Familial non-medullary thyroid cancer: unraveling the genetic maze

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Abstract

Familial non-medullary thyroid cancer (FNMTc) constitutes 3–9% of all thyroid cancers. Out of all FNMTc cases, only 5% in the syndromic form has well-studied driver germline mutations. These associated syndromes include Cowden syndrome, familial adenomatous polyposis, Gardner syndrome, Carney complex type 1, Werner syndrome and DICER1 syndrome. It is important for the clinician to recognize these phenotypes so that genetic counseling and testing can be initiated to enable surveillance for associated malignancies and genetic testing of family members. The susceptibility chromosomal loci and genes of 95% of FNMTc cases remain to be characterized. To date, 4 susceptibility genes have been identified (SRGAP1 gene (12q14), TITF-1/NKX2.1 gene (14q13), FOXE1 gene (9q22.33) and HABP2 gene (10q25.3)), out of which only the FOXE1 and the HABP2 genes have been validated by separate study groups. The causal genes located at the other 7 FNMTc-associated chromosomal loci (TCO (19q13.2), fPTC/PRN (1q21), FTEN (8p23.1-p22), NMTC1 (2q21), MNG1 (14q32), 6q22, 8q24) have yet to be identified. Increasingly, gene regulatory mechanisms (miRNA and enhancer elements) are recognized to affect gene expression and FNMTc tumorigenesis. With newer sequencing technique, along with functional studies, there has been progress in the understanding of the genetic basis of FNMTc. In our review, we summarize the FNMTc studies to date and provide an update on the recently reported susceptibility genes including novel germline SEC23B variant in Cowden syndrome, SRGAP1 gene, FOXE1 gene and HABP2 genes in non-syndromic FNMTc.

Introduction

Thyroid cancer is common, and its prevalence is steadily rising (Davies & Welch 2014). Papillary thyroid cancer (PTC) is the commonest histologic sub-type, accounting for at least 90% of all thyroid cancers (Howlader et al. 2016). More than 90% of thyroid cancer is sporadic, due to somatic genetic alterations (Xing 2013). Only 3-9% of all thyroid cancers are familial non-medullary thyroid cancer (FNMTc) cases, defined by the presence of thyroid cancer in 2 or more first-degree relatives, in the absence of predisposing environmental factors. Among FNMTc, PTC is the commonest (85–91%) histological sub-type, followed by follicular thyroid cancer (6–9.7%), anaplastic thyroid cancer (1.6%) and Hurthle cell cancer (Loh 1997, Vriens et al. 2009, Moses et al. 2010). Out of all FNMTc's, only 5% in the syndromic form has well-defined driver germline mutations. This includes Cowden syndrome and other inheritable syndromes.
Patients with FNMTc have been reported to present at an earlier age, have more multi-focal and aggressive disease with local invasion, lymph node metastases, increased risk of recurrence and decreased survival rates. (Grossman et al. 1995, Loh 1997, Alsanea et al. 2000, Uchino et al. 2002, Lee et al. 2014). However, others have reported no increase in disease aggressiveness compared with sporadic thyroid cancer (Loh 1997, Ito et al. 2009, Robenshtok et al. 2010, Pinto et al. 2014). ‘Anticipation’ has been reported in FNMTc with the second generation having earlier and more severe manifestations of the disease (Capezzone et al. 2008b). Some authors have advised for more intensive treatment including total thyroidectomy with prophylactic central neck dissection (Uchino et al. 2002). The role of prophylactic thyroidectomy is highly debatable. Understanding the genetic basis of this heterogeneous disease entity and the identification of molecular predictors of disease aggressiveness could help better risk stratify, allow predictive screening of family members to identify family members at risk for FNMTc and guide surveillance and management plan.

**Syndromic familial non-medullary thyroid cancer**

As the germline gene mutations accounting for syndromic FNMTc are highly penetrant and actionable, targeted gene testing is recommended when the clinician recognizes the clinical phenotype of the syndrome. The genetic
Table 1 Syndromic familial non-medullary thyroid cancer (data from Vriens et al. 2009).

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Germline gene mutation</th>
<th>Type of thyroid cancer</th>
<th>Extra-thyroidal clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowden syndrome</td>
<td>-PTEN mutation</td>
<td>PTC (classical and follicular variant)</td>
<td>Hamartomas and epithelial tumors of the breast, kidney, colon, endometrium and brain; mucocutaneous lesions; macrocephaly</td>
</tr>
<tr>
<td></td>
<td>-SDHB-D mutation</td>
<td>FTC</td>
<td></td>
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<tr>
<td></td>
<td>-KLLN promoter methylation</td>
<td>FTC</td>
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<tr>
<td></td>
<td>-PIK3CA mutation</td>
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<td></td>
<td>-AKT1 mutation</td>
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<tr>
<td></td>
<td>-SEC23B mutation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial adenomatous polyposis</td>
<td>APC mutation</td>
<td>PTC, including cribriform pattern sub-type</td>
<td>Multiple adenomatous polyps with malignant potential lining mucosa of GI tract, particularly colon</td>
</tr>
<tr>
<td>Gardner’s syndrome</td>
<td>APC mutation</td>
<td>PTC, including cribriform pattern sub-type</td>
<td>FAP variant with extra-colonic manifestations: supernumerary teeth, fibrous dysplasia of the skull, osteomas of the mandible, fibromas, desmoid tumors, epithelial cysts; hypertrophic retinal pigment epithelium; upper GI tract hamartomas; hepatoblastomas</td>
</tr>
<tr>
<td>Carney complex</td>
<td>PRKAR1A mutation</td>
<td>PTC, FTC, follicular adenoma</td>
<td>Myxomas of soft tissues; skin and mucosal pigmentation (blue nevi); schwannomas; tumors of the adrenal and pituitary glands and testicle</td>
</tr>
<tr>
<td>Werner’s syndrome</td>
<td>WRN mutation</td>
<td>FTC, PTC, ATC</td>
<td>Premature aging; scleroderma-like skin changes; cataracts; premature greying and/or thinning of scalp hair; short stature</td>
</tr>
<tr>
<td>DICER1 syndrome</td>
<td>DICER1 mutation</td>
<td>PTC (and MNG)</td>
<td>Familial pleuropulmonary blastoma; cystic nephroma; Ovarian Sertoli-Leydig cell tumors</td>
</tr>
</tbody>
</table>

ATC, anaplastic thyroid cancer; FAP, familial adenomatous polyposis; FTC, follicular thyroid cancer; GI, gastrointestinal; MNG, multinodular goiter; PTC, papillary thyroid cancer.

and clinical features of syndromic FNMTC have been summarized in Table 1.

Cowden syndrome

This is an autosomal dominant disorder characterized by hamartomatous changes and epithelial tumors of breast, thyroid, kidney, colon and endometrium. The International Cowden Consortium established the operational criteria of Cowden syndrome (CS) in 1995 (revised in 2000) to identify individuals and families for genetic testing. The characteristics are categorized to those fulfilling pathognomonic, major and minor criteria (Table 2) (Nelen et al. 1996, Pilarski & Eng 2004, Pilarski et al. 2013). This was initially found to be predominantly associated with a mutation in the PTEN tumor suppressor gene on chromosome 10q22-23. In earlier studies that selected for familial cases with exaggerated phenotypes, 81% of CS patients were found to have germline PTEN mutations (Marsh et al. 1998). A subsequent study of 3042 probands diagnosed with relaxed International Cowden Consortium operational criteria for Cowden syndrome (full Cowden syndrome criteria minus one criterion; Cowden-like syndrome (CSL)) showed that only 25% of these patients harbored germline PTEN mutations (Tan et al. 2011a). Up to 25% of CS and CSL patients have thyroid cancer, and 60% of CS and CSL patients have thyroid nodules (Ngeow et al. 2011, Tan et al. 2011a).

