Association of NF-κB polymorphisms with clinical outcome of non-medullary thyroid carcinoma

Theo S Plantinga[1,2], Mirela S Petrulea[3], Marije Oosting[1], Leo A B Joosten[1], Doina Piciu[4], Johannes W Smit[1], Romana T Netea-Maier[1] and Carmen E Georgescu[3]

[1]Department of Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands
[2]Department of Pathology, Radboud University Medical Center, Nijmegen, The Netherlands
[3]Department of Endocrinology, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[4]Oncology Institute, Cluj-Napoca, Romania

Correspondence should be addressed to C E Georgescu
Email
c_e_georgescu@yahoo.com

Abstract

The NF-κB inflammatory pathway plays a major role in cancer development and clinical progression. Activation of NF-κB signaling is promoted by NFKB1 and inhibited by NFKBIA. The present study aimed to determine the relevance of *NFKB1* rs4648068 and *NFKBIA* rs2233406 genetic variants for non-medullary thyroid cancer (NMTC) susceptibility, progression and clinical outcome. This case–control and cohort study consists of a Romanian discovery cohort (157 patients and 258 controls) and a Dutch validation cohort (138 patients and 188 controls). In addition, patient cohorts were analyzed further for the association of genetic variants with clinical parameters. Functional studies were performed on human peripheral blood mononuclear cells. No associations were observed between the studied genetic variants and TC susceptibility. Although no statistically significant associations with clinical parameters were observed for *NFKB1* rs4648068, the heterozygous genotype of *NFKBIA* rs2233406 was correlated with decreased radioactive iodide sensitivity requiring higher cumulative dosages to achieve clinical response. These findings were discovered in the Romanian cohort ($P<0.001$) and confirmed in the Dutch cohort ($P=0.01$). Functional studies revealed that this *NFKBIA* rs2233406 genotype was associated with elevated TLR4-mediated IL-1β production. In conclusion, genetic variation in *NFKBIA*, an inhibitor of NF-κB signaling, is associated with clinical response to RAI therapy and with increased production of the pro-inflammatory cytokine IL-1β, providing a potential mechanism for the observed clinical associations. These data suggest that NF-κB signaling is involved in NMTC pathogenesis and that the inflammatory tumor microenvironment could contribute to RAI resistance.

Key Words
- non-medullary thyroid cancer
- NF-κB
- inflammation
- IL-1β
- radioactive iodide therapy

Endocrine-Related Cancer (2017) **24**, 307–318

Introduction

Non-medullary thyroid cancer (NMTC), including papillary (PTC) and follicular (FTC) histological subtypes, is the most common endocrine cancer with rising incidence (Jemal *et al.* 2011, Morris *et al.* 2013, Pellegriti *et al.* 2013). Although the majority of patients with NMTC have a good prognosis, patients with advanced NMTC are rarely cured,

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0033

Published by Bioscientifica Ltd.

mainly because their tumors lose the ability to accumulate radioactive iodide (RAI). This is due to loss of sodium iodide symporter (NIS) expression (Riesco-Eizaguirre *et al.* 2006, Durante *et al.* 2007, Mian *et al.* 2008). Inhibition of inflammatory pathways, including signaling through nuclear factor κB (NF-κB), has been proposed as a potential therapeutic target for patients with RAI refractory disease (Pacifico & Leonardi 2010, Li *et al.* 2013).

NF-κB proteins represent a family of transcription factors including five members: RelA (p65), RelB, c-Rel, NF-κB1 (p50) and NF-κB2 (p52). After protein synthesis, homo- or heterodimers of these proteins are assembled in the cytoplasm. In the inactive state, NF-κB protein dimers are conjugated with IκBα (encoded by the *NFKBIA* gene) to prevent nuclear translocation of NF-κB. However, upon the encounter of a wide variety of extracellular or intracellular stimuli, nuclear translocation and transcriptional activity of NF-κB is enabled by proteasomal degradation of IκBα (Pacifico & Leonardi 2010, Li *et al.* 2013).

Besides its function in inflammatory pathways, the NF-κB signaling cascade is one of the most important intracellular pathways in cancer development and progression by influencing cell survival, carcinogenesis, proliferation and anticancer drug resistance. Previous studies have shown that NF-κB is upregulated in many different cancer types and is associated with poor outcome (Hoesel & Schmid 2013, Xia *et al.* 2014). In NMTC, it has also been reported that NF-κB signaling can play an important role in carcinogenesis through its contributions to inhibition of apoptosis (Bravo *et al.* 2003, Pacifico *et al.* 2004, Starenki *et al.* 2004), induction of carcinogenesis by chronic inflammation (Kato *et al.* 2006, Pacifico & Leonardi 2010) and promotion of tumor invasiveness (Palona *et al.* 2006, Bauerle *et al.* 2010, Cras *et al.* 2012). Furthermore, NF-κB signaling has been shown to intertwine with other pathways promoting NMTC pathogenesis, including BRAF, JNK, PI3K, RET and TGFβ (Namba *et al.* 2007, Bommarito *et al.* 2011, Neely *et al.* 2011).

Besides its effects on tumorigenesis and tumor progression, NF-κB is also an important factor that can contribute to resistance to anticancer therapies. Studies into NMTC have shown that active NF-κB may be associated with decreased therapeutic efficacy of RAI, the standard adjuvant therapy administered to patients after surgery (Meng *et al.* 2012*a*,*b*). However, the underlying mechanism of this effect has not been completely elucidated.

Gene polymorphisms of the NF-κB pathway have been related to the susceptibility of various cancer types including multiple myeloma (Parker *et al.* 2002), non-Hodgkin lymphoma (Giachelia *et al.* 2012), melanoma (Bu *et al.* 2007), colorectal cancer (Seufert *et al.* 2013) and lung cancer (Shiels *et al.* 2012) and have been linked to therapeutic response (Bu *et al.* 2007, Giachelia *et al.* 2012) and patient survival (Ungerback *et al.* 2012). This association could be racial-specific (Nian *et al.* 2014), at least for some cancer subtypes (Lehnerdt *et al.* 2008, Zhou *et al.* 2009). In Table 1, a complete list of all genetic associations between polymorphisms in NF-κB-related genes with endocrine-related cancers is provided, including thyroid cancer, breast cancer, ovarian cancer and prostate cancer. This list indicates that no studies have been performed into the role of NF-κB polymorphisms in relation to therapy response.

Although previous studies have established an important role for NF-κB in cancer, and in NMTC in particular, the clinical relevance of genetic variation in genes encoding components of the NF-κB pathway for NMTC pathogenesis and clinical outcome has been hardly investigated so far. Only one previous study has examined

Table 1 Complete overview of previously reported genetic associations of NF-κB polymorphisms with endocrine-related cancers.

Type of endocrine-related malignancy	References	NF-κB polymorphisms	Associated with
Thyroid cancer	(Wang *et al.* 2015)	rs28362491	Susceptibility
Breast cancer	(Wang *et al.* 2014, Eskandari-Nasab *et al.* 2016)	rs28362491, *rs*2233406	Susceptibility
	(Jamshidi *et al.* 2015)	rs5996080, rs7973914, rs17243893, rs57890595	Survival
	(Murray *et al.* 2013)	rs230532, rs3774932	Clinical outcome
Ovarian cancer	(Fan *et al.* 2011, Huo *et al.* 2013, Chen *et al.* 2015*a*, Lu *et al.* 2016)	rs230528, rs230521, rs4648068, rs3774964, rs3774968, rs28362491	Susceptibility
Prostate cancer	(Zhang *et al.* 2009, Kopp *et al.* 2013, Cui *et al.* 2015, Han *et al.* 2015)	rs28362491, *rs*2233406, *rs*3138053, rs28362491	Susceptibility

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0033

Published by Bioscientifica Ltd.

the rs28362491 insertion/deletion polymorphism in *NFKB1* and has identified this polymorphism as a susceptibility factor for PTC (Wang *et al.* 2015). We hypothesize that NF-κB-related genetic variants could represent important risk factors in development or clinical progression of NMTC and could improve our understanding of the mechanisms responsible for the effects of NF-κB on RAI responsiveness. In the present study, single-nucleotide polymorphisms in *NFKB1* (rs4648068) and *NFKBIA* (rs2233406) genes were evaluated for their role in susceptibility, progression and clinical outcome of NMTC patients. These genes and genetic variants were selected based on population frequency and previously published associations with human diseases and/or known functional effects on protein function or gene expression(Bu *et al.* 2007, Lu *et al.* 2012, Ali *et al.* 2013, Tan *et al.* 2013, Hua *et al.* 2014, Zhang *et al.* 2014, 2015, Chen *et al.* 2015*a*,*b*). Further, functional consequences of genetic variation in NF-κB genes were investigated for the production of pro-inflammatory cytokines that are important factors in the tumor microenvironment and could therefore be involved in both tumorigenesis and response to therapy.

