1C: Phosphatidylinositol-4-phosphate 5-kinase type-1 gamma gene; PXN: Paxillin; ZYX: Zyxin; TERC: Telomerase RNA component; hTERT: Human telomerase reverse transcriptase.

with cell oxyphilia. However, when a linkage analysis on 22 FNMTC families was performed, 1 family with linkage to the TCO locus was found and the thyroid cancers in this family did not display cell oxyphilia [39]. Importantly, linkage to the TCO locus has been subsequently confirmed in independent studies [16,39]. Furthermore, analysis of additional families has provided evidence for the genetic interaction between the TCO at 19p132 and NMTC1 at 2q21 loci (another potential genetic locus), resulting in a significantly increased risk of NMTC in patients carrying both susceptibility loci [39].

5.1.3. fPTC/PRN 1q21 locus

It was firstly identified on Chromosome 1q21 in an American family with five members affected by PTC, one by colon cancer and two by papillary renal neoplasm (PRN) [40]. Thirty-one members of this family were genotyped and haplotype analysis revealed that all affected subjects carried the same phenotype within the linkage region. To date, the relationship of this locus with FNMTC has not been confirmed in any other independent study and no further families with a PTC and PRN association have been reported. These findings suggest that the fPTC/PRN locus may harbour a susceptibility gene for a unique and rare FNMTC phenotype where PTC is associated with PRN. However no association to this locus is suggested for the majority of FNMTC.

5.1.4. NMTC1 2q21 locus

The existence of a susceptibility locus for FNMTC (NMTC1) on chromosome 2q21 was first identified in a large Tasmanian pedigree with recurrence of PTC [41]. An extensive genome-wide scan followed by haplotype analysis revealed that seven out of eight subjects with PTC shared a common haplotype on chromosome 2q21. These observations were subsequently confirmed with a linkage analysis performed on additional 80 pedigrees with FNMTC. Moreover, a linkage analysis was carried out on a further 10 FNMTC families, 9 of which contained thyroid cancers with cell oxyphilia. This study revealed a significant evidence in favour of a two locus inheritance model between TCO and NMTC1 [42], suggesting that interaction between the TCO and NMTC1 loci may increase the risk of FNMTC in patients who inherit both susceptibility loci [39,42]. Furthermore, the loss of heterozygosity at the TCO and NMTC1 loci was demonstrated in some tumour specimens from patients with FNMTC [43]. Together, all these findings suggest that alterations at TCO and NMTC1 could be important in a fraction of cases with FNMTC.

5.1.5. FTEN 8p23.1-p22 locus

It was discovered in a clinical screening of a Portuguese family with 11 cases of benign thyroid disease and 5 cases of thyroid cancer using higher genomic resolution techniques such as single-nucleotide polymorphism (SNP) followed by microsatellites [44]. A genome-wide significant evidence of linkage, to a single region on chromosome 8p23.1-p22 was obtained and recombination events delimited the minimal region to a 7.46-Mb span. The authors excluded seventeen suggestive candidate genes located in the minimal region as susceptibility genes by mutational analysis. Allelic losses in the 8p23.1-p22 region were absent in seven thyroid tumours from family members, suggesting that the inactivation of a putative tumour suppressor gene may have occurred [44]. Further analyses are warranted before this locus is considered to harbour an FNMTC susceptibility gene.

Remarkably, all these studies showed the main limitation of being performed in individual families, with distinct variants of FNMTC (e.g., papillary renal neoplasia, oxyphilic tumours) not existing in the vast majority of families. For that reason, some of these loci still remain to be confirmed in other families. To address this problem, two new loci have been recently linked to FNMTC susceptibility using genome-wide association study (GWAS) technology. Two common gene polymorphisms associated with thyroid cancer have been detected at chromosomes 9q22.33 and 14q13.3 in a GWAS conducted in 378 cases and 37,196 Icelandic controls [45]. The estimated risk of thyroid cancer in homozygous carriers was 5.7-fold greater than that of non-carriers. These variants involved, respectively, the FOXE1 and the NKX2-1 genes, which encode for two thyroid transcription factors. It is important to corroborate these results in different familiar cohorts to confirm the role of these polymorphisms in FNMTC. Additionally, a SNP array-genotype analysis from 38 FNMTC families across the United States and Italy identified significant linkage between FNMTC phenotype and 2 SNP markers on chromosomes 1q21 and 6q22 [46]. These 2 regions may possibly encompass heretofore undiscovered genes that predispose to FNMTC. Some genes potentially associated with FNMTC reside on these regions, such as the neuroblastoma breakpoint family; however, the exact genes have not been yet identified.

