REVIEW

Association studies in thyroid cancer susceptibility: are we on the right track?

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Abstract

It is widely accepted that thyroid cancer is strongly determined by the individual genetic background. In this regard, it is expected that sporadic thyroid cancer is the result of multiple low- to moderate-penetrance genes interacting with each other and with the environment, thus modulating individual susceptibility. In the last years, an important number of association studies on thyroid cancer have been published, trying to determine this genetic contribution. The aim of this review is to provide a comprehensive and critical evaluation of the associations reported so far in thyroid cancer susceptibility in case-control studies performed in both non-medullary (papillary and follicular) and medullary thyroid cancers, including their potential strengths and pitfalls. We summarize the genetic variants reported to date, and stress the importance of validating the results in independent series and assessing the functional role of the associated loci.

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Introduction

Thyroid cancer accounts for more than 1% of all malignancies, with an estimated annual incidence of 212 000 cases worldwide (Stewart *et al.* 2003, Ferlay *et al.* 2010). This number has been rapidly increasing in recent years (Liu *et al.* 2001), probably due to the improvement in diagnostic techniques (Davies & Welch 2006) and to the effect of environmental factors (Chen *et al.* 2009).

‘Thyroid cancer’ is a general term that includes two main groups of neoplasias, depending on the cell type affected by the malignant transformation. The most common thyroid cancers arise from follicular cells or thyrocytes, the thyroid hormone-producing cell lineage in the gland (DeLellis 2004). Among them, papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC) represent the two most common subtypes (85 and 10% respectively; Kondo *et al.* 2006), generally displaying a good prognosis with relatively few deaths (Ferlay *et al.* 2010), in contrast with their poorly differentiated counterparts that, although less frequent (5%), show a poor outcome. PTC displays a sex ratio female:male of around 3:1, suggesting the influence of unknown hormonal factors. The only well-established environmental factor related to PTC is exposure to ionizing radiation (Williams 2009). The PTC incidence

is remarkably high in developed countries, and when it spreads it usually metastasizes to local lymph nodes (DeLellis 2004). FTC lacks the morphological nuclear features of PTC, tends to be more aggressive, produces distant metastasis rather than lymph node invasion, and displays a more equilibrated sex ratio (around 2:1, female:male). It has been associated with a deficiency in iodine intake, thus being more frequent in developing countries (Woodruff *et al.* 2010).

The second group, referred to as medullary thyroid carcinoma (MTC), represents a minority of thyroid tumors (5%) and affects a different cell lineage, the parafollicular or C-cells, which are involved in calcitonin secretion (Randolph & Maniar 2000). This entity is histopathologically much more homogeneous than PTC and FTC, not only since the latter are subdivided into several morphological subtypes, but also with regard to the underlying genetic mechanisms driving the disease. MTC has a poor prognosis: up to 50% of patients develop local metastases to cervical and mediastinal nodal groups, and around 20% display distant metastases to the lung, liver, or bone at diagnosis (DeLellis 2004). Around 25% of MTC tumors are hereditary, with an autosomal dominant model of inheritance. Familial MTC (FMTC) appears as part of a rare inherited syndrome, multiple endocrine neoplasia 2 (MEN2; MIM#171400), which is subdivided

into three clinically distinct forms called MEN type 2A, MEN2B, and FMTC. These familial forms have germline gain-of-function mutations in the *RET* proto-oncogene in common. Since there is a variable clinical expression among *RET* germline mutation carriers, the existence of additional genetic risk factors acting as modulators of phenotype has been suggested (Cranston & Ponder 2003). Actually, it is accepted that some risk factors could act both as phenotype modifiers in FMTC and as genetic determinants of the sporadic forms of MTC.

Aim and statistical approach

The aim of this review is to provide a comprehensive overview of the association studies published on sporadic thyroid cancers. An exhaustive search of case-control studies in follicular cell-derived tumors (PTC and FTC) and MTC was performed in PubMed, including papers published in English up to 1 October 2010. The present review is focused on reported positive associations, preferably replicated, of individual SNPs and disease, rather than on combinations of SNPs or haplotype analyses. For an exhaustive review of both positive and negative results published until May 2008, we strongly recommend reading the review by Adjadj *et al.* (2009).

Since an insufficient sample size is one of the main pitfalls of association studies (Houlston & Peto 2004, McCarthy *et al.* 2008, Kere 2010), we estimated the statistical power of each study reviewed in this paper, using 'PS Power and Sample Size Calculations', version 2.1.31 (Dupont & Plummer 1998). Given that the attributable risk of common susceptibility alleles is expected to be around 1.1- to 1.6-fold (Fletcher & Houlston 2010), we calculated the power for detecting an odds ratio (OR) of 1.5, at a significance level of $\alpha=0.05$ in a χ^2 test. The variables influencing the power of each study considered in this analysis were the number of cases included (sample size, n) and the frequency of the variant in the general population (minor allele frequency (MAF)). We subsequently divided the published articles into two categories: those with enough statistical power to detect an OR of 1.5 and those that lacked this power.

Follicular cell-derived thyroid cancer

Different genetic alterations involving the axis RET/PTC-RAS-BRAF of the MAP kinase signaling pathway have been described as causal somatic changes in PTC and FTC (Kondo *et al.* 2006). Although involving the same pathway, specific and mutually exclusive changes have been described in each subtype,

involving rearrangements of *RET* and *NTRK1* with several partners (Greco *et al.* 1992, Santoro *et al.* 1992, Nikiforov 2002) and point mutations in *BRAF* (Davies *et al.* 2002) in PTC, and *PPARG* rearrangements (Kroll *et al.* 2000), and mutations in *RAS* (Manenti *et al.* 1994, Nikiforova *et al.* 2003) associated with FTC.

In addition, it is well established that follicular cell-derived thyroid cancer has a strong genetic component, since it displays the highest risk in first-degree relatives of probands among neoplasias not displaying regular Mendelian inheritance (Goldgar *et al.* 1994, Pal *et al.* 2001, Hemminki *et al.* 2004). Familial risk ratio calculations for these individuals range from 8.6- to 10.3-fold increased susceptibility to develop a thyroid tumor, strongly suggesting an inherited genetic background predisposing to the development of the disease. This observation, along with the fact that around 5% of these so-called non-MTC (NMTC) show familial aggregation, first led to the idea that familial NMTC could be explained by mutations in high-penetrance genes. Several linkage studies, using non-syndromic families displaying PTC in several members, have explored this possibility, reporting putative susceptibility loci at 14q31 (Bignell *et al.* 1997), 19p13.2 (Canzian *et al.* 1998, Prazeres *et al.* 2008), 1q21 (Malchoff *et al.* 2000), 2q21 (McKay *et al.* 2001), 8p23.1-p22 (Cavaco *et al.* 2008), 8q24 (He *et al.* 2009), and 6q22 (Suh *et al.* 2009). These findings were not uniformly replicated, and it is interesting to note that no high-penetrance gene has been identified so far in these putative loci suggested by linkage studies, probably due to the heterogeneity of the disease. In fact, it seems that the commonly used terms 'familial PTC' or 'familial NMTC' include different entities. This is reinforced by the specific phenotypic features displayed in each family, including several histological subtypes of PTC (follicular variant of PTC (FVPTC), oxyphilic tumors) and different additional manifestations (e.g. multinodular goiters, melanoma). Overall, whether familial NMTC is a distinct entity (Capezzone *et al.* 2008) that could be explained by mutations in high-penetrance genes or alternatively is the result of the combined effect of low- to moderate-penetrance loci remains unclear. The genetic contribution to familial NMTC, its controversies and clinical implications have been extensively reviewed in the past couple of years (Nose 2008, Vriens *et al.* 2009, Bonora *et al.* 2010, Khan *et al.* 2010) and will not be further commented on here.

