Association of IRAK1 Gene Polymorphism rs3027898 With Papillary Cancer Restricted to the Thyroid Gland: A Pilot Study

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Abstract. Background/Aim: The incidence of thyroid cancer has increased predominantly due to an increase in papillary thyroid cancer (PTC). Alteration of toll-like receptor function has been reported to play a crucial role in carcinogenesis. The aim of the present study was to investigate predisposition to PTC associated with genetic markers of toll-like receptor and interleukin-1 receptor pathways involving nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) stimulation. Specifically, the study focused on the following genes: interleukin-1 receptor-associated kinase 1 (IRAK1, rs3027898), NF-κB inhibitor alpha (NFKBIA, rs696), NF-κB subunit 1 (NFKB1, rs28362491), and microRNA-146a (miR-146a, rs2910164). Patients and Methods: Forty-eight unrelated patients with papillary cancer restricted to the thyroid gland and 93 healthy volunteers were enrolled in the study. Results: A strong statistically significant difference was observed between patients with PTC and controls for IRAK1 rs3027898 variant. When the statistical analysis was replicated taking into account patient’s sex, the rs3027898 A allele was revealed to be the risky variant in males. Conclusion: Additional studies in larger groups of patients of various origins are needed to validate these preliminary findings.

Thyroid cancer is the most common neoplasia of the endocrine system and accounts for 1% of all newly diagnosed cancer cases. Papillary thyroid carcinoma (PTC) is the most common malignant thyroid neoplasm, comprising up to 80% of all thyroid carcinomas (1). Notable progress has been made in the application of molecular markers to cancer diagnosis in thyroid nodules fine-needle aspirates (2). The most common genetic alteration found in 40-45% of PTCs is the B-Raf proto-oncogene mutation resulting in a valine-to-glutamate change at the residue 600 (V600E) of the BRAF protein, which is a serine/threonine kinase (3). However, even though the BRAF V600E mutation is a sensitive marker, it is not a specific index of PTC susceptibility and lethality (2). Recently, the advancements in genotyping methods have permitted more efficient patient management based on the combination of cytological and molecular testing techniques (2). Nevertheless, additional knowledge of the genetic basis of PTC is expected to improve the individualized management of patients with thyroid nodules and cancer.

Even though an increasing number of publications have shown that nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) plays a role in thyroid cancer, little is known on the role of genetic variants in genes of NF-κB pathway in PTC susceptibility (4). NF-κB is a transcription factor of genes related to apoptosis, inflammation and immune response. Interleukin-1 (IL1) receptor-activated kinase1 (IRAK1) plays a significant role in toll-like receptor and IL1 receptor pathways of NF-κB activation (5, 6). Specifically, NF-κB is inactivated by cytoplasmic trapping through the inhibitors of kappa B (IκB) proteins (e.g. NF-κB inhibitor alpha, NFKBIA). Kinases phosphorylate serine residues of the IκB proteins and lead them for destruction via the ubiquitination pathway. Subsequently, NF-κB factor is activated and regulates the expression of a variety of genes implicated in various biological events.
Recently, the −94 insertion/deletion ATTG polymorphism in the NFκB1 promoter (rs28362491) was associated with an increased risk of PTC in Chinese patients (7). In addition, toll-like receptor-associated polymorphisms were associated with PTC in Koreans (8, 9). Furthermore, it was reported that a common variant in miR-146a reduces mature miRNA expression and predisposes to PTC (10). It is worth mentioning that miR-146a is a microRNA that targets IRAK1 gene, of which the variant rs3027898 has been associated with immune diseases by our and other groups (10-14).

Additionally, an emerging body of literature shows that altered IRAK1 expression predisposes to inflammatory diseases, autoimmune diseases, solid tumors and hematological malignancies, which may explain the focus on IRAK1 inhibitors in preclinical and clinical studies (15, 16).

In the present study, we aimed to genotype DNA from patients suffering from PTC confined to the thyroid gland. Specifically, we explored the role of polymorphisms of four genes belonging to the toll-like receptor/IL1 receptor pathways, namely IRAK1 [rs3027898, in the 3'-untranslated region (3'-UTR)], NFKB1 (rs28362491), NFKBIA (rs696, in the 3' UTR), and miR-146a (rs2910164, in the sequence of miRNA precursor).