Study subjects and methods

Study subjects

Patients with histologically confirmed NMTC who visited the Endocrinology Department at the Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca or the Oncology Institute Cluj-Napoca, Romania or the outpatient clinic at the Division of Endocrinology of the Department of Medicine, Radboud University Medical Center, Nijmegen, the Netherlands, were asked to provide blood for genetic testing. In total, 157 NMTC Romanian patients (collected between 2014 and 2015, discovery cohort) and 138 consecutive NMTC Dutch patients (collected between 2009 and 2010, validation cohort) were enrolled in the study. Total thyroidectomy was performed in all cases in addition to modified radical lymph node neck dissections in patients with confirmed nodal metastases. RAI (^{131}I) ablation of residual thyroid tissue was performed 4–6 weeks after surgery. After this initial treatment, patients were considered in remission if they had a TSH-stimulated thyroglobulin (Tg) < 1 pM/l in the absence of anti-Tg antibodies and no evidence of loco-regional disease or distant metastasis on the whole body iodine scans (WBS) and/or neck ultrasonographic examinations at 6–9 months after the ablation. If this was not the case, patients were repeatedly treated with RAI to reach remission, if indicated. In fact, all patients who were not cured at the last follow-up had received more than 200 mCi RAI during the course of their disease. Tumor recurrence was defined as new evidence of loco-regional disease or distant metastasis after successful primary therapy. Current disease status was defined as remission (or cured) in case of undetectable Tg in the absence of anti-Tg antibodies and no evidence of loco-regional disease or distant metastases at the last follow-up visit. Persistent or recurrent disease was defined as detectable Tg and/or evidence of loco-regional disease or distant metastases. For all definitions based on Tg levels, the absence of anti-Tg antibodies was required. Histological, clinical and follow-up data were retrieved from the patients' medical records and are shown in Table 2. In the Romanian cohort, significantly more female patients were included as compared to the Dutch cohort, and T-stage, M-stage and cumulative RAI dose distribution were also significantly different between the cohorts. In addition, 258 Romanian and 188 Dutch healthy, genetically unrelated individuals, having no evidence of NMTC or other malignancies, were recruited as population-based control subjects from either the Dutch (Nijmegen area) or the Romanian population (Cluj-Napoca area) by local advertising. These control cohorts are healthy population–based groups not knowingly been affected by any type of malignancy or other type of disease that was selected to fit the patient cohorts with respect to age and gender. All control subjects are of adult age ranging from 18 to 65 years. Control subjects were not individually matched with patients; however, a similar distribution of age and gender in all cohorts was reached by selection of appropriate control subjects. Furthermore, gender has a minor influence on the genetic susceptibility analysis, since genetic variants in NFκB genes are located on autosomal chromosomes 4 or 14, which are therefore inherited irrespective of gender.

Ethics statement

The study has been performed in accordance with the Declaration of Helsinki, and approval was obtained from the Ethics Committees of Iuliu Haţieganu University of Medicine and Pharmacy Cluj-Napoca, Romania and Radboud University Medical Center, Nijmegen, the Netherlands. Informed consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0033

Published by Bioscientifica Ltd.

Table 2 Distribution of clinicopathological characteristics and treatment in two European non-medullary thyroid carcinoma (NMTC) populations.

Variable	No. (%)		*P* value
	Romanian NMTC cohort	Dutch NMTC cohort	
Patients	157	138	
Age (years)	55 (14–76)	39 (8–65)	0.76
Gender (F/M)	137/20 (87.3/12.7)	104/34 (75.4/24.6)	0.008
Tumor histology			
PTC	111 (70.7)	99 (71.7)	0.47
FTC	37 (23.6)	34 (24.6)	
FVPTC	9 (5.7)	4 (2.9)	
PDTC	0 (0)	1 (0.7)	
T stage			
T1	76 (48.4)	48 (34.8)	<0.001
T2	26 (16.6)	53 (38.4)	
T3	48 (30.6)	26 (18.9)	
T4	7 (4.5)	11 (8.0)	
N stage			
N0	24 (15.3)	26 (18.8)	0.18
N1	41 (26.1)	46 (33.4)	
Nx	92 (58.6)	66 (47.8)	
M stage			
M0	26 (16.6)	45 (32.6)	0.002
M1	11 (7.0)	3 (2.2)	
Mx	120 (76.4)	90 (65.2)	
Cumulative RAI dose (mCi)			
≤100	105 (66.9)	36 (26.1)	<0.001
101–200	23 (14.6)	49 (35.5)	
>200	29 (18.5)	53 (38.4)	
Persistent or recurrent disease	39 (24.8)	31 (22.5)	0.63

P values are calculated by χ^2 tests.

Genotyping

Single-nucleotide polymorphisms (SNPs) were selected based on population frequency and previously published associations with human diseases and/or known functional effects on protein function or gene expression (Zhang *et al.* 2014, 2015, Chen *et al.* 2015*a*,*b*). After obtaining informed consent, blood was drawn from the cubital vein of participants into EDTA collection tubes and subjected to DNA extraction using the GeneJET Whole Blood Genomic DNA Purification Mini Kit (Fermentas, Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Until further analysis, DNA samples were stored at –20°C. TaqMan SNP Genotyping assays (Life Technologies, Carlsbad, CA, USA) designed with two specific probes, and primers for each variant were utilized for genotyping the SNPs in *NFKB1* (rs4648068, C_11345289_10) and *NFKBIA* (rs2233406, C_73867_10). Ten nanograms of genomic DNA were amplified by quantitative polymerase chain reaction (qPCR) in a 7300 Real-Time PCR System (Life Technologies, Carlsbad, CA, USA), under standard conditions. The real-time PCR included an initial denaturation step at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and then at 60°C for 1 min. Quality control was performed by duplicating samples within and across plates and by the incorporation of positive and negative control samples.

Cytokine production by peripheral blood mononuclear cells (PBMC) and NMTC cell lines

For PBMC isolation, venous blood was drawn from the cubital vein of healthy volunteers into 10 mL EDTA tubes (Monoject). The mononuclear cell fraction was obtained by density centrifugation of blood diluted 1:1 in pyrogen-free saline over Ficoll-Paque (Pharmacia Biotech, PA, USA). Cells were washed twice in saline and suspended in culture medium (RPMI, Invitrogen, CA, USA) supplemented with gentamicin 10 µg/mL, L-glutamine 10 mM and pyruvate 10 mM. NMTC cell lines BC-PAP and FTC133 were obtained from the sources previously described and were authenticated by short tandem repeat profiling (Schweppe *et al.* 2008). BC-PAP and FTC133 cell

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0033

Published by Bioscientifica Ltd.

Table 3 Distribution of *NFKB1* (rs4648068) and *NFKBIA* (rs2233406) gene single-nucleotide polymorphisms (SNPs) in the Romanian cohort of non-medullary thyroid carcinoma (NMTC) patients and controls.