Searching for low-penetrance genes by association studies in follicular cell-derived thyroid cancer

The great majority of NMTC, around 95% of cases, behaves as a sporadic entity. In this regard, NMTC is widely considered as a complex disease, in which

common genetic variants in low-penetrance genes (LPGs) may interact with each other and with the environment, modulating the individual susceptibility to this cancer (Adjadj *et al.* 2009, Sturgis & Li 2009). In this scenario, linkage analysis does not have the power to identify these LPGs. Thus, association studies, either genome-wide or carefully designed candidate gene approaches, may be more appropriate strategies to define genetic risk factors (Houlston & Peto 2004, Milne & Benitez 2008).

Moderate sample sizes lead to few replicated associations

The identification of LPGs related to follicular cell-derived carcinoma is an arduous task due to the heterogeneity and relative rarity of the disease. Indeed, many of the studies reviewed here were rather small in sample size, and only a few polymorphisms were replicated in independent cohorts of cases and controls. A summary of the positive associations published for individual polymorphisms and PTC/FTC susceptibility is shown in Tables 1 and 2 and Fig. 1A, and will be expanded throughout this review.

Models of inheritance and tumor types

Almost half of the studies reported a significant association fitting a recessive model, that is, when both alleles of the associated variant are present in homozygosis. This brings concern about the strength of some of these associations, since in several cases the number of polymorphic homozygous carriers was relatively small, conditioning the power of the studies and their reproducibility. Furthermore, and also influenced by sample size requirements, almost all studies reviewed here describe associations with PTC (accounting for 85% of cases) or with PTC+FTC considered together. Only one single study specifically reports an association between FTC and *VDR* variants (Penna-Martínez *et al.* 2009), although the low number of cases considered ($n=40$) requires a cautious interpretation.

Biases in population ancestry

In concordance with other diseases, most of the reported associations come from studies performed in populations of Caucasian ancestry. Some exceptions to this tendency are two papers published by a group studying patients from Taiwan (Hsiao *et al.* 2007, Chiang *et al.* 2008), who reported significant associations for *XRCC1* (see below) and *VEGFA*, respectively, in PTC. Other examples are a study in a cohort from southern Tunisia (Rebai *et al.* 2009), and two reports

based on Saudi Arabian populations (Siraj *et al.* 2008a,b), the latter reporting a significant twofold increased risk for a non-synonymous variant of the *CYP1A1* gene in a group of 202 PTC patients.

Genotyping methods

Regarding genotyping methods, in contrast with other cancers and polygenic diseases, only a few studies relied on high throughput and/or array-based technologies (Hsiao *et al.* 2007, Chiang *et al.* 2008, Akulevich *et al.* 2009, Gudmundsson *et al.* 2009, Landa *et al.* 2009, Takahashi *et al.* 2010), and a restriction fragment length polymorphism approach was the predominantly used technique (more than 15 studies, see Tables 1 and 2). This fact supports the notion that much remains to be explored in the thyroid cancer susceptibility field.

Candidate gene versus genome-wide association studies approaches

Overall, the great majority of case-control studies are candidate gene or pathway-driven approaches, with the exception of two recently published genome-wide association studies (GWAS) in thyroid cancer (Gudmundsson *et al.* 2009, Takahashi *et al.* 2010), which will be commented on later on in this review. Among the pathways and metabolic processes studied, the most common ones are DNA repair due to the known relation between thyroid cancer and ionizing radiation (Ron *et al.* 1995, Williams 2002), cell cycle control along with the RET signaling pathway, and detoxification processes (related to the cytochrome P450 (*CYP*) and glutathione-S-transferase (*GST*) gene families).

In this regard, several studies have reported associations between polymorphisms in DNA damage response genes and PTC. One of the validated variants in this pathway is rs861539, which leads to a p.Thr241Met amino acid change in the homologous recombination gene *XRCC3*; this gene is also associated with other malignancies, especially breast cancer (Economopoulos & Sergentanis 2010). The association with thyroid cancer was first reported in a hospital-based study that included 134 patients and 161 controls, reporting a 2.1-fold increased risk (95% confidence interval (CI) = 1.3–3.4; $P=0.004$) for polymorphic homozygous carriers (Sturgis *et al.* 2005). These results were recently validated in another hospital-based study with 109 NMTC patients and 217 controls (OR = 2.0; 95% CI = 1.1–3.6; $P=0.026$; Bastos *et al.* 2009). Although the ORs for rs861539 are probably overestimated due to the relatively small sample size of both studies, the fact that two independent groups reported similar risks fitting the same model supports the involvement of *XRCC3* in

Table 1 Variants associated with the development of NMTC in well powered and/or validated studies