On the other hand, recent technical advances in molecular genetics, such as multiple germline mutation analyses have excluded the most common somatic mutations in genes associated with sporadic thyroid cancers, including RET, RET/PTC, MET, MEK1, MEK2, APC, PTEN and NTRK, as candidate genes for FNMTC [44]. However, somatic mutations of BRAF and RAS were also identified in Portuguese families with several individuals affected with NMTC [43]. The authors suggest that these somatic genetic alterations may be involved in the FNMTC tumour progression.

5.2. miRNAs in FNMTC

MicroRNAs (miRNAs) are endogenous, conserved, single stranded, small (approximately 22 nucleotides in length), noncoding RNAs that repress gene expression at the post-transcriptional level by targeting mRNA [47]. Lin-4, the first miRNA discovered (1993), was found to regulate *Caenorhabditis elegans* development by inhibiting the protein expression of lin-14 via binding to the 3′UTR of its mRNA [48]. Since then, and according to the miRNA database miRBase, the human genome encodes more than 1500 miRNA sequences, which may target approximately 60% of human protein-coding genes [49]. miRNA anneals to complementary sequences in the 3′-untranslated regions (3′UTR) of target mRNAs of protein-coding genes, causing mRNA to be cleaved or to repress the translational machinery needed for protein synthesis. Thus, miRNA can either inhibit translation or induce degradation of its target mRNA or both, depending upon the overall degree of complementarity of the binding site, the number of binding sites, and the accessibility of those binding sites [50]. The stronger its complementarity with the prospective target RNA, the more likely the miRNA will degrade the target mRNA, and those miRNAs that display imperfect sequence complementarities with target mRNAs primarily, result in translational inhibition. Two classes of microRNAs relevant to cancer are distinguished: 'onco-miRs' with tumour promoting effects versus 'tumour-suppressive miRNAs' that restrain cancer progression.

As far as we know, there is only one study comparing the miRNA profile of FNMTC with their sporadically occurring counterparts [51]. In this study, miRNA expression profile of familial vs sporadic nonmedullary thyroid cancer tumour samples was analyzed using whole genome miRNA microarrays. The authors found two miRNAs, miR-886-3p and miR-20a, differentially expressed between the FNMTC and the sporadic NMTC groups. Importantly, they were validated to be differentially expressed by 3- and 4-fold by quantitative real time RT-PCR, respectively. Moreover, miR-886-3p and miR-20a were also downregulated in NMTC as compared to normal thyroid tissue by 3.5–4-fold. Both miR-20a (13q31.3) and miR-886-3p (5q31.2) are not located in chromosomal loci previously identified as susceptibility loci by linkage studies in kindreds with FNMTC, nevertheless, this issue is not surprising given the small nucleotide lengths of miRNAs.

Recently, miR-886-3p has been observed to be downregulated in squamous cell lung cancer compared with normal lung tissue, suggesting a potential tumour suppressor role for this miRNA [52]. These authors demonstrated that overexpression of miR-886-3p caused dramatic decrease (more than 4-fold) in the expression of genes that

regulate DNA replication (CDC6) and focal adhesion (PIP5K1C, PXN, ZYX) in two different well-characterized thyroid cancer cell lines. Overexpression of miR-886-3p significantly inhibited cell proliferation to 79% ($p < 0.001$) by increasing the number of cells in S phase (12–16%) while decreasing the number of cells in G0/G1 (11–22%) ($p < 0.001$). On the other hand, miR-886-3p overexpression decreased the number and size of spheroids in thyroid cancer cell lines when cultured in ultra low adherent culture flasks and significantly inhibited migration of thyroid cancer cell lines using the scratch wound healing assay ($p < 0.001$) [51].

Dysregulation of miR-20a expression has been previously observed in prostate, ovarian and head and neck squamous cells among others [53,54]. miR-20a promotes cellular proliferation and invasion, and higher expression levels have been associated with tumour dedifferentiation [53]. The biology of miRNAs represents a relatively new research area and is still an emerging field [55]. For that reason, further analyses are guaranteed to identify the role of new miRNAs in the FNMTC. Future studies aimed at understanding how miRNAs are integrated into FNMTC are a prerequisite for their development as potential therapeutic targets.

