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CLINICAL STUDY

Risk genetic polymorphism and haplotype associated with papillary thyroid cancer and their relation to associated diseases in Slovak population

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ABSTRACT

BACKGROUND: Risk for developing papillary thyroid carcinoma (PTC), the most common endocrine malignancy, is thought to be mediated by lifestyle, environmental exposures and genetic factors. Recent progress in the genome-wide association studies of thyroid cancer leads to the identification of several genetic variants conferring risk to this malignancy across different ethnicities.

METHODS AND RESULTS: We set out to elucidate the impact of selected single nucleotide polymorphisms (SNPs) on papillary thyroid carcinoma risk and to evaluate the interactions of these genetic variants with associated diseases for the first time in the Slovak population. Six SNPs (rs966423, rs2439302, rs965513, rs116909374, rs1537424 and rs944289) were genotyped in 86 patients with PTC and 99 healthy control subjects. The association analysis and multivariable modelling of PTC risk by the genetic factors, supplemented with a rigorous statistical validation, were performed. One of the six SNPs rs966423 (DIRC3, OR = 1.51, p = 0.03) was significantly associated with PTC. Next two SNPs rs965513 (PTCSC2, OR = 1.34) and rs116909374 (MBIP, OR = 0.44) showed a suggestive association. Haplotype TTC (SNPs located on chromosome 14q13) showed a suggestive association with PTC (p = 0.07, OR = 1.55). In the PTC group, significant associations were observed between rs966423 (DIRC3) and ischemic heart diseases (p = 0.009), rs965513 (PTCSC2) and diabetes mellitus (p = 0.04) and haplotype 14q13 and musculoskeletal diseases. Next three associations rs966423 (DIRC3) and arterial hypertension; rs116909374 (MBIP) and other benign diseases; rs1537424 (MBIP) and disorder lipid metabolism, rs965513 (PTCSC2) and anti-Tg (thyroglobulin antibody) showed suggestive associations.

CONCLUSION: These results indicate that germline variants not only predispose to PTC, but may also be related to other risk factors, including associated diseases. However, these associations were only moderate, and further multi-ethnic studies are required to evaluate the usefulness of these germline variants in the clinical stratification of PTC patients *(Tab. 8, Ref. 37).* Text in PDF *www.elis.sk*

KEY WORDS: papillary thyroid cancer, SNPs, haplotype, associated diseases interaction.

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Introduction

In Europe, thyroid cancer (TC) is relatively rare, accounting for only 0.7 % (males) to 2.5 % (females) of all cancer estimates and 0.2 % (males) and 0.6 % (females) of all estimated cancer-related deaths in 2012. A rise in TC incidence, especially of the papillary type, has been reported in many countries, the Slovak Republic included, during recent decades. This increasing trend may be attributed partly to an improved detection of smaller (<2 cm) tumours via more frequent and better ultrasound detection, fine-needle aspiration biopsies and the increased pathological reporting of incidental microcarcinomas (1). There are multiple types of thyroid cancer with papillary thyroid carcinoma (PTC) being the most common, accounting for ~80 % of all the cases (2).

In general, well-differentiated PTC is regarded as a fairly indolent tumour with long-term survival rates >95 % (3), however there are certain variants of PTC that are more aggressive in nature

Tab. 1. SNP characteristics and genotyping information.

Tag SNP	Annotation	Nearest gene	Chr	Conting position	SNP function	Forward/reverse	bp	MAF
rs966423	Intron	DIRC3	2q35	217445617C>T	NE	TGGAGAGGTGAGAAAAGTAGGG/ CCTGCCTCCTCAGTCTTCAG	59	0.29
rs2439302	Intron	NRG1	8p12	32574851G>C	Risk allele decreasesNRG1 expression	TGCAAGAATGGCCTAACACA/ GAGAGTTAGGTGGCAAAGCTG	74	0.4
rs965513	Intron	FOXE1, PTCSC2	9q22.33	100556109A>G	Regulation of PTCSC2 and FOXE1 expression	GTGGCTGGAATGGAACAGAT/ TTTGTTAGCATTGTGAGAACAGACT	75	0.2
rs116909374	Regulatory region	MBIP	14q13.3	36269155C>T	NE	TCCTGTTCCTTCCTAGAACCA/ TGTTGGAAGAATGAGGGTGA	73	0.03
rs1537424	Intron variant	MBIP	14q13.3	36104812C>T	NE	TTATCAGACCAAGGGGAGCA/ GCTCTCCAGTTGGTGTTCAG	52	0.46
rs944289	Intergenic variant	PTCSC3, NKX2–1	14q13.3	36180040C>T	Risk allele decreases promoter activation PTCSC3	TCACCAACTTATGCCAATTCTC/ AAGGCTGACTTTCCAGACAA	60	0.89