Chr location	Gene	Studied variant (synonyms) ^a	Cases	Controls	MAF cases; MAF controls (%)	OR (95% CI) ^b	P value ^b	Best fitting model	Power to detect an OR=1.5	Power to detect an OR=2.0	Genotyping platform	Functional assessment	Matching or adjustment criteria	Ref. ^c	Replicated?
9q22.33	Near FOXE1 (57 kb)	rs65513 (g.29720641A>G)	962 (PTC + FTC)	38 923	48.0; 34.1	1.75 (1.59–1.94)	1.7 × 10 ^{−27}	M	100.0	100.0	Illumina, centaurus, SNaPshot	Associated with lower serum TSH and T ₄ and higher T ₃	N/A	1	2, 3
9q22.33	Near FOXE1 (57 kb)	rs65513 (g.29720641A>G)	660 (Rx PTC)	1268	47.4; 35.7	1.65 (1.43–1.91)	4.8 × 10 ^{−12}	M	98.5	100.0	Illumina	N/A	Age, gender, origin, radiation exposure	3	1
9q22	FOXE1	rs1867277 (c.−283 G>A)	984 (PTC)	1028	51.5; 42.8	1.49 (1.30–1.70)	5.9 × 10 ^{−9}	M	99.5	100.0	Illumina + TaqMan	Determines binding of USF1/2 factors	Age, gender, origin	2	1, 3
9q22	FOXE1	rs1867277 (c.−283 G>A)	660 (Rx PTC)	820	N/A	1.46 (1.27–1.71)	4.5 × 10 ^{−7}	M	N/A	N/A	+KASPar	N/A	Age, gender, origin, radiation exposure	3	2
14q13.3	Near NKX-1 (249 kb)	rs44289 (g.17649246C>T)	1006 (PTC + FTC)	38 345	35.7; 42.7	1.37 (1.24–1.52)	2.0 × 10 ^{−9}	M	100.0	100.0	Illumina, centaurus, SNaPshot	Associated with lower serum TSH	N/A	1	No
8q24.21	Gene desert	rs6983267	485 (PTC)	1910	51.4; 47.6	1.37 (1.02–1.82)	0.04	R	97.8	100.0	RFLP (NnuCI)	N/A	N/A	4	Multisite cancer associated region
5q34	Pre-miR-146a	rs2910164 (c.60G>C)	608 (PTC)	901	26.2; 23.9	1.62 (1.30–2.00)	7.0 × 10 ^{−6}	O	93.3	100.0	Sequencing and/or SNaPshot	Influences miR-146a and its target genes	N/A	5	No
19q13.2	XRCC1	rs25487 (p.Arg399Gln)	255 (PTC)	596	32.7; 36.2	0.70 (0.59–0.93)	0.03	D	76.1	99.6	RFLP (MspI)	N/A	N/A	6	7
19q13.2	XRCC1	rs25487 (p.Arg399Gln)	251 (PTC + FTC)	503	27.3; 34.8	0.70 (0.50–1.00)	0.049	D	72.9	99.3	RFLP (NciI)	N/A	Age	7	6
19q13.2	XRCC1	rs1799782 (p.Arg194Trp)	283 (PTC + FTC)	469	34.1; 28.9	1.85 (1.11–3.07)	0.044	R	72.4	99.3	TaqMan	N/A	Origin	8	No
8q24	TG	rs1133076 (p.Arg2530Gln)	304 (NMTC)	396	44.7; 51.8	1.60 (1.10–2.30)	0.015	M	74.7	99.3	RFLP (TaqI)	N/A	Age, gender, ancestry	9	No
11p11.2	PTPRJ	rs4752904 (p.Asp872Glu)	299 (PTC)	339	41.1; 46.2	1.61 (1.15–2.25)	0.0053	R	70.7	98.8	Sequencing	N/A	N/A	10	No
14q32.3	XRCC3	rs661539 (p.Thr241Met)	109 (PTC + FTC)	217	44.0; 40.4	2.00 (1.10–3.60)	0.026	R	40.6	83.7	RFLP (NlaIII)	N/A	Age, gender, origin	11	12
14q32.3	XRCC3	rs661539 (p.Thr241Met)	134 (NMTC (PTC + FTC + Hürthle))	161	40.7; 29.8	2.10 (1.30–3.40)	0.004	R	37.6	81.5	RFLP (NlaIII)	N/A	Age, gender, ancestry, radiation exposure, smoking and alcohol consumption	12	11
17p13.1	TP53	rs1042522 (p.Arg272Pro)	123 (PTC)	197	39.2; 23.0	1.70 (1.17–2.46)	0.006	M	35.2	78.8	TaqMan	N/A	Origin, age (± 3 years)	6	13, 14
17p13.1	TP53	rs1042522 (p.Arg272Pro)	98 (PTC + FTC)	153	37.2; 34.3	7.02 (1.93–25.59)	0.0015	R	33.5	75.2	Allele-specific PCR	N/A	Age, gender	13	6, 14
17p13.1	TP53	rs1042522 (p.Arg272Pro)	68 (Rx PTC)	313	39.0; 33.1	2.00 (1.16–3.45)	<0.05	R	32.4	72.9	RT-PCR + sequencing	N/A	N/A	14	6, 13

The table is sorted by power of the study, replication status, gene and variant: ^ars code for each SNP is always provided, as well as other aliases; ^bcalculated by us when not provided. MAF, minor allele frequency; OR, odds ratio; 95% CI, confidence interval 95%; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; Rx PTC, radiation-induced PTC; NMTC, non-medullary thyroid cancer. M, multiplicative; R, recessive; D, dominant; Ref., reference. ^cReferences: 1, Gudmundsson *et al.* (2009); 2, Landa *et al.* (2009); 3, Takahashi *et al.* (2010); 4, Wokorczyk *et al.* (2008); 5, Jazdzewski *et al.* (2008); 6, Akulevich *et al.* (2009); 7, Ho *et al.* (2009); 8, Chiang *et al.* (2008); 9, Matakidou *et al.* (2004); 10, Iuliano *et al.* (2010); 11, Bastos *et al.* (2009); 12, Sturgis *et al.* (2009); 13, Granja *et al.* (2004); 14, Rogounovitch *et al.* (2006).

Table 2 Additional variants associated with the development of NMTC, which should be further validated