5.3. Telomeres and telomerase in FNMTC

Telomeres are non-coding regions at the end of eukaryotic chromosomes consisting of hundreds of copies of a simple tandem repeat sequence (TTAGGG in vertebrates) that serves to stabilize the chromosome for replication through cell division. Telomeres progressively shorten with each cell replication due to incomplete lagging of DNA strand synthesis and oxidative damage. When telomeres become critically short, the cells undergo senescence or apoptosis [56]. Telomerase is a specialized ribonucleoprotein with reverse transcriptase activity that counteracts telomere shortening by adding telomeric repeats to the G-rich strand. It is composed of a telomerase RNA component (TERC) that serves as a template for the addition of repeats, and a protein component, the telomerase reverse transcriptase (hTERT) [56]. In humans, telomerase activity is abundant in germ cells, adult stem cells, and activated immune cells, whereas it is absent or low in adult differentiated cells and resting immune cells. In the absence of telomerase or when the activity of the enzyme is low, apoptosis is triggered. Interestingly, although most cells die by apoptosis when telomeres become critically short, rare cells survive and maintain stable short telomere lengths through the reactivation of telomerase facilitating cell immortalization. This suggests that maintenance of telomere length is necessary for continued cell division and immortalization and both have been implicated in the control of the proliferate capacity of normal and malignant cells [57]. Thus, patients who have inherited or acquired genetic defects in telomere maintenance seem to have an increased risk of developing familial benign diseases and malignant diseases such as head, neck, lung, breast, and renal cancers [58].

The strong association of telomerase re-activation with cancer provides evidence that this mechanism plays an important role in cancer development. Moreover, telomerase activity (TA) can be regarded as a marker for human cancers [59]. In normal thyroid samples, TA is almost absent whereas among thyroid cancer, increased TA was found in all histotypes (papillary, follicular, medullary and anaplastic), with large variations in different series, but approximately approaching more than 50% of the samples [27]. This issue was firstly reported in 1997, where the presence of TA was observed in 100% of FTC and its absence in 76% of benign thyroid lesions [60]. These authors stated that TA may provide a potential diagnostic marker distinguishing benign from malignant follicular thyroid tumours. Some studies showed that TA correlated significantly with the progression of the clinical stage suggesting that telomerase expression could be important in defining the clinical behaviour of thyroid carcinomas [61,62].

Recently, several telomeric abnormalities, such as telomeric associations and telomeric fusions driving to chromosomal fragilities have been discovered in patients with FNMTC compared with healthy subjects and sporadic cases [63]. In addition, the presence of an imbalance of the telomere–telomerase complex has been reported in a study with 34 patients with FNMTC, validated in a second series with 18 FNMTC patients and compared with the expression in sporadic cases of NMTC [64]. The authors observed that FNMTC patients display shorter telomeres, increased amplification in hTERT gene copy number, and higher telomerase activity, compared with sporadic NMTC patients. High telomerase activity found in FNMTC patients together with exaggerated hTERT activity and the increase in hTERT gene copy number represents genetic abnormalities associated with genomic instability that allow DNA-damaged cells escape from apoptosis and contributes to genomic instability and immortalization [65]. These observations suggest that patients born with short telomeres might reach earlier in life the threshold telomere length sufficient to trigger cancer development and/or progression. Importantly, patients of the second generation were always diagnosed with thyroid cancer at an earlier age, compared with their affected relative in the first generation [64]. These findings are in agreement with the definition of "genetic anticipation" reinforcing the hypothesis that FNMTC is a true familial disease rather than the fortuitous association of the same disease in a family.

6. Conclusions

Evidence of a familial inheritance of NMTC has been accumulating over the last years with prevalence from 5–10% in different series. Since the first description of FNMTC in 1955, numerous cases have been reported recognizing FNMTC as a distinct clinical entity. As heritability of FNMTC is one of the highest of all cancers and there are no genetic tests available, at least all first-degree relatives of affected families, even if asymptomatic, should be examined undergoing a careful history and comprehensive physical examination. This way, preventive screening will allow earlier detection, more timely intervention, and hopefully improved outcomes for patients and their families. Although some controversy exists, a number of reports here reviewed, including large cohort studies, suggest that FNMTC is more aggressive than sporadic NMTC, being associated with early age of onset, an increased incidence of multiple benign thyroid nodules, multifocality, nodal involvement, lymph node metastasis, shorter disease-free survival period and recurrence, among others. Thus, more aggressive postoperative treatment and more rigorous follow-up should be considered for FNMTC patients. On the other hand, a number of potential loci for FNMTC susceptibility have been analyzed; however, the causative genes predisposing to FNMTC have not been yet identified. For that reason, with the advent of new molecular approaches, larger studies to examine the effects of single genetic variants should be conducted. Recent studies have provided increasing evidence that telomeres and telomerase expression and activity can contribute to genomic instability and immortalization of tumour cells from FNMTC compared with healthy subjects and sporadic cases. Moreover, the biology of miRNAs represents a relatively new research area being an emerging field, and future studies aimed at understanding how miRNAs are integrated into FNMTC are guaranteed.

Acknowledgements

Dr. Orenes-Piñero is supported by a postdoctoral contract with number APOYO_041/2014 from Instituto Murciano de Investigación Biosanitaria, IMIB, Murcia, Spain.

