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Molecular genetics of thyroid cancer

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Summary

The pathogenesis of the development and progression of thyroid cancer (TC) is far from being clear at present. Accumulated evidence suggests that it is a complex polygenic disorder for which genetic factors play an important role in disease aetiology. Here we review the literature to report the genetic variations and alterations that have been described in the aetiology of TC. The functional effects of some mutations and single nucleotide polymorphisms on TC are validated, establishing the role of sequence variations in this cancer. However, large prospective studies are still required to evaluate the diagnostic and prognostic value of these genetic determinants in clinical practice.

1. Introduction

Thyroid cancer (TC) is the most prevalent endocrine malignancy having an incidence two to three times higher in women (Farahati *et al.*, 2004). It can occur at any age although it is more frequent after the age of 30 years and significantly more aggressive in older subjects (Leenhardt *et al.*, 2005). TC is classified into four main histology groups: papillary (PTC), follicular (FTC), medullary (MTC), and undifferentiated or anaplastic thyroid carcinomas. The great majority of malignant thyroid tumours are nonmedullary, either PTC (80–85%) or FTC (10–15%) (DeLellis *et al.*, 2006; Kondo *et al.*, 2006).

Molecular analysis of thyroid tumours has indicated that many genetic alterations are found to be involved in cancer development. The most affected genes are those involved in DNA repair, signal transduction and cell cycle control (Kang *et al.*, 2002). In fact, in recent years, a multitude of genetic variations such as mutations and single nucleotide polymorphisms (SNPs) have been identified and associated with TC risk. The most frequent genetic alterations that have been described in papillary and follicular cancers, are *BRAF* and *RAS* point mutations and *RET/PTC* and *PAX8/PPARγ* rearrangements (Nikiforova & Nikiforov, 2009). Recently, it has been reported that point mutations in the *TERT* and *TP53* genes are an important event in TC cancer diagnosis or progression (Vinagre *et al.*, 2013; Pita *et al.*, 2014). Yet, recent studies have demonstrated a strong association of some SNPs with TC risk. Among sequence variants that have been highly implicated in the aetiology of TC are the variants at 8q24 (rs6983267), 9q22·23 (rs965513) and 14q13·3 (rs944289, rs116909374) loci (Gudmundsson *et al.*, 2009; 2012; Sahasrabudhe *et al.*, 2015). These polymorphic sites may affect the enhancer activity of genes or gene regulation, but how they influence these outcomes is not precisely acknowledged.

In a complex polygenic disease such TC, which may be a result of the interaction between a number of genetic and epigenetic factors, analysis of multiple gene polymorphisms and mutations is beginning to be necessary in order to study the susceptibility of the disease. Therefore, in this report, we have reviewed the literature to describe the molecular genetics status of TC.

2. Thyroid cancer and genetic polymorphisms

Genome wide association studies (GWAS) have identified some chromosomal regions as new hotspots for TC susceptibility (Table 1 and Fig. 1). These regions are localized at 9q22·33, 14q13·3, 2q35 and 8p12. In

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Table 1. *SNPs strongly associated with thyroid cancer in genome wide association studies.*

SNP	Region	Location	Gene	Functional class	Allele risk	Sample seize (cases/controls)	Population	*p*-value	OR	Reference
rs965513	9q22·33	97793827	Near *FOXE1*		A	192/37196 90/1343 342/384	Icelandic Spanish Columbus	2×10^{-27}	1·75	Gudmundsson *et al.*, 2009
						2240/2218 468/470 509/1118 446/420	Italian Polish UK Spanish	3×10^{-10}	1·78	Köhler *et al.*, 2013
						194/179 214/448	Belarusian Russian	5×10^{-12}	1·65	Takahashi *et al.*, 2010
rs944289	14q13·3	36180040	Near *NKX2-1*		T	192/37196 432/1727 90/1343 342/384	Icelandic European Spanish Columbus	2×10^{-9}	1·37	Gudmundsson *et al.*, 2009
rs966423	2q35	217445617	*DIRC3*	Intron	C	222/24189 90/1399 365/383 151/832	Icelandic Spanish Columbus Dutch	1×10^{-9}	1·34	Gudmundsson *et al.*, 2012
rs2439302	8p12	32574851	*NRG1*	Intron	G	222/24189 90/1399 365/383 151/832	Icelandic Spanish Columbus Dutch	2×10^{-9}	1·36	Gudmundsson *et al.*, 2012
rs116909374	14q13·3	36269155	*MBIP*		T	222/24189 90/1399 365/383 151/832	Icelandic Spanish Columbus Dutch	5×10^{-11}	2·09	Gudmundsson *et al.*, 2012
rs6759952	2q35	217406996	*DIRC3*	Intron	T	2240/2218 468/470 509/1118 446/420	Italian Polish UK Spanish	6×10^{-10}	1·25	Köhler *et al.*, 2013