	***NFKB1* rs4648068**					***NFKBIA* rs2233406**				
Model	Genotype	NMTC (*N*=157)	Controls (*N*=258)	OR (95%CI)	*P*	Genotype	NMTC (*N*=157)	Controls (*N*=258)	OR (95%CI)	*P*
GDD	AA	82 (52.3%)	140 (54.3%)	1.00#	0.90	GG	90 (57.3%)	143 (55.4%)	1.00#	0.84
	AG	60 (38.2%)	96 (37.2%)	1.07 (0.70–1.63)		GA	56 (35.7%)	99 (38.4%)	0.90 (0.59–1.37)	
	GG	15 (9.6%)	22 (8.5%)	1.16 (0.57–2.37)		AA	11 (7.0%)	16 (6.2%)	1.09 (0.49–2.46	
Dominant	AA	82 (52.3%)	140 (54.3%)	1.08 (0.73–1.61)	0.69	GG	90 (57.3%)	143 (55.4%)	0.93 (0.62–1.38)	0.71
	AG/GG	75 (47.7%)	118 (45.7%)			GA/AA	67 (42.7%)	115 (44.6%)		
Recessive	AA/AG	142 (90.4%)	236 (91.5%)	1.13 (0.57–2.26)	0.72	AA/GA	146 (93.0%)	242 (93.8%)	1.14 (0.51–2.53)	0.75
	GG	15 (9.6%)	22 (8.5%)			AA	11 (7.0%)	16 (6.2%)		
HWE *P* value		0.41	0.34				0.57	0.84		

P values are calculated by χ^2 tests to test the overall association between genotype and disease status. OR – Odds Ratio (by binary logistic regression); GDD – Gene Dose-Dependent; HWE – Hardy–Weinberg Equilibrium; #reference.

lines were cultured in RPMI or DMEM culture medium, respectively (Invitrogen, CA, USA), both supplemented with gentamicin 10 µg/mL, L-glutamine 10 mM and pyruvate 10 mM. Cells were counted in a Coulter counter (Coulter Electronics), and the number was adjusted to 5×10^6 cells/mL. A total of 5×10^5 cells in a 100 µL volume was added to 96-well plates (Greiner) and incubated with either 100 µL of culture medium (negative control) or with *E.coli* lipopolysaccharide (LPS, 10 ng/mL, Sigma, MO, USA). Cytokine measurements of IL-1β, TNFα and IL-6 were performed in the supernatants after 4 and/or 24 h incubation, using a commercial ELISA kit (R&D Systems, MN, USA).

Statistical analysis

Genotype and allele frequencies were calculated, and the Hardy–Weinberg equilibrium was assessed using a goodness-of-fit χ^2-test for biallelic markers. The odds ratios (ORs) and 95% confidence intervals (95% CI) of the association between genotype frequencies and NMTC susceptibility in addition to clinicopathological characteristics and treatment outcomes were analyzed using binary logistic regression models. In addition, χ^2 analysis and Fisher's exact tests were applied to determine whether histology, TNM staging, cumulative RAI dose (subdivided as 0–100 mCi (0–3.8 GBq), 101–200 mCi (3.8–7.4 GBq) or >7.4 GBq (>200 mCi)) and disease status

Table 4 Distribution of *NFKB1* (rs4648068) and *NFKBIA* (rs2233406)gene single-nucleotide polymorphisms (SNPs) inthe Dutch cohort of non-medullary thyroid carcinoma (NMTC) patients and controls.

	***NFKB1* rs4648068**					***NFKBIA* rs2233406**				
Model	Genotype	NMTC (*N*=138)	Controls (*N*=188)	OR (95%CI)	*P*	Genotype	NMTC (*N*=138)	Controls (*N*=188)	OR (95%CI)	*P*
GDD	AA	69 (50.0%)	82 (43.6%)	1.00#	0.52	GG	74 (53.6%)	89 (47.3%)	1.00#	0.50
	AG	55 (39.9%)	85 (45.2%)	0.77 (0.48–1.23)		GA	52 (37.7%)	78 (41.5%)	0.80 (0.50–1.28)	
	GG	14 (10.1%)	21 (11.2%)	0.79 (0.37–1.68)		AA	12 (8.7%)	21 (11.2%)	0.69 (0.32–1.49)	
Dominant	AA	69 (50.0%)	82 (43.6%)	0.77 (0.50–1.20)	0.25	GG	74 (53.6%)	89 (47.3%)	0.78 (0.50–1.21)	0.26
	AG/GG	69 (50.0%)	106 (56.4%)			GA/AA	64 (46.4%)	99 (52.7%)		
Recessive	AA/AG	124 (89.9%)	167 (88.8%)	0.90 (0.44–1.83)	0.77	AA/GA	126 (91.3%)	167 (88.8%)	0.76 (0.36–1.60)	0.46
	GG	14 (10.1%)	21 (11.2%)			AA	12 (8.7%)	21 (11.2%)		
HWE *P* value		0.54	0.88				0.51	0.53		

P values are calculated by χ^2 tests to test the overall association between genotype and disease status. OR – odds ratio (by binary logistic regression); GDD – gene dose-dependent; HWE – Hardy–Weinberg Equilibrium; #reference.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0033

Published by Bioscientifica Ltd.

Table 5 Summary of *NFKB1* and *NFKBIA* genetic variants in relation to phenotype and clinical outcomes in Romanian non-medullary thyroid carcinoma (NMTC) patients.

	***NFKB1* rs4648068** (*N* = 157)				***NFKBIA* rs2233406** (*N* = 157)				
Variable	AA (%), *N* = 82	AG (%), *N* = 60	GG (%), *N* = 15	*P*	GG (%), *N* = 90	GA (%), *N* = 56	AA (%), *N* = 11	*P*	OR* (95% CI)
Histology									
PTC	57 (69.5%)	43 (71.7%)	11 (73.3%)	0.64	61 (67.8%)	41 (73.2%)	9 (81.8%)	0.55	
FTC	21 (25.6%)	14 (23.3%)	2 (13.3%)		25 (27.8%)	11 (19.6%)	1 (9.1%)		
FVPTC	4 (4.9%)	3 (5.0%)	2 (13.3%)		4 (4.4%)	4 (7.1%)	1 (9.1%)		
PDTC	0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)		
T stage									
T1	40 (48.8%)	30 (50.0%)	6 (40.0%)	0.98	39 (43.3%)	28 (50.0%)	9 (81.8%)	0.24	
T2	12 (14.6%)	11 (18.3%)	3 (20.0%)		16 (17.8%)	10 (17.9%)	0 (0%)		
T3	26 (31.7%)	17 (28.3%)	5 (33.3%)		29 (32.2%)	17 (30.4%)	2 (18.2%)		
T4	4 (4.9%)	2 (3.3%)	1 (6.7%)		6 (6.7%)	1 (1.8%)	0 (0%)		
N stage									
N0	9 (11.0%)	11 (18.3%)	4 (26.7)	0.07	13 (14.4%)	9 (16.1%)	2 (18.2%)	0.54	
N1	22 (26.8%)	12 (20.0%)	7 (46.7)		22 (24.4%)	18 (32.1%)	1 (9.1%)		
Nx	51 (62.2%)	37 (61.7%)	4 (26.7)		55 (61.1%)	29 (51.8%)	8 (72.7%)		
M stage									
M0	13 (15.9%)	9 (15.0%)	4 (26.7)	0.87	14 (15.6%)	11 (19.6%)	1 (9.1%)	0.74	
M1	6 (7.3%)	4 (6.7%)	1 (6.7%)		7 (7.8%)	4 (7.1%)	0 (0%)		
Mx	63 (76.8%)	47 (78.3%)	10 (66.7)		69 (76.7%)	41 (73.2%)	10 (90.9%)		
Cumulative RAI dose (mCi)									
≤100	54 (65.9%)	40 (66.7%)	11 (73.3%)	0.52	85 (94.4%)	12 (21.4%)	8 (72.7%)	<0.001	1.00#
101–200	13 (15.9%)	10 (16.7%)	0 (0%)		4 (4.4%)	16 (28.6%)	3 (27.3%)		17.7 (6.1–51.8)
>200	15 (18.3%)	10 (16.7%)	4 (26.7%)		1 (1.1%)	28 (50.0%)	0 (0%)		217.0 (27.0–1742.7)
Disease status									
Persistent or recurrent	18 (22.0%)	15 (25.0%)	6 (40.0%)	0.33	25 (27.8%)	13 (23.2%)	1 (9.1%)	0.38	
Remission	64 (78.0%)	45 (75.0%)	9 (60.0%)		65 (72.2%)	43 (76.8%)	10 (90.9%)		