Chr location	Gene	Studied variant (synonyms) ^a	Cases	Con.	MAF cases; MAF controls (%)	OR (95% CI) ^b	P value ^b	Best fitting model	Power to detect an OR=1.5	Power to detect an OR=2.0	Genotyping platform	Functional assessment	Matching or adjustment criteria	Ref. ^d	Rep.?
10q11.2	RET	rs1800861 (p.Leu769Leu)	247 PTC	319	22-0; 24-0	2.22 (N/A)	<0.05	R	57.5	96.4	RFLP (TaqI)	N/A	Age, gender, origin	1	No
10q11.2	RET	rs1800860 (p.Ala432Ala)	101 NMTC	174	29-2; 26-4	1.64 (1.00-2.69)	0.049	D	32.7	74.6	RFLP (AseI)	N/A	Age, gender, ancestry	2	No
11q22-q23	ATM	rs1801516 (p.Asp1853Asn)	255 PTC	596	11-6; 15-7	0.69 (0.45-0.86)	0.03	M	57.4	96.4	RFLP (AflII)	N/A	N/A	3	No
11q22-q23	ATM	rs664677 (rs222-77 T>C)	132 PTC	398	42-8; 38-9	1.84 (1.10-3.24)	0.03	R	52.0	93.1	RFLP (RsaI)	N/A	N/A	3	No
15q24.1	CYP1A1	rs1048943 (p.Ile462Leu)	202 PTC	511	30-9; 19-4	2.07 (1.49-2.88)	<0.0001	D	55.0	95.3	RFLP (BsaI)	N/A	Age, ancestry	4	No
15q24.1	CYP1A1	rs4646903 (CYP1A1m1, CYP1A1*2A)	136 PTC	277	13-2; 20-2	0.56 (0.36-0.89)	0.015	D	39.0	83.4	RFLP (MspI)	N/A	Age, gender, origin	5	No
6p12	VEGFA	rs699947 (CYP1A1*2A)	297 (PTC +FTC)	249	28-1; 23-3	1.36 (1.02-1.81)	0.039	A	55.0	95.8	TaqMan	N/A	Age, gender	6	No
12p13.1-p12	CDKN1B	rs34330 (-2578C>A)	129 FVPTC	385	31-0; 25-3	2.12 (1.09-4.15)	0.023	R	45.7	89.3	TaqMan	Reduces CDKN1B mRNA levels	Age, gender	7	No
22q13.1	CYP2D6	rs3892097 (CYP2D6*4)	187 PTC	256	13-4; 21-7	0.28 (0.09-0.93)	0.037	R	45.3	89.9	RFLP (BstNI)	N/A	N/A	8	No
8p22	NAT2	rs1801280 (p.Ile114Thr, NAT2*5)	152 (PTC +FTC)	148	44-1; 53-0	0.58 (0.35-0.98)	0.04	R	41.2	83.3	LightCycler real-time PCR	N/A	N/A	9	No
12p13-p12.2	RAD52	rs11226 (c.744C>T)	210 PTC	228	31-2; 18-0	1.53 (1.03-2.28)	0.036	D	41.0	86.6	RFLP (HaeIII)	N/A	Age, ancestry	10	No
12p13-p12.2	RAD52	rs4987206 (p.Gln221Glu)	210 PTC	217	18-6; 1-4	17.58 (7.44-41.58)	<0.0001	D	8.5	16.7	RFLP (BlnA1)	N/A	Age, ancestry	10	No
22q11.23	GSTT1	GSTT1 null	47 FVPTC	247	25-3; 42-7	0.31 (0.10-0.97)	0.044	R	24.8	58.2	Multiplex PCR	N/A	N/A	8	4
22q11.23	GSTT1	GSTT1 null	223 PTC	513	N/A	3.48 (2.48-4.88)	<0.0001	R	N/A	N/A	Multiplex PCR	N/A	Age, ancestry	4	8
12q13.11	VDR	rs2228570 (rs10735810 ^c , c.2T>A)	40 FTC	321	50-0; 34-6	3.75 (1.45-9.70)	0.04	R	23.2	54.7	RFLP (FokI)	N/A	N/A	11	No
12q13.11	VDR	rs7975232 (c.1025-49G>T)	40 FTC	321	57-5; 42-5	3.80 (1.45-9.98)	0.04	D	22.9	53.9	RFLP (ApaI)	N/A	N/A	11	No
22q12.1	CHEK2	rs17879961 (p.I157T)	173 NMTC	4000	4-3; 2-4	1.90 (1.08-3.24)	0.04	D	20.9	48.1	RFLP (PstI)	N/A	Origin	12	No
1p13.3	GSTM1	GSTM1 null	42 PTC (stage III/IV)	247	63-6; 76-1	0.50 (0.26-0.96)	0.036	R	13.4	28.9	Multiplex PCR	N/A	N/A	8	4
1p13.3	GSTM1	GSTM1 null	223 PTC	513	N/A	0.72 (0.52-0.99)	0.047	R	N/A	N/A	Multiplex PCR	N/A	Age, ancestry	4	8

The table is sorted by power of the study, replication status, gene and variant. ^ars code for each SNP is always provided, as well as other aliases. ^bcalculated by us when not provided. ^cThis SNP was originally reported by the author, but was later merged into rs2228570. MAF, minor allele frequency; OR, odds ratio; 95% CI, confidence interval 95%; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; FVPTC, follicular variant of PTC; NMTC, non-medullary thyroid cancer; RFLP, restriction fragment length polymorphism. Con., controls; R, recessive; D, dominant; M, multiplicative; A, additive; Ref., reference; Rep., replicated. ^dReferences: 1, Lesueur *et al.* (2002); 2, Ho *et al.* (2005); 3, Akulevich *et al.* (2009); 4, Siraj *et al.* (2008a); 5, Bufalo *et al.* (2006); 6, Hsiao *et al.* (2007); 7, Landa *et al.* (2010); 8, Lemos *et al.* (2007); 9, Hernandez *et al.* (2008); 10, Siraj *et al.* (2008b); 11, Penna-Martinez *et al.* (2009); 12, Cybulski *et al.* (2004).

Figure 1 Schematic representation of the genetic contribution of gene variants to thyroid cancer, depending on the odds ratio and allele frequency of the reported variants. (A) Genes associated with non-medullary thyroid cancer; (B) Genes associated with medullary thyroid cancer.

PTC susceptibility. Similarly, two groups reported an association between rs25487 (p.Arg399Gln amino acid change) in the *XRCC1* gene and NMTC. Both groups reported a similar protective effect under a dominant inheritance model, in well-powered cohorts of 255 PTC versus 596 controls (OR=0.70; 95% CI=0.59–0.93; $P=0.03$; Akulevich *et al.* 2009) and 251 NMTC versus 503 controls (OR=0.70; 95% CI=0.50–1.00; $P=0.049$; Ho *et al.* 2009). Surprisingly, Akulevich *et al.* (2009) did not find any association with *XRCC1*, involved in the base excision repair pathway, when considering only radiation-exposed PTC cases and controls, but this could be an effect of the small sample size after stratification ($n=123$ vs 197). Another non-synonymous polymorphism (rs1799782, p.Arg194Trp) in *XRCC1* was also associated with thyroid cancer in a cohort of 283 cases and 469 controls from Taiwan (OR=1.85; 95% CI=1.11–3.07; Chiang *et al.* 2008). The difference between this risk-conferring SNP and the protection associated with rs25487 could be due to a different effect of each amino acid change or to differences in population-specific allele frequencies

(Consortium 2003). For additional comments about these associations between DNA repair genes and thyroid cancer, we recommend reading a recent editorial written by some of the authors who originally reported some of these results (Sturgis & Li 2009).

Another group of genes frequently studied in NMTC codes for proteins involved in cell cycle regulation. Common germline variations within the *TP53* tumor suppressor gene were extensively studied (Granja *et al.* 2004, Bufalo *et al.* 2006, Rogounovitch *et al.* 2006, Akulevich *et al.* 2009) and focused on the polymorphism rs1042522 (p.Arg72Pro). First, arginine homozygosity was described as protective (OR=0.50; 95% CI=0.29–0.86) in a group of 68 radiation-induced PTC (Rogounovitch *et al.* 2006), that later was expanded ($n=123$) by the same group, reporting an increased risk (OR=1.70; 95% CI=1.17–2.46) for homozygous proline carriers (Akulevich *et al.* 2009). A risk associated to proline homozygosity had been already reported (Granja *et al.* 2004), although this was overestimated due to the low sample size and a very significant Hardy–Weinberg disequilibrium among controls. Overall, although confirmatory studies in *TP53* with larger sample sizes may be desirable, there is evidence suggesting that variation at this locus could play a role in PTC pathogenesis. Furthermore, it is widely known that somatic mutations inactivating p53 are frequently found in thyroid tumors that become undifferentiated and have an aggressive clinical behavior (Kondo *et al.* 2006).

