SPANDIDOS PUBLICATIONS

The role of common variants of non-homologous end-joining repair genes *XRCC4*, *LIG4* and *Ku80* in thyroid cancer risk

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Abstract. Variations, such as single nucleotide polymorphisms (SNPs) in DNA damage repair genes have been pointed out as possible factors to cancer predisposition. Ionizing radiation (IR) induces DNA double strand breaks (DSBs) and is the main recognized risk factor for thyroid cancer. However, most of the patients do not show chronic contact with IR and the other factors have non-concordant data. Thus, thyroid cancer could be due to gene variations in association with certain exogenous factors. One of the pathways that repair DSBs is DNA non-homologous end-joining (NHEJ) that comprises several polymorphic genes. We intend to study the role of polymorphic variants in *XRCC4*, *LIG4* and *Ku80* genes, since there is scarcity of data on the role of these genes in thyroid cancer susceptibility. We carried out a hospital-based case-control study in a Caucasian Portuguese population (109 patients and 217 controls) to estimate the potential role of the *XRCC4* (N298S and T134I), *LIG4* (T9I) and *Ku80* (Ex21-238G→A, Ex21+338T→C, Ex21-352C→A, Ex21+466A→G) polymorphisms in the individual susceptibility for this disease. The results here reported do not associate these polymorphisms with susceptibility for non-familial thyroid cancer. However, when the data were analyzed according to the type of tumour, significant results for *Ku80* Ex21-238G→A and Ex21+466A→G were found for papillary tumours (adjusted OR = 2.281; 95% CI = 1.063-4.894; P=0.034). Taken together these results suggest that some of these variants in NHEJ genes can contribute to thyroid cancer susceptibility. However, further studies with a larger sample size will be needed to support our results.

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Introduction

Thyroid carcinoma is one of the most frequent endocrine neoplasia, even though it accounts for <2% of all cancers diagnosed worldwide (1). It is three times more frequent in the female gender than in males and despite the fact that it can occur in any age group, it is most common in those above 30 years (2). The most common histological varieties are non-familiar papillary and follicular thyroid carcinomas, being the first one the most frequent of all thyroid follicular-cell malignancies with 85-90% of incidence (3). The only verified cause of thyroid carcinogenesis is exposure to ionizing radiation (IR), though other risk factors have been pointed out as candidates, such as dietary iodine deficiency, hormonal factors, lymphocytic thyroiditis and familiar history (3-5). Since most of the patients do not show chronic contact with IR and the other factors have non-concordant data, thyroid cancer could be due to sporadic mutations in association with certain exogenous factors.

The double-strand breaks (DSBs) are probably the most detrimental type of DNA damage since it may cause chromosomal abnormalities, leading to inactivation of tumour suppressor genes or activation of oncogenes and ultimately tumourigenesis (6). DSBs can have exogenous and endogenous origin such as IR and reactive oxygen species (ROS), respectively. They can also be generated during V(D)J recombination, DNA replication and when DNA single-strand breaks are encountered. The termini of chromosomes can also be recognised as DSB due to defective metabolism of