Forward, Reverse and bp represent the sequence of the forward and reverse primers and amplicon length, respectively, in a highresolution melting analysis. SNPs – single nucleotide polymorphisms; SNP – single nucleotide polymorphism; Chr. – chromosome; NE – not established; *DIRC3* – disrupted in renal carcinoma 3; *NRG1* – neuregulin 1; *PTCSC2* – papillary thyroid carcinoma susceptibility candidate 2; *FOXE1* – forkhead box E1; MBPI – MAP3K12 binding inhibitor protein 1; *PTCSC3* – papillary thyroid carcinoma susceptibility candidate 3; bp base pair; MAF – minor allelic frequencies

with less-favourable disease-free survival and overall survival. These variants differ in histology, cytology, molecular markers, treatment strategies, and outcomes (4). The majority of PTC has an excellent prognosis in terms of long-term survival (5) however, the incidence and recurrence rate of PTC are increasing worldwide, especially among women (6).

There is a lot of evidence that PTC development is associated with two groups risk factors – modifiable and non-modifiable. Group of modifiable factors include lifestyle (diabetes in women and obesity), iodine intake, iodine deficiency, environmental pollutants (exposure to ionizing radiation, medical exposures). Non-modifiable risk factors, including the age from 40 to 70 years, female gender, ethnicity, noncancerous benign thyroid conditions (Hashimoto's thyroiditis), family history of thyroid disease (goiter, thyroiditis, or adenoma) thyroid-stimulating hormone concentrations, anti-thyroglobulin antibodies positivity, Lower platelet distribution width and higher platelecrit values and genetic predisposition (7, 8, 9, 10, 11, 12, 13, 14, 15, 16). Moreover, numerous reports showed that genetic factors may involve in development of PTC. The transformation of thyroid follicular cells may result in differentiated or undifferentiated TC, through a multistep process that is the most accepted theory of follicular cell carcinogenesis. Differentiated TC, accounting for more than 90 % of thyroid malignancies, comprises PTC and follicular thyroid carcinoma (FTC). In the last 30 years, the availability of the genome sequence has produced much progress in elucidating the molecular mechanisms underlying TC. TC is a genetically simple disease with a relatively low somatic mutation burden in each tumour. Driver mutations, i.e., mutations that provide a selective growth advantage thus promoting cancer development, are identified in more than 90 % of TC (17).

It is therefore crucial to elucidate the pathological mechanism of PTC, to develop a new diagnostic strategy for the disease and to intervene in the progression of thyroid neoplasms to malignant carcinoma.

Patients and methods

Subjects and data collection

A total of 86 patients with the main diagnosis of papillary thyroid cancer (C73, according to The International Classification of Diseases (ICD)) were included in the study. Included were all the patients at the clinic, who agreed and signed an informed consent. The control group consisted of 99 patients. All the subjects were Caucasians of European origin. The study included patients, who underwent thyroid surgery at the Surgical clinic and Otorhinolaryngological clinic of the Central military hospital (CMH) in Ružomberok (Slovakia) in the years 2015 to 2019. Cases were unselected for age and family history. The diagnosis of papillary thyroid cancer was confirmed through histopathological examination. Clinicopathological information was obtained from the medical records, and tumour, lymph nodes, metastasis (TNM) staging was recorded. This study was approved by the Human Subjects Committees of the Jessenius Faculty of Medicine in Martin at Comenius University, Bratislava and CMH in Ružomberok.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Genes and tagged single nucleotide polymorphisms (tag DNPs)

In this study, we sought to analyze the possible association of six SNPs variants with PTC in the Slovak population. The data were generated with the 1000 Genomes Project (Phase 3) and an adopted algorithm available in Haploview 4.2 using CEU population, a r^2 threshold of 0.8, MAF >0.1, and pair-wise tagging to select SNPs for tagging (18, 19).