In a study of 664 patients with Cowden syndrome or Cowden-like syndrome, majority of the thyroid
cancers were of classical papillary sub-type (55.1%), followed by follicular variant papillary sub-type (19.5%). Follicular thyroid cancer (FTC) only constitutes 10.0% of the thyroid cancer cases. Interestingly, FTC was over-represented in patients who had positive PTEN germline mutation, which was also associated with a higher risk of pediatric-onset thyroid cancer, as early as at the age of 7 years. As such, it has been suggested that children, aged 6 and above, with germline PTEN mutations should undergo a baseline surveillance thyroid ultrasound at the time of diagnosis. In this cohort, 5.4% of the CS and CSL patients had PTEN germline mutation and 13.4% had PTEN variant of unknown significance. The PTEN mutation-negative patients tested positive for SDHB-D variants (3.9%) and KILLIN (KLLN) promoter methylation (2.3%) (Ngeow et al. 2011). SDHB-D genes encode for succinate dehydrogenase (SDH), and their germline variants result in the upregulation of the AKT and MAPK pathways, similar to PTEN mutations, and also result in hypoxia inducible factor HIF1a stabilization/ hyperactivation and FAD/NAD-dependent loss of p53 function that could lead to tumor formation (Ni et al. 2008, 2012). KILLIN (KLLN) is a p53 target gene, upstream of PTEN sharing a common promoter with it (Cho & Liang 2008). The methylation of KILLIN, a tumor suppressor, downregulates its transcription and disrupts p53 activation of KILLIN (Bennett et al. 2010). Based on the later age of onset of CS and CSL manifestations in patients with SDHB-D mutations and KLLN promoter methylation, the current recommendations of thyroid ultrasound surveillance from 18 years of age (or 5 years before the earliest age of onset in a family member) should be adequate (Ngeow et al. 2011). The corresponding age and gender-standardized incidence rates of thyroid cancer for each of the described mutations were as follows: 72 for pathogenic PTEN mutation (95% confidence interval (CI), 51–99; P<0.001), 63 (95% CI: 42–92; P<0.001) for SDHB-D variants and 45 (95% CI: 26–73; P<0.001) for KLLN promoter methylation (Ngeow et al. 2011).

In another study of 91 CS and CSL probands without PTEN, SDHB-D and KLLN mutations, targeted sequencing revealed germline PIK3CA mutations in 8.8% and germline AKT1 mutations in 2.2% (Orloff et al. 2013). PIK3CA encodes p110a, the catalytic subunit of PI3K, which adds a phosphate to phosphatidylinositol-4,5-bisphosphate (PIP2) to form phosphatidylinositol-3,4,5-trisphosphate (PIP3) at the cellular membrane. PIP3 recruits AKT1 to the cell membrane (Vanhaesebroeck et al. 2001). Activated AKT phosphorylates downstream protein effectors, including the mammalian target of rapamycin (mTOR),

### Table 2  International Cowden Consortium operational criteria for the diagnosis of Cowden syndrome (revised in 2000).

<table>
<thead>
<tr>
<th>Pathognomonic criteria</th>
<th>Major criteria</th>
<th>Minor criteria</th>
</tr>
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<tbody>
<tr>
<td>Mucocutaneous lesions:</td>
<td>Breast cancer</td>
<td>Other thyroid lesions (for example, goiter)</td>
</tr>
<tr>
<td>Trichilemmomas, facial</td>
<td>Thyroid cancer, especially follicular thyroid cancer</td>
<td>Mental retardation (IQ ≤ 75)</td>
</tr>
<tr>
<td>Acral keratoses</td>
<td>Macrocephaly (occipital frontal circumference ≥ 97th percentile)</td>
<td>Hamartomatous intestinal polyps</td>
</tr>
<tr>
<td>Papillomatous lesions</td>
<td>Lhermitte–Duclos disease defined as presence of a cerebellar dysplastic gangliocytoma</td>
<td>Fibrocytic disease of the breast</td>
</tr>
<tr>
<td>Mucosal lesions</td>
<td>Endometrial carcinoma</td>
<td>Lipomas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fibromas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genito-urinary tumors (for example, uterine fibroids, renal cell carcinoma) or genito-urinary malformation</td>
</tr>
</tbody>
</table>

An operational diagnosis of Cowden syndrome is made if an individual meets any one of the following criteria:

1. Pathognomonic mucocutaneous lesions alone if there are:
   - Six or more facial papules, of which three or more must be trichilemmoma or cutaneous facial papules and oral mucosal papillomatosis or oral mucosal papillomatosis and acral keratoses, or six or more palmo-plantar keratoses

2. Two major criteria but one must be either macrocephaly or Lhermitte–Duclos disease

3. One major and three minor criteria

4. Four minor criteria

In a family in which one individual meets the diagnostic criteria for Cowden syndrome, other relatives are considered to have a diagnosis of Cowden syndrome if they meet any of the following criteria:

1. A pathognomonic mucocutaneous lesion

2. Any one major criterion with or without minor criteria

3. Two minor criteria

### Notes

- **SDHB-D** and **KLLN** are known to be tumor suppressor genes, and their mutations can lead to the development of familial non-medullary thyroid cancer.
- **Pathogenic** mutations in **SDHB-D** and **KLLN** can lead to the development of Cowden syndrome.
- **PTEN** is a tumor suppressor gene located on chromosome 10q23.3, and its mutations are associated with Cowden syndrome.
- **PIK3CA** is a gene encoding the catalytic subunit of PI3K, and its mutations are associated with Cowden syndrome.
- **AKT1** is a gene encoding the catalytic subunit of AKT, and its mutations are associated with Cowden syndrome.
- **mTOR** is a gene encoding mammalian target of rapamycin, and its mutations are associated with Cowden syndrome.
which has an established role in human cancers. PTEN is a prominent negative regulator of the PI3K/Akt signaling pathway (Furnari et al. 1998).

Recently, whole exome sequencing of a CS proband, from a CS family enriched for thyroid cancer across 4 generations, who had tested negative for PTEN, SDHB-D and KLLN hypermethylation, was performed. Several genes were identified on whole exome sequencing; these were confirmed by Sanger sequencing in 7 other family members to identify the CS candidate genes segregating in the proband's family. All family members with CS shared 3 genes with heterozygous missense variants, C16orf72 (c.253T>C, p.Ser85Pro), PTPN2 (c.1204G>A, p.Ala402Thr) and SEC23B (c.1781T>G, p.Val594Gly). No variant was noted in PIK3CA and AKTI. All 3 genes were sequenced in 96 unrelated CS probands with thyroid cancer, and germline heterozygous SEC23B variant was detected in 3 probands (3.1%). SEC23B encodes Sec23 Homolog B (S. cerevisiae), a component of coat protein complex II (COPII), and it transports proteins from endoplasmic reticulum (ER) to Golgi apparatus. When the p.Val594Gly variant was tested in a normal thyroid cell line, it was shown to be a functional mutation that resulted in ER stress-mediated cell colony formation, growth and invasion. These suggest that germline heterozygous SEC23B variants predispose to cancer potentially through ER stress (Yehia et al. 2015).

In summary, in the presence of a germline PTEN mutation, surveillance for thyroid cancer should be performed given that up to a quarter of cases have an increased risk for thyroid cancer (Ngeow et al. 2011, Tan et al. 2011a). The Cleveland Clinic score, based on clinical manifestations, could be used as a CS risk predictor. A score of 10 or more is associated with a pretest probability of 3%, and a referral to the geneticist for genetic counseling and germline genetic testing for PTEN mutation is recommended (Tan et al. 2011b). In PTEN wild-type CS cases, the utility of testing for other genetic mutation remains to be validated in future studies.