telomeres (7). After DSB detection the cell cycle is arrested in DNA damage checkpoints and repair machinery is recruited. These can be repaired by homologous recombination (HRR) and non-homologous end-joining (NHEJ) (8). NHEJ is predominant in G_0 and G_1 phase of cell cycle and is often described as an inaccurate mechanism since it does not use homologous sequences to perform the repair, bringing the DNA termini together in a DNA-protein complex (9). Although this mechanism, in some cases, is susceptible to introduce errors within DNA while executing the repair, it may pose a lower threat than the risk of the cell entering in S phase or mitosis with an unrepaired DSB (10). The core genes implicated in NHEJ repair are: *Ku70* and *Ku80*, also known as *XRCC6* and *XRCC5*, respectively, both coding for DNA end binding proteins; *PRKDC* coding for the DNA-dependent protein kinase catalytic subunit (DNA-PK_{cs}); *LIG4* and *XRCC4* coding for non-homologous end-joining proteins; and *NHEJ1* (*XLF*/*Cernnunos*) coding for a co-factor which interacts with the complex XRCC4/LIG4, in order to promote the DNA end joining (11,12). Other genes are implicated in the pathway with the purpose of DNA ends processing, being the best well known *DCLRE1C* (Artemis) and DNA polymerases μ and λ. These are not considered core genes because in some situations (e.g., blunt ends) the DNA can be rejoined without processing (13). The NHEJ repair mechanism starts with the recruitment of Ku proteins to the DNA ends. Once these are bound to the DNA, the DNA-PK_{cs} recruitment is facilitated and the serine/threonine kinase activity of this complex is activated. A synaptic complex is then formed with the intent of preparing the DNA ends for processing by Artemis and DNA polymerases, if necessary or to DNA rejoining by XRCC4/LIG4 complex (9).

Recent evidence that some DNA repair functions are haploinsufficient, adds weight to the notion that variants in DNA repair genes constitute part of the spectrum of defects contributing to cancer risk. Several single nucleotide polymorphisms (SNPs) have been described in all repair genes including those that repair DSBs, in particularly NHEJ. Some reports have described several SNPs in this repair pathway genes as possible biomarkers of susceptibility to diverse cancers, such as lung, breast, bladder, myeloma and glioma (14-20). To our knowledge, no study concerning polymorphic variants of NHEJ genes has been done in thyroid cancer.

Over the past years, the screening of polymorphic genes has been the main approach to describe inter-individual differences and its implication to cancer predisposition. In thyroid cancer, some studies concerning this approach were already made using the Portuguese population, namely in glutathione S-transferase genes (21), *ERCC2* (4) and genes of the homologous recombination repair (22). Due to the fact that we have previously studied the role of DSBs repair, namely homologous recombination repair (22), that NHEJ core genes have many polymorphic variants and owing to the scarcity of data on the association of these polymorphic genes and thyroid cancer, we carried out an hospital-based case-control study in a Portuguese Caucasian population in order to estimate the potential role of the *XRCC4* (Asn298Ser, rs1805377 and Thr134Ile, rs28360135), *LIG4* (Thr9Ile, rs1805388) and *Ku80* (Ex21-238G→A, rs2440; Ex21+338T→C, rs1051677; Ex21-352C→A, rs6941; Ex21+466A→G, rs1051685) polymorphisms in the individual susceptibility for this disease.

Materials and methods

Study subjects. This study comprises 109 Caucasian Portuguese thyroid cancer patients without familial history of thyroid cancer or previous neoplasic pathology. Patients were recruited in the Department of Nuclear Medicine of the Portuguese Oncology Institute of Lisbon, where they receive Iodine-131 treatment. Histological diagnosis was confirmed for all the cases. For all but one case, two control individuals matched for age (±1 year) and gender were recruited. The control population (n=217), with no previous or current malignant disease or thyroid pathology, was recruited at São Francisco Xavier Hospital (Department of Laboratorial Medicine), where they were observed for non-neoplastic pathology. The anonymity of the patients and control population was guaranteed, and all studies were conducted with the written informed consent of all those involved, obtained prior to blood withdrawal. This study was also conducted with approval by the institutional ethics board of the Faculty of Medical Sciences, UNL. Information on demographic characteristics, family history of cancer, life style habits (e.g., smoking) was collected using a questionnaire administered by trained interviewers. The response rate was >95% for cases and controls.

DNA extraction. Blood samples of all patients and controls were collected into 10 ml heparinised tubes and stored at -20˚C until use. Genomic DNA was obtained from 250 μl of whole blood using a commercially available kit according to the manufacturer instructions (QIAamp DNA extraction kit; Qiagen, Hilden, Germany). Each DNA sample was stored at -20˚C until analysis.