References

[1] R. Siegel, C. DeSantis, K. Virgo, K. Stein, A. Mariotto, T. Smith, D. Cooper, T. Gansler, C. Lerro, S. Fedewa, C. Lin, C. Leach, R.S. Cannady, H. Cho, S. Scoppa, M. Hachey, R. Kirch, A. Jemal, E. Ward, Cancer treatment and survivorship statistics, 2012, CA Cancer J. Clin. 62 (2012) 220, http://dx.doi.org/10.3322/caac.21149.

[2] L. Davies, H.G. Welch, Increasing incidence of thyroid cancer in the United States, 1973–2002, JAMA 295 (2006) 2164, http://dx.doi.org/10.1001/jama.295.18.2164.

[3] L.C. Sakoda, P.L. Horn-Ross, Reproductive and menstrual history and papillary thyroid cancer risk: the San Francisco Bay Area thyroid cancer study, Cancer Epidemiol. Biomarkers Prev. 11 (2002) 51.

[4] K. Hemminki, X. Li, Familial risk of cancer by site and histopathology, Int. J. Cancer 103 (2003) 105, http://dx.doi.org/10.1002/ijc.10764.

[5] R.A. DeLellis, R.V. Lloyd, P.U. Heitz, C. Eng (Eds.), Pathology and Genetics of Tumours of Endocrine Organs, World Health Organization Classification of TumoursIARC Press, Lyon, 2004.

[6] S.L. Woodruff, O.A. Arowolo, O.O. Akute, A.O. Afolabi, F. Nwariaku, Global variation in the pattern of differentiated thyroid cancer, Am. J. Surg. 200 (2010) 462.

[7] E. Bonora, G. Tallini, G. Romeo, Genetic predisposition to familial nonmedullary thyroid cancer: an update of molecular findings and state-of-the-art studies, J. Oncol. (2010) 385206, http://dx.doi.org/10.1155/2010/385206.

[8] R.F. Grossman, S.H. Tu, Q.Y. Duh, A.E. Siperstein, F. Novosolov, O.H. Clark, Familial nonmedullary thyroid cancer. An emerging entity that warrants aggressive treatment, Arch. Surg. 130 (1995) 892, http://dx.doi.org/10.1001/archsurg.1995.01430080094015.

[9] D.W. Robinson, T.G. Orr, Carcinoma of the thyroid and other diseases of the thyroid in identical twins, Arch. Surg. 70 (1955) 923, http://dx.doi.org/10.1001/archsurg.1955.01270120131015.

[10] K.C. Loh, Familial nonmedullary thyroid carcinoma: a metareview of case series, Thyroid 7 (1997) 107, http://dx.doi.org/10.1089/thy.1997.7.107.

[11] H. He, R. Nagy, S. Liyanarachchi, H. Jiao, W. Li, S. Suster, J. Kere, A. de la Chapelle, A susceptibility locus for papillary thyroid carcinoma on chromosome 8q24, Cancer Res. 69 (2009) 625, http://dx.doi.org/10.1158/0008-5472.CAN-08-1071.

[12] M.R. Vriens, I. Suh, W. Moses, E. Kebebew, Clinical features and genetic predisposition to hereditary nonmedullary thyroid cancer, Thyroid 19 (2009) 1343, http://dx.doi.org/10.1089/thy.2009.1607.

[13] J.R. Burgess, A. Duffield, S.J. Wilkinson, R. Ware, T.M. Greenaway, J. Percival, L. Hoffman, Two families with an autosomal dominant inheritance pattern for papillary carcinoma of the thyroid, J. Clin. Endocrinol. Metab. 82 (1997) 345, http://dx.doi.org/10.1210/jcem.82.2.3789.

[14] F. Cetta, G. Montalto, M. Gori, M.C. Curia, A. Cama, S. Olschwang, Germline mutations of the APC gene in patients with familial adenomatous polyposis-associated thyroid carcinoma: results from a European cooperative study, J. Clin. Endocrinol. Metab. 85 (2000) 286, http://dx.doi.org/10.1210/jcem.85.1.6254.

[15] S. Uchino, S. Noguchi, H. Kawamoto, H. Yamashita, S. Watanabe, H. Yamashita, S. Shuto, Familial nonmedullary thyroid carcinoma characterized by multifocality and a high recurrence rate in a large study population, World J. Surg. 26 (2002) 897, http://dx.doi.org/10.1007/s00268-002-6615-y.

[16] O. Alsanea, N. Wada, K. Ain, M. Wong, K. Taylor, P.H. Ituarte, P.A. Treseler, H.U. Weier, N. Freimer, A.E. Siperstein, Q.Y. Duh, H. Takami, O.H. Clark, Is familial nonmedullary thyroid carcinoma more aggressive than sporadic thyroid cancer? A multicenter series, Surgery 128 (2000) 1043, http://dx.doi.org/10.1067/msy.2000.110848.