Familial adenomatous polyposis (FAP) and Gardner syndrome

This is an autosomal dominant disease caused by inactivating mutations of adenomatous polyposis coli (APC) tumor suppressor gene on chromosome 5q21. It is characterized by young-onset multiple gastrointestinal adenomatous polyps, especially of the colon, with malignant potential. Thyroid cancers have been associated with FAP, and these are usually papillary thyroid cancers. The unique cribriform histological pattern is also observed in 20–40% of FAP-associated thyroid cancers, occurring more commonly below age 30 in females (Cetta et al. 2000a, 2011, Lee et al. 2004a, Levy et al. 2014). It is important for the clinicians and endocrinologists to be aware that PTC may be the first clinical manifestation in 25-30% of FAP cases (Cetta et al. 2000b, Chikkamuniyappa & Jagirdar 2004, Donnellan et al. 2009).

Gardner syndrome is a subset of FAP in which patients also develop extra-colonic manifestations. The initial triad of FAP, osteoma and soft tissue lesions is now recognized to include other abnormalities also (Bell & Mazzaferr 1993) (Table 1). The overall absolute risk of developing thyroid cancer in FAP patients is estimated to be 0.4–2.0% based on retrospective review of registries (Bulow et al. 1988, Giardiello et al. 1993, Houlston & Stratton 1995, Perrier et al. 1998, van der Linde et al. 1998). These findings were reported in symptomatic patients. In studies that used screening ultrasound thyroid in FAP patients, the incidence of cribriform-morula variant PTC was 0.6–6.2%, and incidence of thyroid cancer (including all sub-types of PTC) was 2.6–8.5% (Jarrar et al. 2011, Uchino et al. 2016).

The APC is part of the regulatory beta-catenin destruction complex in the canonical Wnt/beta-catenin pathway. In the non-stimulated form, this complex stimulates the degradation of beta-catenin; in the stimulated form with the activation of Wnt pathway, this destruction complex disassociates, allowing beta-catenin to enter the nucleus to promote gene transcription leading to cell proliferation. The inactivating APC mutation leads to loss of beta-catenin destruction complex, impeding beta-catenin degradation, increasing beta-catenin translocation into the nucleus (Veeman et al. 2003, Giannelli et al. 2014, Uchino et al. 2016).

In summary, the detection of cribriform-morular variant of PTC should prompt the clinician to screen for FAP as a third of FAP cases first manifest as PTC (Cetta et al. 2000b, Chikkamuniyappa & Jagirdar 2004, Donnellan et al. 2009). Established FAP cases should be screened for thyroid cancer even though the risk is overall low (0.4–2.0%) (Bulow et al. 1988, Giardiello et al. 1993, Houlston & Stratton 1995, Perrier et al. 1998, van der Linde et al. 1998). The utility of thyroid ultrasound screening in FAP remains to be validated in further studies.

Carney complex

This is an autosomal dominant disease characterized by the following features listed as major criteria for diagnosis:
spotty skin pigmentation with a typical distribution (lips, conjunctiva and canthi, and vaginal and penile mucosa), myxoma (cutaneous and mucosal), cardiac myxoma, breast myxomatosis or fat-suppressed magnetic resonance imaging findings suggestive of this diagnosis, primary pigmented nodular adrenocortical disease or paradoxical positive response of urinary glucocorticosteroids to dexamethasone administration during Liddle's test, acromegaly due to GH-producing adenoma, large-cell calcifying Sertoli cell tumor or characteristic calcification on testicular ultrasonography, thyroid carcinoma or multiple hypoechoic nodules on thyroid ultrasonography in a young patient, psammomatous melanotic schwannoma, blue nevus, breast ductal adenoma (multiple), and osteochondro-myxoma. A patient is considered to have Carney complex if two major criteria are present or if one major criterion is present and a first-degree relative has Carney complex or an inactivating PRKAR1A mutation. The prevalence of thyroid nodule and cancer in a series of 338 patients is 5%. Out of these 11 cases, 6 were follicular adenomas (hyperfunctioning in 2), 3 were papillary thyroid carcinomas (with a case with follicular variant sub-type) and 2 were follicular thyroid carcinomas (Stratakis et al. 1996, 1997, 2001).

An earlier linkage analysis study of families with Carney complex showed a genetic locus on 2p16 with an aggregate logarithm of odds (LOD) score of 5.97, although no single family had a LOD score greater than 1.8 for the locus (Stratakis et al. 1996). A LOD score of +3 or more is accepted as confirmation of linkage and a score of −2 or less is indicative of non-linkage. In families whose linkage analysis did not segregate with 2p16 markers, linkage analysis segregated to the 17q22-24 locus (Casey et al. 1998). Loss of heterozygosity was detected in the region of 17q22-24 locus around the PRKAR1A gene in Carney complex kindreds (Kirschner et al. 2000a). Subsequently, on performing targeted sequencing of 53 kindreds with Carney complex, the PRKAR1A gene mutation was identified in 40.7% of cases. This gene encodes the type 1A regulatory subunit of protein kinase A (PKA). PRKAR1A is postulated to be a tumor suppressor, and its loss-of-function mutation leads to enhanced signaling by PKA (Kirschner et al. 2000b). In a recent study, on screening 353 patients from the international Carney complex consortium, the PRKAR1A gene mutation was found in 73% of patients. Penetration of 97.5% was observed in PRKAR1A gene mutation carriers. Most of the PRKAR1A gene mutations (82%) resulted in nonsense mRNA that failed to translate into protein (nonsense-mediated mRNA decay) (Bertherat et al. 2009, Almeida & Stratakis 2010).

Forlino and coworkers described a case report of Carney complex with acromegaly from GH-secreting pituitary adenoma, skin pigmentation and myxomas in a 19-year-old female who tested negative for PRKAR1A gene mutation. Genome-wide sequencing showed a 1.6-Mb triplication of chromosome 1p31.1, including PRKACB that codes for catalytic subunit beta (Cβ) of PKA. Her lymphocytes, fibroblasts and myxoma displayed increased levels of Cβ, but not Cα. Just like in Carney complex from PRKAR1A gene mutation, in this patient's lymphocytes, cAMP increased kinase activity. This revealed the potential putative role of PRKACB gene defects in the Carney complex phenotype (Forlino et al. 2014). This remains to be validated in other patients with Carney complex.

Patients with clinical phenotype of Carney complex should be screened for thyroid cancer even though the prevalence of thyroid nodule and cancer is not high (5%) (Stratakis et al. 1996, 1997, 2001). Suspected cases can be tested for germline PRKAR1A mutation.

**Werner syndrome**

This autosomal recessive disease is associated with mutations of the WRN gene on chromosome 8p11-21 (Yu et al. 1996). It is characterized by cardinal features of premature aging, scleroderma-like skin changes, cataracts, short stature, premature graying and/or thinning of scalp hair and parental consanguinity or affected sibling, which onset over 10 years of age. Other manifestations include diabetes mellitus, hypogonadism, soft tissue calcification, premature atherosclerosis and a high incidence of neoplasms. The presence of all cardinal features and 2 further signs would indicate 'definite' diagnosis based on the International Registry of Werner syndrome diagnostic criteria (http://www.wernersyndrome.org/registry/diagnostic.html). Thyroid cancer was observed in 16% of 189 patients in a case series, and it occurred at a younger age. Follicular thyroid cancer was more common, followed by papillary and anaplastic thyroid cancers (Ishikawa et al. 1999, Lauper et al. 2013).

Patients with the clinical phenotype of Werner syndrome should be screened for thyroid cancer, and genetic testing for WRN gene mutation can be performed.

**DICER1 syndrome**

MicroRNAs (miRNAs) are single-stranded, short (22-nucleotides long) and non-coding RNAs that regulate gene expression at the post-transcriptional level by inhibiting mRNA (Bartel 2004). Based on the miRBase
miRNAs with complete complementary match to the mRNA sequence induce mRNA cleavage, whereas those with incomplete mRNA match lead to inhibition of translation and protein synthesis. The latter incomplete match allows for a single miRNA to regulate multiple genes. In the setting of oncology, miRNAs could have either tumor-promoting (oncomir) or tumor-suppressive effects.

