

Association of Polymorphisms in *ERCC2* Gene with Non-Familial Thyroid Cancer Risk

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Abstract

The *ERCC2* protein is an evolutionary conserved ATP-dependent helicase that is associated with a TFIIH transcription factor complex and plays an important role in nucleotide excision repair. Mutations in this gene are responsible for xeroderma pigmentosum and also for Cockayne syndrome and trichothiodystrophy. Several single nucleotide polymorphisms have been identified in the *ERCC2* locus. Among them, a G23591A polymorphism in the codon 312 results in an Asp → Asn substitution in a conserved region and a A35931C polymorphism in the codon 751 results in a Lys → Gln substitution. Because these polymorphisms have been associated with an increased risk for several types of cancers, we carried out an hospital based case-control study in a Caucasian Portuguese population to evaluate the potential role of these polymorphisms on the individual susceptibility to thyroid cancer. The results obtained did not reveal a significant

association between each individual polymorphism studied (G23591A and A35931C) and an increased thyroid cancer risk, but individuals homozygous for non-wild-type variants are overrepresented in patients group. The evaluation of the different haplotypes generated by these polymorphisms showed that individuals simultaneously homozygous for rare variants of both polymorphisms have an increased risk for thyroid cancer [adjusted odds ratio (OR) 3.084; 95% confidence interval (95% CI), 1.347-7.061; $P = 0.008$] and for papillary thyroid-type tumors (adjusted OR, 2.997; 95% CI, 1.235-7.272; $P = 0.015$) but not for follicular thyroid-type tumors. These results suggest that genetic polymorphisms in this gene might be associated with individual susceptibility towards thyroid cancer, mainly papillary-type tumors, but larger studies are required to confirm these results. (Cancer Epidemiol Biomarkers Prev 2005;14(10):2407–12)

Introduction

Thyroid cancer accounts for <1% of all human cancers but is the most frequent endocrine neoplasia. Thyroid tumors are unusual in children and adolescents, and their incidence increases with age in adults (1) with the majority of cases occurring between 25 and 65 years of age (2). The non-familial papillary and follicular thyroid carcinomas are the most common histologic varieties, with a long-term (~10-year) survival rate of >90% (3). Exposure to ionizing radiation is the only verified cause of thyroid carcinogenesis in humans, especially when exposure occurs at young age, although dietary iodine deficiency has also been linked to this pathology (4-6). Exposure to ionizing radiation could lead to the formation of radical DNA reactive species in target tissues that are related with acute and late radiation effects (7). However, individuals without previous exposure to ionizing radiation can also develop thyroid cancers, usually defined as sporadic tumors (8), suggesting that other risk factors could also be involved in the etiology of sporadic tumors. In fact, papillary tumors associated with radiation exposure usually have different forms of the activated *RET* proto-oncogene and at a higher frequency than that observed in spontaneous non-radiation-induced tumors (8) suggesting the existence of other

risk factors for thyroid papillary tumors. Additionally, the high frequency of cancer among family members of thyroid cancer patients supports the hypothesis that hereditary factors are important in the etiology of thyroid cancer. Thus, the identification of susceptibility factors associated with individual predisposition to thyroid cancer could possibly give further insight into the etiology of this malignancy.

Inherited differences in the capacity to metabolize environmental carcinogens and to repair DNA lesion induced by endogenous and exogenous carcinogens, as a consequence of individual genetic polymorphisms, have been suggested to modify individual susceptibility for cancer. These genetic polymorphisms are usually less penetrant than dominant mutations seen in retinoblastoma, Wilms' tumor, and cancers of the Li-Fraumeni syndrome, but its study in cancers is important taking account its higher prevalence and consequently its higher possible attributable risk (9). Various environmental xenobiotics that lead to an enlargement of the thyroid gland in experimental animals exert their action probably as a consequence of their cooxidation by thyroid peroxidase to form reactive intermediates that covalently bind to amino acid residues critical to peroxidase activity. The thyroid peroxidase inactivation causes thyroid-stimulating hormone levels to increase thus inducing thyroid peroxidase and NADPH oxidase synthesis. The resulting prolonged growth stimulus selects for proliferative/transformed thyroid cells and increases the risk of thyroid carcinogenesis. The suicidal inactivation of thyroid peroxidase by some thyroid carcinogens in experimental animals has been related with the production of radical species that could react with thyroid peroxidase (10). It cannot be excluded, however, that the reactive compounds produced by these mechanisms could also react with DNA leading to mutations.