[17] M. Capezzone, S. Marchisotta, S. Cantara, G. Busonero, L. Brilli, K. Pazaitou-Panayiotou, A.F. Carli, G. Caruso, P. Toti, S. Capitani, A. Pammolli, F. Pacini, Familial non-medullary thyroid carcinoma displays the features of clinical anticipation suggestive of a distinct biological entity, Endocr. Relat. Cancer 15 (2008) 1075, http://dx.doi.org/10.1677/ERC-08-0080.

[18] F. Pitoia, G. Cross, M.E. Salvai, E. Abelleira, H. Niepomniszcze, Patients with familial non-medullary thyroid cancer have an outcome similar to that of patients with sporadic papillary thyroid tumors, Arq. Bras. Endocrinol. Metab. 55 (2011) 219, http://dx.doi.org/10.1590/S0004-27302011000300007.

[19] D. Navas-Carrillo, A. Ríos, J.M. Rodríguez, P. Parrilla, E. Orenes-Piñero, Screening escalation of familiar papillary thyroid cancer for early detection of thyroid disease, 2014. Unpublished results.

[20] R.S. Sippel, N.R. Caron, O.H. Clark, An evidence-based approach to familial nonmedullary thyroid cancer: screening, clinical management, and follow-up, World J. Surg. 31 (2007) 924, http://dx.doi.org/10.1007/s00268-006-0847-1.

[21] A. Ríos, J.M. Rodríguez, M. Canteras, P.J. Galindo, M.D. Balsalobre, P. Parrilla, Risk factors for malignancy in multinodular goitres, Eur. J. Surg. Oncol. 30 (2004) 58, http://dx.doi.org/10.1016/j.ejso.2003.10.021.

[22] S. Uchino, S. Noguchi, H. Yamashita, T. Murakami, S. Watanabe, T. Ogawa, A. Tsuno, S. Shuto, Detection of asymptomatic differentiated thyroid carcinoma by neck ultrasonographic screening for familial nonmedullary thyroid carcinoma, World J. Surg. 28 (2004) 1099, http://dx.doi.org/10.1007/s00268-004-7676-x.

[23] H. Miki, K. Oshimo, H. Inoue, M. Kawano, K. Tanaka, K. Komaki, T. Uyama, T. Morimoto, Y. Monden, Incidence of ultrasonographically detected thyroid nodules in healthy adults, Tokushima J. Exp. Med. 40 (1993) 43.

[24] J.M. Stulak, C.S. Grant, D.R. Farley, G.B. Thompson, J.A. van Heerden, I.D. Hay, C.C. Reading, J.W. Charboneau, Value of preoperative ultrasonography in the surgical management of initial and reoperative papillary thyroid cancer, Arch. Surg. 141 (2006) 489, http://dx.doi.org/10.1001/archsurg.141.5.489.

[25] J.L. Roh, J.Y. Park, J.M. Kim, C.J. Song, Use of preoperative ultrasonography as guidance for neck dissection in patients with papillary thyroid carcinoma, J. Surg. Oncol. 99 (2009) 28, http://dx.doi.org/10.1002/jso.21164.

[26] A.C. Calvete, J.M. Rodríguez, J. de Dios Berná-Mestre, A. Ríos, D. Abellán-Rivero, M. Reus, Interobserver agreement for thyroid elastography: value of the quality factor, J. Ultrasound Med. 32 (2013) 495.

[27] M. Capezzone, S. Marchisotta, S. Cantara, F. Pacini, Telomeres and thyroid cancer, Curr. Genomics 10 (2009) 526, http://dx.doi.org/10.2174/138920209789503897.

[28] T.J. McDonald, A.A. Driedger, B.M. Garcia, S.H. Van Uum, I. Rachinsky, V. Chevendra, D. Breadner, R. Feinn, S.J. Walsh, C.D. Malchoff, Familial papillary thyroid carcinoma: a retrospective analysis, J. Oncol. (2011) 948786, http://dx.doi.org/10.1155/2011/948786.

[29] C. Leux, T. Truong, C. Petit, D. Baron-Dubourdieu, P. Guénel, Family history of malignant and benign thyroid diseases and risk of thyroid cancer: a population-based case–control study in New Caledonia, Cancer Causes Control 23 (2012) 745, http://dx.doi.org/10.1007/s10552-012-9944-7.

[30] D. Navas-Carrillo, A. Ríos, J.M. Rodríguez, P. Parrilla, E. Orenes-Piñero, Prognostic differences between sporadic and familiar papillary thyroid cancer, 2014. Unpublished results.