The \textit{Dicer} gene is located on 14q32.13, and its mutation increases the risk of familial pleuroperulmonary blastoma (FPB), cystic nephroma and ovarian Sertoli-Leydig cell tumors (SLCT) (Slade et al. 2011). It has been associated with both familial MNG and MNG with SLCT, independent of FPB. Differentiated thyroid cancer (PTC and FTC) is infrequently observed in DICER syndrome, and most cases had prior exposure to radiation and chemotherapy for the treatment of associated malignancy. Somatic \textit{Dicer} mutations in papillary thyroid cancer were identified in 3 such cases (Kock et al. 2014). Recently, papillary thyroid cancer had been reported in a family with germline \textit{Dicer} mutation, who had not undergone any prior chemotherapy. Some of the PTC cases in this family were found to have somatic \textit{Dicer} mutations (Rutter et al. 2015).

Germline \textit{Dicer} mutations are associated with the dysregulation of miRNA expression patterns. Five miRNAs’ (miR-345, let-7a, miR-99b, miR-133 and miR-194) expression were reduced from peripheral blood and \textit{Dicer}-related MNG tissues. Of these, let-7a and miRNA-345 were downregulated in \textit{Dicer}-related goiter when compared with normal thyroid tissue and a follicular thyroid cancer. Let-7a has not been implicated in thyroid disease, whereas miR-345 is known to be highly expressed in thyroid tissue (Río Frio et al. 2011).

The prevalence of thyroid cancer in DICER1 syndrome seems to be low and evaluation for goiter should be guided by clinical examination by the clinician. The clinical utility of surveillance thyroid ultrasound remains to be validated.

**Non-syndromic familial non-medullary thyroid cancer**

Efforts to identify candidate cancer predisposition genes in non-syndromic FNMTMC have yielded mainly low-to-moderate penetrance genes. Further studies to characterize their penetrance and function are required, and routine genetic testing for these genes is not recommended. The details of genetic and clinical features of non-syndromic FNMTMC studies have been summarized in Table 3.

**FOXE1**

Forkhead box E1 (\textit{Foxe1}) gene is located at chromosome 9q22.33 and encodes for the FOXE1 transcription factor (also known as thyroid transcription factor 2, TTF-2), which regulates thyroglobulin and thyroperoxidase gene expression and plays a role in the thyroid precursor migration from pharynx to neck (De Felice et al. 1998). The FOXE1 protein consists of a forkhead/winged helix DNA-binding domain and polyalanine (polyAla) tract, with a variable length ranging from 11 to 22 alanine (Ala) residues, with 14-Ala being the most frequently occurring allele of this gene in the population. This common length polymorphism could lead to a gain-of-function mutation (Macchia et al. 1999, Hishinuma et al. 2001, Carré et al. 2007, Santarpia et al. 2007, Szczepanek et al. 2011, Bullock et al. 2012).

In a genome-wide association study (GWAS) of Icelandic, Columbus and Spanish cohort with sporadic PTC and FTC cases, 2 single-nucleotide polymorphisms (SNP) that were associated with increased risk of PTC and FTC included rs944289 (located on chromosome 14q13.3 near NKX2-1) and rs965513 (located on chromosome 9q22.33 near \textit{Foxe1}) (Gudmundsson et al. 2009). The association of these 2 SNPs with sporadic PTC cases was replicated in targeted SNP analysis studies in another study of Spanish and Italian cohorts of sporadic PTC and FTC. The latter study mainly found strong associations between \textit{Foxe1} gene and PTC cases, as well as associations with rs1867277. It also demonstrated in a functional assay of rs1867277 within the \textit{Foxe1} 5′UTR that this variant regulates \textit{Foxe1} transcription (Landa et al. 2009). In another study of sporadic PTC in Australia, \textit{Foxe1} was shown to be associated with PTC.

(miRNA database), the human genome encodes for 1881 miRNA sequences (http://www.mirbase.org/) (Kozomara & Griffiths-Jones 2014). Primary miRNA is first transcribed from miRNA gene by RNA polymerase II (Lee et al. 2004b). This is then assembled to a 70-nucleotide-long precursor miRNA by RNAase III enzyme Drosha and its RNA binding counterpart DGCR8 before its transfer out of the nucleus by Exportin-5 (Lee et al. 2003, Yi et al. 2003, Lund et al. 2004, Borchert et al. 2006). In the cytoplasm, the RNase endonuclease enzyme Dicer processes precursor miRNA to mature miRNA that is then incorporated into RNA-induced silencing complex, binding to sequences in 3′-untranslated regions of target mRNA (Hutvagner & Zamore 2002). miRNAs with complete complementary match to the miRNA sequence induce mRNA cleavage, whereas those with incomplete mRNA match lead to inhibition of translation and protein synthesis. The latter incomplete match allows for a single miRNA to regulate multiple genes. In the setting of oncology, miRNAs could have either tumor-promoting (oncomir) or tumor-suppressive effects.

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Efforts to identify candidate cancer predisposition genes in non-syndromic FNMTC have yielded mainly low-to-moderate penetrance genes. Further studies to characterize their penetrance and function are required, and routine genetic testing for these genes is not recommended. The details of genetic and clinical features of non-syndromic FNMTC studies have been summarized in Table 3.

**FOXE1**

Forkhead box E1 (\textit{Foxe1}) gene is located at chromosome 9q22.33 and encodes for the FOXE1 transcription factor (also known as thyroid transcription factor 2, TTF-2), which regulates thyroglobulin and thyroperoxidase gene expression and plays a role in the thyroid precursor migration from pharynx to neck (De Felice et al. 1998). The FOXE1 protein consists of a forkhead/winged helix DNA-binding domain and polyalanine (polyAla) tract, with a variable length ranging from 11 to 22 alanine (Ala) residues, with 14-Ala being the most frequently occurring allele of this gene in the population. This common length polymorphism could lead to a gain-of-function mutation (Macchia et al. 1999, Hishinuma et al. 2001, Carré et al. 2007, Santarpia et al. 2007, Szczepanek et al. 2011, Bullock et al. 2012).

In a genome-wide association study (GWAS) of Icelandic, Columbus and Spanish cohort with sporadic PTC and FTC cases, 2 single-nucleotide polymorphisms (SNP) that were associated with increased risk of PTC and FTC included rs944289 (located on chromosome 14q13.3 near NKX2-1) and rs965513 (located on chromosome 9q22.33 near \textit{Foxe1}) (Gudmundsson et al. 2009). The association of these 2 SNPs with sporadic PTC cases was replicated in targeted SNP analysis studies in another study of Spanish and Italian cohorts of sporadic PTC and FTC. The latter study mainly found strong associations between \textit{Foxe1} gene and PTC cases, as well as associations with rs1867277. It also demonstrated in a functional assay of rs1867277 within the \textit{Foxe1} 5′UTR that this variant regulates \textit{Foxe1} transcription (Landa et al. 2009). In another study of sporadic PTC in Australia, \textit{Foxe1} was shown to be associated with PTC.
### Table 3  Non-syndromic familial non-medullary thyroid cancers.