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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. This variation in DNA repair capacity has characteristics expected of cancer susceptibility genes. The proteins encoded by those alleles might exhibit reduced function rather than absence of function, which causes disease (11, 12). Several polymorphisms in genes that encode for DNA repair proteins have been described, and most of these are participants in the four major DNA repair pathways: base excision repair, nucleotide excision repair (NER), mismatch repair, and double-strand break/recombination repair.

The NER pathway is the major cellular repair pathway for a large variety of DNA lesions (UV-induced photoproducts, bulky chemical adducts, and also oxidative DNA damage) and is also associated with the transcription coupled repair pathway (13). NER involves 25 proteins that locate the damaged strand, introduce incisions on each side of lesions, excise an oligonucleotide of 24 to 32 residues, and fill in the gap by repair synthesis and ligation. The DNA helicase ERCC2 (XPD), subunit of the basal transcription factor TFIIH that is required for transcription initiation by RNA polymerase II, is also one of the major proteins involved in NER (11). Germ line mutations in the coding region of the ERCC2 gene are correlated with different clinical phenotypes such as xeroderma pigmentosum, trichothiodystrophy, and Cockayne's syndrome (14). Several ERCC2 polymorphisms in the coding regions with relatively high allele frequencies were recently identified. The data available from the Cancer Genome Anatomy Project SNP500 Cancer Database (<http://snp500.cancer.nci.nih.gov/>) show that for the nine single nucleotide polymorphisms described in ERCC2, only six were validated. From the validated single nucleotide polymorphisms, two of them, IVS19 -70C>T and IVS19 -26C>T, are located deep within intron and the predicted relative risk of the phenotype is considered low according to Tabor et al. (15). The IVS6 +9A>C polymorphism is located within 10 bp of the exon, and according to Tabor et al., the predicted relative risk of the phenotype could be significant, but the frequency of this polymorphism in Caucasians is low (<5%), and for that reason, this polymorphism was not evaluated in this study. From the remaining three polymorphisms, one (Ex6 -10A>C) is synonymous and only the nonsynonymous polymorphisms Ex10 -16G>A (Asp³¹²Asn) and Ex23 +61A>C (Lys⁷⁵¹Gln) were evaluated, based on their biological plausibility. Because putative risk factors for thyroid cancer may also produce genotoxicants (e.g., lipoperoxidation products formed as a consequence of radiation exposure) and the lesions arising from exposure to those compounds are usually repaired by enzymes involved in the NER pathway, we carried out a hospital-based case-control study in a Caucasian Portuguese population to evaluate the potential modifying role of the ERCC2 polymorphisms on the individual susceptibility to non-familial thyroid cancer.

Materials and Methods

Study Subjects. The study population was from a hospital-based case-control study done by the Department of Nuclear Medicine of the Portuguese Oncology Institute of Lisbon and the Faculty of Medical Sciences (Departments of Genetics and Laboratorial Medicine). The study includes 108 thyroid cancer patients without familial history of thyroid cancer, 77 with papillary carcinoma (71%), 28 with follicular carcinoma (26%), and three with poorly differentiated thyroid carcinoma (3%). Mean age was 52.5 years (17 males and 91 females). Patients were recruited in the Department of Nuclear Medicine of the Portuguese Oncology Institute of Lisbon where they receive Iodine-131 treatment. Histologic diagnosis was confirmed in all

cases. All the patients were Caucasian Portuguese born with Portuguese ascendants with no previous neoplastic pathology. For all but three cases, two control individuals matched for age (± 1 year), sex, and ethnicity were recruited. For the remaining three cases, only one matched control was recruited.

The control population, 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 nonmalignant pathology. These were also Caucasian Portuguese born in Portugal and with Portuguese ascendants. Thirty-one percent of control individuals had no significant pathology. The main diagnosis in control population was NIDDM (21%), cardiovascular (20%), nononcological surgical patients (11%), gastrointestinal (4%), orthopedic (3%), gynecologic (3%), and other nonmalignant (7%) diseases. The anonymity of the patients and control population was guaranteed, and all studies were conducted with the informed consent of all the individuals involved. Written informed consent was obtained before blood withdrawal. Information on demographic characteristics, family history of cancer, life-style habits (smoking), and exposure to ionizing radiation was collected using a questionnaire administered by trained interviewers. Former smokers were defined as those who gave up smoking 2 years before thyroid cancer diagnosis or 2 years before the inclusion date as corresponding matched case. The response rate was higher than 95% for cases and controls.