P-values are calculated by χ^2 tests. *Binary logistic regression by comparing heterozygous genotype with both homozygous genotypes (AA and GG) combined. PTC = papillary thyroid cancer; FTC = follicular thyroid cancer; FVPTC = follicular variant papillary thyroid cancer; PDTC = poorly differentiated thyroid cancer. #reference.

after thyroidectomy plus radioablation were associated with the genotype of the analyzed genes. All statistical analyses were carried out with SPSS v22.0 for statistical computing. Differences in cytokine production capacity between groups were analyzed using the Kruskal–Wallis test. Overall, statistical tests were two-sided, and a *P* value below 0.05 was considered statistically significant.

Results

NF-κB pathway SNPs and susceptibility to non-medullary thyroid cancer

To assess the effects of genetic variation in NF-κB genes on susceptibility to NMTC, two SNPs were selected based on previously published associations with human diseases and/or known functional effects on protein function or gene expression: the intronic SNP rs4648068 in *NFKB1* and the 5′ UTR SNP rs2233406 (also known as -839A/G) in *NFKBIA*. The genotypes corresponding to these SNPs were determined in the Romanian discovery cohort (157 patients, 258 healthy controls) and in the Dutch validation cohort (138 patients, 188 healthy controls). Table 2 summarizes the main clinical and demographical characteristics of the selected Romanian and Dutch NMTC patients. Of note, the distribution of age and sex was not significantly different between patient and control groups, since the controls were selected to fit the patient cohorts (data not shown). The distribution of *NFKB1* and *NFKBIA* genotypes among the Romanian and Dutch cohorts is presented in Tables 3 and 4, respectively. The results indicate that no significant associations were observed between the selected SNPs in either *NFKB1* or *NFKBIA* and susceptibility to develop NMTC, irrespective

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0033

Published by Bioscientifica Ltd.

Table 6 Summary of *NFKB1* and *NFKBIA* genetic variants in relation to phenotype and clinical outcomes in Dutch non-medullary thyroid carcinoma (NMTC) patients.

	***NFKB1* rs4648068** (*N* = 138)				***NFKBIA* rs2233406** (*N* = 138)				
Variable	AA (%), *N* = 69	AG (%), *N* = 55	GG (%), *N* = 14	*P*	GG (%), *N* = 74	GA (%), *N* = 52	AA (%), *N* = 12	*P*	OR* (95%CI)
Histology									
PTC	46 (66.7%)	43 (78.2%)	10 (71.4%)	0.44	52 (70.3%)	40 (76.9%)	7 (58.3%)	0.29	
FTC	18 (26.1%)	12 (21.8%)	4 (28.6%)		18 (24.3%)	11 (21.2%)	5 (41.7%)		
FVPTC	4 (5.8%)	0 (0%)	0 (0%)		4 (5.4%)	0 (0%)	0 (0%)		
PDTC	1 (1.4%)	0 (0%)	0 (0%)		0 (0%)	1 (1.9%)	0 (0%)		
T stage									
T1	25 (36.2%)	19 (34.5%)	4 (28.6%)	0.24	24 (32.4%)	20 (38.5%)	4 (33.3%)	0.63	
T2	31 (44.9%)	16 (29.1%)	6 (42.9%)		26 (35.1%)	22 (42.3%)	5 (41.7%)		
T3	8 (11.6%)	14 (25.5%)	4 (28.6%)		17 (23.0%)	6 (11.5%)	3 (25.0%)		
T4	5 (7.2%)	6 (10.9%)	0 (0%)		7 (9.5%)	4 (7.7%)	0 (0%)		
N stage									
N0	10 (14.5%)	13 (23.6%)	3 (21.4%)	0.51	13 (17.6%)	10 (19.2%)	3 (25.0%)	0.96	
N1	27 (39.1%)	16 (29.1%)	3 (21.4%)		25 (33.8%)	18 (34.6%)	3 (25.0%)		
Nx	32 (46.4%)	26 (47.2%)	8 (57.1%)		36 (48.6%)	24 (46.2%)	6 (50.0%)		
M stage									
M0	25 (36.2%)	17 (30.9%)	3 (21.4%)	0.74	22 (29.7%)	19 (36.5%)	4 (33.3%)	0.74	
M1	2 (2.9%)	1 (1.8%)	0 (0%)		1 (1.4%)	2 (3.9%)	0 (0%)		
Mx	42 (60.9%)	37 (67.3%)	11 (78.6%)		51 (68.9%)	31 (59.6%)	8 (66.7%)		
Cumulative RAI dose (mCi)									
≤100	19 (27.5%)	15 (27.3%)	2 (14.3%)	0.78	22 (29.7%)	12 (23.1%)	2 (16.7%)	0.01	1.00#
101–200	23 (33.3%)	21 (38.2%)	5 (35.7%)		29 (39.2%)	12 (23.1%)	8 (66.7%)		0.65 (0.25–1.68)
>200	27 (39.1%)	19 (34.5%)	7 (50.0%)		23 (31.1%)	28 (53.8%)	2 (16.7%)		2.24 (0.93–5.39)
Disease status									
Persistent or recurrent	12 (17.4%)	8 (14.5%)	2 (14.3%)	0.90	13 (17.6%)	6 (11.5%)	3 (25.0%)	0.44	
Remission	57 (82.6%)	47 (85.5%)	12 (85.7%)		61 (82.4%)	46 (88.5%)	9 (75.0%)		

P values are calculated by χ^2 tests. *Binary logistic regression by comparing heterozygous genotype (GA) with both homozygous genotypes (AA and GG) combined. PTC = papillary thyroid cancer; FTC = follicular thyroid cancer; FVPTC = follicular variant papillary thyroid cancer; PDTC = poorly differentiated thyroid cancer. #reference.

of the chosen genetic association model (i.e. dominant, recessive or gene dose-dependent model). Also, no statistically significant associations were observed after stratifying for gender, as was reported previously for the *NFKBIA* rs2233406 polymorphism in colorectal cancer patients (Tan *et al.* 2013) (data not shown). Of note, genotype frequencies in both NMTC patients and controls study populations were in accordance with that expected under the Hardy–Weinberg equilibrium(Tables 3 and 4).

NF-κB pathway SNPs and clinical outcome of NMTC

Within the NMTC study populations recruited in Romania and the Netherlands, the impact of *NFKB1* or *NFKBIA* genotypes on the clinical postoperative treatment response and outcome of NMTC patients was investigated. With regard to the *NFKB1* rs4648068 SNP, no statistically significant differences were found in any of the patient cohorts for any of the selected clinical parameters. Interestingly, however, the *NFKBIA* rs2233406 SNP was significantly associated with the required cumulative RAI dose to reach remission, reaching statistical significance in both the Romanian and Dutch cohorts. These genetic associations indicate that in both cohorts especially the heterozygous GA genotype is overrepresented in the patient group receiving a relatively high cumulative RAI dose above 200 mCi, whereas in patients with either homozygous genotype (AA or GG), a lower RAI dose was more likely to be sufficient to achieve clinical response. Nevertheless, between the *NFKBIA* rs2233406 genotype groups, no differences were observed concerning the rate of disease persistence or disease recurrence as measure of patient outcome, neither in the Romanian nor in the Dutch patient cohorts (Table 5 and 6). Irrespective of *NFKB1* and *NFKBIA* genotypes, 45–55% of patients (16 out of 29 Romanian patients and 24 out of 53 Dutch patients)

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0033

Published by Bioscientifica Ltd.