<table>
<thead>
<tr>
<th>Chromosomal loci</th>
<th>Gene</th>
<th>Type of thyroid cancer</th>
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<tr>
<td>9q22.33</td>
<td>FOXE1 gene</td>
<td>PTC</td>
<td>Targeted DNA sequencing of germline FOXE1 gene of 60 FNMTC families</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>PTC and FTC</td>
<td>Genotyping of 23 SNP at 11 candidate loci in 133 FNMTC pedigrees (SNPs near FOXE1 gene are associated with NMT risk, using family-based association test, modified quasi-likelihood score, and logistic-normal model)</td>
<td>Bonora et al. (2014)</td>
</tr>
<tr>
<td>10q25.3</td>
<td>HABP2 gene</td>
<td>PTC</td>
<td>Whole exome sequencing of FNMC kindred with 7 affected members</td>
<td>Gara et al. (2015)</td>
</tr>
<tr>
<td>TCO (19q13.2)</td>
<td>Not known</td>
<td>PTC with cell oxyphilia (Hurtle cell changes) MNG</td>
<td>Linkage analysis using STRP- 1 kindred of 7 families</td>
<td>Canzian et al. (1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FA</td>
<td>MNG</td>
<td></td>
</tr>
<tr>
<td>fPTC/ PRN (1q21)</td>
<td>Not known</td>
<td>PTC</td>
<td>Linkage analysis using STRP- 1 family with 7 affected members</td>
<td>Bevan et al. (2001), McKay et al. (2004)</td>
</tr>
<tr>
<td>FTEN (8p23.1-p22)</td>
<td>Not known</td>
<td>PTC</td>
<td>Linkage analysis using SNP- 38 families with PTC but no papillary renal neoplasm (LOD score +3.04)</td>
<td>Suh et al. (2009)</td>
</tr>
<tr>
<td>NMTC1 (2q21)</td>
<td>Not known</td>
<td>PTC (classical and follicular variant)</td>
<td>Linkage analysis using SNP- 1 family with 11 affected members (maximum LOD score +4.41)</td>
<td>Cavaco et al. (2008b)</td>
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<tr>
<td></td>
<td></td>
<td>MNG</td>
<td>Linkage analysis using STRP- 1 kindred, 80 pedigrees (multi-point heterogeneity LOD score +3.07; if select for pedigrees with at least 1 case of fPVPTC, heterogeneity LOD score +4.17)</td>
<td>McKay et al. (2001)</td>
</tr>
<tr>
<td>MNG1 (14q32)</td>
<td>Not known</td>
<td>PTC</td>
<td>Linkage analysis using STRP- 10 families with PTC and MNG (LOD score +2.85)</td>
<td>McKay et al. (2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MNG</td>
<td>LOH study- 2 out of 14 families had LOH (14%)</td>
<td>Prazeres et al. (2008)</td>
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<td>Bignell et al. (1997)</td>
</tr>
<tr>
<td>6q22</td>
<td>Not known</td>
<td>PTC</td>
<td>Linkage analysis using SNP- 38 families with PTC (LOD score +4.88)</td>
<td>Suh et al. (2009)</td>
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<tr>
<td>8q24</td>
<td>Not known</td>
<td>PTC</td>
<td>Linkage analysis using SNP- 26 patients with maximum LOD score of 1.3</td>
<td>He et al. (2009)</td>
</tr>
<tr>
<td>12q14</td>
<td>SRGAPI1 gene</td>
<td>PTC</td>
<td>Linkage analysis using SNP- 21 families (PPL score 0.3 implying a 30% probability of linkage)</td>
<td>He et al. (2013a)</td>
</tr>
<tr>
<td>14q13</td>
<td>TITF-1/ NXX2.1 gene</td>
<td>PTC</td>
<td>Targeted DNA sequencing of germline TITF-1/NXX2.1 gene of 20 PTC patients with history of MNG</td>
<td>Ngan et al. (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MNG</td>
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</table>

Using SNP analysis including rs1867277. FOXE1 with 16 alanine repeats (FOX116Ala) was also associated with PTC with an odds ratio of 2.23 (CI: 1.42–3.50, P=0.0005) (Bullock et al. 2012).

On sequencing the FOXE1 gene in 60 Portuguese FNMTC families and 80 sporadic NMTC cases, 10 germline variants in the promoter and coding sequence of the gene were detected. These include 9 polymorphisms and 1 variant (c.743C>G, p.A248G) that was not previously described. This variant segregated with PTC in one FNMTC family and was also detected in a case of sporadic PTC (Tomaz et al. 2012, Pereira et al. 2015). FOXE1 polyalanine tract expansions consisting of more than 14 alanine residues were associated with both FNMTC and sporadic NMTC (Tomaz et al. 2012). Functional studies using rat normal thyroid cell clones and human papillary thyroid carcinoma cell line pools, expressing the wild-type and mutant forms of FOXE1 demonstrated that the mutant variant promoted cell proliferation and migration, suggesting that it may be involved in thyroid

FA, follicular adenoma; LOD score, log 10 of odds score; MNG, multinodular goiter; PPL, posterior probability of linkage; PTC, papillary thyroid cancer; SNP, single nucleotide polymorphism; STRP, short tandem repeat polymorphisms.
tumorigenesis (Pereira et al. 2015). This study supports the involvement of a germline FOXE1 variant in FNMTC etiology in these familial cases (Pereira et al. 2015).

Another study genotyped 23 SNPs at 11 candidate loci (including chromosome loci 9q22.33, 14q13.3, 1p12-13, 1q21, 6q22, 8p22, 8p23 and 8q24 in pre-miR-146a, in the tumor suppressor gene WWOX and in the PDE8B gene) in 672 patients from 133 FNMTC kindreds. SNPs (rs965513 and rs10759944) at 9p22.33 near FOXE1 showed the most consistent association with FNMTC using family-based association test (FBAT), modified quasi-likelihood score (MQLS) and logistic-normal model (LNM) (Bonora et al. 2014). The previously reported SNP rs944289 from sporadic PTC and FTC GWAS (Gudmundsson et al. 2009) was not shown to be associated with FNMTC in this study (Bonora et al. 2014). The previously reported SNP rs1867277, falling in the 5'UTR of FOXE1 (Landa et al. 2009), only showed association with FNMTC on using the LN method but did not show consistent association with FNMTC on using the FBAT and MQLS methods in this study (Bonora et al. 2014). In a subset of 95 index FNMTC cases carrying the risk alleles for the 3 associated SNPs (rs965513, rs10759944, rs1867277) at the 9q22.33 locus, the entire coding sequence of FOXE1 was screened for mutations. However, no deleterious missense mutations were found. The polyalanine stretch length polymorphism was not shown to be associated with FNMTC in this study (Bonora et al. 2014).

In summary, FOXE1 gene variant might be a low-penetrant susceptibility gene for FNMTC. Literature supporting this association is not entirely consistent, and further validation studies are required before FOXE1 gene screening can be advocated in FNMTC cases.

**HABP2**

The Hyaluronan-Binding Protein 2 (HABP2) gene is located on chromosome 10q25.3. On performing whole exome sequencing of peripheral blood in 7 affected members of a FNMTC kindred (PTC and follicular adenoma) and unaffected spouses as controls, a germline G534E variant was identified in the HABP2 gene. This was subsequently validated on Sanger sequencing. Increased HABP2 protein expression was demonstrated in FNMTC samples compared with normal adjacent thyroid tissue and samples from sporadic thyroid cancers. All affected cases were heterozygous for this variant. Functional studies revealed that the G534E variant resulted in increased colony and foci formation and cellular migration, suggesting a loss of tumor suppression function. Expressing equal amount of HABP2 and the G534E variant in the same cells still resulted in increased foci number, supporting that the G534E variant has a dominant-negative tumor suppressive effect (Gara et al. 2015). Of note, the filtering criterion used to identify the mutation in the kindred in this study was an allele frequency of 1% or less for variant prioritization. The 2 databases used were the 1000 Genomes Project (phase 3, version 5 [20130502]; http://www.1000genomes.org) and the HapMap3 (release3; http://hapmap.ncbi.nlm.nih.gov/) databases that included general healthy population. There were subsequent correspondences (Sponziello et al. 2015, Tomsic et al. 2015, Zhou et al. 2015) commenting on higher reported frequency of the HABP2 G534E variant from other public databases including the Exome Aggregation Consortium database, with an average allele frequency of 2.2% (www.exac.broadinstitute.org). However, this database included data from 7601 patients with cancer from TCGA out of the total of 60,706 persons (12.5%), likely contributing to the higher gene frequency reported compared with the 1000 Genome Project and HapMap3 databases (Gara et al. 2015). The NHLBI Grand Opportunity Exome Sequencing Project database (that does not include cancer patients) showed an allele frequency of 3.88% in Americans of European descent, which corresponded to approximately 8% of persons with a European background (http://evs.gs.washington.edu/EVS/).

