Thyroid nodules, polymorphic variants in DNA repair and *RET-*related genes, and interaction with ionizing radiation exposure from nuclear tests in Kazakhstan

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Abstract

Risk factors for thyroid cancer remain largely unknown except for ionizing radiation exposure during childhood and a history of benign thyroid nodules. Because thyroid nodules are more common than thyroid cancers and are associated with thyroid cancer risk, we evaluated several polymorphisms potentially relevant to thyroid tumors and assessed interaction with ionizing radiation exposure to the thyroid gland. Thyroid nodules were detected in 1998 by ultrasound screening of 2997 persons who lived near the Semipalatinsk nuclear test site in Kazakhstan when they were children (1949-62). Cases with thyroid nodules (n=907) were frequency matched (1:1) to those without nodules by ethnicity (Kazakh or Russian), gender, and age at screening. Thyroid gland radiation doses were estimated from fallout deposition patterns, residence history, and diet. We analyzed 23 polymorphisms in 13 genes and assessed interaction with ionizing radiation exposure using likelihood ratio tests (LRT). Elevated thyroid nodule risks were associated with the minor alleles of *RET* S836S (rs1800862, p = 0.03) and *GFRA1* -193C>G (rs not assigned, p = 0.05) and decreased risk with *XRCC1* R194W (rs1799782, p-trend = 0.03) and *TGFB1* T263I (rs1800472, p = 0.009). Similar patterns of association were observed for a small number of papillary thyroid cancers (n=25). Ionizing radiation exposure to the thyroid gland was associated with significantly increased risk of thyroid nodules (age and gender adjusted excess odds ratio/Gy = 0.30, 95% confidence interval 0.05-0.56), with evidence for interaction by genotype found for *XRCC1* R194W (LRT p value = 0.02). Polymorphisms in RET signaling, DNA repair, and proliferation genes may be related to risk of thyroid nodules, consistent with some previous reports on thyroid cancer. Borderline support for gene-radiation interaction was found for a variant in XRCC1, a key base excision repair protein. Other pathways, such as genes in double strand break repair, apoptosis, and genes related to proliferation should also be pursued.

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Thyroid nodules; single nucleotide polymorphisms; epidemiology; thyroid cancer; ionizing radiation; interaction

Introduction

There are few putative risk factors for thyroid cancer except a previous history of benign thyroid adenoma and ionizing radiation exposure during childhood (1-4). It is likely that genetic susceptibility affects radiation-related thyroid cancer risk in view of the inter-individual variation in response to radiation dose and familial aggregation of thyroid disease and thyroid cancer (5-8). Genetic factors associated with thyroid tumorigenesis have been reported (9), but most studies have been constrained by small sample sizes. Benign thyroid nodules are approximately 10 times more frequent than thyroid cancer, with about 300,000 diagnosed in the US each year (10) and a strong link between benign thyroid nodules and thyroid cancer has been demonstrated (11). Thus, single nucleotide polymorphism (SNP) and haplotype comparisons between persons with and without prevalent nodules may be informative about thyroid cancer risk.

Polymorphic variation (either SNPs or repeat length polymorphisms) associated with increased or decreased thyroid cancer risk have been described for several genes including the proto-oncogene rearranged-during-transfection (*RET*), (12-14) and selected DNA repair genes (15-16) although not all studies were consistent. No study of papillary thyroid cancer has been of sufficient size to conduct analysis of gene-radiation interaction.

Recently thyroid gland radiation doses were retrospectively estimated among persons who lived downwind of the Semipalatinsk Nuclear Test Site (SNTS) in northeastern Kazakhstan when they were children and who participated as adults in a 1998 thyroid nodule screening study (17). Nuclear fallout doses around the SNTS are believed to have been about an order of magnitude greater than those from the Nevada Test Site in the United States (18). We evaluated 23 polymorphic variants using DNA from archived Guthrie cards in 13 genes suspected to be involved in thyroid tumorigenesis, including RET signaling, thyroid stimulating hormone receptor, DNA repair, and growth stimulation and to assess interaction with ionizing radiation dose to the thyroid gland.

Materials and Methods

Study population and screening

In the early 1960's, the Kazakhstan Research Institute of Radiation Medicine and Ecology assembled a cohort of approximately 10,000 people in northeast Kazakhstan who lived in villages that were downwind of the Semipalatinsk Nuclear Test Site (SNTS) and 10,000 residents of less exposed settlements (19). In 1998, thyroid disease prevalence was evaluated by ultrasound in a sub-set of the original exposed cohort plus other village residents (20) and forms the parent study of the present genetic sub-study. The parent study comprised current residents in eight villages who were under age 20 at the time of one or more of the major atmospheric nuclear weapons tests conducted during 1949-1962 and were either cohort members (N=1989) or selected comparison subjects (N=1008). A total of 2,997 people were screened and interviewed. The screened population was approximately 40% Russian and 60% Kazakh. Questionnaire-based interviews were used to collect information on ethnicity, age, gender, smoking, residential history, general medical information, and selected dietary information. The screening protocol included thyroid ultrasound examination, fine-needle aspiration (FNA) of suspicious nodules with determination of malignancy by cytopathological

results of the FNA, height/weight measurement, and finger-stick phlebotomy dried on Guthrie cards for thyroid stimulating hormone (TSH) analysis (for full study details, see (17)).