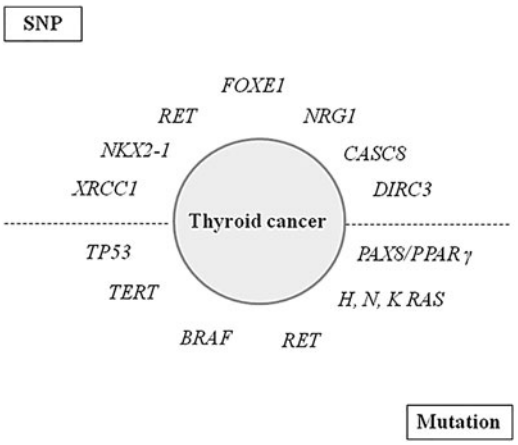

Fig 1. Genes implicated in thyroid cancer.

fact, the first GWAS has identified 9q22·33 (rs965513) in *FOXE1* and 14q13·3 (rs944289) with *NKX2-1* as one of the nearby genes (Gudmundsson *et al.*, 2009). The second GWAS focused on radiation-induced papillary TC and has confirmed the strong association with *FOXE1* (Takahashi *et al.*, 2010). The third GWAS, centred on the analysis of genetic determinants of TSH levels, not only has confirmed the 9q22·33 and 14q13·3 associations but also led to the identification of novel susceptibility loci at 2q35 (rs966423 in *DIRC3*) and 8p12 (rs2439302 in NRG1; Gudmundsson et al., *2012*). A more recent GWAS has confirmed the association with *DIRC3* loci by identifying additional common differentiated thyroid cancer (DTC) susceptibility loci at 2q35 (Köhler *et al.*, 2013). Since then, many studies have been performed to evaluate these associations as well as other genomic regions in independent populations.

(i) *FOXE1*

Forkhead box E1 (*FOXE1*) is a single exon gene located at chromosome 9q22·33 encoding the transcription factor *FOXE1* (or *TTF-2*), which likely plays a crucial role in thyroid morphogenesis. This transcription factor regulates thyroglobulin and thyroid peroxidase expression. Many association studies have been performed to investigate the role of *FOXE1* polymorphisms in TC susceptibility. Several variants of this gene have been positively correlated with thyroid malignancy (Table S1). However, two common variations (rs1867277 and rs71369530) have been associated with TC risk in various ethnic groups (Matsuse *et al.*, 2011; Gudmundsson *et al.*, 2012; Jones *et al.*, 2012).

The SNP rs1867277 is caused by an A/G transition in the 5′-UTR region of the gene, whereas the rs71369530 polymorphism is a polymorphic polyalanine tract (rs71369530, polyAla) just distal to its DNA-binding domain. Recently, it has been shown that the variant rs1867277 affects *FOXE1* transcription. In fact, it has been reported that the A allele of this SNP increases the transcriptional activity of the *FOXE1* gene promoter, by the recruitment of leucine zipper upstream stimulatory factors 1 and 2 (Landa *et al.*, 2009). Regarding the *FOXE1* polyAla tract, it has been reported that it has 11–22 alanine residues, although *FOXE1* 14Ala and *FOXE1* 16Ala account for greater than 98% of reported alleles (Kallel *et al.*, 2010). Some studies have suggested a functional consequence for the presence of polyAla expansions (>14) but not for contractions (⩽14). *FOXE1* with 16Ala induced a stronger transactivation of the thyroglobulin promoter than the 14Ala variant (Carré *et al.*, 2007). However, a recent study has reported a modest transcriptional impairment of 16Ala *FOXE1*, when compared with the function of the 14Ala variant, on *FOXE1* responsive promoters, which was not attributable to differences in DNA binding (Liyanarachchi *et al.*, 2013).