Subsequently, Zhao and coworkers performed targeted DNA testing for HABP2 mutation in probands of 12 FNMTC families for germline mutation and 217 sporadic PTC cases for somatic mutation, but did not detect any HABP2 genetic variants in the Chinese cohort, suggesting ethnic differences in this variant (Gara et al. 2015, Zhao et al. 2015).

In a US study that performed targeted DNA sequencing of HABP2 gene in 64 subjects from 29 kindreds with FNMTC, including 43 PTC cases, 5 benign thyroid neoplasm cases and 16 normal controls, 6 subjects from 4 kindreds tested positive for HABP2 G534E genetic variant. All cases had PTC and were heterozygous for the gene mutation. Three of the cases that tested positive for HABP2 G534E variant were from the same kindred and demonstrated an autosomal dominant inheritance pattern. However, some kindreds only had 1 PTC subject available for genetic tasting (Zhang & Xing 2016).

Another US study reported the presence of the HABP2 G534E variant in 6.1% of familial cases (out of 179 FNMTC families), 8.0% of 1160 sporadic PTC cases and 8.7% of 1395 controls. The corresponding minor
allele frequencies were 3.1, 4.1 and 4.3%, respectively, with no statistical differences among the 3 groups. It was also demonstrated that HABP2 expression was higher in liver compared with that in kidney, brain and breast tissues and was not detectable in normal thyroid tissues and PTC tissues (both with or without the variant) (Tomsic et al. 2016).

The HABP2 G534 genotype was screened in 2105 NMTC cases in the TCUKIN study, a multicenter population-based study in the British Isles, and in 5172 UK controls from the 1958 Birth Cohort and the National Blood Donor Service study. The frequency of HABP2 G534E was 4.2% in cases and 4.6% in controls. There was no association between this variant and NMTC risk across all histological sub-types (PTC, FTC and Hurthle cell) (Sahasrabudhe et al. 2015).

This was also studied in a Middle Eastern population including 11 FNMT cases (from 4 families), 509 sporadic NMTC patients and 190 controls. None of the FNMT cases carried the germline HABP2 mutation. Only 1 sporadic case of NMTC (0.2%) and 1 control case (0.5%) carried the HABP2 G534E variant (Alzahrani et al. 2016).

HABP2 G534 variant frequency seems to vary with different ancestries, being of low-to-moderate frequency in European ancestry and low frequency in Asian and Middle Eastern ancestry. The association of the HABP2 G534E variant with affected FNMT members, if it is not pathogenic, might be due to its linkage with the causative candidate gene for FNMT in these cases. Its role in the pathogenesis of FNMT remains to be validated in larger FNMT studies.

Telomere–telomerase complex

Telomeres are non-coding chromosomal ends consisting of tandem repeat TTAGGG sequences. They are important in maintaining the chromosomal stability and shorten with each cell replication. Cells undergo apoptosis when telomeres reach a critically short length. Telomere length is usually maintained by telomerase complex that includes the telomerase reverse transcriptase (TERT) and the telomerase RNA complex. At the end of a chromosome, the shelterin complex binds to the telomere and regulates the telomerase activity. It is composed of 6 proteins: telomere repeat-binding factor 1 (TRF1), TRF2, TRF1-interacting nuclear factor 2 (TIN2), tripeptidyl peptidase1 (TPP1) and repressor activator protein 1 (RAP1) (Fu & Collins 2007). The maintenance of telomere length is important for cell division and immortalization. Shortened telomere length is associated with chromosomal instability, including chromosomal rearrangement, chromosomal arm gain or loss, chromosomal fusion, deletions or amplification that play a role in cancer evolution (De Lange 2005).

It was reported that FNMT cases (mainly PTC subtype) had significantly shorter germline telomere length compared with sporadic PTC cases (Capezzone et al. 2008a). The neoplastic and non-neoplastic surrounding thyroid tissues in FNMT cases were also noted to have shorter telomere length compared with sporadic thyroid cancer cases and their corresponding normal thyroid tissues, suggesting that FNMT has a genetic predisposition to tumor development (Capezzone et al. 2011). Some of these studies also showed a significant correlation between FNMT cases with higher telomerase reverse transcriptase gene amplification and mRNA expression compared with sporadic PTC cases (Capezzone et al. 2008a), as well as associations with chromosomal instability in the form of spontaneous telomeric associations and telomeric fusions compared with sporadic PTC cases and healthy subjects (Cantara et al. 2012). However, another study did not show any differences in telomere length and TERT activity between affected FNMT cases compared with sporadic PTC cases, even though it demonstrated a shorter telomere length in affected FNMT cases compared with unaffected FNMT family members. It did not find any significant differences in TERT and shelterin component gene copy number and mRNA expression level in affected FNMT cases compared with unaffected family members and sporadic PTC cases (He et al. 2012). On the contrary, 1 study did not show any significant differences in telomere length between FNMT and sporadic PTC cases (Jendrzejewski et al. 2011).

In summary, some studies have demonstrated that FNMT cases have shorter germline and somatic tumoral telomere length suggesting the role of telomere shortening in the development of FNMT. However, there is inconsistent literature on the differential value of telomere length in distinguishing FNMT from sporadic PTC cases, and further studies are required before clinical application is considered.

Enhancer at chromosome 4q32

Genome-wide linkage analysis with SNP arrays was performed in a US family pedigree with 13 affected family members across 3 generations with 11 cases of PTC and 2 cases of anaplastic thyroid carcinoma (ATC). This revealed a locus on chromosome 4q32; on multi-point non-parametric linkage (NPL) analysis, the maximum
NPL Z-score was 18.5 (He et al. 2013b). The haplotype was found in all except for 1 affected family member with PTC, as well as 4 members with benign thyroid disease.

Transcription factors bind to regulatory sequences, including enhancer elements to regulate gene expression. An enhancer element was noted in the linkage peak; the sequence in the 4q32A>C region is highly conserved among mammals supporting regulatory elements in the region. This single nucleotide point mutation (4q32 A>C) was shown to affect the binding of transcription factors POU2F1 and YY1 to the enhancer. Functional studies revealed that this genetic alteration led to decreased level of enhancer RNA and reduced transcription in the presence of POU2F1 and YY1 transcription factors, suggesting reduced enhancer activity in 4q32 A>C mutation.

In summary, mutation of the enhancer at 4q32 seemed to be highly penetrant for thyroid cancer in this pedigree. However, this mutation is extremely rare and is not detected in 38 other familial NMTC kindreds, sporadic thyroid cancer patients (800 US cases and 1876 Polish cases), controls (820 US controls and 1650 Polish controls) or public databases (dbSNP and 1000 Genomes Project).

**MNG1 locus**

The multinodular goiter 1 (MNG1) locus is located on 14q32 and was described in a Canadian pedigree with 18 cases of MNG and 2 cases of papillary thyroid cancer. The mode of inheritance was autosomal dominant. Overall, 34 members of this family were genotyped leading to the identification of this locus, with a maximum 2-point LOD score of 3.8 and multi-point LOD score of 4.88. However, on linkage analysis of other kindreds with MNG and NMTC in the same study (Bignell et al. 1997) and in other subsequent studies, linkage between MNG1 locus and NMTC could not be demonstrated (Lesueur et al. 1999, McKay et al. 1999, Bevan et al. 2001, Tsilchorozidou et al. 2005, Cavaco et al. 2008a,b, Na et al. 2012).

In summary, the MNG1 locus had only been associated with FNMC in 1 kindred of 2 PTC cases and 18 MNG cases. It may rarely account for FNMC associated with MNG, or it may harbor a gene for MNG alone, and not FNMC.