One or more thyroid nodules (discrete masses $\geq$ 3 mm in the largest diameter) were found in 920 subjects; 491 persons with nodules $\geq$ 1 cm underwent FNA (all but two agreed to FNA) and 25 papillary carcinomas were diagnosed. Cases were study participants with nodules detected by ultrasound. Controls were selected from among those without nodules and were frequency matched to cases using a 1:1 matching ratio on ethnicity (Kazakh or Russian), gender, age at the time of screening in 5 year strata, and cohort sub-group (described by Land et al (17) to be roster and non-roster subjects plus non-roster individuals from an eighth village). For some strata there were insufficient control numbers to retain the 1:1 ratio, so all possible controls in each such stratum were selected. Human subject protection review was obtained by the parent study from the institutional review boards at the National Cancer Institute in Bethesda, Maryland, USA, and the Semipalatinsk State Medical Academy in Kazakhstan. All subjects provided informed consent for finger-stick phlebotomy and ultrasound screening. The sub-study was based on anonymized Guthrie card material remaining after TSH analysis, for which a human subjects exemption was obtained. Anonymization entailed linking the epidemiologic and clinical data (stripped of personal identifiers) with the genotyping results and then the link was destroyed.

Genotyping

Following TSH assays performed in 1998, the Guthrie cards were stored at room temperature in a dry container until June 2003 when 3 mm samples from the cards were punched and processed. Laboratory investigators were blinded to case and control status. Approximately 10 ng of genomic DNA extracted from the Guthrie cards were used as template in Taqman 5′-nuclease assays for all SNPs. Taqman assays were performed using 450 nanomolar primer concentrations and 100 nanomolar probe concentrations and Taqman Universal Master Mix (Applied Biosystems, Foster City, CA). Probes specific for each SNP were designed with Primer Express software (Applied Biosystems, Foster City, CA) and labeled with either 6-FAM, TET, or VIC as reporter dyes with either Black Hole Quencher-1 (IDT, Inc., Coralville, IA) or MGB-NFQ (Applied Biosystems, Foster City, CA) as quenchers. Most assays were performed in 20 microliter reactions in 96-well trays using an ABI 7700 instrument (Applied Biosystems, Foster City, CA), but some were performed in 5 microliter reactions in 384-well trays using an ABI 7900HT instrument (Applied Biosystems, Foster City, CA).

Subjects with each of the possible genotypes for each SNP (excluding those for which no homozygous variant subject had ever been identified) were confirmed by sequencing and included on each genotyping tray. As a quality control check for genotyping, approximately 2.4% of samples (2 to 8 aliquots for each of 13 individuals) were duplicates and to which laboratory investigators were completely blinded. Of the approximately 800 genotypes scored for these duplicates, 99.6% were concordant with other results from the same individual. The inconsistent genotypes occurred for 3 separate SNPs in 2 individuals and probably do not reflect a systematic error with any single assay.

Thyroid gland radiation dose reconstruction

Ionizing radiation dose reconstruction has been described in detail elsewhere (17). Briefly, individual radiation doses to the thyroid gland were based on fallout deposition patterns from 11 different nuclear tests conducted at the SNTS between August 1949 and September 1962, residential histories from personal interviews, and childhood diet. Milk consumption was especially important because of the Iodine 131 (^{131}I) fallout-pasture-animal grazing-milk pathway. Thyroid doses were calculated as the sum of gamma radiation from external sources and beta radiation from ingested ^{131}I, given that the epidemiologic study found little evidence

to suggest that the relative biologic effectiveness of the two components of dose differed (17).

Statistical Methods

Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CI) for thyroid nodules by genotype. We assumed a co-dominant mode of inheritance unless the homozygote numbers for the minor allele were ≤ 1% of the total, in which case we combined them with the heterozygotes. Tests for linear trend were calculated across all genotypes unless there were no persons homozygous for the minor allele. Potential confounding factors considered were thyroid volume, body weight, and TSH, as well as the matching variables of age, ethnicity, sub-group, and gender, but these factors did not appreciably affect the odds ratios and were not included in the models. Deviation from the expectation of Hardy-Weinberg Equilibrium (HWE) was determined by chi-square testing in controls because deviation among cases is presumptive of an association with disease. In analyses of thyroid nodules, the 25 individuals with thyroid cancer were excluded and analyzed separately. Haplotypes for the four *RET* SNPs were estimated using SNPHAP (http://www-gene.cimr.cam.ac.uk/clayton/software/), as implemented in HapScope (21). Associations between haplotypes and disease status were evaluated with SAS PROC HAPLOTYPE. We also used quasi-likelihood hierarchical regression modeling (22-23) to account for the potential correlation of multiple SNPs and to increase the accuracy and precision of SNP effect estimates over conventional methods (24-26). All tests were two-sided. Analyses were done using SPSS version 13.0 (Chicago, IL), SAS/GLIMMIX version 8.02 and SAS/GENETICS version 9.0 (SAS Institute, Inc., Cary, NC).

The main effect of thyroid radiation dose was assessed by modeling the odds ratio as a linear function in logistic regression models:

$$OR = 1 + \beta^* D$$

where *D* is the continuous radiation dose and *β* is the excess odds ratio (EOR) per unit dose in Gray (Gy). To test whether SNPs modified the relation between radiation and thyroid nodule risk, we allowed the radiation-related EOR to vary by genotype while adjusting for the genotype effect. EOR heterogeneity across genotype categories was assessed using likelihood ratio tests (LRT). Confidence intervals for radiation risk estimates were derived using the profile likelihood method. We used EPICURE software (Hirosoft, Seattle, WA) for the linear dose-response analyses.

Results

There were 1840 individuals eligible for inclusion, with genotyping attempted for 1827. Guthrie cards were missing for seven cases and three controls, and exhaustion of controls within some age/gender/ethnic strata further reduced numbers available by three. Among the 1827 individuals who were genotyped, six had missing nodule information, leaving 907 cases and 914 controls available for analysis. Due to missing dose information, the sample size for gene-radiation interaction analyses was slightly reduced to 879 cases and 884 controls. For any given SNP, variable numbers of samples were successfully genotyped, ranging from 99.7% to 91.2%. Among the controls, significant deviation from HWE was found for *RAD18* R302Q (p= 0.006).