From gene desert regions to scarcity of functional studies

There are some other examples of positive associations for NMTC found in genes related to cell cycle control. In this regard, two apparently independent variants in the cell cycle checkpoint kinase *ATM* were associated with PTC (Akulevich *et al.* 2009). Another checkpoint regulator, *CHEK2*, was also associated with PTC in a multicancer study, mostly focused on breast and prostate neoplasias but also including 173 thyroid cancer cases, in which the change p.Ile157Thr seemed to confer a 1.90-fold increased risk (Cybulski *et al.* 2004). The same group also reported an association with PTC for rs6983267 (OR=1.37; 95% CI=1.02–1.82), located in the gene desert region 8q24, in a well-powered study that included an expanded series of 485 cases (Wokolorczyk *et al.* 2008).

Unfortunately, despite this tendency of studying genes and variants that a priori are supposed to have a straightforward biological effect, very few studies have assessed the functional role of the associated alleles (Jazdzewski *et al.* 2008, Landa *et al.* 2009, 2010).

In this regard, a study in a Spanish population found an association between the promoter variant rs34330

(c. -79C>T) of the cyclin-dependent kinase inhibitor *CDKN1B* (p27) and the risk of developing a specific subtype of thyroid cancer: the FVPTC (Landa *et al.* 2010). The relevance of this study mostly relies on the functional assays performed, which demonstrated an effect of the associated variant on the transcription rate of this gene, providing insight into the underlying mechanism that could drive the reported risk.

The role of polymorphic variants in non-coding RNA sequences, or microRNAs, has been explored in a single and elegant study, which associated in a large group of 608 cases the heterozygous status of SNP rs2910164, located in the pre-miR-146a, with an increased risk of developing PTC (Jazdzewski *et al.* 2008). This paper also provided a comprehensive view of the effect of this variant, which influences the stability and processing of pri-miR, the expression of mature miR-146a, and the levels of its relevant target genes, *TRAF6*, *IRAK1*, and *CCDC6*.

The importance of biological interpretation

As mentioned above, other pathways frequently assessed for NMTC are those involved in the detoxification of endogenous or xenobiotic compounds (related to *CYP* and *GST* gene families), and the *RET* signaling pathway.

Several detoxification genes have been associated with thyroid cancer, and some of the studies are worth mentioning. Regarding phase I enzymes, significant associations have been reported for rs4646903 of *CYP1A1* in a group of 136 PTC cases (Bufalo *et al.* 2006) and for rs3892097 in *CYP2D6* in 187 PTC cases (Lemos *et al.* 2007). About phase II enzymes, one study associated the *GSTM1* null genotype to PTC of stages III/IV ($n=42$), and *GSTT1* null status to FVPTC ($n=47$; Lemos *et al.* 2008). Both associations were also reported in a much larger study, with 223 Saudi Arabian PTC patients (Siraj *et al.* 2008b), although in an opposite direction for *GSTT1*. Combinations of *GSTM1*+*T1* null genotypes have also been described in NMTC, although they should be interpreted with caution, since the consideration of multiple variants led to an important reduction in sample size (Morari *et al.* 2002, Granja *et al.* 2004, Ho *et al.* 2006). Finally, the genotype status of *N*-acetyltransferase (*NAT2*) also seemed to be associated with PTC (Hernandez *et al.* 2008, Guilhen *et al.* 2009). Overall, although there is some evidence for association of detoxification enzyme polymorphisms with thyroid cancer, the biological link remains unclear.

On the contrary, key thyroid follicular cell pathways such as the *RET* signaling pathway and iodine metabolism appear obvious processes to explore the genetic susceptibility to thyroid cancer. In fact, two studies have assessed the role of SNPs in *RET*, which is frequently

rearranged in PTC tumors (Santoro *et al.* 1992, Nikiforov 2002), reporting associations for the synonymous SNPs rs1800861 and rs1800860 in 247 PTC (Lesueur *et al.* 2002) and 101 NMTC (Ho *et al.* 2005) patients respectively. Another example of how useful molecular knowledge can be for searching genetic variants is provided by a very recent study, which linked rs4752904 (p.Asp872Glu) in the *PTPRJ* gene with PTC susceptibility (OR=1.61; 95% CI=1.15–2.25; Iuliano *et al.* 2010). Interestingly, *PTPRJ* encodes a tyrosine phosphatase which is known to be able to dephosphorylate RET and which has an established role as a tumor suppressor gene in thyroid carcinogenesis (Trapasso *et al.* 2000, Iervolino *et al.* 2006). Similarly, the thyroglobulin gene (*TG*), due to its fundamental role in iodine metabolism and thyroid hormone production, is a priori a good candidate to study, and has been consequently associated with NMTC (Matakidou *et al.* 2004). In this work, performed in a group of 304 patients, the non-synonymous variant rs1133076 (p.Arg2530Gln) conferred a 1.6-fold increased risk of developing the disease.

FOXE1: thyroid related, extensively validated, and functionally assessed

Finally, in the last 2 years, three multistage, well-powered case-control studies, including two GWAS (Gudmundsson *et al.* 2009, Takahashi *et al.* 2010) and a candidate gene approach (Landa *et al.* 2009), have strongly pointed out the involvement of the forkhead box E1 factor, encoded by the *FOXE1* gene, in PTC susceptibility. These findings probably represent the best validated genetic factor identified in PTC so far, and represent a good example of an a priori biologically relevant locus that has been extensively identified as a LPG. FOXE1 (formerly known as ‘thyroid transcription factor 2’) is a pioneer transcription factor, whose action is essential for the development, differentiation, and hormone responsiveness of the thyroid gland (Cuesta *et al.* 2007). FOXE1 is also necessary for the maintenance of the differentiated state of the thyroid, as it is involved in regulating the transcription of thyroid-specific genes such as the *TG* and *TPO* (Zannini *et al.* 1997).