(ii) *RET*

The rearranged during transfection (*RET*) proto-oncogene is one of the receptor tyrosine kinase, cell-surface molecules that transduce signals for cell growth and differentiation. A wide panel of SNPs in the *RET* gene have been reported to the curated databases but only a limited, and perhaps selected, number of these *RET* variants have been tested for an association (Table S1). Among them are the nonsynonymous variant G691S (rs1799939) in exon 11, the synonymous variants L769L (rs1800861) in exon 13, S836S (rs1800862) in exon 14 and S904S (rs1800863) in exon 15. These SNPs have been repeatedly implicated in the increase of MTC risk (Figlioli *et al.*, 2013; Lantieri *et al.*, 2013). However, the effect of *RET* polymorphisms on other types of TC remains unclarified. Only the SNP S836S was positively correlated with PTC but not with FTC. Despite the presence of some studies suggesting a possible role of the *RET* polymorphisms in MTC susceptibility, the data about the true association between MTC and *RET* variants were conflicting and extremely variable. The cause of this incoherence has been assigned to the limited number of patients enrolled (underpowered studies) and/or to the genetic variability among different populations (Weber & Eng, 2005; Fugazzola *et al.*, 2008). To avoid this high discrepancy in observed data, Weber & Eng (2005) in their study have suggested the application of rigorous parameters to dissect out the few true *RET* modifiers among a vast amount of random associations and proposed an approach termed reverse phenotyping. Here, the genotype/haplotype is identified in a cohort study and then associated with phenotypical factors, analogous to an unsupervised analysis used in global gene

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expression analysis that avoids assumptions based on the phenotype to identify hidden patterns.

On the other hand, it has been shown that a synonymous polymorphism in exon 2 (Ala45Ala, rs1800858), which occurred at a lower frequency among cases of MTC, may confer a protective allele against the development of MTC (Cebrian *et al.*, 2005). However, a recent study has shown that the G allele of this SNP remarkably increases the risk of TC (Huang & Yang, 2015). The mechanism by which the silent polymorphisms may act in the development of TC may include transcript stability, mRNA structure, and DNA protein binding and protein folding (Ho *et al.*, 2005). In the case of the nonsynonymous SNP G691S, the two amino acids, glycine in the wild-type RET protein and serine in the polymorphic *RET* variant, confer different electrochemical and conformational structures to the RET protein, and consequently influence the processing, folding, subcellular localization or function of the protein (Robledo *et al.*, 2003). More recently, the functional activity of this variant has been investigated by western blot analyses and the result has showed that the fraction of Ret9-G691S protein located at the plasma membrane level was overrepresented when compared to Ret9-WT, suggesting facilitated targeting at the cell membrane for this variant (Fugazzola *et al.*, 2008).

The relationship between the *RET* variants and the clinicopathological features of the disease is, yet, unclarified. In the study of Ceolin *et al.* (2012), no significant correlation has been observed between the *RET* variants analysed (G691S, L769L, S836S and S904S) and the clinicopathological parameters of the patients. Nevertheless, patients carrying haplotypes with three or four risk alleles had increased risk for lymph node and distant metastases at diagnosis. More recently, two *RET* SNPs (G691S and S904S) have been significantly associated with an increase in tumour size at diagnosis and a more aggressive disease (Santos *et al.*, 2014).

(iii) *XRCC1*

The X-ray repair cross complementing group 1 (*XRCC1*) gene encodes a scaffold protein involved in the repair of DNA single-strand breaks formed by ionizing radiation and alkylation damage. Numerous validated SNPs in the *XRCC1* gene have been identified, only three of which were most widely investigated including Arg194Trp on exon 6 (rs1799782, C/T), Arg280His on exon 9 (rs25489, G/A) and Arg399Gln on exon 10 (rs25487, G/A; Table S1). Over the last decade, several epidemiological studies have reported the association regarding *XRCC1* polymorphisms and TC risk (Fard-Esfahani *et al.*, 2011; Ryu *et al.*, 2011; Santos *et al.*, 2012). However, a significant heterogeneity among studies has been observed. In fact, increased risks for DTC have been shown for Arg194Trp and Arg280His genotypes while a mild reduction of risk has been estimated for the Arg399Gln polymorphism (Fard-Esfahani *et al.*, 2011). Nevertheless, it has been suggested in a meta-analysis involving eight different populations that the Arg399Gln polymorphism may be associated with decreased TC risk among the Caucasian population while the Arg194Trp SNP may be associated with a tendency of increased TC risk in the two larger sample size trials. But, no significant association has been found between the Arg280His polymorphism and TC (Qian *et al.*, 2012). On the other hand, in a more recent study only the Arg194Trp SNP has been associated with increased risk of TC (Wang *et al.*, 2015).