Descriptive characteristics of the subjects with thyroid nodules (cases) and their frequency matched controls without nodules are shown in Table 1. Ethnic Kazakhs and Russians made up 64.1% and 35.9% of the study group, respectively. Age and gender distributions were similar

among cases and controls owing to the frequency matched design, although cases among the Kazakhs tended to be younger than those among the Russians. The proportions of individuals with one versus two or more ultrasound-detected nodules and with papillary thyroid cancer were equivalent between Kazakhs and Russians. TSH levels were similar between cases and controls (not shown). For 12 of the 23 SNPs evaluated, the frequency of genotypes between Russian controls and Kazakh controls differed significantly (Table 2).

The age and gender adjusted excess odds ratio per Gy (EOR/Gy) was 0.30, (95% confidence interval (CI), 0.05-0.56). Increased thyroid nodule risk was associated with *RET* S836S (rs1800862; CT vs. CC (referent), OR = 1.4; 95% CI, 1.0-2.0) and *GFRA1* -193C>G (rs not assigned; CG vs. CC, OR=1.8; 95% CI, 1.0-3.1, p=0.051) and decreased risk with *XRCC1* R194W (rs1799782; *p*-trend=0.03 for increasing minor alleles) and *TGFB1* T263I (rs1800472; CT vs CC, OR=0.5; 95% CI, 0.3-0.8) (Table 3). When we analyzed each SNP stratified by ethnicity (Table 4), the associations with the above four SNPs tended to be consistent and strongest among Kazakh individuals and the directions of the risk estimates were similar among Russian individuals. Associations between genotypes and papillary thyroid cancer risk are shown in Table 5. Two of the four SNPs that were associated with increased risk of thyroid nodules were suggestively associated with increased risk of thyroid cancer: *RET* S836S (OR=4.4, 95% CI, 1.7-11.4) and *GFRA1* -193C>G (OR=4.0, 95% CI, 0.9-18.4). Thyroid cancer risk was decreased for the minor allele of *XRCC1* R194W and there were no carriers of the *TGFB1* T263I minor allele among papillary thyroid cancer cases. Increased thyroid cancer risk was associated with *TSHR* D727E and decreased risk with *ZNF350* L66P and S472P. Haplotyping and hierarchical regression did not add any information to that gained by single SNP analyses (not shown). Analyses of thyroid nodules stratified by gender showed similar risk patterns for *GFRA1* -193C>G, *XRCC1* R194W, and *TGFB1* T263I, however the increased risk of thyroid nodules associated with *RET* S836S was limited to women only (data not shown).

Among genotypes consistent with HWE in controls, two interactions between thyroid gland radiation dose from fallout and genotype were statistically significant (Table 3). *XRCC1* R194W and *BRIP1* P919S both had LRT p values = 0.02. The common homozygous *XRCC1* R194W alleles were associated with increased radiation-related risk of thyroid nodules (EOR/Gy = 0.51, 95% CI, 0.18-0.98) and the minor allele was consistent with no elevated radiation-related risk. For *BRIP1* P919S, the common homozygous and heterozygous variant alleles were associated with increased radiation-related risk of thyroid nodules and the homozygous variant alleles were consistent with no elevated risk.

Discussion

Thyroid carcinogenesis is hypothesized to result from mutational events combined with growth stimulation (4), a hypothesis consistent with the marked inverse association observed between radiation dose-response and exposure age. All the SNPs and genes were selected because they could affect the likelihood of mutations from DNA misrepair or might constitutively influence cellular proliferation. In this study we identified two polymorphisms, *RET* S836S and *GFRA1* -193 C>G, the variant forms of which were associated with an increased risk of both thyroid nodules and papillary thyroid cancer. Two other polymorphisms were associated with decreased risk of thyroid nodules, *TGFB1* T263I and *XRCC1* R194W, with *TGFB1* T263I also associated with decreased risk of papillary thyroid cancer. *XRCC1* R194W and *BRIP1* P919S modified the radiation-related risk of thyroid nodules. The results suggest that, at least in the population studied here, several SNPs may be related to thyroid nodule and cancer risk, or that other variants in linkage disequilibrium, are risk alleles.

The *RET* S836S polymorphism has been associated with both differentiated thyroid cancer and benign thyroid disease, with adjusted odds ratios of 2.5 (95% CI 1.0-6.6) and 2.2 (0.7-6.8) observed, respectively, for the minor allele (13). However, Lesueur et al (12) and Lönn et al (14) did not find an association with *RET* S836S and sporadic papillary thyroid cancer, but did observe an increased risk associated with *RET* L769L and *RET* G691S, respectively. It may be that the synonymous *RET* polymorphisms exert their effects by affecting protein folding or altering RNA secondary structures resulting in reduced protein function (reviewed in (27)). Two *RET* haplotypes have been associated with risk of benign thyroid disease, but not with differentiated thyroid cancer (13). In our *RET* haplotype analysis, we found no additional information beyond that obtained from analyzing SNPs individually.

RET activation requires binding with one of the four RET ligands that interact preferentially with four high-affinity glycosyl-phosphatidylinositol co-receptors called GFRA1 through GFRA4 (reviewed in (28)). Polymorphic variants in the genes *GFRA1-GFRA4* have been analyzed in relation to sporadic medullary thyroid cancer, (29-31) but medullary thyroid cancer is histologically distinct from and probably uninformative for comparisons with papillary thyroid cancer. To our knowledge, *GFRA1* gene polymorphisms have not been evaluated previously in persons with benign thyroid nodules.