Selection of the tag SNPs. SNP tagging approach allows identifying a set of SNPs in linkage disequilibrium that efficiently captures all the known SNPs and any unknown SNPs in the gene. The tagging SNPs were identified from GVS: Genome Variation Server (http://gvs-p.gs.washington.edu/GVS/). Using this tool we defined a set of tagging SNPs such that all known common SNPs in each of the genes studied had an estimated r^2 >0.8. The SNPs included in the selection should be considered for Caucasian Population and minor allele frequencies (MAF) had to be >5%. Thus, we have studied 7 SNPs in 3 genes of NHEJ repair pathway. These tag SNPs represent a total of 30 SNPs with an r^2 in the order of 1.00. The tagged SNPs are mainly located in intronic regions of the 3 genes. The polymorphisms selected were *XRCC4* (Asn298Ser, rs1805377 and Thr134Ile, rs28360135), *LIG4* (Thr9Ile, rs1805388) and *Ku80* (Ex21-238G→A, rs2440; Ex21+338T→C, rs1051677; Ex21-352C→A, rs6941; Ex21+466A→G, rs1051685).

Genotyping and allelic discrimination. All gene polymorphisms were genotyped by Real-Time PCR (AB7300) using TaqMan® SNP Genotyping Assays from Applied Biosystems (ABI Assays reference: rs1805377, C__11685997_10; rs28360135, C__25618660_10; rs1805388, C__11427969_20; rs1805389, C__11427971_20; rs1051685, C__8838368_1_;

__8838374_10; rs1051677, C__8838367_1_; rs2440, 1046_10). To carry out the allelic discrimination for these gene polymorphisms the DNA samples were quantified by PicoGreen dsDNA Quantification Reagent (Molecular Probes, Eugene, OR, USA) according to the manufacturer's recommendations.

The Real-Time PCR amplification was performed in 10 μl reactions containing 10 ng of genomic DNA, 1X SNP Genotyping Assay Mix (containing two primer/probe pairs in each reaction and two fluorescent dye detectors - FAM® and VIC®) and 1X TaqMan Universal PCR Master Mix containing the AmpliTaqGold® DNA polymerase, dNTPs and optimized buffer components. The amplification conditions consisted of an initial AmpliTaq Gold activation at 95˚C during 10 min, followed by 40 amplification cycles consisting of denaturation at 92˚C for 15 sec and annealing/extension at 60˚C for 1 min. Ten to 15% of the genotype determinations were carried out twice in independent experiments and 100% concordance was obtained between the experiments.

Statistical analysis. Hardy-Weinberg frequencies for the different polymorphisms studied, in the control and patients populations, were carried out using exact probability tests available in Mendel software (v8.0.1) (23).

The Chi-square (χ^2) test was used to evaluate the differences in genotype frequency, smoking status, age and gender distributions between thyroid cancer patients and controls.

The Kolmogorov-Smirnov and Shapiro-Wilk tests were used in order to verify the normality of the continuous variables (age) and the Levene test was used to analyze the homogeneity of variances. The statistical analysis of the homogeneity of age distributions between cases and controls was carried out using the Student's t-test.

The crude and adjusted odds ratio (OR) and the corresponding 95% confidence intervals (CI) were calculated using unconditional multiple logistic regression. The model for adjusted OR included terms for age at diagnosis (≤30, 31-49, 50-69 and ≥70 years), being the lower age group the referent class; gender, being male cases the referent class; and smoking habits, being non-smokers the referent group. All analyses were performed with an SPSS statistical package (version15, SPSS Inc., Chicago, IL).

The degree of linkage disequilibrium and the haplotype estimation between the different SNPs in the same genes, and the allelic frequencies were calculated using SNPStats software (24).