Healthcare services in Portugal are mainly public and assist generally all the population. The demographic pattern of Lisbon Caucasian population is homogeneously spread through all the city area; thus, the use of control and cancer population from different Lisbon hospital does not seem to be a source of bias because both populations are comparable. Additionally, the Oncology Institute where the cases were collected is the only specialized center for thyroid cancer treatment in Lisbon; therefore, all thyroid cancer patients diagnosed in Lisbon hospitals are referred to this center for treatment.

DNA Extraction. Blood samples of all patients and controls were collected into 10-mL heparinized 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.

Genotyping. The ERCC2 Lys⁷⁵¹Gln and Asp³¹²Asn polymorphisms were determined by PCR followed by RFLP. DNA samples for A35931C polymorphism were amplified with the following primers: 5'-CCCTCTCCCTTCTCTGTTCTCTGC-3'

Table 1. General characteristics for the thyroid cancer cases (n = 108) and control population (n = 213)

Characteristics	Cases, n (%)	Controls, n (%)	P*
Gender			
Men	17 (16)	31 (16)	0.869 [†]
Woman	91 (84)	182 (84)	
Age ^{‡§}			
≤ 30	4 (4)	9 (4)	0.944 [†]
31-49	39 (36)	76 (36)	
50-69	51 (47)	102 (48)	
≥ 70	14 (13)	26 (12)	
Smoking habits			
Never	96 (89)	173 (82)	0.142 [†]
Current	12 (11)	37 (18)	
Missing	0 (0)	3 (1)	

*See Materials and Methods.

[†]Cases versus control group.

[‡]Age of diagnosis for cases.

[§]Age of control population at the time of diagnosis for the matched case.

Table 2. Distribution of genotypes in control population (n = 213) and thyroid cancer cases (n = 108)

	ERCC2 polymorphisms G23591A (Asp ³¹² Asn)		
	Asp/Asp, n (%)	Asp/Asn, n (%)	Asn/Asn, n (%)
Control population	103 (48.4)	91 (42.7)	19 (8.9)
Thyroid cancer patients	45 (41.7)	46 (42.6)	17 (15.7)

	ERCC2 polymorphisms A35931C (Lys ⁷⁵¹ Gln)		
	Lys/Lys, n (%)	Lys/Gln, n (%)	Gln/Gln, n (%)
Control population	93 (43.7)	92 (43.2)	28 (13.1)
Thyroid cancer patients	40 (37.0)	48 (44.4)	20 (18.5)

(upstream) and 5'-GGAACAGTGCAGGAGGGATGGG-3' (downstream). The PCR was done in a 50- μ L reaction containing 1 \times PCR buffer, 2.5 mmol/L MgCl₂, 0.8 mmol/L deoxynucleotide triphosphate, 1.0 μ mol/L of each primer, and 1.25 units of AmpliTaq Gold (Applied Biosystems, Foster City, CA). After an initial denaturation at 94°C for 7 minutes, there were 35 cycles of 30 seconds at 94°C, 30 seconds at 62°C, and 30 seconds at 72°C and a final extension step of 10 minutes at 72°C.

Ten microliters of amplified fragment was digested with 5 units of *Pst*I (Fermentas, Vilnius, Lithuania) during 90 minutes at 37°C. The fragments obtained were analyzed in a 2% agarose gel with ethidium bromide (0.5 mg/mL). The homozygous wild-type allele (Lys⁷⁵¹) produced one restriction site (28 and 331 bp); whereas the homozygous variant allele (Gln⁷⁵¹) produced three bands (28, 63, and 268 bp), and heterozygous (Lys/Gln) displayed all four bands (28, 63, 268, and 331 bp).

The amplification fragment for G23591A polymorphism was obtained with 5'-CTGTTGGTGGGTGCCCGTATCTGTTGGTCT-3' (upstream) and 5'-TAATATCGGGGCTCACCCTGCAGCACTTCCT-3' (downstream), as described by Spitz et al. (16), with minor modifications. The restriction analysis was done with 10 μ L of PCR product and digested with 5 units of *Eco*130I (Fermentas) during 120 minutes at 37°C. The fragments obtained were analyzed in a 2% agarose gel with ethidium bromide (0.5 mg/mL). The homozygous wild-type allele (Asp³¹²) produced one restriction site (245 and 506 bp); whereas the homozygous variant allele (Asn³¹²) produced three bands (32, 245, and 474 bp), and heterozygous (Asp/Asn) displayed all four bands (32, 245, 474, and 506 bp).