Several of the villages studied in Kazakhstan are in mild to moderately iodine deficient areas (17,32). In geographic regions of iodine deficiency, the thyroid glands of residents are exposed to mild chronic TSH stimulation that could increase H_2O_2 and free radical production (reviewed in (33)), causing damage to DNA. We evaluated several gene variants in DNA damage repair pathways as we hypothesized these would be important among persons with marginal iodine intake and exposure to radioactive fallout. We included the base excision repair (BER) pathway because oxidative and some radiation damage to nucleotides would be repaired by this mechanism. In radiation challenge assays, frequencies of chromatid breaks per cell after lymphoctye γ-radiation were higher among papillary thyroid cancer cases than controls and benign thyroid disease cases were intermediate between the two groups (34), suggesting higher radiation sensitivity or compromised radiation-induced damage repair ability was a risk factor for thyroid tumors. XRCC1 is a key BER scaffolding protein and the *XRCC1* R194W minor allele was associated with a statistically significantly decreased risk of thyroid nodules and the common allele with a suggestive radiation-related increased thyroid nodule risk. In other words, the more common C allele appeared to be the risk allele, associated both with thyroid nodules and nodules related to radiation exposure to the thyroid gland. BRIP1 is a BRCA1 interacting protein for which one SNP (P919S) modified the radiation-related thyroid nodule risk. However, absent a main effect, it is more difficult to ascribe importance to the possible gene-radiation interaction observed for *BRIP1* P919S when a chance finding is also possible.

Transforming growth factor β is a cytokine with multiple regulatory actions and responses to cellular damage (including from radiation) and can promote or suppress carcinogenesis depending on the timing and specific function mediated by its extracellular signaling (reviewed in (35)). We found that the *TGFB1* T263I minor allele was associated with statistically significantly decreased risk of thyroid nodules; the minor allele was not represented among any thyroid cancer cases compared to 4.9% of controls. Further detailed evaluation of the polymorphic variation in the *TGFβ1* gene and thyroid tumors, such as could be obtained from collaborative efforts, should be considered.

Strengths of this study were the relatively large sample size, having 84% power to detect an OR of 1.5 with alpha set at 0.002 (Bonferroni correction for 23 tests) if the allele frequency was ≥ 30%, and that the thyroid nodules were detected within a screened population under standard conditions. We were able to test for variation across two diverse populations and for two thyroid disease endpoints. While not an exhaustive set of risk factors, we were able to

assess the impact of selected co-variates such as TSH levels, thyroid volume, body weight, and the matching variables. We found several SNP and thyroid nodule associations for which one might argue the papillary thyroid cancer results provided some corroboration of the findings. Confounding by population stratification is unlikely because thyroid nodule prevalence did not differ by ethnicity and no inconsistencies were found when we conducted our analyses stratified by ethnicity.

Several limitations should also be considered, including the large number of tests conducted and the relatively modest genotypic associations seen. Using a Bonferroni correction, none of the p-values for the genotype associations with thyroid nodule risk would be considered significant ($p < 0.002$). Power for detecting less common variants was low: 38% to detect an OR of 1.5 with alpha set at 0.002 and an allele frequency of 10%. However, the gene-radiation interaction analyses were more exploratory in nature and require replication as the primary safeguard against false positive associations. The radiation doses to the thyroid gland are uncertain and efforts have been made to re-contact village residents to collect additional key data on milk consumption besides from cows, housing construction, and patterns of animal husbandry (location of grazing areas around the villages). Analyses of uncertainties are planned after the doses are revised based on incorporating the new information.

In summary, the associations found with thyroid nodules and papillary cancer suggest polymorphic variation in *RET* S836S, *GFRA1* -193 C>G, and *TGFB1* T263I, were related to risk. The common allele of *XRCC1* R194W was related to risk of thyroid nodules and suggestively modified the radiation-associated nodule risk. Other pathways, such as genes in double strand break repair, apoptosis, and additional genes related to proliferation should also be pursued.

Acknowledgments

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Table 1

Descriptive characteristics for those residents found to have thyroid nodules (cases) and those without thyroid nodules (controls) at ultrasound examination by ethnicity among residents near Semipalatinsk, Kazakhstan, 1998

Characteristic at time of examination and interview (August 1998)	Kazakh				Russian			
	Thyroid nodules detected (cases) n=574		Thyroid nodules not detected (controls) n=593		Thyroid nodules detected (cases) n=333		Thyroid nodules not detected (controls) n=321	
	N	%	N	%	N	%	N	%
Age in years								
43-49	113	19.7	116	19.6	44	13.2	46	14.3
50-54	119	20.7	121	20.4	57	17.1	60	18.7
55-59	177	30.8	186	31.4	73	21.9	74	23.1
60-64	124	21.6	130	21.9	85	25.5	75	23.4
65-70	41	7.1	40	6.7	74	22.2	66	20.6
Gender								
Female	437	76.1	455	76.7	255	76.6	242	75.4
Male	137	23.9	138	23.3	78	23.4	79	24.6
Number of ultra-sound detected nodules								
One	363	63.2	NA*	NA	199	59.8	NA	NA
Two	211	36.8	NA	NA	134	40.2	NA	NA
Papillary cancer found								
No	556	96.9	NA	NA	326	97.9	NA	NA
Yes	18	3.1	NA	NA	7	2.1	NA	NA