### Patients and Methods

Forty-eight unrelated patients with PTC (13 males; mean age=51±12 years, range=20-74 years) were enrolled in the study. All patients had a PTC of less than 4 cm [up to T2 according to the American Cancer Society (17)] that was confined into the thyroid gland (no microscopic or macroscopic extra-thyroidal extension) and had no apparent lymph node spread (clinical or imaging-negative lymph nodes). Additionally, 93 healthy volunteers (59 males; mean age=50±18 years, range=17-85 years) with no personal or family history of neoplasia, chronic autoimmune or infectious diseases, were studied. This study adhered to the tenets of the declaration of Helsinki (version 2002) and approved was by the Ethics Committees of AHEPA University Hospital and Aristotle University of Thessaloniki (approval numbers 237/7-12-2015 - 351/1-3-2017). Written consent was obtained from each participant in the study.

Genomic DNA was extracted from peripheral blood lymphocytes using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Polymorphisms rs3027898 IRAK1, rs28362491 NFKB1, rs696 NFKBIA, and rs2910164 miR-146a were studied with restriction fragment length polymorphism assay (RFLP) as described previously (13, 18). All samples were run twice and random samples were analyzed by sequencing to qualify the genotyping data by the RFLP analysis.

SPSS statistical package (SPSS Inc., Chicago, IL, USA) was used to test differences in distribution of each polymorphism between patients with PTC and controls (Pearson’s chi-square, Yates’ chi-square if any expected frequency was below 1 or if the expected frequency was less than 5 in more than 20% of cells). Furthermore, the odds ratio (OR) with a confidence interval (CI) of 95% was calculated (reference allele versus the variant allele). A difference at p=0.05 was considered as statistically significant.

### Results

Polymorphisms rs696 and rs2910164 were in Hardy–Weinberg equilibrium in the control group (p=0.0967 and p=0.8020; respectively), while the rs28362491 variant was not (p=0.0001). The latter is in accordance with other European control groups which were not or were only approximately in equilibrium for the rs28362491 variant (19). Finally, the X-chromosome linked polymorphism rs3027898 was in Hardy–Weinberg equilibrium in the female control group, where the three genotypes existed (p=0.6615). More specifically, in the control group, 15 females carried the AA genotype, 16 the AC genotype, and 3 the CC genotype, while 39 males carried the A allele and 20 the C allele. Concerning the patients, 13 females carried the AA genotype, 19 the AC genotype, and 3 the CC genotype, while 13 male patients carried the A allele and none the C allele (Table I).

The distribution of the other studied polymorphisms rs28362491 (NFKB1), rs696 (NFKBIA), and rs2910164 (miR-146a) did not differ significantly between patients with PTC and controls (Table I). Strong statistically significant difference was observed between patients with PTC and controls in genotype’s distribution of polymorphism rs3027898 located in the 3' UTR of IRAK1 gene (p=0.002, Table I).

Taking into consideration the hemizygous state of rs3027898 polymorphism (X-linked) in males and the X-inactivation process in females (one active X-chromosome in female cells), we replicated the analysis of rs3027898 genotype’s distribution in male patients versus male controls, and in female patients versus female controls. Regarding the female groups, the analysis of rs3027898 was not expanded to alleles, but restricted to genotypes because non-random X inactivation patterns were also reported for various other X-linked genes (20) which might cause biased grouping of heterozygous alleles in females. In these analyses, strong statistical difference was observed in the allelic distribution between male PTC patients versus male controls with the A allele being the risky variant (p=0.033, Table I). However, the confidence interval includes the value 1, which may seem to cancel the observed association. Nevertheless, we also mention the small sample size of our groups which may have affected the confidence interval width and be misleading (21).