Results

This study comprised 109 thyroid cancer patients and 217 age- and gender-matched controls. The histological classification in this study was as follows: 71.6 % papillary tumours (78 patients), 25.7% follicular tumours (28 patients) and 2.7% poorly differentiated thyroid tumours (3 patients). Table I shows the main characteristics of case-control populations. The age distribution, smoking habits and gender, in control and case population is not statistically different (Table I). The frequency of females was significantly higher, being in accordance with a worldwide estimation for thyroid cancer (1).

Table I. General characteristics for the thyroid cancer cases (n=109) and control population (n=217).

Characteristics	Cases n (%)	Controls n (%)	P-value[c]
Gender			
Male	17 (15.6)	32 (14.7)	0.84
Female	92 (84.4)	185 (85.3)	
Age[a,b]			
≤30	4 (3.7)	9 (4.2)	0.99
31-49	39 (35.8)	77 (35.5)	
50-69	52 (47.7)	104 (47.9)	
≥70	14 (12.8)	27 (12.4)	
Smoking habits			
Never	97 (89)	177 (81.6)	0.12
Current	12 (11)	38 (17.5)	
Missing	0 (0)	2 (0.9)	

[a]Age of diagnosis for cases. [b]Age of control population at the time of diagnosis for the matched case. [c]P-value obtained by χ^2 (cases vs. control group).

We have genotyped 7 SNPs in 3 of all the NHEJ pathway core genes: 2 for *XRCC4* (Asn298Ser and Thr134Ile), 1 for *LIG4* (Thr9Ile) and 4 for *Ku80* 3'UTR (Ex21-238G→A, Ex21+338T→C, Ex21-352C→A, Ex21+466A→G). The genotypic frequencies of all SNPs are in Hardy-Weinberg equilibrium for control and thyroid cancer cases.

The results of genotypic and allelic analysis for all SNPs are reported in Table II. Concerning the genotypic frequencies, the polymorphisms show no statistically significant difference between case and control populations. Allelic frequencies from control population were in accordance with the ones described in dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/). In this table we can also observe, when control frequencies were considered, that *Ku80* Ex21+338T→C and Ex21-352C→A polymorphisms seems to be in linkage disequilibrium. In order to evaluate this incidence, linkage disequilibrium tests using SNPStats were performed for *Ku80* polymorphisms. A strong value of D' (1.00) was found between *Ku80* Ex21+338T→C and Ex21-352C→A. Since tag SNP selection put these polymorphisms in separate groups we reviewed the tag SNPs. In this re-evaluation, *Ku80* Ex21+338T→C and Ex21-352C→A were placed in the same group. Thus, an update of the data for the Caucasian population was done in our selection, leading us to carry out the genotyping of two SNPs in strong linkage disequilibrium. Consequently, all conclusions taken for one SNP will be valid for the other one.

Haplotype analysis was also performed in the genes with more than one SNP studied. The results obtained (data not shown) do not reveal any significant association with thyroid cancer.

Gene-gene interaction was also studied, since *XRCC4* and *LIG4* form a tight complex. However, all possible interactions showed no statistical significance (data not shown).

Table II. Distribution of genotypes in case and control populations, minor allele frequencies (MAF) and ORs (95% CI).