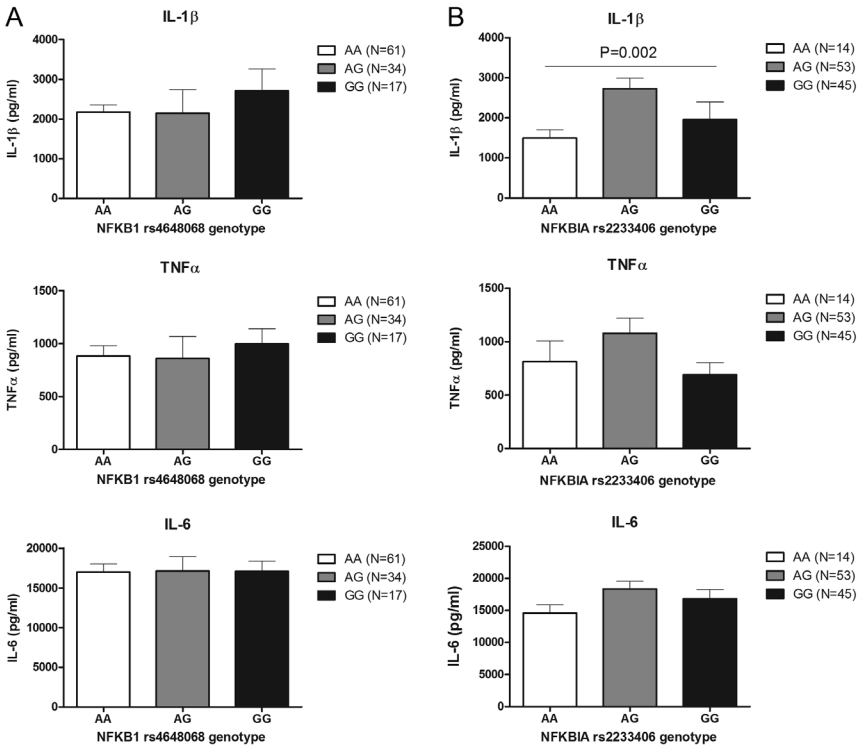

Figure 1
(A) Cytokine production capacity of IL-1β, TNFα and IL-6 by PBMCs obtained from healthy volunteers after stimulation for 24 h with LPS, stratified for *NFKB1* rs4648068 genotype. Data are mean ± S.E.M. (B) Cytokine production capacity of IL-1β, TNFα and IL-6 by PBMCs obtained from healthy volunteers after stimulation for 24 h with LPS, stratified for *NFKBIA* rs2233406 genotype. Data are mean ± S.E.M (*P* value is generated by Kruskal–Wallis test).

that received a cumulative RAI dose of ≥200 mCi by repeated treatments eventually reached remission. Within these subgroups, no significant associations with either *NFKB1* or *NFKBIA* genotype were observed. Furthermore, after genotype stratification, no statistically significant differences were observed between Dutch and Romanian patients for any of the included clinical parameters (data not shown). Since for some statistical analyses the Fisher's exact test is also appropriate, differences in statistical output of Fisher's exact tests were assessed in relation to chi-square tests. Importantly, no important differences in statistical significance were observed between the results of the two tests.

Functional consequences of NF-κB SNPs for LPS-induced pro-inflammatory cytokine production by peripheral blood mononuclear cells

NF-κB is a well-established transcription factor for the expression of proteins involved in inflammatory pathways including pro-inflammatory cytokines as important mediators of inflammatory signals. In order to study the functional effects of *NFKB1* rs4648068 and *NFKBIA* rs2233406 polymorphisms for the inflammatory response, PBMCs obtained from healthy volunteers with different *NFKB1*/*NFKBIA* genotypes were stimulated with lipopolysaccharide (LPS) for 24 h and IL-1β, TNFα and IL-6 production was measured in the supernatants. No differences were observed between different *NFKB1* rs4648068 genotypes for production of LPS-induced IL-1β,

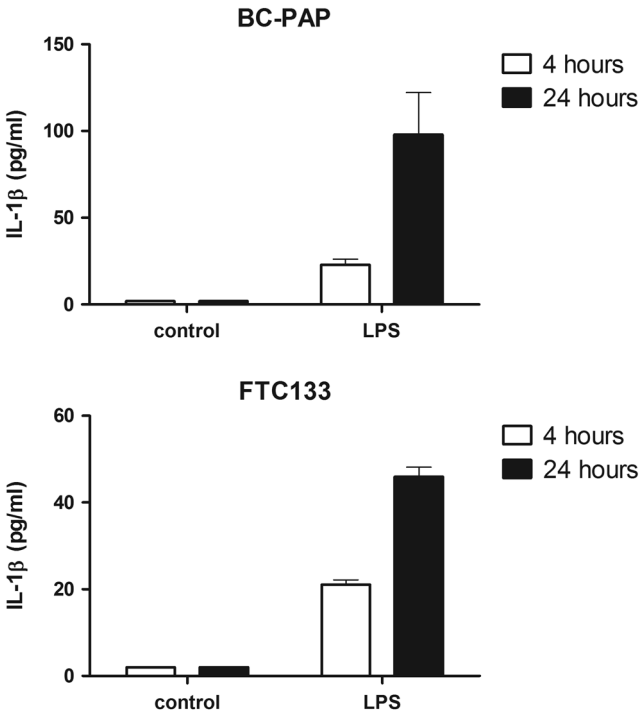

Figure 2
IL-1β production capacity of BC-PAP and FTC133 NMTC cell lines after stimulation with LPS for either 4 or 24 h. Data are mean ± S.E.M (*N* = 6).

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0033

Published by Bioscientifica Ltd.

TNFα or IL-6 (Fig. 1A). In contrast, after stratifying for the *NFKBIA* rs2233406 genotype, statistically significant differences were obtained for the production of LPS-induced IL-1β, which was specifically higher in PBMCs from GA heterozygous individuals as compared to cells with either AA or GG homozygous genotypes. Similar trends were observed for the production of TNFα and IL-6, although no statistically significant differences were reached (Fig. 1B). In the unstimulated conditions, cytokine concentrations were below detection limit (data not shown).

IL-1β production by NMTC cell lines

To assess whether NTMC cell lines are responsive to LPS, NMTC cell lines BC-PAP and FTC133 were cultured in the presence of LPS for 4 and 24 h. Measurement of the supernatants revealed detectable production of IL-1β by LPS-stimulated cells, whereas IL-1β was not measurable in the negative control conditions (Fig. 2).

Discussion

The present study was performed to investigate whether frequent genetic variants in human genes encoding NF-κB pathway components are associated with NMTC susceptibility, severity and/or clinical outcome in a Romanian discovery cohort and a Dutch validation cohort. Interestingly, one of the selected genetic variants, the *NFKBIA* rs2233406 single-nucleotide polymorphism, was significantly associated with the clinical response of NMTC patients to RAI therapy, which was discovered in the Romanian cohort and confirmed in the Dutch cohort. In both cohorts, the heterozygous GA genotype conferred relative resistance to RAI therapy, whereas carriers of the AA and GG homozygous genotypes exhibited a better clinical response to RAI treatment. In the Dutch validation cohort, a statistically less significant association of the *NFKBIA* rs2233406 genotype with cumulative RAI dose was observed as compared to the Romanian discovery cohort. These differences in significance between the Dutch and Romanian cohorts could originate from the observed differences between the Dutch and Romanian patient cohorts listed in Table 2 with respect to distribution of gender, T-stage, M-stage and cumulative RAI dose. Nevertheless, we are confident that this represents a true genetic association, since the same *NFKBIA* rs2233406 GA genotype is associated with decreased RAI responsiveness in both cohorts. This is further corroborated by functional data demonstrating aberrant cytokine production in the presence of the *NFKBIA* GA genotype. In contrast, no significant associations were observed between NF-κB genetic variants and susceptibility to develop NMTC. Furthermore, for the other selected polymorphism, *NFKB1* rs4648068, no associations were observed with either susceptibility for NMTC, severity of the disease or clinical outcome. Also, after genotype stratification, no statistically significant differences were observed between Dutch and Romanian patients for any of the included parameters.