### Discussion

Thyroid cancer is a common malignancy of the endocrine system that requires early pre-operative diagnosis, especially in cases when the tumor is small and confined within the gland. In this context, we tried to identify in the blood, possible gene polymorphisms that might be used in the future as biomarkers in the earlier diagnosis and management...
controls: The rs3027898 C variant was found in the 20 females. The strong statistical association with the A allele repeated according to sex due to the hemizygous state of the X-chromosome in males and the X-inactivation process. The risky variant was confirmed in male patients, Graves’ disease (24). Taking into account that this variant is located on the X-chromosome, the statistical analysis was observed between PTC patients and controls. Previously, the expression of inflammatory cytokines. On the other hand, miR-146a which subsequently inhibits the expression of IRAK1 protein, causes a negative feedback loop (23).

In the present study, a significant difference in the distribution of IRAK1 polymorphism rs3027898 was observed between PTC patients and controls. Previously, the rs3027898 A allele was reported to be the risky variant in autoimmune thyroid diseases and in predisposition to Graves’ disease (24). Taking into account that this variant is located on the X-chromosome, the statistical analysis was repeated according to sex due to the hemizygous state of the X-chromosome in males and the X-inactivation process in females. The strong statistical association with the A allele found to be the risky variant was confirmed in male patients, but not between female patients and controls. This may be accounted for by the difference in the expression of the protective rs3027898 C allele between male versus female controls: The rs3027898 C variant was found in the 20 hemizygous C male controls out of the 59 male controls and in 3 homozygous CC female controls out of 34 female controls. In heterozygous AC female controls (n=19), due to X inactivation process, which allele is eventually expressed is unknown. The potential high expression of the protective rs3027898 C allele in male controls due to the hemizygous state may also explain why PTC is three-times higher in women than in men (25).

Additionally, in previous studies, the expression of IRAK1 mRNA was reported significantly lower in PTC clinical tissue samples than normal adjacent thyroid specimens, while that of miR-146a was reportedly increased (10, 26-28). It is worth mentioning that in a genetic association study, the rs2910164 GC heterozygous state was associated with PTC showing higher expression than the homozygous rs2910164 GG state, while the homozygosity for rs2910164 CC was extremely rare among patients with PTC (10). However, the rs2910164 association with PTC was not replicated by our study, nor by other studies of Caucasian and Asian origin, nor in a recent meta-analysis (29-31).

In contrast, we revealed the association of IRAK1 rs3027898 A allele with PTC in males. This finding might be compatible with the low IRAK1 gene expression in PTC clinical tissue sample if we take into account that the rs3027898 A allele is

| Gene | SNP       | Genotype | Patients (N=48; male=13) | Controls (N=93; male=59) | p-Value | OR (95% CI) | p-Value |
|------|-----------|----------|-------------------------|--------------------------|---------|-------------|---------|
| NFKB1 | rs3027898 | Genotype | Ins                      | 9 (19%)                  | 11 (12%) | 0.359       |         |
|      |           |          | Ins/Del                 | 34 (71%)                 | 66 (71%) |             |         |
|      |           |          | Del                      | 5 (10%)                  | 16 (17%) |             |         |
|      | Allele    | Ins       | 52 (54%)                 | 88 (47%)                 |         |             |         |
|      |           | Del       | 44 (46%)                 | 98 (53%)                 |         |             |         |
| NFKBIA | rs696     | Genotype | GG                       | 15 (31%)                 | 24 (26%) | 0.79        |         |
|      |           | GA        | 26 (54%)                 | 54 (58%)                 |         |             |         |
|      |           | AA        | 7 (15%)                  | 15 (16%)                 |         |             |         |
|      | Allele    | G         | 56 (58%)                 | 102 (55%)                |         |             |         |
|      |           | A         | 40 (42%)                 | 84 (45%)                 |         |             |         |
| miR-146a | rs2910164| Genotype | GG                       | 26 (54%)                 | 53 (57%) | 0.942       |         |
|      |           | GC        | 19 (40%)                 | 35 (38%)                 |         |             |         |
|      | Allele    | G         | 25 (74%)                 | 141 (76%)                |         |             |         |
| IRAK1 | rs3027898 | Genotype | CC                       | 3 (6%)                   | 23 (25%) | 0.002       |         |
|      |           | AC        | 19 (40%)                 | 16 (17%)                 |         |             |         |
|      | Allele    | C         | 26 (54%)                 | 54 (58%)                 |         |             |         |
|      | Allele    | A         | 13 (100%)                | 39 (66%)                 | 0.961   | (0.515-1.956) | 0.734 |
|      | Genotype  | Male      | C                        | 0 (0%)                   | 20 (34%) |             |         |
|      |           | Female    | CC                       | 3 (9%)                   | 3 (9%)   | 0.872*      |         |
|      |           |           | AC                       | 19 (54%)                 | 16 (47%) |             |         |
|      |           |           | AA                       | 13 (37%)                 | 15 (44%) |             |         |