**TCO locus**

The thyroid tumors with cell oxyphilia (TCO) locus is located on 19p13.2 and was first mapped in a three-generation French family with 6 cases of multinodular goiter and 2 cases of papillary thyroid cancer with cell oxyphilia (Hurtle cell changes) (Canzian et al. 1998). On subsequent FNMC studies, families with linkage to the TCO locus were identified. However, the phenotype of cell oxyphilia was only observed in some of these cases, and the LOD score was in the range of +1.5 (Bevan et al. 2001, McKay et al. 2004, Prazeres et al. 2008). In a linkage analysis study of a Greek family with FNMC without cell oxyphilia, linkage with TCO locus was excluded (Tsilchorozidou et al. 2005). Loss of heterozygosity (LOH) at the TCO locus had been shown in both sporadic oxyphilic tumors (Stankov et al. 2004) and familial clusters of FNMC (57%), suggesting that inactivation of the TCO gene, acting as a tumor suppressor, may contribute to tumorigenesis (Prazeres et al. 2008).

In summary, a minority of FNMC cases might arise due to a susceptibility gene at the TCO locus though only some of these tumors demonstrated tumor cell oxyphilia. It is an uncommon type of FNMC and in some sporadic tumors with this phenotype, the TCO locus had been associated.

**fPTC/PRN locus**

This locus on 1q21 had been described in 3 generations of a U.S. kindred with 5 cases of papillary thyroid cancer and 2 cases of papillary renal neoplasm (PRN), observed to be inherited in an autosomal dominant pattern. After genotyping 31 family members, this locus gave a maximum 3-point LOD score of +3.58 (Malchoff et al. 2000). To date, no further family with the phenotype of PTC and PRN has been described. In another linkage analysis study of 38 FNMC families using genome-wide single-nucleotide polymorphism (SNP) array, significant linkage was identified between chromosomal locus 1q21 and FNMC (PTC sub-type) with LOD score of +3.04. Of note, there was an absence of PRN manifestations in this study cohort (Suh et al. 2009). Further linkage analysis studies of FNMC families without PRN did not reveal an association between this locus and FNMC (Bevan et al. 2001, Cavaco et al. 2008b).

In summary, these findings suggest that the fPTC/PRN locus might harbor a candidate gene for FNMC that may or may not be associated with PRN. The phenotype where PTC is associated with PRN is extremely rare and had only been observed in 1 kindred to date.
**NMTC1 locus**

This locus on 2q21 was reported in 7 out of 8 cases (4 classical and 4 follicular variant papillary thyroid cancers) from a Tasmanian pedigree. Further linkage analysis of this locus was tested in a separate set of 80 FNMTc pedigrees, and the multi-point heterogeneity LOD score was +3.07. In a stratification from the same study cohort based on the presence of at least 1 case of follicular variant papillary thyroid cancer (fvPTC), 17 pedigrees were identified, and the multi-point heterogeneity LOD score was +4.17, suggesting that the locus had a more significant association with fvPTC (McKay et al. 2001). Similarly, a linkage analysis of 10 FNMTc families demonstrated a linkage with this locus with LOD score of +2.85 (McKay et al. 2004). Another study showed that 2 out of 14 cases of FNMTc demonstrated LOH at the NMTC1 locus (Prazeres et al. 2008). However, other studies did not show linkage of FNMTc to this locus (Tsilchorozidou et al. 2005, Cavaco et al. 2008a).

In summary, a few studies have demonstrated an association of FNMTc with the NMTC1 locus. The strength of this association seemed stronger with fvPTC.

**FTEN locus**

This locus was reported to be at 8p23.1-p22 in a linkage analysis study of Portuguese kindred with 11 cases of benign thyroid diseases and 5 cases of thyroid cancers (4 classical and 1 follicular variant of PTC), with a maximum LOD score of +4.41. Single-nucleotide polymorphism (SNP) was tested along with microsatellite analysis. There was no LOH demonstrated in the cases. A bioinformatics search was performed in the region of chromosome 8p23.1-p22. Out of the 32 genes identified, 17 genes were tested for sequence changes in coding region and splice sites, but no potentially pathogenic changes were identified (Cavaco et al. 2008b). This locus was not detected on further linkage analysis of another 6 families with FNMTc (Cavaco et al. 2008b).

The association of FTEN locus with FNMTc had been reported in only 1 kindred. It may rarely account for FNMTc and remains to be further studied in other FNMTc families.

**6q22 locus**

In a linkage analysis study of 38 FNMTc families, with 110 relatives including 49 PTC cases, using genome-wide SNP array, significant linkage was identified between chromosomal locus 6q22 and FNMTc with LOD score of +3.30 (Suh et al. 2009).

This association has not been replicated in other FNMTc studies and requires further validation in other FNMTc cohorts.

**8q24 locus**

In a linkage analysis study of 26 FNMTc (PTC) patients using SNP arrays, the largest peak localized to 8q24 locus with a maximum LOD score of +1.3. The first family studied consisted of 3 generations with 8 cases of PTC, of which 2 had concurrent melanoma, another 2 members had melanoma and 10 members had MNG. In the other 25 FNMTc families studied, co-existing melanoma was not reported. Sequencing of genes in the region of locus did not reveal any known candidate mutations. Gene expression analysis indicated that AK023948 (PTCS1), one of the non-coding RNA genes in the region of the locus, which is shown to be downregulated in PTC, could be a candidate susceptibility gene (He et al. 2009).

This association has not been replicated in other FNMTc studies, and the locus is likely to account for FNMTc rarely.

**SRGAP1**

A genome-wide linkage analysis, using SNP genotyping, performed in 38 FNMTc families with PTC identified this 12q14 locus in 21 families with a posterior probability of linkage (PPL) score of 0.3 (He et al. 2013a). This implies that there is a 30% probability of linkage of FNMTc to 12q14 locus. In association analyses of 2 Ohio sporadic PTC cohorts (consisting of 269 and 289 patients, respectively), SNPs in the locus region were identified. SNP rs2168411 showed a significant association with PTC. The association of this SNP with PTC was further validated in a Polish sporadic PTC cohort (consisting of 906 cases). Overall, the association was modest (odds ratio 1.2, P=0.0008) indicating that this SNP is unlikely to be causative for sporadic PTC.

SNP rs2168411 is located in the Slit-Robo Rho GTPase-activating protein 1 (SRGAP1) (Wong et al. 2001). The FNMTc families with linkage to 12q14 locus underwent re-sequencing of all exons and exon–intron boundaries of SRGAP1 gene. Four germline missense variants (Q149H, A275T, R617C and H875R) co-segregated with PTC phenotype in 1 FNMTc family each. These 4 variants were then screened in the sporadic PTC sample sets from Ohio and Poland. Two missense variants, Q149H
and A275T, localized in the Fes/CIP4 homology domain, were not detected in the sporadic PTC cases and normal controls. One missense variant, R617C, located in the Rho GAP domain, was detected in <1% of sporadic PTC cases and controls. H875 variant was detected in >10% of sporadic PTC and control cases and did not show any significant differences between PTC cases and controls (He et al. 2013a).

**SRGAP1** regulates the small G-protein CDC42 in neurons and affects cell mobility (Wong et al. 2001). Functional assays demonstrated that the ability to inactivate CDC42, a key function of SRGAP1, was severely impaired by the Q149H and R617C variants (He et al. 2013a). These 2 missense variants in SRGAP1 could lead to loss of function affecting CDC42 activity. CDC42 acts as a signal transduction convergence point in intracellular signaling networks, mediates multiple signaling pathways and plays a role in tumorigenesis (Etienne-Manneville 2004).

In summary, the SRGAP1 gene might be a low-penetrant susceptibility gene in FNMTC. The Q149H and R617C variants could lead to loss-of-function changes leading to inability to inactivate CDC42. Further studies to validate the association of this gene in other FNMTC cohorts are needed before this can be used in clinical practice in the screening of FNMTC cases.