All the genotype determinations were carried out twice in independent experiments and all the inconclusive samples were reanalyzed.

Statistical Analysis. The analysis of Hardy-Weinberg frequencies for ERCC2 alleles in the control and patients population were carried out using Genepop (V3.4) software (17). Genotypic frequencies for each population were tested for Hardy-Weinberg proportions using exact probability tests available in Genepop (V3.4) and the difference in Hardy-Weinberg across population for ERCC2 polymorphisms was carried out using Fisher exact test. Independent segregation of alleles between loci was assessed by exact test of genotypic linkage disequilibrium, also available in Genepop (V3.4; ref. 17).

The χ^2 test was used to evaluate the distribution of gender in case groups after stratification (papillary and follicular cases), the differences in genotype frequency in the populations studied, and the heterogeneity between papillary and follicular patients.

The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to verify the normality of the continuous variables (e.g., 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 *t* test. The statistical analysis of the homogeneity of sex and smoking status distributions between cases and controls was carried out using χ^2 test.

The crude and adjusted odds ratio (OR) and the corresponding 95% confidence intervals (95% CI) were calculated using unconditional multiple logistic regression. The model for adjusted OR included terms for age at diagnosis (<30, 30-49, 50-69, and \geq 70 years), being the lower age group the reference class; sex, being male cases the reference class; and smoking habits, being nonsmokers the reference group. All analyses were done with an SPSS statistical package (version 10.5, SPSS, Inc., Chicago, IL).

Table 3. Genotypic frequencies of ERCC2 polymorphisms G23591A (Asp³¹²Asn) and A35931C (Lys⁷⁵¹Gln) in Caucasian populations

ERCC2 polymorphisms G23591A (Asp ³¹² Asn)			
Asp/Asp (%)	Asp/Asn (%)	Asn/Asn (%)	Population
52.5, 44, 44	37, 40.5, 46	19, 7, 10	American Caucasians (refs. [16, 24, 33] respectively)
42	43	15	Germans (34)

ERCC2 polymorphisms A35931C (Lys ⁷⁵¹ Gln)			
Lys/Lys (%)	Lys/Gln (%)	Gln/Gln (%)	Population
38, 44, 40, 44	52, 44, 46, 45	10, 12, 13, 11	American Caucasians (refs. [16, 24, 33, 35] respectively)
41	45	14	Germans (34)
39, 43	46, 40	15, 17	Swedish (36) and (31) respectively.
37, 33	46, 51	17, 17	Italians (37) and (30) respectively.

Table 4. Distribution of genotypes according to gender in thyroid cancer cases ($n = 108$) and in papillary ($n = 77$) and follicular tumors ($n = 28$)

	ERCC2 polymorphisms G23591A (Asp ³¹² Asn)		
	Asp/Asp: men, n (%) versus women, n (%)	Asp/Asn: men, n (%) versus women, n (%)	Asn/Asn: men, n (%) versus women, n (%)
All cases	6 (35.3) versus 39 (42.9)	8 (47.1) versus 38 (41.8)	3 (17.6) versus 14 (15.4)
Papillary tumors	5 (35.7) versus 28 (44.4)	6 (42.9) versus 26 (41.3)	3 (21.4) versus 9 (14.3)
Follicular tumors	1 (50.0) versus 10 (38.5)	1 (50.0) versus 11 (42.3)	0 (0.0%) versus 5 (19.2)
	ERCC2 polymorphisms A35931C (Lys ⁷⁵¹ Gln)		
	Lys/Lys: men, n (%) versus women, n (%)	Lys/Gln: men, n (%) versus women, n (%)	Gln/Gln: men, n (%) versus women, n (%)
All cases	3 (17.6) versus 37 (40.7)	42 (70.6) versus 6 (39.6)	2 (11.8) versus 18 (19.8)
Papillary tumors	3 (21.4) versus 27 (42.9)	9 (64.3) versus 23 (36.5)	2 (14.3) versus 13 (20.6)
Follicular tumors	0 (0.0) versus 9 (34.6)	2 (100.0) versus 13 (50.0)	0 (0.0) versus 4 (15.4)