* Not applicable

Table 2

Number genotyped and allele frequencies among Kazakhs and Russians, residents near Semipalatinsk, Kazakhstan, 1998

| Gene Polymorphism | rs number[*] | Kazakh | | Russian | | p-value[§] |
		Total[†] number	MAF[‡] in controls	Total[†] number	MAF[‡] in controls	Controls
RET signaling						
RET A45A	1800858	1140	0.37	642	0.28	<0.001
RET G691S	1799939	1152	0.18	648	0.20	0.68
RET L769L	1800861	1151	0.41	649	0.28	<0.001
RET S836S	1800862	1167	0.03	652	0.03	0.87
GFRA1 -193 C>G	---	1155	0.01	647	0.01	0.87
GFRA3 IVS7 +39G>A	---	1132	0.05	625	0.07	0.27
EPAC G332S	12422983	1081	0.07	589	0.16	<0.001
TSH receptor						
TSHR D727E	1991517	1075	0.18	625	0.13	0.065
TSHR IVS1 +8651A>G	2284716	1082	0.20	623	0.14	0.002
TSHR N187N	2075179	1111	0.32	638	0.19	<0.001
DNA Repair Genes						
XRCC1 R194W	1799782	1162	0.20	649	0.07	<0.001
XRCC1 R280H	25489	1150	0.06	640	0.05	0.75
XRCC1 R399Q	25478	1140	0.27	637	0.34	0.009
APEX D148E	1130409	1143	0.39	638	0.48	0.001
RAD18 R302Q	373572	1136	0.47	637	0.28	<0.001
BRCA1 interacting proteins						
BRCA2 N372H	144848	1158	0.24	647	0.21	0.23
BRIP1 -64 G>A	2048718	1159	0.71	650	0.49	<0.001
BRIP1 P919S	4986764	1094	0.39	603	0.42	
ZNF350 L66P	2278420	1164	0.22	653	0.20	0.36
ZNF350 S472P	4986771	1143	0.02	643	0.03	0.13
ZNF350 R501S	2278415	1157	0.20	653	0.17	0.13
Growth factor and cell cycle genes						
TGFB1 T263I	1800472	1127	0.02	629	0.04	0.004

| Gene Polymorphism | rs number [*] | Kazakh | | Russian | | p-value[§] |
		Total[†] number	MAF[‡] in controls	Total[†] number	MAF[‡] in controls	Controls
CHEK2 1100delC	---	1066	0.000	599	0.001	0.006

[*]
rs: referent sequence; --- indicates the rs number has not been assigned.

[†]
Numbers vary because not all cases and controls could be genotyped due to technical issues with the sample.

[‡]
Minor allele frequency

[§]
χ^2 test p-values for genotype frequency differences between Russian and Kazakh controls

Note: Slight deviation from expectation of Hardy-Weinberg equilibrium was observed for *XRCC1* R399Q (p=0.05) and R194W (p=0.02) among Kazakh controls.

Table 3

Risk of thyroid nodules associated with single nucleotide polymorphisms (SNP) and interaction with ionizing radiation exposure to the thyroid gland in a Kazakh and Russian population residing near Semipalatinsk, Kazakhstan, 1998.

Gene	Entrez SNP ID*	AA or nt variant ID†	Genotype	Number		Main Effect for Genotype			Ionizing Radiation from Fallout Interaction		
				Cases	Cont.	OR	95% Confidence Interval	p trend‡	EOR/Gy§	95% Confidence Interval	p value‖
RET	rs1800858	A45A	GG	412	392	1.0	Referent		0.35	0.004 0.93	
			GA	346	402	0.8[2]	0.7 1.0		0.11	<0 0.51	
			AA	101	101	1.0	0.7 1.3	0.25	1.19	0.12 4.09	0.20
RET	rs1799939	G691S	AA	562	588	1.0	Referent		0.41	0.09 0.88	
			AG	277	283	1.0	0.8 1.3		0.06	<0 0.49	
			GG	32	29	1.2	0.7 1.9	0.63	2.36	<0 14.0	0.10
RET	rs1800861	L769L	TT	355	374	1.0	Referent		0.43	0.08 0.98	
			TG	375	391	1.0	0.8 1.2		0.15	<0 0.54	
			GG	139	137	1.1	0.8 1.4	0.67	0.42	<0 1.6	>0.50
RET	rs1800862	S836S	CC	796	850	1.0	Referent		0.30	0.07 0.62	
			CT	83	61	1.4**	1.0 2.0	ND††	<0	<0 0.93	0.10
GFRA1	Not assigned	-193 C>G	CC	839	883	1.0	Referent		0.31	0.07 0.65	
			CG	32	19	1.8	1.0 3.1	ND	<0	<0 1.33	0.33
GFRA3	Not assigned	IVS7 +39G>A	GG	762	783	1.0	Referent		0.27	0.03 0.61	
			GA/AA	85	98	0.9	0.7 1.2	0.92	0.13	-0.19 1.09	>0.50
EPAC	rs12422983	G332S	CC	662	671	1.0	Referent		0.29	0.03 0.66	
			CT/TT	149	155	1.0	0.8 1.2	0.95	0.21	-0.14 1.14	>0.50
TSHR	rs1991517	D727E	CC	585	601	1.0	Referent		0.12	<0 0.43	
			CG	219	213	1.1	0.9 1.7		0.44	<0 1.41	
			GG	24	29	1.0	0.6 1.7	0.60	0.16	<0 3.91	>0.50
TSHR	rs2284716	IVS1 +8651A>G	AA	553	569	1.0	Referent		0.10	<0 0.44	
			AG	241	259	1.0	0.8 1.2		0.25	<0 0.89	
			GG	30	24	1.3	0.7 2.2	0.89	2.02	<0 23.49	0.47
TSHR	rs2075179	N187N	TT	469	481	1.0	Referent		0.42	0.11 0.89	
			TA	303	314	1.0	0.8 1.2		<0	<0 0.3	
			AA	75	78	1.0	0.7 1.4	0.95	0.2	<0 1.53	0.20