References for tag SNPs as well as captured SNPs, positions, and locations in the human genome (asseblyGRCh38.p7) and genotyping information are presented in the Table 1.

DNA analysis

Whole blood samples were taken from each of the participants. Genomic DNA was extracted from 200 µl whole blood using a MagNA Pure LC DNA Isolation Kit I (F. Hoffmann-La Roche Ltd, Basel, Switzerland) according to the manufacturer's instructions. The concentration of each DNA sample was set to 30 µg/ml. DNA quantification was performed using an Invitrogen Qubit Fluorometer (Thermo Fisher Scientific inc., Waltham, USA) and an Invitrogen Qubit dsDNA BRAssay Kit (Thermo Fisher Scientific inc., Waltham, USA). High-resolution melting analysis (HRMA) was the main method used for genotyping of selected SNPs. A LightCycler 480 II, LightCycler 480 High Resolution Melting Master Mix and LightCycler 480 Gene scanning Software V1.5.1 (all: F. Hoffmann-La Roche Ltd, Basel, Switzerland) were used for HRMA. Primers (Tab. 3) for all genotyping reactions were designed using Primer3Plus software (20). Positive and negative controls were included in all reactions. Approximately 10=of all samples were used as blinded duplicates for „in-house" reaction quality control.

Statistical analysis

Single marker analyses and haplotype analyses were conducted using SNP & Variation Suite v8.3 (Golden Helix, Inc., Bozeman, MT, www.goldenhelix.com). Fisher's exact test was used to estimate the significance of deviation from Hardy-Weinberg equilibrium and to execute basic allelic associations. Pearson's chi-squared test for contingency tables was used to examine haplotype associations. A haplotype frequency was estimated using the EM algorithm. Association tests were confirmed by a logistic regression with case/control status as the dependent variable and suspected haplotype as the independent variable in dominant genetic model. Logistic regressions were performed with the PASW statistical package in SPSS Statistics 18 (SPSS Inc., released 2009; PASW statistics for Windows, Version 18.0. Chicago; SPSS, inc.). Odds ratios (ORs) with 95% confidence intervals (95% CI) were used to assess genetic effects.

Tab. 2. Characteristics of the study group.

Feature	Total n=86
Gender n (%)	
Female	52 (60.5%)
Male	34 (39.5%)
Age at diagnosis, years (range)	47.1 (17-85)
TNM clinical stage n (%)	
I	61 (70.9%)
II	7 (7.6%)
III	13 (15.2%)
IVa	5 (6.3%)
Associated diseases	
No associated disease	30 (34.8%)
Diabetes mellitus	12 (14%)
Arterial hypertension	22 (25.6%)
Ischemic heart disease	5 (5.8%)
Lipid metabolism disorder	5 (5.8%)
Other malignant disease	5 (5.8%)
Musculoskeletal disorder	8 (9.3%)
Other benign operations	12 (14%)
Other benign diseases	29 (33.7%)

Among benign operations we include procedures as: appendectomy, cholecystectomy, cataract, etc.
The group of benign diseases includes diseases such as psoriasis vulgaris, hepatopathy, benign prostatic hyperplasia and the like.

Results

In this pilot study we genotyped 86 patients having the average age at diagnosis of 47.1 years (age at onset from 17 to 85 years). The study cohort included 34 males (39.5 %) and 52 females (60.5 %). Because of the minor allele frequency (MAF) greater than 10 % we consider the sample size to be adequate to give enough power to detect common gene haplotypes.

A personal history of cancer at other sites and other associated diseases were also collected. We monitored the occurrence of Arterial Hypertension, Diabetes Mellitus, Ischemic Heart Diseases, Disorder of Lipid Metabolism, Other Benign Diseases, Musculoskeletal Disorders, Other Benign Operations, Other Malignant Diseases and Serum thyroglobulin IgG antibodies (antiTG). The clinical characteristics for the papillary thyroid cancer subjects are presented in the Table 2.

Tab. 3. Allele association analysis of study subjects.