Results

This study comprised 108 thyroid cancer patients and 213 age- and sex-matched controls. Among controls, 66 (31%) were healthy individuals and 147 (69%) were hospital patients without previous or current cancer pathology. Histologic classification of thyroid tumors was as follows: 71% papillary tumors (77 patients), 26% follicular tumors (28 patients), and 3% poorly differentiated thyroid tumors (three patients). All the cases denied previous exposure to ionizing radiation sources (either accidental or therapeutic). Table 1 shows the main characteristics of cancer and control population. The frequency of females was significantly higher in the case population and also in the groups of papillary and follicular cancer ($P < 0.0001$), which is in agreement with the gender distributions usually observed in this type of cancers (1).

The age distribution in control and case population is not statistically different ($P = 0.944$) and the same applies to smoking habits ($P = 0.142$) and gender ($P = 0.869$; Table 1).

The frequencies of ERCC2 genotypes [G192A (Asp³¹²Asn) of exon 10 and A35931C (Lys⁷⁵¹Gln) of exon 23] in control and cancer population were in agreement with the Hardy-Weinberg expectations ($P > 0.10$, exact probability test). When both populations were pooled (cancer patients and controls), there was still no significant deviation from Hardy-Weinberg equilibrium, indicating that for both genotypes of ERCC2, they represent the same pool ($P > 0.5$, Fisher exact test).

The test for genetic linkage disequilibrium between the two loci was highly significant ($P < 0.0001$), indicating that the two polymorphisms are tightly linked in our population, as previously observed in other populations (18, 19). The frequencies of the ERCC2 genotypes observed in the control

Table 5. Number of cases/controls and ORs (95% CI) of thyroid cancers in relation to the ERCC2 G23591A (Asp³¹²Asn) and A35931C (Lys⁷⁵¹Gln) genotypes

Genotypes	n (%)	Crude OR (95% CI)	Adjusted OR (95% CI) [†]
All thyroid cancers ($n = 108^*$)			
G23591A (Asp ³¹² Asn)			
Asp/Asp	45 (41.7%)	1 (reference)	1 (reference)
Asp/Asn	46 (42.6%)	1.157 (0.703-1.905)	1.110 (0.666-1.850)
Asn/Asn	17 (15.7%)	2.048 (0.975-4.302)	2.009 (0.949-4.253)
A35931C (Lys ⁷⁵¹ Gln)			
Lys/Lys	40 (37.0%)	1 (reference)	1 (reference)
Lys/Gln	48 (44.4%)	1.213 (0.729-2.018)	1.176 (0.697-1.984)
Gln/Gln	20 (18.5%)	1.661 (0.839-3.288)	1.600 (0.802-3.191)
Papillary tumors			
G23591A (Asp ³¹² Asn)			
Asp/Asp	33 (42.9)	1 (reference)	1 (reference)
Asp/Asn	32 (41.6)	1.098 (0.626-1.926)	1.032 (0.582-1.832)
Asn/Asn	12 (15.6)	1.971 (0.866-4.486)	1.855 (0.804-4.252)
A35931C (Lys ⁷⁵¹ Gln)			
Lys/Lys	30 (39.0)	1 (reference)	1 (reference)
Lys/Gln	32 (41.6)	1.078 (0.606-1.917)	1.024 (0.569-1.845)
Gln/Gln	15 (19.5)	1.661 (0.784-3.516)	1.538 (0.721-3.248)
Follicular tumors			
G23591A (Asp ³¹² Asn)			
Asp/Asp	11 (39.3)	1 (reference)	1 (reference)
Asp/Asn	12 (42.9)	1.235 (0.520-2.934)	1.307 (0.531-3.219)
Asn/Asn	5 (17.9)	2.464 (0.769-7.899)	2.484 (0.750-8.227)
A35931C (Lys ⁷⁵¹ Gln)			
Lys/Lys	9 (32.1)	1 (reference)	1 (reference)
Lys/Gln	15 (53.6)	1.685 (0.702-4.042)	1.916 (0.769-4.772)
Gln/Gln	4 (14.3)	1.476 (0.422-5.159)	1.542 (0.432-5.509)

*Also including three thyroid cancers nonclassifiable as papillary or follicular thyroid cancers.

[†]ORs were adjusted for sex (male and female), age (<30, 30-49, 50-69, and ≥ 70 y), and smoking status (never, former, and current smokers). Female; <30 y, and never smokers are reference classes.