Gene	Entrez SNP ID [*]	AA or nt variant ID [†]	Genotype	Number Cases	Number Cont.	Main Effect for Genotype OR	Main Effect 95% Confidence Interval	p trend [‡]	Ionizing Radiation from Fallout Interaction EOR/Gy [§]	95% Confidence Interval	p value [‖]
XRCC1	rs1799782	R194W	CC	674	665	1.0	Referent		0.51	0.18 – 0.98	
			CT	187	209	0.9	0.7 – 1.1	0.03	< 0	< 0 – 0.38	0.02
			TT	15	32	0.5	0.3 – 0.9		< 0	< 0 – 0.26	
XRCC1	rs25489	R280H	GG	776	800	1.0	Referent		0.22	0.0009 – 0.54	
			GA/AA	89	96	1.0	0.7 – 1.3	0.96	0.93	0.05 – 3.14	0.20
XRCC1	rs25478	R399Q	GG	415	460	1.0	Referent		0.42	0.11 – 0.87	
			GA	366	343	1.2	1.0 – 1.4	0.52	0.04	< 0 – 0.43	0.30
			AA	76	89	0.9	0.7 – 1.3		0.59	< 0 – 2.78	
APEX	rs1130409	D148E	GG	275	309	1.0	Referent		0.47	0.04 – 1.14	
			GT	432	411	1.2	1.0 – 1.5	0.46	0.23	< 0 – 0.63	> 0.50
			TT	158	167	1.1	0.8 – 1.4		0.10	< 0 – 0.77	
RAD18 [§§]	rs373572	R302Q	AA	329	337	1.0	Referent		0.51	0.13 – 1.11	
			AG	384	387	1.0	0.8 – 1.2	0.62	0.09	< 0 – 0.45	0.30
			GG	146	163	0.9	0.7 – 1.2		0.43	< 0 – 1.65	
BRCA2	rs144848	N372H	AA	492	534	1.0	Referent		0.22	< 0 – 0.63	
			AC	328	319	1.1	0.9 – 1.4	0.16	0.40	0.08 – 0.90	> 0.50
			CC	55	48	1.2	0.8 – 1.9		0.06	< 0 – 1.93	
BRIP1	rs2048718	-1918 G>A	GG	155	130	1.0	Referent		0.30	< 0 – 1.43	
			GA	354	397	0.7 [**]	0.6 – 1.0	0.38	0.10	< 0 – 0.50	0.30
			AA	368	376	0.8	0.6 – 1.1		0.59	0.14 – 1.34	
BRIP1	rs4986764	S919P	CC	295	304	1.0	Referent		0.47	0.11 – 1.05	
			CT	377	417	0.9	0.7 – 1.1	0.69	0.32	0.008 – 0.80	0.02
			TT	144	134	1.1	0.8 – 1.5		< 0	< 0 – 0.03	
ZNF350	rs2278420	L66P	TT	542	567	1.0	Referent		0.34	0.02 – 0.83	
			TC	300	300	1.0	0.9 – 1.3	0.88	0.21	< 0 – 0.70	> 0.50
			CC	35	44	0.8	0.5 – 1.3		0.45	< 0 – 3.42	
ZNF350	rs4986771	S472P	TT	827	860	1.0	Referent		0.30	0.07 – 0.63	
			TC	35	36	1.0	0.6 – 1.6	ND	< 0	< 0 – 2.03	0.20
ZNF350	rs2278415	R501S	AA	566	597	1.0	Referent		0.29	0.002 – 0.73	
			AT	281	274	1.1	0.9 – 1.3		0.22	< 0 – 0.73	> 0.50

Gene	Entrez SNP ID*	AA or nt variant ID†	Genotype	Number		Main Effect for Genotype			Ionizing Radiation from Fallout Interaction		
				Cases	Cont.	OR	95% Confidence Interval	p trend‡	EOR/Gy§	95% Confidence Interval	p value‖
			TT	27	36	0.8	0.5 1.3	0.94	0.75	<0 6.11	> 0.50
			CC	829	835	1.0	Referent		0.24	0.03 0.54	
TGFB1	rs1800472	T263I	CT	21	43	0.5	0.3 0.8	ND	0.21	<0 4.56	
			CC	805	830	1.0	Referent		Model would not converge		
CHEK2	Not assigned	1100delC	C/-	2	4	0.5	0.1 2.8	ND			

*
Entrez SNP reference ID number (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=snp)

†
Amino acid sequence variation (regular font), nucleotide sequence variation (italics)

‡
Excess Odds Ratio for total (external plus internal) thyroid gland radiation dose from fallout; significance of EOR/Gy is denoted when the 95% CI (profile likelihood bounds) exclude the null value of zero

§
Test for ordinal trend across genotypes

‖
Likelihood ratio test comparing the genotype-specific EORs

**
Excludes null value of 1.0

††
ND-Not done

‡‡
Model would not converge unless heterozygous and homozygous variant carriers were combined

§§
Test for expectation of Hardy Weinberg Equilibrium among controls, p = 0.006

Table 4

Risk of thyroid nodules associated with single nucleotide polymorphisms by ethnicity in a Kazakh and Russian population residing near Semipalatinsk, Kazakhstan, 1998