Genotype	Cases n (%)	Control n (%)	MAF		Crude OR (95% CI)	Adjusted OR (95% CI)[a]
			Cases	Controls		
XRCC4 polymorphism Asn298Ser						
Asn/Asn	93 (85.3)	166 (76.5)	Ser: 0.08	Ser: 0.13	1 (Reference)	1 (Reference)
Asn/Ser	15 (13.8)	45 (20.7)			0.60 (0.32-1.13)	0.60 (0.31-1.14)
Ser/Ser	1 (0.9)	6 (2.8)			0.30 (0.04-2.51)	0.31 (0.04-2.63)
XRCC4 polymorphism Thr134Ile						
Thr/Thr	96 (88.1)	195 (90.3)	Ile: 0.06	Ile: 0.05	1 (Reference)	1 (Reference)
Thr/Ile	13 (11.9)	21 (9.7)			1.26 (0.60-2.62)	1.24 (0.59-2.59)
Ile/Ile	-	-			-	-
LIG4 polymorphism Thr9Ile						
Thr/Thr	83 (76.1)	158 (72.8)	Ile: 0.14	Ile: 0.15	1 (Reference)	1 (Reference)
Thr/Ile	22 (20.2)	54 (24.9)			0.78 (0.44-1.36)	0.77 (0.44-1.36)
Ile/Ile	4 (3.7)	5 (2.3)			1.52 (0.40-5.82)	1.35 (0.34-5.36)
Ku80 polymorphism 3'UTR (Ex21-238G→A)						
G/G	31 (29.0)	76 (35.2)	A: 0.45	A: 0.40	1 (Reference)	1 (Reference)
G/A	56 (52.3)	107 (49.5)			1.28 (0.76-2.18)	1.34 (0.78-2.28)
A/A	20 (18.7)	33 (15.3)			1.49 (0.74-2.98)	1.56 (0.77-3.17)
Ku80 polymorphism 3'UTR (Ex21+338T→C)						
T/T	81 (74.3)	169 (78.2)	C: 0.13	C: 0.12	1 (Reference)	1 (Reference)
T/C	27 (24.8)	44 (20.4)			1.28 (0.74-2.21)	1.28 (0.29-4.36)
C/C	1 (0.9)	3 (1.4)			0.70 (0.07-6.79)	0.70 (0.07-6.98)
Ku80 polymorphism 3'UTR (Ex21-352C→A)						
C/C	80 (74.8)	169 (78.2)	A: 0.13	A: 0.12	1 (Reference)	1 (Reference)
C/A	26 (24.3)	44 (20.4)			1.25 (0.72-2.17)	1.19 (0.68-2.07)
A/A	1 (0.9)	3 (1.4)			0.70 (0.07-6.88)	0.702 (0.70-7.01)
Ku80 polymorphism 3'UTR (Ex21+466A→G)						
A/A	91 (83.5)	170 (78.7)	G: 0.09	G: 0.11	1 (Reference)	1 (Reference)
A/G	16 (14.7)	43 (19.9)			0.70 (0.37-1.30)	1.19 (0.68-2.07)
G/G	2 (1.8)	3 (1.4)			1.25 (0.20-7.59)	0.70 (0.07-7.01)

[a]ORs were adjusted for gender, age (≤30, 31-49, 50-69 and ≥70 years) and smoking status (never and current smokers). Male, ≤30 years and never smokers are reference classes.

Stratification by tumour type was made for all SNPs genotyped. Statistical significance was not observed when cases and controls were compared. However, when unconditional multiple logistic regression test was applied, significant ORs associated with the variant genotype of *Ku80* 3'UTR Ex21-238G→A polymorphism were detected (adjusted OR = 2.28; 95% CI = 1.06-4.89; P=0.03; Table III) for papillary tumours and not follicular ones. This result seems thus to indicate that *Ku80* 3'UTR Ex21-238G→A may confer an increased risk for papillary thyroid cancer.

In order to evaluate molecular differences between female and male population, stratification by gender was made as well. Statistical significance was found for the male population using χ^2 test (P=0.03) in *Ku80* polymorphisms Ex21+

Distribution of genotypes in case and control populations when stratified by histological type and ORs (95% CI) to TR (Ex21-238G→A) polymorphism.

	Genotype	Cases n (%)	Control n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
Ku80 polymorphism 3'UTR (Ex21-238G→A)					
Papillary tumour	G/G	20 (26.0)	76 (35.2)	1 (Reference)	1 (Reference)
	G/A	38 (49.4)	107 (49.5)	1.35 (0.73-2.50)	1.40 (0.75-2.61)
	A/A	19 (24.7)	33 (15.3)	**2.12 (1.03-4.63)[b]**	**2.28 (1.06-4.89)[c]**
Follicular tumour	G/G	10 (37)	76 (35.2)	1 (Reference)	1 (Reference)
	G/A	16 (59.3)	107 (49.5)	1.14 (0.49-2.64)	1.12 (0.48-2.64)
	A/A	1 (3.7)	33 (15.3)	0.23 (0.03-1.87)	0.236 (0.03-1.94)

[a]ORs were adjusted for gender, age (≤30, 31-49, 50-69 and ≥70 years) and smoking status (never and current smokers). Male, ≤30 years and never smokers are reference classes; [b]P=0.04; [c]P=0.03.