Chr	Marker	Minor Allele (D)	Frequency PTC (n=84)	Frequency Controls (n=99)	Fisher's HWE p PTC (n=84)	Fisher's HWE p Controls (n=99)	Fisher's Exact p allelic association
2	rs966423	C	0.55	0.44	0.67	**0.04**	**0.05**
8	rs2439302	G	0.43	0.47	0.51	0.69	0.46
9	rs965513	A	0.42	0.35	1.00	0.51	0.19
14	rs116909374	T	0.03	0.07	1.00	1.00	0.15
14	rs1537424	C	0.33	0.40	1.00	0.21	0.16
14	rs944289	C	0.34	0.39	0.48	0.29	0.33

PTC – Papillary Thyroid Cancer; n – number of individuals *per* group; *Fisher´s HWE P* – Fisher´s exact test- Hardy Weinberg Equilibrium; *Fisher´s Exact P* – Fisher´s exact test

Tab. 4. Genotyping association analysis of study subjects.

	Minor allele	Major allele	n (%)			OR	CI (95%)	Fisher's Exact p	Exact Armitage p
Marker	Da	db	DD	Dd	dd	Minor allele		genotypic association	genotypic additive association
rs966423	C	t	24 (0.28)	45 (0.53)	16 (0.19)	1.51	1.00–2.29	0.05	0.03
rs2439302	G	c	17 (0.20)	39 (0.46)	28 (0.33)	0.88	0.56–1.28	0.73	0.44
rs965513	A	g	15 (0.18)	41 (0.49)	28 (0.33)	1.34	0.88–2.04	0.37	0.18
rs116909374	T	c	0 (0.00)	5 (0.06)	79 (0.94)	0.44	0.15–1.25	0.14	0.10
rs1537424	C	t	9(0.11)	37 (0.44)	39 (0.45)	0.73	0.48–1.13	0.28	0.17
rs944289	C	t	8 (0.10)	40 (0.48)	35 (0.42)	0.79	0.51–1.21	0.24	0.29

A – Minor Allele (D); b – Major Allele (d); OR – odds ratio; CI – confidence interval; n – number of individuals per group

Tab. 5. Haplotype association analysis of study subjects.

Chr		EM Cases	EM Controls	p	χ^2	OR	CI (95%)
14q13	TTC	0.624	0.530	0.073	3.223	**1.545**	**1.016 –2.351**
	CCC	0.299	0.354	0.268	1.226	0.787	0.505 – 1.227
	TCC	0.030	0.031	0.965	0.002	0.982	0.294 – 3.276
	TTT	0.019	0.045	0.170	1.884	0.253	0.054 –1.188
	CTC	0.018	0.027	0.542	0.372	0.496	0.126 –1.949
	CCT	0.011	0.013	0.830	0.046	0.882	0.195 –3.997

SNP order in haplotypes: rs137424, rs944289, rs116909374; EM, cases – expectation-maximization cases; EM controls, expectation-maximization controls; chi^2 – Chi square test; OR – Odds Ratio; CI – confidence interval

Association allele variants with PTC

The observed genotype distributions for all tagSNPs were in Hardy-Weinberg equilibrium for the patient groups. Results for the allele frequency analysis and genotyping analysis of all targeted genetic variants are reported in Tables 3 and 4. One of the six analyzed genetic variants (rs116909374) had a MAF < 0.1. For the rs966423 (DIRC3), there was a statistically significant difference in the MAF between PTC and the control group (Tab. 3) (55 % PTC vs 44 % control; p=0.05; OR=1.51). For the genotyping additive association analysis one of the six SNPs rs966423 (DIRC3) was significantly associated with PTC (Tab. 4) (p=0.03; OR (95% CI)=1.51 (1.00–2.29). Next two SNPs rs965513 (PTCSC2, OR=1.34) and rs116909374 (MBIP, OR=0.44) showed a suggestive association (Tab. 4). The presence of a minor allele in the rs116909374 genotype indicates a protection against PTC (Tab. 4) (OR = 0.04).

Haplotypes

Haplotype analysis was performed to further evaluate the role of the tested genes in papillary thyroid cancer susceptibility. Six common haplotypes were estimated for 3 tag SNPs located in chromosome 14q13. The results of the haplotype analysis are shown in the Table 5. Haplotype TTC showed a suggestive association with PTC (p=0.07, OR=1.55).