NFKB1: Nuclear factor kappa-light-chain-enhancer of activated B-cells subunit 1; NFKBIA: nuclear factor kappa-light-chain-enhancer of activated B-cells inhibitor alpha; IRAK1: interleukin-1 receptor-associated kinase 1; OR: odds ratio, 95% CI: 95% Confidence interval. *Yates’ correction.
much more frequent compared to the rs3027898 C allele (0.8 A vs. 0.2 C in Caucasians derived from NCBI database according to HapMap-CEU project). Therefore, in the majority of IRAK1 mRNA 3′-UTR sequences, where miR-146a acts and restricts IRAK1 mRNA expression, the rs3027898 A allele is expected to exist. However, even though further studies are needed to confirm this hypothesis, the targeting of miR-146a/IRAK1 axis as a potential approach for PTC management has already been suggested (32).

In conclusion, to our knowledge, this is the first study to report an association of the IRAK1 gene polymorphism rs3027898 with PTC. The present study, which was designed to include only small tumors (less than 4 cm) with no extrathyroidal extension nor lymph node spread, indicates that IRAK1 rs3027898 might have potential use as a biomarker for the early identification of PTC risk before its local spread. However, this was only a pilot study (33) restricted to small patient and control groups of Greek origin, and therefore, we highlight the need for additional studies in larger groups of patients of various origins to validate this finding. Additionally, functional analyses, which evaluate IRAK1 mRNA/protein levels in patients with PTC and controls are needed so as to clarify the potential use of IRAK1 in early diagnosis or management of this disease.

Conflicts of Interest

The Authors declare that they have no conflicts of interest in regard to this study.

Authors’ Contributions

A. Chatzikyriakidou conceived and designed the study, performed the research, the data analysis and the literature review, and wrote the manuscript. A. Chorti performed sample collection. Th. Papavramidis pointed out cases and controls, and edited and reviewed the article.