Table 6. Combined ERCC2 G23591A (Asp³¹²Asn) and A35931C (Lys⁷⁵¹Gln) genotypes in the studied populations

ERCC2 35931	ERCC2 23591		
	Asp/Asp, n (%)	Asp/Asn, n (%)	Asn/Asn, n (%)
Control population			
Lys/Lys	82 (38)	18 (8)	3 (2)
Lys/Gln	9 (4)	68 (32)	14 (7)
Gln/Gln	2 (1)	6 (3)	11 (5)
Cases			
Lys/Lys	35 (32)	8 (7)	2 (2)
Lys/Gln	5 (5)	38 (35)	3 (3)
Gln/Gln	0 (0)	2 (2)	15 (14)*

* $P < 0.007$ when compared with the frequency of the same haplotype in control population.

population (Table 2) are similar to those reported in other Caucasian populations (see Table 3).

In spite of the higher thyroid cancer incidence in women, the frequency of the different genotypes studied was not statistically different (χ^2 test with Yates correction) between male and female thyroid carcinoma patients, even when stratified for cancer type (Table 4). This similarity of genotype frequencies in cases according to gender does not justify a further gender stratification of the population for further statistical analysis.

The results obtained showed that the frequency of each individual ERCC2 genotypes was not significantly different (χ^2 test with Yates correction) for all the cases studied and also for the groups of papillary and follicular tumors, when compared with control group (Table 2). In addition, for each of the genotypes studied, no significant increase in crude or adjusted ORs was observed (Table 5), suggesting that individual ERCC2 polymorphisms alone are not associated with a significant risk increase for thyroid cancer. However, after logistic regression analysis, a consistent increase in the OR values for each of the polymorphisms related with the number of variant alleles was observed (Table 5).

Because the two polymorphisms evaluated are in linkage disequilibrium, and for both polymorphisms, a higher frequency of individuals homozygous for the variant alleles in thyroid cancer patients (8.9% versus 15.7% and 13.1% versus 18.5%, for G23591A and A35931C, respectively) was observed, we have evaluated the frequency of the different genotypes combination in the populations studied. The results obtained showed that the frequency of homozygous for both variant alleles is significantly higher in thyroid cancer population ($P < 0.007$; Table 6). Logistic regression analysis showed that the presence of homozygosity for both variant alleles is significantly associated with thyroid cancer risk in all cases (adjusted OR, 3.084; 95% CI, 1.347-7.061; $P =$

0.008) and in papillary tumors (adjusted OR, 2.997; 95% CI, 1.235-7.272; $P = 0.015$), and in follicular tumors, almost reach the significance (adjusted OR, 3.485; 95% CI, 0.967-12.555; $P = 0.056$; Table 7). Analysis for both histologic types of tumors did not reveal a significant heterogeneity, suggesting that the observed marginal significance for follicular-type tumors ($P = 0.056$) could be related with the low number of individuals in this group.

Discussion

Because the ERCC2 gene is involved in the repair of bulky DNA adducts and UV light-induced DNA damage, the role of the ERCC2 G23591A and A35931C polymorphism was evaluated in several cancer case control studies. Indeed, it was reported that the presence of variant alleles are associated with increased risk of several types of cancer such as melanoma (20), prostate (21), bladder (22), esophagus (23), among others.

In this article, we report the role of genetic polymorphisms in the ERCC2 gene in thyroid cancer. Our results show that the ERCC2 polymorphisms G23591A (Asp³¹²Asn) in exon 10 and A35931C (Lys⁷⁵¹Gln) in exon 23 are not separately associated with a significant increase for thyroid cancer (Table 5). The presence of each variant alleles leads to a nonsignificant increase in the adjusted OR, for all cases and also for papillary- and follicular-type tumors (Table 5), this effect being more pronounced for the variant form of the G23591A polymorphism. Nonetheless, and in spite of the nonsignificant adjusted OR values, in several situations, the P almost reach the significance [e.g., adjusted OR for all cases (Asn/Asn), 2.009; 95% CI, 0.949-4.253; $P = 0.07$].

Because both polymorphisms are in linkage disequilibrium, a statistically significant proportion carrying the variant allele G23591A also had the A35931C variant allele. Our results showed that subjects homozygous for both variant alleles are overrepresented in thyroid cancer group (Table 6), and the combination of the two variant forms of both polymorphisms (Asn³¹²Asn and Gln⁷⁵¹Gln) are associated with a significant increase risk to thyroid cancer suggesting an interaction between these two polymorphisms.