It has been suggested that the functional significance of the Arg194Trp polymorphism is due to its location in an evolutionarily conserved region, and the occurrence of chromosomal breaks is largely increased among cases with the Arg/Arg genotype (Vodicka *et al.*, 2007). For the Arg399Gln polymorphism, which is located within a relatively nonconserved region between conserved residues of the BRCA1 COOH terminus domain, it may be associated with higher sister chromatid exchange frequency and prolonged cell cycle delay in response to ionizing radiation (Hu *et al.*, 2001; Matullo *et al.*, 2006). Although in the same gene, these two different SNPs may exert influence on the XRCC1 activity in different ways, thus they might have different effects on cancer risk. Regarding the Arg280His polymorphism, which is located in the PCNA-binding region of the XRCC1 protein, it has been suggested that this variant could potentially alter the structure of *XRCC1* and its ability to interact with apurinic/apyrimidinic endonuclease (Yan *et al.*, 2009).

(iv) *Other genomic regions*

(a) *8q24*

SNPs in the 8q24 chromosomal gene-poor region, known as the 'gene desert', have been consistently implicated in GWAS as susceptibility loci for several cancer sites including TC (Neta *et al.*, 2012). Most of these SNPs are located in the area known as cancer susceptibility candidate 8 (*CASC8*; Table S1). Among the important SNPs of the 8q24 chromosomal region, there is the SNP rs6983267 which has been identified as a multi-cancer SNP. This polymorphic site has been investigated in a large and multi-ethnic study and the results showed a significant association between the rs6983267 G allele and TC. In fact, it has been suggested that this variant increases disease risk by ~13% (Sahasrabudhe *et al.*, 2015). However, the precise function of this locus is still under investigation.

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(b) *Near FOXE1 (9q22·33)*

Since it has been shown that the SNP rs965513 (located 57 kb upstream of the *FOXE1* gene on chromosome 9q22) was a new hot spot for TC susceptibility (Gudmundsson *et al.*, 2009; 2012), the relationship between this polymorphic site and TC risk has been independently replicated by subsequent studies (Wang *et al.*, 2013). It has been suggested to tag a functional variation near the *FOXE1* gene that contributes to an increased risk of developing TC. Besides, the variant has also been associated with low serum concentrations of thyroid stimulating hormone, and free thyroxin (Gudmundsson *et al.*, 2012). More recently, a significant correlation has been observed between this polymorphic site and more aggressive disease (advanced stage) (Penna-Martinez *et al.*, 2014)

(c) *14q13*

Among sequence variants that have been highly implicated in the aetiology of TC are the SNPs rs944289 and rs116909374, located on 14q13 in regions containing no annotated genes. These two polymorphic sites are located within two distinct but neighbouring linkage disequilibrium regions and have been associated with low serum concentration of thyroid stimulating hormone (Gudmundsson *et al.*, 2012). For the rs944289 polymorphism, it has been suggested that the functional significance of this variant is due to is location in the binding site for the CCAAT/enhancer binding proteins (C/EBP) α and β which activated the PTCSC3 (a thyroid-specific lincRNA gene) promoter. The presence of the risk allele of this SNP decreases promoter activation by weakening the binding affinity of the p42 C/EBPα and C/EBPβ transcription factors. (Jendrzejewski *et al.*, 2012).

3. Thyroid cancer and gene mutations

Mutation phenomenon is a rare event in TC compared to other types of malignancies where the mean percentage of mutations does not exceed one mutation per mega base. The signatures of mutational processes (mutational processes that leave idiosyncratic patterns of mutations) have been attributed mostly to unknown aetiologies (about 80%); however, the CpG deamination and off-target modification of DNA by the APOBEC proteins have also been involved but with relatively low frequencies (Martincorena & Campbell, 2015). According to the COSMIC database (COSMIC database, 2015) and the Cancer Gene Census database (www.sanger.ac.uk/CGP/census), few genes across the recurrently mutated cancer genes (79 genes among 198 genes in mutated cancer genes) have been affected by somatic mutations (non-synonymous coding substitution or small insertion deletion) and the most affected gene was the *BRAF* gene (Martincorena & Campbell, 2015).