References

1 Gimm O: Thyroid cancer. Cancer Lett 163(2): 143-56, 2001. PMID: 11165748.
2 Nikiforov YE: Role of molecular markers in thyroid nodule management: Then and now. Endocr Pract 23(8): 979-988, 2017. PMID: 28534687. DOI: 10.4158/EP171805
3 Xing M: BRAF mutation in thyroid cancer. Endocr Relat Cancer 12(2): 245-62, 2005. PMID: 15947100.
4 Pacifico F and Leonardi A: Role of NF-kappaB in thyroid cancer. Mol Cell Endocrinol 321(1): 29-35, 2010. PMID: 19879919. DOI: 10.1016/j.mce.2009.10.010
5 Janssens S and Beyaert R: Functional diversity and regulation of different interleukin-1 receptor-associated kinase (IRAK) family members. Mol Cell 11(2): 293-302, 2003. PMID: 12620219.
6 Dunne A and O’Neill LA: The interleukin-1 receptor/Toll-like receptor superfamily: signal transduction during inflammation and host defense. Sci STKE 2003(171): re3, 2003. PMID: 12606705.
7 Wang X, Peng H, Liang Y, Sun R, Wei T, Li Z, Gong Y, Gong R, Liu F, Zhang L and Zhu J: A functional insertion/deletion polymorphism in the promoter region of the NFkB1 gene increases the risk of papillary thyroid carcinoma. Genet Test Mol Biomarkers 19(3): 167-171, 2015. PMID: 25692306. DOI: 10.1089/gtmb.2014.0271
8 Kim MK, Park SW, Kim SK, Park HJ, Eun YG, Kwon KH and Kim J: Association of Toll-like receptor 2 polymorphisms with papillary thyroid cancer and clinicopathologic features in a Korean population. J Korean Med Sci 27(11): 1333-1338, 2012. PMID: 23166414. DOI: 10.3346/jkms.2012.27.11.1333
9 Kim SK, Park HJ, Hong IK, Chung JH and Eun YG: A missense polymorphism (rs11466653, Met326Thr) of toll-like receptor 10 (TLR10) is associated with tumor size of papillary thyroid carcinoma in the Korean population. Endocrine 43(1): 161-169, 2013. PMID: 23124277. DOI: 10.1007/s12020-012-9783-z
10 Jazdzewski K, Murray EL, Franssila K, Jarzab B, Schoenberg DR and de la Chapelle A: Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci USA 105(20): 7269-7274, 2008. PMID: 18474871. DOI: 10.1073/pnas.0802682105
11 Yang XK, Li P, Zhang C, Leng RX, Li S, Liu J, Li BZ, Pan HF and Ye DQ: Association between IRAK1 rs3027898 and miRNA-499 rs3746444 polymorphisms and rheumatoid arthritis: A case-control study and meta-analysis. Z Rheumatol 76(7): 622-629, 2017. PMID: 27581002. DOI: 10.1007/s00393-016-0169-0
12 Li C, Huang S, Mo S, Zhang N, Zhou L, Mao Z, Lv W, Li J and Zhou Y: Susceptibility of autoimmune diseases in three polymorphisms of infection-associated gene IRAK1. J Infect Dev Ctries 9(6): 614-623, 2015. PMID: 26142671. DOI: 10.3855/jidc.6776
13 Chatzikyriakidou A, Voulgari PV, Georgiou I and Drosos AA: A polymorphism in the 3′-UTR of interleukin-1 receptor-associated kinase (IRAK1), a target gene of miR-146a, is associated with rheumatoid arthritis susceptibility. Joint Bone Spine 77(5): 411-413, 2010. PMID: 20870441. DOI: 10.1016/j.jbspin.2010.05.013
14 Chatzikyriakidou A, Voulgari PV, Georgiou I and Drosos AA: The role of microRNA-146a (miR-146a) and its target IL-1R-associated kinase (IRAK1) in psoriatic arthritis susceptibility. Scand J Immunol 71(5): 382-385, 2010. PMID: 20500689. DOI: 10.1111/j.1365-3083.2010.02381.x
15 Singer JW, Fleischman A, Al-Fayoumi S, Mascarinos HO, Yu Q and Aagarwal A: Inhibition of interleukin-1 receptor-associated kinase 1 (IRAK1) as a therapeutic strategy. Oncotarget 9(70): 33416-33439, 2018. PMID: 30279971. DOI: 10.18632/oncotarget.26058
16 Zhu J and Mohan C: Toll-like receptor signaling pathways--therapeutic opportunities. Mediators Inflamm 2010: 781235, 2010. PMID: 20981241. DOI: 10.1155/2010/781235
17 Perrier ND, Brierley JD, and Tuttle RM: Differentiated and anaplastic thyroid carcinoma: Major changes in the American Joint Committee on Cancer Eighth Edition Cancer Staging Manual. CA Cancer J Clin 68(1): 55-63, 2018. PMID: 29092098. DOI: 10.3322/caac.21439