[31] G. Lupoli, G. Vitale, M. Caraglia, M.R. Fittipaldi, A. Abbruzzese, P. Tagliaferri, A.R. Bianco, Familial papillary thyroid microcarcinoma: a new clinical entity, Lancet 20 (1999) 637, http://dx.doi.org/10.1016/S0140-6736(98)08004-0.

[32] N.G. Ranen, O.C. Stine, M.H. Abbott, M. Sherr, A.M. Codori, M.L. Franz, N.I. Chao, A.S. Chung, N. Pleasant, C. Callahan, Anticipation and instability of IT-15 (CAG)n repeats in parent–offspring pairs with Huntington disease, Am. J. Hum. Genet. 57 (1995) 593.

[33] T. Vulliamy, A. Marronem, R. Szydlo, A. Walne, P.J. Mason, I. Dokal, Disease anticipation is associated with progressive telomere shortening in families with dyskeratosis congenita due to mutations in TERC, Nat. Genet. 36 (2004) 447, http://dx.doi.org/10.1038/ng1346.

[34] E. Robenshtok, G. Tzvetov, S. Grozinsky-Glasberg, I. Shraga-Slutzky, R. Weinstein, L. Lazar, S. Serov, J. Singer, D. Hirsch, I. Shimon, C. Benbassat, Clinical characteristics and outcome of familial nonmedullary thyroid cancer: a retrospective controlled study, Thyroid 21 (2011) 43, http://dx.doi.org/10.1089/thy.2009.0406.

[35] M.A. Kouvaraki, S.E. Shapiro, N.D. Perrier, G.J. Cote, R.F. Gagel, A.O. Hoff, S.I. Sherman, J.E. Lee, D.B. Evans, RET proto-oncogene: a review and update of genotype–phenotype correlations in hereditary medullary thyroid cancer and associated endocrine tumors, Thyroid 15 (2005) 531, http://dx.doi.org/10.1089/thy.2005.15.531.

[36] G.R. Bignell, F. Canzian, M. Shayeghi, M. Stark, Y.Y. Shugart, P. Biggs, J. Mangion, R. Hamoudi, J. Rosenblatt, P. Buu, S. Sun, S.S. Stoffer, D.E. Goldgar, G. Romeo, R.S. Houlston, S.A. Narod, M.R. Stratton, W.D. Foulkes, Familial nontoxic multinodular thyroid goiter locus maps to chromosome 14q but does not account for familial nonmedullary thyroid cancer, Am. J. Hum. Genet. 61 (1997) 1123, http://dx.doi.org/10.1086/301610.

[37] S. Neumann, H. Willgerodt, F. Ackermann, A. Reske, M. Jung, A. Reis, R. Paschke, Linkage of familial euthyroid goiter to the multinodular goiter-1 locus and exclusion of the candidate genes thyroglobulin, thyroperoxidase, and Na^+/I^- symporter, J. Clin. Endocrinol. Metab. 84 (1999) 3750, http://dx.doi.org/10.1210/jcem.84.10.6023.

[38] F. Canzian, P. Amati, H.R. Harach, J.L. Kraimps, F. Lesueur, J. Barbier, P. Levillain, G. Romeo, D. Bonneau, A gene predisposing to familial thyroid tumours with cell oxyphilia maps to chromosome 19p13.2, Am. J. Hum. Genet. 63 (1998) 1743, http://dx.doi.org/10.1086/302164.

[39] S. Bevan, T. Pal, C.R. Greenberg, H. Green, J. Wixey, G. Bignell, S.A. Narod, W.D. Foulkes, M.R. Stratton, R.S. Houlston, A comprehensive analysis of NG1, TCO1, fPTc, PTEN, TSHR, and TRKA in familial nonmedullary thyroid cancer: confirmation of linkage to TCO1, J. Clin. Endocrinol. Metab. 86 (2001) 3701, http://dx.doi.org/10.1210/jcem.86.8.7725.

[40] C.D. Malchoff, M. Sarfarazi, B. Tendler, F. Forouhar, G. Whalen, V. Joshi, A. Arnold, D.M. Malchoff, Papillary thyroid carcinoma associated with papillary renal neoplasia: genetic linkage analysis of a distinct heritable tumour syndrome, J. Clin. Endocrinol. Metab. 85 (2000) 1758, http://dx.doi.org/10.1210/jcem.85.5.6557.