Functional studies into the biological effects of the *NFKBIA* rs2233406 polymorphism revealed that specifically the GA heterozygous genotype, which was significantly associated with a worse clinical response to RAI treatment, was also associated with an increased production of IL-1β elicited by PBMCs stimulated with LPS, a well-known ligand of Toll-like receptor 4 (TLR4). This is in accordance with a previous study demonstrating that the *NFKBIA* rs2233406 heterozygous GA genotype is associated with decreased NFKBIA expression and IkBα protein levels, allowing for elevated production of IL-1β (Ali *et al.* 2013). No differences in cytokine production were observed between LPS-stimulated PBMCs stratified for the *NFKB1* rs4648068 genotype. These functional data are in line with the genetic association data and suggest a potential role for IL-1β production contributing to RAI therapy resistance. In fact, it has been demonstrated previously that IL-1β signaling negatively influences expression of NIS, a critical prerequisite for RAI accumulation in malignant thyroid follicular cells (Yamashita *et al.* 1989, Ohta *et al.* 1996, Spitzweg *et al.* 1999). One might hypothesize that increased production of LPS-induced IL-1β, observed in PBMCs bearing the *NFKBIA* rs2233406 GA heterozygous genotype, could therefore impair clinical responses to RAI by downregulating NIS. Notably, also thyrocytes are known to express TLR4 and are capable of responding to LPS (Nicola *et al.* 2009). Consequently, autocrine and paracrine IL-1β signaling could also be evoked by malignant thyroid follicular cells themselves in response to certain TLR4 agonists, being either LPS or, perhaps more likely, danger-associated molecular patterns that activate TLR4 signaling such as HMGB1, which is present in high amounts in the tumor microenvironment (Yu *et al.* 2006). In this respect, in the present study, we could demonstrate that malignant thyroid follicular cells BC-PAP and FTC133 are capable of recognizing LPS and of eliciting IL-1β production. Moreover, within the tumor microenvironment, tumor-associated macrophages

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0033

Published by Bioscientifica Ltd.

(TAMs) might also be an important source of IL-1β production induced by TLR4 signaling. These TAMs have been found to be abundantly present in advanced NMTC and are associated with a poor prognosis (Ryder *et al.* 2008).

The rs2233406 polymorphism in *NFKBIA*, an inhibitor of NF-κB signaling, is located in the promoter region and could therefore influence the regulation of *NFKBIA* expression. The observation that the GA heterozygous genotype of *NFKBIA* rs2233406 leads to differential regulation of IL-1β production as compared to the AA and GG homozygous genotypes suggests that both homozygous genotypes exhibit similar consequences as a result of different effects on *NFKBIA* expression: either too high or too low expression. Importantly, the direct functional consequences of this polymorphism on *NFKBIA* expression and function remain to be investigated.

These novel data indicate a potential role of NF-κB signaling in the pathogenesis of NMTC and suggest that the inflammatory tumor microenvironment could contribute to RAI resistance. These results warrant further investigations into the role of the tumor microenvironment in the regulation of NIS expression and in the progression of NMTC in order to identify potential therapeutic targets that may enhance RAI sensitivity in patients with advanced therapy refractory disease.

Declaration of interest

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

Funding

This work was supported by the European Social Fund, Human Resources Development Operational Programme 2007–2013, project no. POSDRU/159/1.5/S/138776. TSP was supported by a Veni grant of the Netherlands Organization for Scientific Research (NWO; 016.136.065) and by the Alpe d'HuZes fund of the Dutch Cancer Society (KUN2014-6728).

Author contributions

T S P, M S P and M O performed the experiments and data analysis; L A B J and D P provided protocols, patient material and experimental guidance; T S P, M S P, J W S, R T N M and C E G designed the study and wrote the manuscript. All authors read and approved the final manuscript. All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgements

Theo S Plantinga and Mirela S Petrulea share first authorship; Romana T Netea-Maier and Carmen E Georgescu share senior authorship.

References

Ali S, Hirschfeld AF, Mayer ML, Fortuno ES, 3rd, Corbett N, Kaplan M, Wang S, Schneiderman J, Fjell CD, Yan J, *et al.* 2013 Functional genetic variation in NFKBIA and susceptibility to childhood asthma, bronchiolitis, and bronchopulmonary dysplasia. *Journal of Immunology* **190** 3949–3958. (doi:10.4049/jimmunol.1201015)

Bauerle KT, Schweppe RE & Haugen BR 2010 Inhibition of nuclear factor-kappa B differentially affects thyroid cancer cell growth, apoptosis, and invasion. *Molecular Cancer* **9** 117. (doi:10.1186/1476-4598-9-117)

Bommarito A, Richiusa P, Carissimi E, Pizzolanti G, Rodolico V, Zito G, Criscimanna A, Di Blasi F, Pitrone M, Zerilli M, *et al.* 2011 BRAFV600E mutation, TIMP-1 upregulation, and NF-kappaB activation: closing the loop on the papillary thyroid cancer trilogy. *Endocrine-Related Cancer* **18** 669–685. (doi:10.1530/ERC-11-0076)

Bravo SB, Pampin S, Cameselle-Teijeiro J, Carneiro C, Dominguez F, Barreiro F & Alvarez CV 2003 TGF-beta-induced apoptosis in human thyrocytes is mediated by p27kip1 reduction and is overridden in neoplastic thyrocytes by NF-kappaB activation. *Oncogene* **22** 7819–7830. (doi:10.1038/sj.onc.1207029)

Bu H, Rosdahl I, Sun X-F & Zhang H 2007 Importance of polymorphisms in NF-kappaB1 and NF-kappaBIalpha genes for melanoma risk, clinicopathological features and tumor progression in Swedish melanoma patients. *Journal of Cancer Research and Clinical Oncology* **133** 859–866. (doi:10.1007/s00432-007-0228-7)

Chen LP, Cai PS & Liang HB 2015*a* Association of the genetic polymorphisms of NFKB1 with susceptibility to ovarian cancer. *Genetics and Molecular Research* **14** 8273–8282. (doi:10.4238/2015.July.27.15)

Chen Y, Lu R, Zheng H, Xiao R, Feng J, Wang H, Gao X & Guo L 2015*b* The NFKB1 polymorphism (rs4648068) is associated with the cell proliferation and motility in gastric cancer. *BMC Gastroenterology* **15** 243. (doi:10.1186/s12876-015-0243-0)

Cras A, Politis B, Balitrand N, Darsin-Bettinger D, Boelle PY, Cassinat B, Toubert M-E & Chomienne C 2012 Bexarotene via CBP/p300 induces suppression of NF-kappaB-dependent cell growth and invasion in thyroid cancer. *Clinical Cancer Research* **18** 442–453. (doi:10.1158/1078-0432.CCR-11-0510)

Cui X, Yan H, Ou TW, Jia CS, Wang Q & Xu JJ 2015 Genetic variations in inflammatory response genes and their association with the risk of prostate cancer. *BioMed Research International* **2015** 674039. (doi:10.1155/2015/674039)

Durante C, Puxeddu E, Ferretti E, Morisi R, Moretti S, Bruno R, Barbi F, Avenia N, Scipioni A, Verrienti A, *et al.* 2007 BRAF mutations in papillary thyroid carcinomas inhibit genes involved in iodine metabolism. *Journal of Clinical Endocrinology and Metabolism* **92** 2840–2843. (doi:10.1210/jc.2006-2707)