Polymorphism	Kazakh						Russian					
	Number of cases	Number of controls	Odds Ratio with 95% CI				Number of cases	Number of controls	Odds Ratio with 95% CI			
RET signaling and the TSH receptor												
RET A45A (GC<u>G</u> > GC<u>A</u>)												
GG	237	229	1.0				175	163	1.0			
GA	224	273	0.8	0.6		1.0	122	129	0.9	0.6		1.2
AA	78	78	0.9	0.7		1.4	23	23	1.0	0.5		1.7
p-trend[*]			0.40						0.54			
RET G691S												
GG	347	385	1.0				215	203	1.0			
GS	182	180	1.1	0.9		1.4	95	103	0.9	0.6		1.2
SS	19	17	1.2	0.6		2.4	13	12	1.0	0.5		2.3
p-trend			0.30						0.58			
RET L769L (CT<u>T</u> > CT<u>G</u>)												
TT	181	209	1.0				174	165	1.0			
TG	257	264	1.1	0.9		1.5	118	127	0.9	0.6		1.2
GG	108	110	1.1	0.8		1.6	31	27	1.1	0.6		1.9
p-trend			0.40						0.84			
RET S836S (AG<u>C</u> > AG<u>T</u>)[†]												
CC	498	552	1.0				298	298	1.0			
CT	56	39	1.6²	1.0		2.4	27	22	1.2	0.7		2.2
GFRA1 -193 C>G												
CC	525	573	1.0				314	310	1.0			
CG	23	12	2.1²	1.0		4.2	9	7	1.3	0.5		3.5
GFRA3 IVS7 +39G>A[‡]												
GG	492	516	1.0				270	267	1.0			
GA & AA	44	58	0.8	0.5		1.2	41	40	1.0	0.6		1.6
p-trend			0.29						0.79			

Polymorphism	Kazakh						Russian					
	Number of cases	Number of controls	Odds Ratio with 95% CI				Number of cases	Number of controls	Odds Ratio with 95% CI			
EPAC G332S												
GG	452	471	1.0				210	200	1.0			
GS and SS‡	58	78	0.8	0.5	1.1		91	81	1.1	0.7	1.5	
p-trend			0.16						0.97			
TSHR D727E												
DD	349	371	1.0				236	230	1.0			
DE	146	144	1.1	0.8	1.4		73	69	1.0	0.7	1.5	
EE	20	23	0.9	0.5	1.7		4	6	0.7	0.2	2.3	
p-trend			0.82						0.86			
TSHR IVS1 +8651A>G												
AA	334	342	1.0				219	227	1.0			
AG	159	180	0.9	0.7	1.2		82	79	1.1	0.8	1.5	
GG	24	21	1.2	0.6	2.1		6	3	2.1	0.5	8.4	
p-trend			0.81						0.43			
TSHR N187N (AA<u>T</u>>AA<u>C</u>)												
TT	252	271	1.0				217	210	1.0			
TA	211	227	1.0	0.8	1.3		92	87	1.0	0.7	1.5	
AA	65	63	1.1	0.8	1.6		10	15	0.6	0.3	1.5	
p-trend			0.69						0.59			
DNA repair genes												
XRCC1 R194W§												
RR	382	388	1.0				292	277	1.0			
RW∥	155	168	0.9	0.7	1.2		32	41	0.7	0.5	1.2	
WW∥	15	32	0.5	0.2	0.9							
p-trend			0.07						0.31			
XRCC1 R280H												
RR	498	519	1.0				278	281	1.0			
RH and HH‡	46	65	0.7	0.5	1.1		43	31	1.4	0.9	2.3	
p-trend			0.27						0.20			
XRCC1 R399Q												

Polymorphism	Kazakh Number of cases	Number of controls	Odds Ratio with 95% CI	Russian Number of cases	Number of controls	Odds Ratio with 95% CI
RR	270	321	1.0	145	139	1.0
RQ	225	209	1.3[2] (1.0–1.6)	141	134	1.0 (0.7–1.4)
QQ	42	51	1.0 (0.6–1.5)	34	38	0.9 (0.5–1.4)
p-trend			0.30			0.68
APEX D148E						
DD	197	223	1.0	78	86	1.0
DE	264	258	1.2 (0.9–1.5)	168	153	1.2 (0.8–1.8)
EE	85	94	1.0 (0.7–1.5)	73	73	1.1 (0.7–1.7)
p-trend			0.65			0.64
RAD18 R302Q[6]						
RR	176	172	1.0	153	165	1.0
RQ	256	268	0.9 (0.7–1.2)	128	119	1.2 (0.8–1.6)
QQ	109	134	0.8 (0.6–1.1)	37	29	1.4 (0.8–2.3)
p-trend			0.18			0.19
BRCA1 interacting proteins						
BRCA2 N372H						
NN	305	338	1.0	187	196	1.0
NH	213	211	1.1 (0.9–1.4)	115	108	1.1 (0.8–1.6)
HH	33	36	1.0 (0.6–1.7)	22	12	1.9 (0.9–4.0)
p-trend			0.53			0.12
BRIP1 -64 G>A						
GG	63	47	1.0	92	83	1.0
GA	207	241	0.6 (0.4–1.0[2])	147	156	0.9 (0.6–1.2)
AA	283	296	0.7 (0.5–1.1)	85	80	1.0 (0.6–1.5)
p-trend			0.47			0.83
BRIP1 P919S						
PP	196	210	1.0	99	94	1.0
PS	243	261	1.0 (0.8–1.3)	134	156	0.8 (0.6–1.2)
SS	78	86	1.0 (0.7–1.4)	66	48	1.3 (0.8–2.1)
p-trend			0.89			0.45

Polymorphism	Kazakh Number of cases	Kazakh Number of controls	Kazakh Odds Ratio with 95% CI	Russian Number of cases	Russian Number of controls	Russian Odds Ratio with 95% CI
ZNF 350 L66P						
LL	321	358	1.0	221	209	1.0
LP	205	204	1.1 (0.9–1.4)	95	96	0.9 (0.7–1.3)
PP	25	29	1.0 (0.6–1.7)	10	15	0.6 (0.3–1.4)
p-trend			0.57			0.35
ZNF 350 S472P						
SS	524	561	1.0	303	299	1.0
SP	18	19	1.0 (0.5–2.0)	17	17	1.0 (0.4–2.0)
ZNF 350 R501S						
RR	332	372	1.0	234	225	1.0
RS	195	189	1.2 (0.9–1.5)	86	85	1.0 (0.7–1.4)
SS	22	25	1.0 (0.5–1.8)	5	11	0.4 (0.1–1.3)
p-trend			0.42			0.35
Growth factor genes						
TGFB1 T263I						
TT	529	547	1.0	300	288	1.0
TI	11	19	0.6 (0.3–1.3)	10	24	0.4 (0.2–0.9)
CHEK2 1100delC						
CC	504	543		301	287	
Heterozygote	2	0	--- Undefined	0	4	--- Undefined

*
p-trend was calculated across all three genotypes even when numbers for homozygote variant carriers were small.