[41] J.D. McKay, F. Lesueur, L. Jonard, A. Pastore, J. Williamson, L. Hoffman, J. Burgess, A. Duffield, M. Papotti, M. Stark, H. Sobol, B. Maes, A. Murat, H. Kääriäinen, M. Bertholon-Grégoire, M. Zini, M.A. Rossing, M.E. Toubert, F. Bonichon, M. Cavarec, A.M. Bernard, A. Boneu, F. Leprat, O. Haas, C. Lasset, M. Schlumberger, F. Canzian, D.E. Goldgar, G. Romeo, Localization of a susceptibility gene for familial nonmedullary thyroid carcinoma to chromosome 2q21, Am. J. Hum. Genet. 69 (2001) 440, http://dx.doi.org/10.1086/321979.

[42] J.D. McKay, D. Thompson, F. Lesueur, K. Stankov, A. Pastore, C. Watfah, S. Strolz, G. Riccabona, R. Moncayo, G. Romeo, D.E. Goldgar, Evidence for interaction between the TCO and NMTC1 loci in familial non-medullary thyroid cancer, J. Med. Genet. 41 (2004) 407, http://dx.doi.org/10.1136/jmg.2003.017350.

[43] B.M. Cavaco, P.F. Batista, C. Martins, A. Banito, F. do Rosário, E. Limbert, L.G. Sobrinho, V. Leite, Familial non-medullary thyroid carcinoma (FNMTC): analysis of fPTC/PRN, NMTC1, MNG1 and TCO susceptibility loci and identification of somatic BRAF and RAS mutations, Endocr. Relat. Cancer 15 (2008) 207.

[44] B.M. Cavaco, P.F. Batista, L.G. Sobrinho, V. Leite, Mapping a new familial thyroid epithelial neoplasia susceptibility locus to chromosome 8p23.1-p22 by high density single nucleotide polymorphism genome-wide linkage analysis, J. Clin. Endocrinol. Metab. 93 (2008) 4426, http://dx.doi.org/10.1210/jc.2008-0449.

[45] J. Gudmundsson, P. Sulem, D.F. Gudbjartsson, J.G. Jonasson, A. Sigurdsson, J.T. Bergthorsson, H. He, T. Blondal, F. Geller, M. Jakobsdottir, D.N. Magnusdottir, S. Matthiasdottir, S.N. Stacey, O.B. Skarphedinsson, H. Helgadottir, W. Li, R. Nagy, E. Aguillo, E. Faure, E. Prats, B. Saez, M. Martinez, G.I. Eyjolfsson, U.S. Bjornsdottir, H. Holm, K. Kristjansson, M.L. Frigge, H. Kristvinsson, J.R. Gulcher, T. Jonsson, T. Rafnar, H. Hjartarsson, J.I. Mayordomo, A. de la Chapelle, J. Hrafnkelsson, U. Thorsteinsdottir, A. Kong, K. Stefansson, Common variants on 9q22.33 and 14q13.3 predispose to thyroid cancer in European populations, Nat. Genet. 41 (2009) 460, http://dx.doi.org/10.1038/ng.339.

[46] I. Suh, S. Filetti, M.R. Vriens, M.A. Guerrero, S. Tumino, M. Wong, W.T. Shen, E. Kebebew, Q.Y. Duh, O.H. Clark, Distinct loci on chromosome 1q21 and 6q22 predispose to familial nonmedullary thyroid cancer: a SNP array-based linkage analysis of 38 families, Surgery 146 (2009) 1073, http://dx.doi.org/10.1016/j.surg.2009.09.012.

[47] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281, http://dx.doi.org/10.1016/S0092-8674(04)00045-5.

[48] B. Wightman, I. Ha, G. Ruvkun, Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*, Cell 75 (1993) 855, http://dx.doi.org/10.1016/0092-8674(93)90530-4.