Eskandari-Nasab E, Hashemi M, Ebrahimi M & Amininia S 2016 The functional 4-bp insertion/deletion ATTG polymorphism in the promoter region of NF-KB1 reduces the risk of BC. *Cancer Biomarkers* **16** 109–115. (doi:10.3233/CBM-150546)

Fan Y, Yu W, Ye P, Wang H, Wang Z, Meng Q, Duan Y, Liang X & An W 2011 NFKB1 insertion/deletion promoter polymorphism increases the risk of advanced ovarian cancer in a Chinese population. *DNA & Cell Biology* **30** 241–245. (doi:10.1089/dna.2010.1107)

Giachelia M, Voso MT, Tisi MC, Martini M, Bozzoli V, Massini G, D'Alo F, Larocca LM, Leone G & Hohaus S 2012 Interleukin-6 plasma levels are modulated by a polymorphism in the NF-kappaB1 gene and are associated with outcome following rituximab-combined chemotherapy in diffuse large B-cell non-Hodgkin lymphoma. *Leukemia & Lymphoma* **53** 411–416. (doi:10.3109/10428194.2011.621566)

Han X, Zhang JJ, Yao N, Wang G, Mei J, Li B, Li C & Wang ZA 2015 Polymorphisms in NFKB1 and NFKBIA genes modulate the risk of

Published by Bioscientifica Ltd.

developing prostate cancer among Han Chinese. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research* **21** 1707–1715. (doi:10.12659/MSM.893471)

Hoesel B & Schmid JA 2013 The complexity of NF-kappaB signaling in inflammation and cancer. *Molecular Cancer* **12** 86. (doi:10.1186/1476-4598-12-86)

Hua T, Qinsheng W, Xuxia W, Shuguang Z, Ming Q, Zhenxiong L & Jingjie W 2014 Nuclear factor-kappa B1 is associated with gastric cancer in a Chinese population. *Medicine* **93** e279. (doi:10.1097/MD.0000000000000279)

Huo ZH, Zhong HJ, Zhu YS, Xing B & Tang H 2013 Roles of functional NFKB1 and beta-TrCP insertion/deletion polymorphisms in mRNA expression and epithelial ovarian cancer susceptibility. *Genetics and Molecular Research* **12** 3435–3443. (doi:10.4238/2013.March.11.6)

Jamshidi M, Fagerholm R, Khan S, Aittomaki K, Czene K, Darabi H, Li J, Andrulis IL, Chang-Claude J, Devilee P, *et al*. 2015 SNP-SNP interaction analysis of NF-kappaB signaling pathway on breast cancer survival. *Oncotarget* **6** 37979–37994. (doi:10.18632/oncotarget.4991)

Jemal A, Bray F, Center MM, Ferlay J, Ward E & Forman D 2011 Global cancer statistics. *CA: A Cancer Journal for Clinicians* **61** 69–90. (doi:10.3322/caac.20107)

Kato Y, Ying H, Zhao L, Furuya F, Araki O, Willingham MC & Cheng SY 2006 PPARgamma insufficiency promotes follicular thyroid carcinogenesis via activation of the nuclear factor-kappaB signaling pathway. *Oncogene* **25** 2736–2747. (doi:10.1038/sj.onc.1209299)

Kopp TI, Friis S, Christensen J, Tjonneland A & Vogel U 2013 Polymorphisms in genes related to inflammation, NSAID use, and the risk of prostate cancer among Danish men. *Cancer Genetics* **206** 266–278. (doi:10.1016/j.cancergen.2013.06.001)

Lehnerdt GF, Bankfalvi A, Grehl S, Adamzik M, Lang S, Schmid KW, Siffert W & Riemann K 2008 No association of the NF-kappaB1 -94ins/delATTG promoter polymorphism with relapse-free and overall survival in patients with squamous cell carcinomas of the head and neck region. *International Journal of Immunopathology and Pharmacology* **21** 827–832. (doi:10.1177/039463200802100407)

Li X, Abdel-Mageed AB, Mondal D & Kandil E 2013 The nuclear factor kappa-B signaling pathway as a therapeutic target against thyroid cancers. *Thyroid* **23** 209–218. (doi:10.1089/thy.2012.0237)

Lu R, Gao X, Chen Y, Ni J, Yu Y, Li S & Guo L 2012 Association of an NFKB1 intron SNP (rs4648068) with gastric cancer patients in the Han Chinese population. *BMC Gastroenterology* **12** 87. (doi:10.1186/1471-230X-12-87)

Lu ZH, Gu XJ, Shi KZ, Li X, Chen DD & Chen L 2016 Association between genetic polymorphisms of inflammatory response genes and the risk of ovarian cancer. *Journal of the Formosan Medical Association* **115** 31–37. (doi:10.1016/j.jfma.2015.01.002)

Meng Z, Lou S, Tan J, Xu K, Jia Q & Zheng W 2012*a* Nuclear factor-kappa B inhibition can enhance apoptosis of differentiated thyroid cancer cells induced by 131I. *PLoS One* **7** e33597. (doi:10.1371/journal.pone.0033597)

Meng Z, Lou S, Tan J, Xu K, Jia Q, Zheng W & Wang S 2012*b* Nuclear factor-kappa B inhibition can enhance therapeutic efficacy of 131I on the in vivo management of differentiated thyroid cancer. *Life Sciences* **91** 1236–1241. (doi:10.1016/j.lfs.2012.09.026)

Mian C, Barollo S, Pennelli G, Pavan N, Rugge M, Pelizzo MR, Mazzarotto R, Casara D, Nacamulli D, Mantero F, *et al*. 2008 Molecular characteristics in papillary thyroid cancers (PTCs) with no 131I uptake. *Clinical Endocrinology* **68** 108–116. (doi:10.1111/j.1365-2265.2007.03008.x)

Morris LGT, Sikora AG, Tosteson TD & Davies L 2013 The increasing incidence of thyroid cancer: the influence of access to care. *Thyroid* **23** 885–891. (doi:10.1089/thy.2013.0045)

Murray JL, Thompson P, Yoo SY, Do KA, Pande M, Zhou R, Liu Y, Sahin AA, Bondy ML & Brewster AM 2013 Prognostic value of single nucleotide polymorphisms of candidate genes associated with inflammation in early stage breast cancer. *Breast Cancer Research and Treatment* **138** 917–924. (doi:10.1007/s10549-013-2445-x)

Namba H, Saenko V & Yamashita S 2007 Nuclear factor-kB in thyroid carcinogenesis and progression: a novel therapeutic target for advanced thyroid cancer. *Arquivos Brasileiros de Endocrinologia e Metabologia* **51** 843–851. (doi:10.1590/S0004-27302007000500023)

Neely RJ, Brose MS, Gray CM, McCorkell KA, Leibowitz JM, Ma C, Rothstein JL & May MJ 2011 The RET/PTC3 oncogene activates classical NF-kappaB by stabilizing NIK. *Oncogene* **30** 87–96. (doi:10.1038/onc.2010.396)

Nian X, Zhang W, Li L, Sun Y, Sun E & Han R 2014 Meta-analysis of studies on the association between the NF-kappaB1-94ins/del ATTG promoter polymorphism and cancer. *Tumour Biology* **35** 11921–11931. (doi:10.1007/s13277-014-2470-3)

Nicola JP, Velez ML, Lucero AM, Fozzatti L, Pellizas CG & Masini-Repiso AM 2009 Functional toll-like receptor 4 conferring lipopolysaccharide responsiveness is expressed in thyroid cells. *Endocrinology* **150** 500–508. (doi:10.1210/en.2008-0345)

Ohta K, Pang XP, Berg L & Hershman JM 1996 Antitumor actions of cytokines on new human papillary thyroid carcinoma cell lines. *Journal of Clinical Endocrinology and Metabolism* **81** 2607–2612. (doi:10.1210/jc.81.7.2607)

Pacifico F & Leonardi A 2010 Role of NF-kappaB in thyroid cancer. *Molecular and Cellular Endocrinology* **321** 29–35. (doi:10.1016/j.mce.2009.10.010)