†
Point estimate was significant among Kazakhs, p ≤ 0.05.

‡
Due to small numbers, heterozygote and homozygote variant carriers were combined.

§
Significant deviation from Hardy-Weinberg Equilibrium among Kazakh controls, p = 0.02.

‖
Due to small numbers, heterozygote and homozygote variant carriers were combined for Russians.

Note: All occult papillary thyroid cancers (n=25) were excluded from analysis of nodules. Numbers may not sum to total because not all samples were successfully genotyped.

Table 5

Risk of papillary thyroid cancer associated with single nucleotide polymorphisms in a Kazakh and Russian population, Semipalatinsk, Kazakhstan.

Polymorphism	Number of controls	Number of cases (cancers)	Odds Ratio with 95% CI		
RET signaling and the TSH receptor					
RET A45A (GC<u>G</u> > GC<u>A</u>)					
GG	392	13	1.0		
GA	402	9	0.7	0.3	1.6
AA	101	3	0.9	0.3	3.2
p-trend			0.58		
RET G691S					
GG	588	19	1.0		
GS	283	5	0.5	0.2	1.5
SS	29	1	1.1	0.2	8.2
p-trend			0.37		
RET L769L (CT<u>T</u> > CT<u>G</u>)					
TT	374	12	1.0		
TG	391	5	0.4	0.1	1.1
GG	137	8	1.8	0.7	4.5
p-trend			0.48		
RET S836S (AG<u>C</u> > AG<u>T</u>)					
CC	850	19	1.0		
CT	61	6	4.4	1.7	11.4
GFRA1 193 C>G					
CC	883	23	1.0		
CG	19	2	4.0	0.9	18.4
GFRA3 IVS7 +39G>A[†]					
GG	783	23	1.0		
GA & AA	98	2	0.7	0.2	3.0
p-trend			0.69		
EPAC G332S					
GG	671	19	1.0		
GS	147	4	1.0	0.3	2.9

Polymorphism	Number of controls	Number of cases (cancers)	Odds Ratio with 95% CI		
SS	12	2	5.9	1.2	28.2
p-trend					0.21
TSHR D727E					
DD	601	10	1.0		
DE	213	11	3.1	1.3	7.4
EE	29	4	8.3	2.5	28.0
p-trend					0.001
TSHR IVS1 +8651A>G					
AA	569	18	1.0		
AG	259	6	0.7	0.3	1.9
GG	24	1	1.3	0.2	10.3
p-trend					0.71
TSHR N187N (AA<u>T</u>>AA<u>C</u>)					
TT	481	9	1.0		
TA	314	14	2.4	1.0	5.6
AA	78	2	1.4	0.3	6.5
p-trend					0.17
DNA repair genes					
XRCC1 R194W					
RR	665	20	1.0		
RW and WW[†]	241	5	0.7	0.3	1.9
p-trend					0.34
XRCC1 R280H					
RR	800	24	1.0		
RH and HH[†]	96	1	0.3	0.1	2.6
p-trend					0.30
XRCC1 R399Q					
RR	460	12	1.0		
RQ	343	10	1.1	0.5	2.6
QQ	89	2	0.9	0.2	3.9
p-trend					0.99

Polymorphism	Number of controls	Number of cases (cancers)	Odds Ratio with 95% CI		
APEX D148E					
DD	309	8	1.0		
DE	411	11	1.0	0.4	2.6
EE	167	6	1.4	0.5	4.1
p-trend					0.58
RAD18 R302Q[‡]					
RR	337	6	1.0		
RQ	387	16	2.3	0.9	6.0
QQ	163	1	0.3	0.1	2.9
p-trend					0.89
BRCA1 interacting proteins					
BRCA2 N372H					
NN	534	14	1.0		
NH	319	10	1.2	0.5	2.7
HH	48	1	0.8	0.1	6.2
p-trend					0.87
BRIP1 -64 G>A					
GG	130	1	1.0		
GA	397	14	4.6		
AA	376	10	3.5		
p-trend					0.53
BRIP1 P919S					
PP	304	8	1.0		
PS	417	10	0.9	0.4	2.3
SS	134	4	1.1	0.3	3.8
p-trend					0.91
ZNF350 L66P					
LL	567	21	1.0		
LP and PP[†]	344	4	0.3	0.1	0.9
p-trend					0.03
ZNF350 S472P					

Polymorphism	Number of controls	Number of cases (cancers)	Odds Ratio with 95% CI		
SS	860	25	1.0		
SP	36	0	---	Undefined	
ZNF350 R501S					
RR	597	21	1.0		
RS and SS[†]	310	4	0.4	0.1	1.1
p-trend					0.06
Growth factor and cell cycle genes					
TGFBI T263I					
TT	835	24			
TI	43	0	---	Undefined	
CHEK2 1100delC					
CC	830	22			
Heterozygote	4	0	---	Undefined	

*
p-trend was calculated across all three genotypes even when numbers for homozygote variant carriers were small.

[†]Due to small numbers, heterozygous and homozygous variant carriers were combined among cases and controls to compute the odds ratios and 95% confidence intervals.

[‡]Test for expectation of Hardy Weinberg Equilibrium among controls, p = 0.006.