[49] A. Kozomara, S. Griffiths-Jones, miRBase: integrating microRNA annotation and deep-sequencing data, Nucleic Acids Res. 39 (2011) 152, http://dx.doi.org/10.1093/nar/gkq1027.
[50] B.P. Lewis, I.H. Shih, M.W. Jones-Rhoades, D.P. Bartel, C.B. Burge, Prediction of mammalian microRNA targets, Cell 115 (2003) 787, http://dx.doi.org/10.1016/S0092-8674(03)01018-3.
[51] Y. Xiong, L. Zhang, A.K. Holloway, X. Wu, L. Su, E. Kebebew, MiR-886-3p regulates cell proliferation and migration, and is dysregulated in familial non-medullary thyroid cancer, PLoS One 6 (2011) 24717, http://dx.doi.org/10.1371/journal.pone.0024717.
[52] Z. Hu, X. Chen, Y. Zhao, T. Tian, G. Jin, Y. Shu, Y. Chen, L. Xu, K. Zen, C. Zhang, H. Shen, Serum microRNA signatures identified in a genome-wide serum microRNA expression profiling predict survival of non-small-cell lung cancer, J. Clin. Oncol. 28 (2010) 1721, http://dx.doi.org/10.1200/JCO.2009.24.9342.
[53] M. Pesta, J. Klecka, V. Kulda, O. Topolcan, M. Hora, V. Eret, M. Ludvikova, M. Babjuk, K. Novak, J. Stolz, L. Holubec, Importance of miR-20a expression in prostate cancer tissue, Anticancer Res. 30 (2010) 3579.
[54] A.B. Hui, M. Lenarduzzi, T. Krushel, L. Waldron, M. Pintilie, W. Shi, B. Perez-Ordonez, I. Jurisica, B. O'Sullivan, J. Waldron, P. Gullane, B. Cummings, F.F. Liu, Comprehensive microRNA profiling for head and neck squamous cell carcinomas, Clin. Cancer Res. 16 (2010) 1129, http://dx.doi.org/10.1158/1078-0432.CCR-09-2166.
[55] E. Orenes-Piñero, S. Montoro-García, J.V. Patel, M. Valdés, F. Marín, G.Y. Lip, Role of microRNAs in cardiac remodelling: new insights and future perspectives, Int. J. Cardiol. 167 (2013) 1651, http://dx.doi.org/10.1016/j.ijcard.2012.09.120.
[56] Z. Ju, L. Rudolph, Telomere dysfunction and stem cell ageing, Biochimie 90 (2008) 24, http://dx.doi.org/10.1016/j.biochi.2007.09.006.
[57] E. Gilson, A. Londoño-Vallejo, Telomere length profile in humans, Cell Cycle 6 (2007) 2486, http://dx.doi.org/10.4161/cc.6.20.4798.
[58] X. Wu, C.I. Amos, Y. Zhu, H. Zhao, B.H. Grossman, J.W. Shay, S. Luo, W.K. Hong, M.R. Spitz, Telomere dysfunction: a potential cancer predisposition factor, J. Natl. Cancer Inst. 95 (2003) 1211, http://dx.doi.org/10.1093/jnci/djg011.
[59] S.E. Artandi, S. Alson, M.K. Tietze, N.E. Sharpless, S. Ye, R.A. Greenberg, D.H. Castrillon, J.W. Horner, S.R. Weiler, R.D. Carrasco, R.A. DePinho, Constitutive telomerase expression promotes mammary carcinomas in aging mice, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 8191, http://dx.doi.org/10.1073/pnas.112515399.
[60] C.B. Umbricht, M. Saji, W.H. Westra, R. Udelsman, M.A. Zeiger, S. Sukumar, Telomerase activity: a marker to distinguish follicular thyroid adenoma from carcinoma, Cancer Res. 57 (1997) 2144.
[61] L. Bornstein-Quevedo, M.L. García-Hernández, I. Camacho-Arroyo, M.F. Herrera, A.A. Angeles, O.G. Treviño, A. Gamboa-Domínguez, Telomerase activity in well-differentiated papillary thyroid carcinoma correlates with advanced clinical stage of the disease, Endocr. Pathol. 14 (2003) 213, http://dx.doi.org/10.1007/s12022-003-0013-3.
[62] A.M. Straight, A. Patel, C. Fenton, C. Dinauer, R.M. Tuttle, G.L. Francis, Thyroid carcinomas that express telomerase follow a more aggressive clinical course in children and adolescents, J. Endocrinol. Investig. 25 (2002) 302, http://dx.doi.org/10.1007/BF03344009.
[63] S. Cantara, M. Pisu, D.V. Frau, P. Caria, T. Dettori, M. Capezzone, S. Capuano, R. Vanni, F. Pacini, Telomere abnormalities and chromosome fragility in patients affected by familial papillary thyroid cancer, J. Clin. Endocrinol. Metab. 97 (2012) 1327, http://dx.doi.org/10.1210/jc.2011-2096.
[64] M. Capezzone, S. Cantara, S. Marchisotta, S. Filetti, M.M. De Santi, B. Rossi, G. Ronga, C. Durante, F. Pacini, Short telomeres, telomerase reverse transcriptase gene amplification, and increased telomerase activity in the blood of familial papillary thyroid cancer patients, J. Clin. Endocrinol. Metab. 93 (2008) 3950, http://dx.doi.org/10.1210/jc.2008-0372.
[65] A. Zhang, C. Zheng, C. Lindvall, M. Hou, J. Ekedahl, R. Lewensohn, Z. Yan, X. Yang, M. Henriksson, E. Blennow, M. Nordenskjöld, A. Zetterberg, M. Björkholm, A. Gruber, D. Xu, Frequent amplification of the telomerase reverse transcriptase gene in human tumors, Cancer Res. 15 (2000) 6230.