Pacifico F, Mauro C, Barone C, Crescenzi E, Mellone S, Monaco M, Chiappetta G, Terrazzano G, Liguoro D, Vito P, *et al*. 2004 Oncogenic and anti-apoptotic activity of NF-kappa B in human thyroid carcinomas. *Journal of Biological Chemistry* **279** 54610–54619. (doi:10.1074/jbc.M403492200)

Palona I, Namba H, Mitsutake N, Starenki D, Podtcheko A, Sedliarou I, Ohtsuru A, Saenko V, Nagayama Y, Umezawa K, *et al*. 2006 BRAFV600E promotes invasiveness of thyroid cancer cells through nuclear factor kappaB activation. *Endocrinology* **147** 5699–5707. (doi:10.1210/en.2006-0400)

Parker KM, Ma MH, Manyak S, Altamirano CV, Tang YM, Frantzen M, Mikail A, Roussos E, Sjak-Shie N, Vescio RA, *et al*. 2002 Identification of polymorphisms of the IkappaBalpha gene associated with an increased risk of multiple myeloma. *Cancer Genetics and Cytogenetics* **137** 43–48. (doi:10.1016/S0165-4608(02)00541-1)

Pellegriti G, Frasca F, Regalbuto C, Squatrito S & Vigneri R 2013 Worldwide increasing incidence of thyroid cancer: update on epidemiology and risk factors. *Journal of Cancer Epidemiology* **2013** 965212. (doi:10.1155/2013/965212)

Riesco-Eizaguirre G, Gutierrez-Martinez P, Garcia-Cabezas MA, Nistal M & Santisteban P 2006 The oncogene BRAF V600E is associated with a high risk of recurrence and less differentiated papillary thyroid carcinoma due to the impairment of Na+/I- targeting to the membrane. *Endocrine-Related Cancer* **13** 257–269. (doi:10.1677/erc.1.01119)

Ryder M, Ghossein RA, Ricarte-Filho JCM, Knauf JA & Fagin JA 2008 Increased density of tumor-associated macrophages is associated with decreased survival in advanced thyroid cancer. *Endocrine-Related Cancer* **15** 1069–1074. (doi:10.1677/erc-08-0036)

Schweppe RE, Klopper JP, Korch C, Pugazhenthi U, Benezra M, Knauf JA, Fagin JA, Marlow LA, Copland JA, Smallridge RC, *et al*. 2008 Deoxyribonucleic acid profiling analysis of 40 human thyroid cancer cell lines reveals cross-contamination resulting in cell line redundancy and misidentification. *Journal of Clinical Endocrinology and Metabolism* **93** 4331–4341. (doi:10.1210/jc.2008-1102)

Seufert BL, Poole EM, Whitton J, Xiao L, Makar KW, Campbell PT, Kulmacz RJ, Baron JA, Newcomb PA, Slattery ML, *et al*. 2013 IkappaBKbeta and NFkappaB1, NSAID use and risk of colorectal cancer in the Colon Cancer Family Registry. *Carcinogenesis* **34** 79–85. (doi:10.1093/carcin/bgs296)

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0033

Published by Bioscientifica Ltd.

Shiels MS, Engels EA, Shi J, Landi MT, Albanes D, Chatterjee N, Chanock SJ, Caporaso NE & Chaturvedi AK 2012 Genetic variation in innate immunity and inflammation pathways associated with lung cancer risk. *Cancer* **118** 5630–5636. (doi:10.1002/cncr.27605)

Spitzweg C, Joba W, Morris JC & Heufelder AE 1999 Regulation of sodium iodide symporter gene expression in FRTL-5 rat thyroid cells. *Thyroid* **9** 821–830. (doi:10.1089/thy.1999.9.821)

Starenki DV, Namba H, Saenko VA, Ohtsuru A, Maeda S, Umezawa K & Yamashita S 2004 Induction of thyroid cancer cell apoptosis by a novel nuclear factor kappaB inhibitor, dehydroxymethylepoxyquinomicin. *Clinical Cancer Research* **10** 6821–6829. (doi:10.1158/1078-0432.CCR-04-0463)

Tan SC, Suzairi MSiM, Aizat AAA, Aminudin MM, Nurfatimah MSS, Bhavaraju VMK, Biswal BM & Ankathil R 2013 Gender-specific association of NFKBIA promoter polymorphisms with the risk of sporadic colorectal cancer. *Medical Oncology* **30** 693. (doi:10.1007/s12032-013-0693-6)

Ungerback J, Belenki D, Jawad ul-Hassan A, Fredrikson M, Fransen K, Elander N, Verma D & Soderkvist P 2012 Genetic variation and alterations of genes involved in NFkappaB/TNFAIP3- and NLRP3-inflammasome signaling affect susceptibility and outcome of colorectal cancer. *Carcinogenesis* **33** 2126–2134. (doi:10.1093/carcin/bgs256)

Wang X, Peng H, Liang Y, Sun R, Wei T, Li Z, Gong Y, Gong R, Liu F, Zhang L, *et al*. 2015 A functional insertion/deletion polymorphism in the promoter region of the NFKB1 gene increases the risk of papillary thyroid carcinoma. *Genetic Testing and Molecular Biomarkers* **19** 167–171. (doi:10.1089/gtmb.2014.0271)

Wang Z, Liu QL, Sun W, Yang CJ, Tang L, Zhang X & Zhong XM 2014 Genetic polymorphisms in inflammatory response genes and their associations with breast cancer risk. *Croatian Medical Journal* **55** 638–646. (doi:10.3325/cmj.2014.55.638)

Xia Y, Shen S & Verma IM 2014 NF-kappaB, an active player in human cancers. *Cancer Immunology Research* **2** 823–830. (doi:10.1158/2326-6066.CIR-14-0112)

Yamashita S, Kimura H, Ashizawa K, Nagayama Y, Hirayu H, Izumi M & Nagataki S 1989 Interleukin-1 inhibits thyrotrophin-induced human thyroglobulin gene expression. *Journal of Endocrinology* **122** 177–183. (doi:10.1677/joe.0.1220177)

Yu M, Wang H, Ding A, Golenbock DT, Latz E, Czura CJ, Fenton MJ, Tracey KJ & Yang H 2006 HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. *Shock* **26** 174–179. (doi:10.1097/01.shk.0000225404.51320.82)

Zhang M, Huang J, Tan X, Bai J, Wang H, Ge Y, Xiong H, Shi J, Lu W, Lv Z, *et al*. 2015 Common Polymorphisms in the NFKBIA Gene and Cancer Susceptibility: A Meta-Analysis. *Medical Science Monitor* **21** 3186–3196. (doi:10.12659/MSM.895257)

Zhang P, Wei Q, Li X, Wang K, Zeng H, Bu H & Li H 2009 A functional insertion/deletion polymorphism in the promoter region of the NFKB1 gene increases susceptibility for prostate cancer. *Cancer Genetics and Cytogenetics191* 73–77. (doi:10.1016/j.cancergencyto.2009.01.017)

Zhang Q, Ji XW, Hou XM, Lu FM, Du Y, Yin JH, Sun XY, Deng Y, Zhao J, Han X, *et al*. 2014 Effect of functional nuclear factor-kappaB genetic polymorphisms on hepatitis B virus persistence and their interactions with viral mutations on the risk of hepatocellular carcinoma. *Annals of Oncology* **25** 2413–2419. (doi:10.1093/annonc/mdu451)

Zhou B, Rao L, Li Y, Gao L, Wang Y, Chen Y, Xue H, Song Y, Peng Y, Liao M, *et al*. 2009 A functional insertion/deletion polymorphism in the promoter region of NFKB1 gene increases susceptibility for nasopharyngeal carcinoma. *Cancer Letters* **275** 72–76. (doi:10.1016/j.canlet.2008.10.002)

Received in final form 30 March 2017
Accepted 20 April 2017
Accepted Preprint published online 20 April 2017

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0033

Published by Bioscientifica Ltd.