**TITF-1/NKX2.1**

This gene maps to chromosome 14q13 and encodes thyroid transcription factor-1 (TTF-1) protein that activates the transcription of thyroglobulin, thyroperoxidase and thyrotropin receptor. Targeted DNA sequencing of 20 PTC patients with a history of MNG, 284 PTC patients without a history of MNG and 349 controls revealed the presence of germline TITF-1/NKX2.1 mutation in 4 PTC patients with a history of MNG. A missense mutation led to a mutant TTF-1 protein (A339) in these patients. Of these 4 patients, 2 of them had a positive family history of PTC. In the first family, the index case had 3 family members with this germline mutation, each of whom was affected with PTC and MNG, MNG or PTC with MNG. The latter patient also had colon carcinoma. In the second family, the index case had 2 family members with this germline mutation, each of whom was affected with PTC and MNG or MNG. The pattern of inheritance was autosomal dominant in both families.

When the gene mutation was transfected to rat normal thyroid cells resulting in overexpression of A339, there was increased proliferation, independent of thyrotropin stimulation, activated STAT3 and Akt signaling and increased cyclin D2 expression indicative of the role of A339V TTF-1 mutation in the development of PTC (Ngan et al. 2009). This association could not be replicated in another FNMTC study (Cantara et al. 2010).

In summary, the A339V TTF-1 germline mutation was reported in 2 families and all cases had MNG. Only the index case from the first family had 2 family members affected with PTC. The other index case from the second family only had 1 family member with PTC and that does not meet the commonly adopted FNMTC criteria of having at least 2 first-degree relatives with NMTC. The A339V TTF-1 mutation may be a susceptibility gene for MNG and may predispose some MNG patients to the development of PTC. However, further validation studies are required to demonstrate its association with FNMTC and the clinical utility of testing for this gene mutation in FNMTC cases.

**miR-886-3p and miR-20a**

Through the use of whole genome miRNA microarrays in familial and sporadic NMTC cases, miR-886-3p and miR-20a were noted to be differentially expressed by 3- and 4-fold respectively, as validated by RT-PCR. Both miRNAs were also downregulated in NMTC with respect to normal thyroid tissues by 3.5- to 4-fold. By pathway and target prediction analysis, miR-886-3p might be involved with regulation of genes involved in DNA replication and focal adhesion pathways. Thyroid cancer cell lines with overexpression of miR-886-3p showed inhibition of cellular proliferation and migration (Xiong et al. 2011). miR-20a expression has been reported to be higher in anaplastic thyroid cancer compared with differentiated thyroid cancer, benign thyroid adenoma and normal thyroid tissues. It has been shown to have tumor suppressive properties, with the inhibition of cell proliferation in thyroid cancer cell lines. This could be mediated by downregulation of LIMK1 expression that is associated with decreased cellular invasion (Xiong et al. 2014).

The above studies suggest that miR-886-3p and miR-20a are involved in the regulation of thyroid NMTC growth; they might have differential regulatory role in familial and sporadic NMTC. The role of miR-886-3p and miR-20a in NMTC needs to be further studied in other studies.
Discussion

We have reviewed the genetic basis of both syndromic and non-syndromic FNMTC. Non-syndromic FNMTC appears to be a heterogenous genetic entity. The putative candidate genes described seem to account for only a minority of FNMTC. To date, 4 susceptibility genes have been identified (SRGAP1 gene (12q14), TITF-1/NKX2.1 gene (14q13), FOXE1 gene (9q22.33) and HABP2 gene (10q25.3)), out of which only the FOXE1 and the HABP2 genes have been validated by separate studies (Bonora et al. 2014, Gara et al. 2015, Pereira et al. 2015, Zhang & Xing 2016). Overall, the literature supporting the association between FOXE1 gene variant and FNMTC is not entirely consistent and further validation studies are required before FOXE1 gene screening can be advocated in FNMTC cases. The HABP2 G534E variant has been reported only in a small number of FNMTC cases and has also showed inconsistent associations with FNMTC and needs to be further validated in larger FNMTC cohorts. The SRGAPI gene had been reported by only a single study group and remains to be validated in a larger FNMTC cohort (He et al. 2013a). A validation study of the TITF-1/NKX2.1 gene in a separate FNMTC study cohort did not reveal germline mutation of this gene in the second cohort (Ngan et al. 2009, Cantara et al. 2010). The causal genes located at the other 7 FNMTC-associated chromosomal loci (TCO (19q13.2), βPTC/PRK (1q21), FTEN (8p23.1-p22), NMTC1 (2q21), MNG1 (14q32), 6q22, 8q24) remain to be identified (Bignell et al. 1997, Canzian et al. 1998, Malchoff et al. 2000, McKay et al. 2001, Cavaco et al. 2008b, He et al. 2009, Suh et al. 2009). However, these findings suggest the multigenic factors for FNMTC predisposition. The improvement in genetic testing methods from linkage analysis to targeted sequencing or whole exome sequencing techniques has improved the understanding of the genetic basis of FNMTC. Of note, predisposing genes could be non-coding and regulatory genes; hence, whole exome sequencing techniques would not detect these genetic alterations. With the use of newer sequencing techniques including whole genome sequencing, genomic predisposing factors could be further evaluated, followed by functional studies to elucidate the role of identified candidate genes. The genetic landscape of syndromic FNMTC has been better characterized. Nevertheless, we foresee that with the availability of newer genetic testing techniques, the genetic basis of syndromic FNMTC could be better understood, and further novel driver germline mutations might be unraveled, like in the case of Cowden syndrome where novel germline SEC23B variant was uncovered using whole exome sequencing (Yehia et al. 2015). When faced with a patient with thyroid cancer and characteristic phenotype of syndromic FNMTC, the clinician should perform targeted genetic testing specific to the syndromic FNMTC. Confirmation of the diagnosis of syndromic FNMTC would initiate surveillance for other associated malignancies and facilitate the genetic counseling and targeted genetic testing of family members. When the clinician encounters a patient with 2 or more first-degree relatives with thyroid cancer, the diagnosis of FNMTC should be considered. If no clinical signs of syndromic FNMTC are observed, there is currently no indication for genetic testing as the candidate genes for non-syndromic FNMTC need to be better characterized.

The differing clinical aggressiveness and susceptibility genes reported in literature could be due to variation in study design of FNMTC studies. The inclusion criteria for FNMTC vary from having at least 1 to 3 first-degree relatives with thyroid cancer. Based on a review of the prevalence of FNMTC from Surveillance Epidemiology and End Results (SEER) database, Mayo clinic and mathematical analysis, if the definition of having 2 first-degree relatives with thyroid cancer is used for FNMTC, only 31–38% of members in the family are likely to carry the familial trait, with the rest being sporadic cases. However, in families with three or more members affected with thyroid cancer, the likelihood of possessing the familial trait for FNMTC is 96% (Charkes 2006). Therefore, many studies that use the FNMTC criterion of two affected members might be analyzing sporadic thyroid cancer cases rather than true FNMTC cases. Ideally, future FNMTC studies should focus on families with 3 or more affected members.

Some of the FNMTC studies did not include a control group for comparison of genetic profile. Both affected and non-affected family members should be studied to reduce the possibility of a false-positive finding by identifying a familial trait that is not the driver germline mutation of FNMTC.

A significant proportion of the FNMTC genetic studies include individuals with benign thyroid disease as ‘affected’ cases. However, these cases might not eventually progress to cancer and should not be considered as FNMTC cases. It is however recognized that family members of FNMTC cases might be at increased risk of developing benign thyroid diseases. Hence, it is still important to track these occurrences to allow study of their association with FNMTC and candidate susceptibility gene.
Multi-center studies of large cohort with strict inclusion criteria, detailed sub-categorization of phenotypes and the use of targeted sequencing or whole exome/genome sequencing are needed to better characterize the genetic landscape of FNMTMC and to allow an in-depth understanding of the underlying pathogenesis. This would be useful for the screening, management and surveillance of FNMTMC cases that is considered by some to be a more aggressive disease entity.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding
This work did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

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Received in final form 30 September 2016
Accepted 3 October 2016