Table IV. Distribution of genotypes in case and control populations when stratified by gender and ORs (95% CI) to *Ku80* 3'UTR Ex21+338T→C and Ex21-352C→A polymorphisms.

	Genotype	Cases n (%)	Control n (%)	P-value[a]	Crude OR (95% CI)	Adjusted OR (95% CI)[b]
Ku80 polymorphism 3'UTR (Ex21+338T→C)						
Female	T/T	70 (76.1)	141 (76.2)		1 (Reference)	1 (Reference)
	T/C	21 (22.8)	41 (22.2)	0.94	1.03 (0.57-1.88)	0.97 (0.53-1.78)
	C/C	1 (1.1)	3 (1.6)		0.67 (0.07-6.57)	0.67 (0.07-6.67)
Male	T/T	11 (64.7)	28 (90.3)		1 (Reference)	1 (Reference)
	T/C	6 (35.3)	3 (9.7)	0.03	**5.09 (1.08-24.02)[c]**	**5.95 (1.12-31.61)[d]**
	C/C	-	-		-	-
Ku80 polymorphism 3'UTR (Ex21-352C→A)						
Female	C/C	69 (76.7)	141 (76.2)		1 (Reference)	1 (Reference)
	C/A	20 (22.2)	41 (22.2)	0.95	0.99 (0.54-1.83)	0.94 (0.51-1.73)
	A/A	1 (1.1)	3 (1.6)		0.68 (0.07-6.67)	0.67 (0.07-6.67)
Male	C/C	11 (64.7)	28 (90.3)		1 (Reference)	1 (Reference)
	C/A	6 (35.3)	3 (9.7)	0.03	**5.09 (1.08-24.02)[c]**	**5.95 (1.12-31.61)[d]**
	A/A	-	-		-	-

[a]P-value obtained by χ^2 (cases vs. control group); [b]ORs were adjusted for gender, age (≤30, 31-49, 50-69 and ≥70 years) and smoking status (never and current smokers). Male, ≤30 years and never smokers are reference classes; [c]P=0.04; [d]P=0.04.

338T→C and Ex21-352C→A (Table IV). When unconditional multiple logistic regression was tested for all SNPs, significant ORs were found for the same polymorphisms [adjusted OR = 5.95 (1.12-31.61); P=0.04; Table IV]. Thus, these polymorphisms may be related to an increase in individual risk for this disease, particularly for males. However, due to gender stratification, the number of cancer cases in each gender groups is small. Thus, this last result must be a false positive (type I error).

Discussion

The polymorphisms *XRCC4* (Asn298Ser and Thr134Ile), *LIG4* (Thr9Ile) and *Ku80* (Ex21-238G→A, Ex21+338T→C, Ex21-352C→A, Ex21+466A→G) have been evaluated in several case-control studies analysing individual susceptibility to cancer (14-20). To our knowledge, this is the first comprehensive study to analyze the possible role of 7 SNPs in 3 genes of the NHEJ repair pathway and its influence for

thyroid cancer susceptibility. Moreover, an SNP tagging approach was used, representing a total of 30 SNPs in the 3 genes studied.

The frequency of the different polymorphisms in the Portuguese control population is similar to the ones reported for other Caucasian populations and there is no deviation from the Hardy-Weinberg equilibrium.