The first GWAS ever performed on thyroid cancer reported a top association for tagSNP rs965513, located in an intergenic region at 9q22.33, 57 kb upstream of *FOXE1* (Gudmundsson *et al.* 2009). The authors described an OR=1.75 (95% CI=1.59–1.94; $P=1.7 \times 10^{-27}$) based on 962 PTC+FTC patients, fitting a multiplicative model, that is, a model in which the risk conferred by an allele is increased r -fold for heterozygotes and r^2 -fold for homozygotes (Lewis 2002). They also correlated the risk allele with low concentrations of TSH and thyroxine (T_4), and high levels of tri-

iodothyronine (T_3). In parallel, another group, based on a cohort of 984 PTC, associated the rs1867277 (c.-283G>A) variant, located in the *FOXE1* 5' UTR, with an increased risk of developing the disease (OR=1.49; 95% CI=1.30–1.70; $P=5.9 \times 10^{-9}$; Landa *et al.* 2009). These latter authors also proposed a functional mechanism, in which the associated risk allele of rs1867277 affects *FOXE1* transcription through a differential recruitment of USF1/USF2 transcription factors. Finally, a third study, involving 660 radiation-induced PTC from the Chernobyl accident (Takahashi *et al.* 2010), extended and validated the association of both rs965513 (OR=1.65; 95% CI=1.43–1.91; $P=4.8 \times 10^{-12}$) and rs1867277 (OR=1.48; 95% CI=1.27–1.71; $P=4.5 \times 10^{-7}$). The large sample sets studied, the similar OR and models of inheritance reported in these three studies, as well as the functional assessment of *FOXE1* variants, highlight the importance of this locus as a LPG in PTC. Nevertheless, since those variants are quite common in the general population, it remains a challenge to identify other genetic factors that could complete the picture and elucidate the still unexplained heritability in PTC.

A schematic representation of the multiple factors affecting NMTC is shown in Fig. 2.

Negative results

In addition, some variants were shown not to be associated with NMTC forms. Among them, SNPs in *ERCC2* (Silva *et al.* 2005), *MTF1* (Akulevich *et al.* 2009), *FAS* (Ho *et al.* 2008), *APEX1* (Chiang *et al.* 2008), *PARP1* (Chiang *et al.* 2008), *GSTO1* (Granja *et al.* 2005, Bufalo *et al.* 2006), *GSTP1* (Hernandez *et al.* 2003, Gaspar *et al.* 2004), *TSHR* (Matakidou *et al.* 2004, Lonn *et al.* 2007), *RAD51* (Sturgis *et al.* 2005, Bastos *et al.* 2009), *RAD52* (Sturgis *et al.* 2005), *BRCA1* (Sturgis *et al.* 2005), *BRCA2* (Sturgis *et al.* 2005), and *XRCC7* (Sturgis *et al.* 2005), were included in relatively well-powered studies and seem not to play a role in the disease. On the other hand, studies on *CDKN2A* (Debniak *et al.* 2006), *EGFR* (Rebai *et al.* 2009), and *MYCL1* (Yaylim-Eraltan *et al.* 2008), lacked the necessary power and led to inconclusive results that should be further expanded.

Medullary thyroid cancer

As described above, about 75% of MTC, the thyroid cancer subtype arising from parafollicular cells, appear as a sporadic disease (Randolph & Maniar 2000). Those

Figure 2 Model of genetic and environmental heterogeneity in individual predisposition to non-medullary thyroid cancer. In this model, several factors are necessary for the development of PTC or FTC. The individual genetic background, either monogenic in familial cases or polygenic in sporadic cases, determines a thyroid cancer-prone condition for a given individual. In addition, exogenous harmful agents, and the acquisition of deleterious somatic mutations in specific genes ultimately determine the development of the disease.

forms, for which there are no environmental causes described, could also behave as a complex disease, driven by multiple polymorphic variants that might determine individual susceptibility.

Association studies in MTC

MTC, although very homogeneous, which avoids the need for stratification, is much less frequent than thyroid cancers derived from follicular cells. The resulting difficulty in recruiting enough patients to reach the necessary power has probably determined the relative rarity of association studies on sporadic MTC (sMTC) published so far. Case-control studies on MTC have unanimously adopted candidate gene or pathway-driven approaches, with several studies assessing the role of the same variants in different populations. So far, variations of the *RET* proto-oncogene and its effectors have monopolized the efforts in looking for low-penetrance loci. Positive associations are summarized in Table 3 and Fig. 1B, and commented on below.

RET polymorphisms in sMTC

Given the essential role of both germinal (95%) and somatic (30–60%) *RET* mutations, researchers have focused their efforts on the putative role of polymorphic variants within this locus. Moreover, because polymorphisms are relatively common in the population, they may present a much higher attributable risk in the general population than rare mutations in high-penetrance cancer susceptibility genes such as *RET* (de Groot *et al.* 2006). Several *RET* SNPs have been postulated as modifiers of phenotype in the familial forms of the disease (Gil *et al.* 2002, Robledo *et al.* 2003, Lesueur *et al.* 2006, Tamanaha *et al.* 2009, Shifrin *et al.* 2010). This modifier effect on familial forms has already been reviewed (Weber & Eng 2005) and will not be further commented on here. In addition, some of those variants were studied as LPGs in sMTC.

A major limitation in these studies is the sample size, with only some series reaching a 100 patients, and none of them including an intra-study validation set of the results. Another major pitfall is the few and controversial functional results for these variants. This, along with the LD pattern of the *RET* locus, makes it difficult to interpret if the associated SNPs are in fact markers of other unknown functional variants, or are themselves influencing *RET* function.

In the first work studying *RET* SNPs, the synonymous variant rs1800862 (p.Ser836Ser) was significantly associated with MTC, and correlated with the presence of the *RET* somatic mutation M918T (Gimm *et al.* 1999). This association was reproduced in two additional, rather small, populations (Ruiz *et al.* 2001, Siqueira *et al.* 2010).

Nevertheless, several other groups failed to replicate this signal (Wiench *et al.* 2004, Baumgartner-Parzer *et al.* 2005, Cebrian *et al.* 2005), thus leading to the conclusion that the *RET* rs1800862 variant may only be a risk factor in certain populations.

On the other hand, the non-synonymous variant rs1799939 (p.Gly691Ser) has been associated with about a 1.7-fold risk per allele for developing sMTC in two larger studies (Elisei *et al.* 2004, Cebrian *et al.* 2005). These two studies postulated, through a functional assessment of *RET* transcription and splicing, that rs1799939 could be the functional variant, but the results were inconclusive. Although one study did not replicate this association (Fugazzola *et al.* 2008), others reported significant results for rs1800863 (p.Ser904Ser; Cebrian *et al.* 2005), which is in LD with p.Gly691Ser, thus reinforcing this association. Furthermore, this variant was identified as an independent predictor of a higher basal calcitonin synthesis rate in sMTC patients (Cardot-Bauters *et al.* 2008).

The picture gets even more complicated since studies with other *RET* variants, such as the synonymous rs1800861 (p.Leu769Leu) and 3'UTR rs3026782, have led to contradictory results (Wiench *et al.* 2004, Cebrian *et al.* 2005, Weinhaeusel *et al.* 2008, Sromek *et al.* 2010). In addition, some intronic polymorphisms have been associated with sMTC, such as rs2565206 (IVS1-126 G>T; Borrego *et al.* 2003, Fernandez *et al.* 2004, 2006) and in LD with p.Ser836Ser, and rs2472737 (IVS14-24; Baumgartner-Parzer *et al.* 2005).