18 Chatzikyriakidou A, Kyriakou A, Meltsanidou P, Lambropoulos A and Patsatsi A: Association of NFκB1 −94ATTG ins/del polymorphism (rs28362491) with pemphigus vulgaris. Exp Dermatol, 2019. PMID: 31077459. DOI: 10.1111/exd.13957
19 Fu W, Zhuo ZJ, Chen YC, Zhu J, Zhao Z, Jia W, Hu JH, Fu K, Zhu SB, He J and Liu GC: NFκB1 −94 insertion/deletion ATTG polymorphism and cancer risk: Evidence from 50 case–control studies. Oncotarget 8(6): 9806-9822, 2017. PMID: 28039461. DOI: 10.18632/oncotarget.14190
20 Van den Veyver IB: Skewed X inactivation in X-linked disorders. Semin Reprod Med 19(2): 183-191, 2001. PMID: 11480916.
21 Braun JP, Concordet D, Geffré A, Bourges Abella N and Trumel C: Confidence intervals of reference limits in small reference sample groups. Vet Clin Pathol 42(3): 395-398, 2013. PMID: 23899127. DOI: 10.1111/vcp.12065
22 Cen X, Liu S and Cheng K: The role of Toll-like receptor in inflammation and tumor immunity. Front Pharmacol 9: 878, 2018. PMID: 30127747. DOI: 10.3389/fphar.2018.00878
23 Jain A, Kaczanowska S and Davila E: IL-1 Receptor-associated kinase signaling and its role in inflammation, cancer progression, and therapy resistance. Front Immunol 5: 553, 2014. PMID: 25452754. DOI: 10.3389/fimmu.2014.00553
24 Song RH, Qin Q, Yan N, Muhali FS, Meng S, He ST and Zhang JA: Variants in IRAK1-MECP2 region confer susceptibility to autoimmune thyroid diseases. Mol Cell Endocrinol 399: 244-249, 2015. PMID: 25458699. DOI: 10.1016/j.mce.2014.10.013
25 Yao R, Chiu CG, Strugnell SS, Gill S and Wiseman SM: Gender differences in thyroid cancer: A critical review. Expert Rev Endocrinol Metab 6(2): 215-243, 2011. PMID: 30290447. DOI: 10.1586/eem.11.9
26 Chou CK, Chi SY, Huang CH, Chou FF, Huang CC, Liu RT and Kang HY: IRAK1, a target of miR-146b, reduces cell aggressiveness of human papillary thyroid carcinoma. J Clin Endocrinol Metab 101(11): 4357-4366, 2016. PMID: 27533309. DOI: 10.1210/jc.2016-2276
27 Stokowy T, Gawel D and Wojtas B: Differences in miRNA and mRNA profile of papillary thyroid cancer variants. Int J Endocrinol 2016: 1427042, 2016. PMID: 27656207. DOI: 10.1155/2016/1427042
28 Yip L, Kelly L, Shuai Y, Armstrong MJ, Nikiforov YE, Carty SE and Nikiforova MN: MicroRNA signature distinguishes the degree of aggressiveness of papillary thyroid carcinoma. Ann Surg Oncol 18(7): 2035-2041, 2011. PMID: 21537871. DOI: 10.1245/s10434-011-1733-0
29 Wei WJ, WangYL, Li DS, Wang Y, Wang XF, Zhu YX, Yang YJ, Wang ZY, Ma YY, Wu Y, Jin L, Ji QH and Wang JC: Association between the rs2910164 polymorphism in pre-Mir-146a sequence and thyroid carcinogenesis. PLoS One 8(2): e56638, 2013. PMID: 23451063. DOI: 10.1371/journal.pone.0056638
30 Jones AM, Howarth KM, Martin L, Gorman M, Mihai R, Moss L, Auton A, Lemon C, Mehanna H, Mohan H, Clarke SE, Wadsley J, Macias E, Coatesworth A, Beasley M, Roques T, Martin C, Ryan P, Gerrard G, Power D, Bremmer C; TCUKIN Consortium, Tomlinson I and Carvajal-Carmona LG: Thyroid cancer susceptibility polymorphisms: confirmation of loci on chromosomes 9q22 and 14q13, validation of a recessive 8q24 locus and failure to replicate a locus on 5q24. J Med Genet 49(3): 158-163, 2012. PMID: 22282540. DOI: 10.1136/jmedgenet-2011-100586
31 Chen H, Zhang H, Liu Y, Chen Z, Gu J, Cui D and Yang T: miR-146a rs2910164 polymorphism and risk of papillary thyroid carcinoma: A meta-analysis. Genet Test Mol Biomarkers, 2018. PMID: 30484703. DOI: 10.1089/gtmb.2018.0038
32 Qiu Z, Li H, Wang J and Sun C: miR-146a and miR-146b in the diagnosis and prognosis of papillary thyroid carcinoma. Oncol Rep 38(5): 2735-2740, 2017. PMID: 29048684. DOI: 10.3892/or.2017.5994
33 Althouse AD: Adjust for multiple comparisons? It’s not that simple. Ann Thorac Surg 101(5): 1644-1645, 2016. PMID: 27106412. DOI: 10.1016/j.athoracsur.2015.11.024

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