Of the several mutations that have been found in TC, only few have characterized TC (Fig. 1). These mutations have affected genes involved in cell proliferation, protein synthesis and cell survival pathways, including rearrangements of *RET/PTC* and *PAX8/PPARγ*, activating point mutations in BRAF serine/threonine kinase, in the *RAS* proto-oncogenes, in the *TERT* gene or inactivating mutations in the tumour suppressors gene *TP53* (Table 2).

(i) *BRAF*

The *BRAF* proto-oncogene, a serine/threonine kinase, encodes a protein belonging to the raf/mil family of serine/threonine protein kinases. This protein plays a role in regulating the MAP kinase/ERK signalling pathway, which affects cell division, differentiation and secretion. Mutations in this gene are the most common genetic event in PTC. About 40 mutations have been identified in human thyroid tumours (COSMIC database, 2015) most of which are missense substitutions (36·8%). Most oncogenic *BRAF* mutations are located in the glycine-rich P loop (residues 462–471) and activation segment (residues 593–622) (Wan *et al.*, 2004; Michaloglou *et al.*, 2008).

The Glu586Lys, Val600Glu, Val600Asp, Val600Lys, Val600Arg and Lys601Glu mutations have been classified as a high kinase activity group, while nine other mutations, Gly464Glu, Gly464Val, Gly466Ala, Gly469Glu, Asn581Ser, Phe595Leu, Leu597Val, Leu597Arg and Thr599Ile, have been classified as an intermediate kinase activity group. However, four mutations, Gly466Glu, Gly466Val, Gly596Arg and Asp594Val, have lower kinase activity than basal wild-type BRAF activity (Wan *et al.*, 2004; Michaloglou *et al.*, 2008). The *BRAF* Val600 mutations mimic phosphorylation of Thr599/Ser602 (required for the activation of wild-type BRAF) and destabilize interactions between residues within the hydrogen bond network in BRAF, rendering the BRAF constitutively active (Michaloglou *et al.*, 2008; Fratev & Jonsdottir, 2009).

Over 90% of *BRAF* mutations are T1799A, resulting in a substitution of glutamine for valine at amino acid codon 600 (Val600Glu, $BRAF^{V600E}$), while other *BRAF* mutations are rarely reported. It has been shown that $BRAF^{V600E}$ leads to expression of a constitutively active kinase BRAF and was associated with aggressive clinicopathological characteristics (such as extrathyroidal invasion, lymph node metastasis and advanced tumour stage) (Lee *et al.*, 2009; Lin *et al.*, 2010), high tumour recurrence even with low stage disease (Xing *et al.*, 2005) as well as radioiodine treatment failure in PTC (Mian *et al.*, 2008) and high tumour related mortality (Elisei

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Table 2. *Mutational profile in thyroid cancer.*

Prevalence of common mutations in (%)			
Papillary carcinoma	Follicular carcinoma	Anaplastic carcinoma	Medullary carcinoma
BRAF: 40–45[a] *RET/PTC*: 10–20[a] *RAS*: 10–20[a]	*RAS*: 40–50[a] *PAX8/PPARγ*: 30–35[a] *TERT*: 14–36[b]	*TP53*: 50–80[a] *TERT*: 33–50[b] *BRAF*: 20–40[a] *RAS*: 20–40[a]	*RET/PTC*: 40–50[a] *RAS*: 25[a]

[a]Nikiforov & Nikiforova, 2011; [b]Muzza *et al.*, 2015.

et al., 2008). More recently, the $BRAF^{V600E}$ mutation has been investigated in a large cohort of PTC and it has been suggested that this genetic alteration represents a diverse group of tumours, consisting of at least four molecular subtypes, with variable degrees of thyroid differentiation. In this study, $BRAF^{V600E}$ has been associated with less-differentiated tumours enriched for classical and tall cell histology, and with distinct gene expression and DNA methylation patterns (Cancer Genome Atlas Research Network, 2014).