Overall, it seems that there are two independent association signals in the *RET* gene, which are the carriers of either G691S/S904S or S836S/IVS1-126G>T haplotypes, and which are more prone to develop sMTC through a still unknown mechanism that could involve these and/or other functional variants. Nonetheless, a comprehensive study of unselected *RET* variants and their LD pattern in several unbiased populations will be desirable to better define the associated risks in this locus.

Other genes related to MTC

Besides the *RET* proto-oncogene, some other loci have been studied in sMTC. One of them is the GDNF family receptor α 1, which acts as a co-receptor of RET, and is encoded by the *GFRA1* gene. The promoter variant rs45568534 (c.-193C>G) was first associated with sMTC development in a small group of patients, who also showed a tendency toward a higher expression of tumoral GFRA1 at both the mRNA and the protein levels when carrying the risk alleles (Gimm *et al.* 2001b). This association was not reproduced in larger case-control studies (Borrego *et al.* 2002, Cebrian *et al.* 2005), although another variant in the 3'UTR (rs1061413, c.*946C>G) was significantly associated

Table 3 Variants associated with the development of MTC

Chr location	Gene	Studied variant (synonyms) ^a	Cases	Con.	MAF cases; MAF controls (%)	OR (95% CI) ^b	P value ^b	Best fitting model	Power to detect an OR = 1.5	Power to detect an OR = 2.0	Genotyping platform	Functional assessment	Matching or adjustment criteria	Ref. ^c	Rep.?
10q11.2	RET	rs1800862 (p.Ser836Ser)	32 MTC	250	9.3; 3.6	2.97 (1.08–8.16)	0.04	D	11.6	21.6	RFLP (AluI)	N/A	Race, origin	1	2, 3
10q11.2	RET	rs1800862 (p.Ser836Ser)	49 MTC	70	9.2; 3.7	N/A	0.03	N/A	8.2	14.7	RFLP (AluI)	N/A	Race, origin	2	1, 3
10q11.2	RET	rs1800862 (p.Ser836Ser)	81 MTC	80	10.5; 3.1	3.98 (1.39–11.40)	0.01	D	7.7	14.4	RFLP (AluI)	N/A	Gender, age	3	1, 2
10q11.2	RET	rs1799939 (p.Gly691Ser)	120 MTC	529	27.1; 17.7	1.72 (1.24–2.39)	0.001	C	40.1	83.9	TaqMan	Does not affect splicing of RET mRNA	Age, gender, ethnicity	4	5
10q11.2	RET	rs1799939 (p.Gly691Ser)	106 MTC	106	27.8; 18.8	1.60 (1.03–2.49)	0.039	M	23.0	57.8	RFLP (BamI) + sequencing	Does not influence RET mRNA expression	Age, gender, ethnicity, origin	5	4
10q11.2	RET	rs1800863 (p.Ser804Ser)	125 MTC	528	26.8; 17.7	1.70 (1.23–2.35)	0.001	C	41.0	84.9	TaqMan	N/A	Age, gender, ethnicity	4	No
10q11.2	RET	rs3026782 (c.*388G>A)	123 MTC	522	26.4; 17.5	1.68 (1.21–2.32)	0.002	C	40.3	84.2	TaqMan	N/A	Age, gender, ethnicity	4	No
10q11.2	RET	rs2565206 (IVS1–126 G>T)	104 MTC	100	50.0; 35.0	N/A	0.003	N/A	29.1	68.2	Fluorescent SSCP + RFLP (MspI and NlaIII)	N/A	Age, gender, race	6	7, 8
10q11.2	RET	rs2565206 (IVS1–126 G>T)	58 MTC	100	50.0; 35.0	N/A	0.012	N/A	22.7	54.5	Fluorescent SSCP + RFLP (MspI and NlaIII)	N/A	Age, gender, race	8	6, 7
10q11.2	RET	rs2565206 (IVS1–126 G>T)	51 MTC	100	48.0; 35.0	N/A	0.039	N/A	21.4	51.3	Fluorescent SSCP + RFLP (MspI and NlaIII)	N/A	Age, gender, race	7	6, 8
10q11.2	RET	rs2472737 (IVS14-24)	45 MTC	79	27.7; 6.3	6.04 (2.49–14.63)	<0.001	D	10.2	20.8	Sequencing	N/A	Age, gender	9	No
10q26.11	GFRα1	rs1061413 (c.*946C>G)	129 MTC	523	33.7; 26.1	1.54 (1.04–2.27)	0.029	D	49.1	91.6	TaqMan	N/A	Age, gender, origin	4	No
10q26.11	GFRα1	rs45568534 (c. – 193C>G)	31 MTC	31	14.5; 0.0	N/A	<0.05	N/A	N/A	N/A	Sequencing	Tendency towards higher GFRA1 mRNA and protein expression	Race	10, 11	No
1p33-p32	ARNT	rs3762422 (c. – 797A>T)	132 MTC	520	32.2; 22.5	1.93 (1.31–2.84)	0.0008	D	47.0	90.2	TaqMan	N/A	Age, gender, ethnicity	4	No
9p21	CDKN2B	rs643319 (c.612-11596C>A)	403 MTC	880	39.7; 44.9	0.75 (0.58–0.96)	0.02	D	92.0	100.0	Illumina + TaqMan	N/A	Age, gender, origin	12	No
9p21	CDKN2B	rs2106119 (c.612-11882A>G)	170 MTC	241	55.6; 45.6	1.47 (1.12–1.94)	0.006	M	52.3	93.0	Illumina + TaqMan	Influences CDKN2B transcription through HNF1z binding	Age, gender, origin	12	No
5p15.33	TERT	rs2075786 (c.2654+269T>C)	402 MTC	898	31.8; 36.9	0.69 (0.54–0.87)	0.002	D	91.5	100.0	Illumina + TaqMan	N/A	Age, gender, origin	12	No
20q13.2	AURKA	rs911160 (c.566+478C>G)	400 MTC	888	30.0; 23.0	1.47 (1.21–1.78)	1.07 × 10 ⁻⁴	M	84.9	99.9	Illumina + TaqMan	N/A	Age, gender, origin	12	12
22q11.21	COMT	rs165849 (g.34407G>A)	386 MTC	553	28.5; 34.6	0.76 (0.62–0.92)	0.006	M	84.7	99.9	Illumina + TaqMan	N/A	Age, gender, origin	12	No
7q21-q22	CDK6	rs6 (c.234-4184G>A)	399 MTC	878	15.0; 21.4	0.61 (0.47–0.79)	1.69 × 10 ⁻⁴	D	83.2	99.9	Illumina + TaqMan	N/A	Age, gender, origin	12	12
2q32.2	STAT1	rs10173099 (c.373-1443G>A)	299 MTC	678	37.0; 29.9	1.55 (1.18–2.05)	0.002	D	79.5	99.8	Illumina + TaqMan	N/A	Age, gender, origin	12	No