XRCC4 and LIG4 proteins form a complex in the cell, and the interaction between them is extremely strong and stable (25,26). This complex is particularly important in the DSBs repair by NHEJ, since it aligns and rejoins the DNA ends (27). Any amino acid exchange that results in diminished stability of this complex could result in a lower activity and consequently a defect in the DNA repair. The data here reported are in agreement with previews studies (for different cancer types) conducted by García-Closas *et al* (28), where they failed to show an association between *XRCC4* Asn298Ser and breast cancer in a meta-analysis using two population-based studies in USA and Poland; and Roddam *et al* (17), reporting null results for multiple myeloma and *LIG4* Thr9Ile in an European population. Conversely, there are previews reports conducted by Figueroa *et al* (16), showing an association of *XRCC4* Asn298Ser heterozygous genotype [OR=1.43 (1.14-1.80 CI), P=0.002, n (cases) = 21% vs. n (controls) = 16%] with bladder cancer risk in a Spanish population; Werbrouck *et al* (29), pointed out an association of *LIG4* Thr9Ile heterozygous and variant homozygous genotypes [OR=0.5, P=0.03, n (cases)=19.7% vs. n (controls) = 28% and OR=0.12, P=0.01, n (cases) = 2% vs. n (controls) = 5.1%, respectively] with head and neck cancer in a Belgian population; Tseng *et al* (20) also reported an association between *XRCC4* Asn298Ser [homozygous variant OR = 2.38 (1.05-5.76 CI), P=0.043, n (cases) = 12.7% vs. total n (controls) = 5.96%] and *LIG4* Thr9Ile [heterozygous and homozygous variant association OR = 1.64 (1.03-2.62 CI), P=0.038] with lung cancer in a Chinese population. This disparity of results found might be due to different risk factors in the assorted populations and/or differences in the genetic background. The results of the present report do not associate *XRCC4* and *LIG4* polymorphisms with susceptibility for non-familial thyroid cancer (Table II). Nevertheless, we cannot exclude the possible effect of other polymorphic variants of these genes in thyroid cancer pathogenesis or the effect of these SNPs in other populations.

The first step during NHEJ is carried out by the Ku70-Ku80 protein complex in the recognition of the DNA ends at the site of the lesion (30). Subsequent protein recruitment can be compromised if any structural change disrupts the link between these two monomers. Genetic polymorphisms can also weaken the bounds between protein interaction and DNA. Regarding the possible role of *Ku80* polymorphisms in thyroid cancer, we did not find significant results when all histological types were considered (Table II). After stratification by tumour type (papillary and follicular) significant results for *Ku80* Ex21-238G→A were found for papillary tumours (Table III), showing that different histological types can have different genetic basis. As referred to above, this disease is much more frequent in the female then in the male population. In order to understand if men and women have different molecular biomarkers we have stratified our study population and observed that *Ku80* Ex21+338T→C is associated, in the male population, with an increased individual risk for this pathology (Table IV). When the general population is stratified, men are much fewer than women. Thus, the statistical methodology has less power and we may be committing a type I error. Therefore, further studies with much larger male populations are indispensable to corroborate these results. These results cannot be compared with others since there are no association studies, to our knowledge, for thyroid cancer with these SNPs. Nevertheless, Hayden *et al* (19) reported significant results for *Ku80* Ex21-238G→A and Ex21+466A→G polymorphisms in association with myeloma. The first SNP shows a decreased risk for the variant homozygous individuals [OR=0.58 (0.35-0.96 CI), P=0.044, n (cases) = 19.3% vs. n (controls) = 12.7%] and the second one an increased risk for variant homozygous individuals [OR=8.3 (1.05-65.35 CI), P=0.027, n (cases) = 3% vs. n (controls) = 0.4%]. To our knowledge, this is the only study dealing with the same SNPs in *Ku80* gene as those studied by us.

In summary, our study shows that *Ku80* Ex21-238G→A and Ex21+338T→C polymorphisms might be involved in individual susceptibility to thyroid cancer. Additional studies with larger populations should be done to support the data.

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