Our results are in agreement with the ones reported in other case control studies in lung cancer (18, 24, 25), where a strong interaction between the variant forms of these polymorphisms was also observed.

Studies of DNA repair of cyclobutane pyrimidine dimers in human skin *in situ* showed that individuals with exon 10 AA and exon 23 CC genotypes showed an ~50% decrease in the DNA repair capacity (26), and the presence of these variant alleles were also associated with an increased frequency of smoking-related *p53* mutations (27). Functional studies on G23591A and A35931C ERCC2 polymorphisms have been

Table 7. Number of cases/controls and ORs (95% CI) of thyroid cancers in relation to the ERCC2 G23591A (Asp³¹²Asn) and A35931C (Lys⁷⁵¹Gln) genotypes

Genotypes	n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)*
All thyroid cancers ($n = 108$) [†]			
Other combinations of genotypes	93 (86.1)	1 (reference)	1 (reference)
Asn/Asn and Gln/Gln	15 (13.9)	2.962 (1.310-6.697)	3.084 (1.347-7.061)
Papillary tumors ($n = 78$)			
Other combinations of genotypes	66 (85.7)	1 (reference)	1 (reference)
Asn/Asn and Gln/Gln	11 (14.3)	3.061 (1.269-7.384)	2.997 (1.235-7.272)
Follicular tumors ($n = 27$)			
Other combinations of genotypes	24 (91.9)	1 (reference)	1 (reference)
Asn/Asn and Gln/Gln	4 (8.1)	3.061 (0.903-10.368)	3.485 (0.967-12.555)

*ORs were adjusted for sex (male and female), age (<30, 30-49, 50-69, and ≥ 70 y) and smoking status (never, former, and current smokers). Female, <30 y, and never smokers are reference classes.

[†]Also including three thyroid cancers nonclassifiable as papillary or follicular thyroid cancers.

conflicting. In a small study, for 31 women at risk of breast cancer, the AA genotype in exon 23 was associated with suboptimal DNA repair capacity, because lymphocytes from homozygous individuals for the A allele (wild-type homozygous) showed reduced repair in an X-ray-induced chromosome aberration assay (28). In contrast, two other studies with larger samples sizes and healthy individuals suggest that DNA repair capacity measured by the host reactivation assay using BPDE-treated plasmids in 360 controls (16) or UV-irradiated plasmids in 102 healthy individuals (29) was reduced in subjects carrying two variant alleles (Asn³¹² or Gln⁷⁵¹) compared with those homozygous for the respective wild-type alleles. When both genotypes were combined, the best repair activity was found in cells from individuals homozygous for wild-type alleles at both loci and the lowest repair activity in those carrying at least two variant alleles (16), this larger studies being in agreement with the results reported in these work.

Considering the data arising from the functional role of A35931C ERCC2 polymorphism in the repair of bulky DNA adducts, it was reported that nonsmokers homozygous for the variant allele (CC) have higher levels of aromatic DNA adduct when compared with individuals carrying at least one copy of the A allele (30, 31). However, this effect was not confirmed in other study (5). It was also reported that apart from a significant increase in the level of DNA adducts in peripheral lymphocytes related with the variant forms of the G23591A and A35931C polymorphisms, individuals with the combined exon 10 AA exon 23 CC have significant higher DNA adduct levels in peripheral lymphocytes than individuals with other combinations of genotypes (31). According to this data, with the simultaneous presence of both polymorphisms in heterozygosity, it would be expected that these individuals would have a higher risk for cancer, because aromatic DNA adducts arising from environmental exposures could be less efficiently repaired.

Because it has been shown that several proteins, such as p53, can modulate NER by protein-protein interactions with XPB and XPD (32) and that there are also several other polymorphisms in the ERCC2 gene, we can not exclude that other putative polymorphisms in this gene and/or in other genes associated with the regulation of the NER pathway might, alone or in association, be involved in the individual susceptibility to thyroid cancer.

Apart from a further confirmation of the results reported here in larger studies, our results suggest that genetic polymorphisms in ERCC2 might be associated with individual susceptibility towards thyroid cancer, as a consequence of a defective DNA repair capacity, but the nature of the genetic damage associated with thyroid cancer requires further studies.

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