(continued)

Table 3 Continued

Chr location	Gene	Studied variant (synonyms) ^a	Cases	Con.	MAF cases; MAF controls (%)	OR (95% CI) ^b	P value ^b	Best fitting model	Power to detect an OR = 1.5	Power to detect an OR = 2.0	Genotyping platform	Functional assessment	Matching or adjustment criteria	Ref. ^c	Rep.?
18q21.3	BCL2	rs1801018 (c.21A>G)	260 MTC	378	48.0; 39.6	1.44 (1.14–1.82)	0.002	M	70.4	99.0	Illumina + TaqMan	N/A	Age, gender, origin	12	No
11p15.5	HRAS	rs8176332 (g.4774C>A)	226 MTC	362	14.4; 10.1	1.47 (1.04–2.09)	0.031	M	35.8	80.6	Illumina + TaqMan	N/A	Age, gender, origin	12	No

The table is sorted by gene and variant; ^ars code for each SNP is always provided, as well as other aliases; ^bcalculated by us when not provided. MAF, minor allele frequency; OR, odds ratio; 95% CI, confidence interval 95%; MTC, medullary thyroid cancer; RFLP, restriction fragment length polymorphism; SSCP, single-strand conformational polymorphism. Con., controls; D, dominant; C, codominant; M, multiplicative; Ref., reference; Rep., replicated. ^cReferences: 1, Ruiz *et al.* (2001); 2, Gimm *et al.* (1999); 3, Siqueira *et al.* (2010); 4, Cebrian *et al.* (2004); 5, Elisei *et al.* (2005); 6, Fernandez *et al.* (2004); 7, Borrego *et al.* (2003); 8, Fernandez *et al.* (2006); 9, Baumgartner-Parzer *et al.* (2005); 10, Gimm *et al.* (2001a); 11, Gimm *et al.* (2001b); 12, Ruiz-Llorente *et al.* (2007).

(Cebrian *et al.* 2005). In this study, based on a UK population, an association was found for rs3762422 (c.−797A>T) in the *ARTN* gene, which encodes a ligand of RET.

Finally, a two-step, pathway-driven study including a Spanish ($n=266$) and a British ($n=155$) group of patients, reported several genes associated with sMTC (Ruiz-Llorente *et al.* 2007). Although not uniformly replicated, significant associations were described for variants within the genes *STAT1*, *AURKA*, *BCL2*, *CDKN2B*, *CDK6*, *COMT*, *TERT*, and *HRAS*. In addition, the potential role of *CDKN2B* was confirmed by a functional assay showing a role of the rs7044859 variant in the promoter region in altering the binding of the transcription factor HNF1.

Negative results

Association studies in sMTC reported negative results, apart from for several *RET* SNPs (Fugazzola *et al.* 2008, Fernandez *et al.* 2009), for variants in *GFRA2* (Gimm *et al.* 2001b, Borrego *et al.* 2002, Cebrian *et al.* 2005), *GFRA3* (Gimm *et al.* 2001b, Borrego *et al.* 2002, Cebrian *et al.* 2005, Ruiz-Llorente *et al.* 2007), *GFRA4* (Cebrian *et al.* 2005), *NTRK2* (Gimm *et al.* 2001a), and *NTRK3* (Gimm *et al.* 2001a), among many others, assessed in the study by Ruiz-Llorente *et al.* (2007). Some of these genes should not be completely discarded, however, due to the limited power of some of the studies.

Overall, the potential role of the different polymorphisms in the development of sMTC needs to be further characterized, and the molecular background of these polymorphisms needs to be elucidated. In addition, studying the genetic susceptibility to MTC could benefit from an as yet not performed GWAS, which could potentially unveil new genes, probably not directly linked to the RET pathway, but playing a role in the disease.

Conclusions and future perspectives

In the last 10 years, an important number of case–control studies have been published on thyroid cancer, although few loci have been consistently associated. Some good examples of the achievements in this field are, for example, the involvement of the *FOXE1* gene in PTC, or *RET* variants in MTC.

A vast proportion of risks attributable to genetic factors, however, remain unexplained. An evident solution, especially relevant for thyroid cancer, which is relatively rare, relies on the establishment of international consortia, able to recruit enough patients to reach the required statistical power to detect low- to moderate-penetrance loci, especially those

involving rare (MAF < 10%) variants. It would be interesting to evaluate the joint effect of the top reported variants in a large population, and thus evaluate the proportion of risk that they explain when considered together.

The establishment of international consortia leads us to another key feature: the need for tumor stratification. In fact, the heterogeneity of NMTC has not been properly addressed so far. It seems logical that if 'PTC, FTC', and each of the PTC subtypes ('classic PTC, FVPTC', etc.) are clinically different, and, in some cases, display distinct somatic alterations, they should also involve different germinal low-penetrance loci. Nonetheless, associations in follicular cell-derived thyroid cancer usually refer to 'NMTC' or to the predominant 'PTC'.

Another major pitfall in this field is the lack of functional data that could explain the underlying mechanisms of the reported polymorphisms. This also applies to other complex or multifactorial diseases, where it remains a challenge to elucidate the functional link between the great majority of associated variants and phenotypic traits (Frazer *et al.* 2009, Katsanis 2009). The path between an association signal and the functional variant is hardly ever straightforward, but it is essential to understand the cancer biology, and potentially improve its prognosis and treatment. In this regard, it will be interesting to take advantage of the new massive sequencing technologies, and resequence those regions or genes associated with thyroid cancer, but still unexplained from a functional point of view.

Finally, and keeping in mind the multifactorial nature of these cancers, it is unlikely that they can be explained by single, individual genes. Because of this, a more comprehensive view should be adopted, considering gene–gene and gene–environment interactions. So far, studies on the combination of several genotypes, such as those involving *GST* genes (Morari *et al.* 2002, Hernandez *et al.* 2003, Ho *et al.* 2006), were mainly performed due to the lack of strong individual associations, with the exception of the study by Gudmundsson *et al.* (2009) who showed an OR = 5.7 for individuals carrying the homozygous genotypes for the individually associated variants near *FOXO1* (*TITF2*) and *NKX2-1* (*TITF1*). Still, the epistatic contribution to thyroid cancer, that is, the conditional phenotypic effect that several variants provide, not resulting from the additive individual effects (Dimas & Dermitzakis 2009), remains unexplored. In this regard, a large study, specifically designed to assess epistasis, which takes into account the reported associated genes, but also considers randomly additional loci as well as environmental factors, could provide the necessary integrative view of susceptibility to thyroid cancer.

Declaration of interest

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

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