BRAF fusions have been identified in PTC (2·7%) with diverse gene patterns including *SND1* (0·6%) and *MKRN1* (0·2%). Some of these fusions supported *BRAF* signalling with expression and conservation of its kinase domain (MKRN1/BRAF), while others suggested an alternative activating mechanism (Cancer Genome Atlas Research Network, 2014).

(ii) *RET*

The *RET* proto-oncogene has been involved in the oncogenesis of medullary and papillary thyroid carcinomas by activation of tyrosine kinases either by point mutation or rearrangement.

There are now at least 30 types of *RET/PTC* rearrangements involving *RET* and 10 different genes. *RET/PTC1* (fusion *CCDC6* and *RET*) and *RET/PTC3* (fusion *NCOA4* and *RET*) are by far the most common rearrangements (COSMIC database, 2015). All of the rearrangements are due to DNA damage and result in the fusion of the *RET* tyrosine kinase domain to the 5′-terminal region of heterologous genes. *RET/PTC* rearrangements are very common in radiation-induced tumours but have been detected in variable proportions of sporadic (nonradiation associated) papillary carcinomas. Little is known about the clinical and pathologic features of the tumours featuring *RET/PTC* rearrangements. Several reports have failed to show correlation of *RET/PTC* rearrangements with clinicopathological markers of increased morbidity (Soares *et al.*, 1998; Tallini *et al.*, 1998). However, *RET/PTC1* and *RET/PTC3* have been associated with less and more aggressive tumours, respectively (Nikiforov *et al.*, 1997; Tallini *et al.*, 1998). A recent study performed on PTC has shown that chromosomal rearrangements including *RET* fusions are associated with younger age at diagnosis but not with risk of recurrence (Cancer Genome Atlas Research Network, 2014).

Activating somatic mutations in the *RET* proto-oncogene have been found in sporadic MTC with a frequency ranging from 23 to 69% of patients' tumours. The most frequent somatic mutation is Met918Thr in exon 16 (COSMIC database, 2015). This mutation has been involved in the initial event of the development of MTC and has a poor prognosis (Zedenius *et al.*, 1995; Romei *et al.*, 1996). Some other somatic missense point mutations as well as small oligonucleotide deletions or insertions in exons 10, 11, 13 and 15 have also been reported in sporadic MTC tumours but with a considerably lower frequency (Marsh *et al.*, 1996; Alemi *et al.*, 1997; Kalinin & Frilling, 1998; Uchino *et al.*, 1999; Bugalho *et al.*, 2000).

(iii) *RAS*

RAS family proteins are small GTPases that play a role in cellular growth, differentiation, adhesion and migration. *RAS* mutations are among the most common genetic alterations that have been observed in thyroid tumours and that lead to constitutive activation of the *RAS* proteins. Recent studies have reported that 10–20% of PTC and 40–50% of FTC harbour *RAS* mutations (Nikiforova *et al.*, 2003; Nikiforov, 2008; Nikiforov & Nikiforova, 2011). They have been associated with higher malignancy, poor prognoses, distant metastasis and shorter overall survival rate in poorly differentiated thyroid carcinomas (Garcia-Rostan *et al.*, 2003; Volante *et al.*, 2009). *RAS* mutations have also been reported in MTC (25%) and constitute a frequent molecular event in *RET*-negative sporadic MTC (Nikiforov & Nikiforova, 2011). However, their role in MTC tumourigenesis remains unclear (Oczko-Wojciechowska *et al.*, 2015).

In the Cancer Genome Atlas project result (Cancer Genome Atlas Research Network, 2014), *RAS* mutations have been identified as driver mutations for PTC and have been significantly associated with highly

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differentiated tumours enriched for follicular histology and low risk of recurrence.

The *RAS* genes consist of three families: *NRAS, HRAS* and *KRAS. RAS* point mutations mostly occur in codons 12, 13 and 61 (exons 2 and 3) (Lee *et al.*, 2013). The *NRAS* mutation at codon 61 (exon 3) accounted for 67–88% of all *RAS* mutations (Vasko *et al.*, 2003).

(iv) *PAX8/PPARγ*

PAX8/PPARγ is formed through the translocation t(2;3)(q13;p25) that fuses the promoter and 5′-coding portion of the thyroid-specific transcription factor *PAX8* gene to the full-length coding sequence of the nuclear receptor peroxisome proliferator-activated receptor-gamma 1 gene (Kroll *et al.*, 2000).

PAX8/PPARγ was presumed to be specific for FTC (Kroll *et al.*, 2000). Subsequent studies have confirmed *PAX8/PPARγ* presence in 30–40% of FTC and also found it in 2–13% of follicular adenomas (Dwight *et al.*, 2003; French *et al.*, 2003; Nikiforova *et al.*, 2003). Additional studies have reported the occurrence of *PAX8/PPARγ* rearrangement in the follicular variant of PTC, typically with low frequency (1–5%) and have reported occurrence occasionally in PTC (1·1%) (Armstrong *et al.*, 2014; Cancer Genome Atlas Research Network, 2014). The clinical course of the *PAX8/PPARγ* rearrangement has been indolent and the disease free survival was near 100% at 5 years (Yip *et al.*, 2015).

(v) *TERT*

Telomerase reverse transcriptase (*TERT*) is a ribonucleoprotein polymerase that maintains telomere repeat TTAGGG at the ends of chromosomes and consists of a protein component with reverse transcriptase activity and a RNA component that serves as a template (Harrington *et al.*, 1997). Recently, highly frequent mutations in the promoter region of *TERT* have been reported in many malignancies including TC (Horn *et al.*, 2013; Huang *et al.*, 2013; Killela *et al.*, 2013; Vinagre *et al.*, 2013). These mutations occur in two hot spot positions, located at -124 bp and -146 bp upstream from the ATG start site and confer enhanced *TERT* promoter activity putatively by generating a consensus binding site (GGAA) for E-26 transcription factors within the *TERT* promoter region (Huang *et al.*, 2013). In the Cancer Genome Atlas Project results, *TERT* mutations have been identified in 9·4% of the informative papillary tumours with 7% for C228T, 0·3% for C228A and 2·1% for C250T substitutions (Cancer Genome Atlas Research Network, 2014).

TERT promoter mutations are an indicator of clinically aggressive tumours. In fact, these mutations have been significantly associated with distant metastases, higher stage and persistent disease. Patients with DTC harbouring *TERT* promoter mutations have been submitted to more radioiodine treatments with higher cumulative dose and to more treatment modalities (Melo *et al.*, 2014). Also, *TERT* mutations have been correlated with disease specific mortality (Melo *et al.*, 2014). These associations were consistent with the published results of the Cancer Genome Atlas Research Project, where *TERT* mutations have been associated with older age as well as higher risk of recurrence and it has been suggested that these mutations may be used to identify high risk patients (Cancer Genome Atlas Research Network, 2014). Recently, it has been shown that the prognostic value of *TERT* mutations is significantly stronger than that of $BRAF^{V600E}$ (Muzza *et al.*, 2015).

(vi) *TP53*

Tumour suppressor protein (TP53) is a transcription factor that regulates the expression of target genes in response to diverse cellular stresses, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair or changes in metabolism. Mutations in the *TP53* gene are a rare event in well DTC (PTC and FTC), while they are frequent in more advanced forms of carcinoma. In fact, *TP53* mutations are a major event in poor DTC and anaplastic TC having a prevalence of 27 and 48%, respectively (Guerra *et al.*, 2013; Pita *et al.*, 2014). Virtually, all the mutations that have been reported are located in the hot spot region located between exons 5 and 9, where codon 273 is the one that is more often affected (Donghi *et al.*, 1993; Fagin *et al.*, 1993).

4. Conclusion

In summary, the genetic predisposition of TC has been highlighted in several studies. Numerous mutations and polymorphisms have been associated with this type of malignancy. The most affected genes are those involved in DNA repair, signal transduction and cell cycle control. Also, the importance of some genetic elements located on gene-poor regions was highlighted. These and other emerging molecular markers may provide additional approaches to thyroid tumour classification (such as suggested in the Cancer Genome Atlas Project), and may stimulate the development of novel approaches to tumour diagnosis and additional parameters for prognostic assessment as well as potential biologic therapeutic strategies. However, large prospective studies are required to further evaluate the diagnostic and prognostic power of these genetic elements.

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Declaration of interest

None.

Supplementary material

The online supplementary material can be found available at http://dx.doi.org/10.1017/S0016672316000057

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