Interactions between the combined germline variants and associated diseases

We also monitored the relationship of selected SNPs and haplotypes derived from them with the associated diseases in the patients with PTC. The following associated diseases were monitored: Arterial Hypertension, Diabetes Mellitus, Ischemic Heart Diseases, Disorder of Lipid Metabolism, Other Benign Diseases, musculoskeletal Disorders, Other Benign Operations, Other Malignant Diseases and Serum thyroglobulin IgG antibodies (antiTG) (Tabs 6, 7, 8). For clarity, Tables 7 and 8 shows the percentage (distribution) of interactions between SNPs and haplotypes with the associated diseases, and we present only the results obtained in our study, which should be given further attention. In the PTC group, significant associations were observed between rs966423 (DIRC3) and ischemic heart diseases (p=0.009), rs965513 (PTCSC2) and diabetes mellitus (p=0.04) and haplotype 14q13 and musculoskeletal diseases. Next three associations rs966423 (DIRC3) and arterial hypertension; rs116909374 (MBIP) and other benign diseases; rs1537424 (MBIP) and disorder lipid metabolism, rs965513 (PTCSC2) and anti-Tg (thyroglobulin antibody) showed a suggestive association.

Discussion

Genetic prognostic factors in PTC are still poorly understood, but increasing evidence suggests that multiple low-penetrance genetic variants, rather than a few high-penetrance variants, can better explain the risk of PTC.

The variant rs966423 on chromosome 2q35 is located in the DIRC3 gene, within a long non-coding RNA (lncRNA); whose function is not established, but it is thought to be a putative tumour suppressor. In literature, there are only a few reports demonstrating the prognostic significance of the rs966423 polymorphism in the DIRC3 (disrupted in renal carcinoma 3) gene, and its relationship with susceptibility to PTC or relationship with unfavourable histopathologic features and mortality in patients diagnosed with PTC. The rs966423 DIRC3 was first identified in 2003 as a fusion transcript involved in familial renal carcinoma (21). Due to the ambiguous role of the rs966423 polymorphism in DIRC3 as a prognostic factor in PTC, we assessed the correlation between this variant and the clinical course of PTC.

In a GWAS, *DIRC3* variants were associated both with TC risk and thyroid stimulating hormone levels. It is thus possible that genetic variants in *DIRC3* alter thyroid stimulating hormone production, and therefore indirectly promote TC development as the result of a decreased thyroid epithelium differentiation (22).

Tab. 6. Association of SNPs and haplotypes with associated diseases.

Marker	AH		DM		IHD		DLM		OBD		MD		OBO		OMD		antiTG	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
rs966423	5.484	0.064	0.661	0.719	9.530	0.009	1.988	0.370	1.782	0.410	1.988	0.370	1.403	0.496	0.827	0.661	1.071	0.585
rs2439302	2.942	0.230	0.888	0.641	3.282	0.194	0.390	0.823	0.163	0.922	0.390	0.823	0.288	0.866	1.339	0.512	0.291	0.864
rs965513	0.389	0.823	6.102	0.047	0.529	0.768	1.633	0.442	4.458	0.108	0.014	0.993	0.501	0.778	0.528	0.768	5.288	0.071
rs116909374	0.318	0.573	1.979	0.160	0.543	0.461	1.079	0.299	2.949	0.086	0.459	0.498	1.004	0.316	0.220	0.639	0.125	0.724
rs1537424	0.092	0.955	0.147	0.929	2.794	0.247	5.028	0.081	0.762	0.683	0.773	0.680	1.690	0.430	3.659	0.161	1.450	0.484
rs9444289	0.139	0.933	0.298	0.862	3.477	0.176	1.551	0.461	4.330	0.115	0.961	0.618	2.103	0.349	0.461	0.794	1.617	0.446
14q13_haplo	3.021	0.697	3.122	0.681	3.835	0.573	7.437	0.190	8.433	0.134	10.949	0.052	4.016	0.547	7.415	0.192	0.281	0.998
Haplo all group	2.061	0.357	2.164	0.339	3.835	0.147	2.501	0.286	0.732	0.694	4.884	0.087	0.313	0.855	0.652	0.722	0.281	0.869

AH – arterial hypertension; DM – diabetes mellitus; IHD – ischemic heart diseases; DLM – disorder of lipid metabolism; OBD – other benign diseases; MD – musculoskeletal disorders; OBO – other benign operations; OMD – other malignant diseases; antiTG – serum thyroglobulin IgG antibodies

Recently, Świerniak et al. published the results of the study indicating a relationship between the TT variant of the rs966423 polymorphism, and a worse prognosis and increased overall mortality in patients with DTC (23).

In our study, we confirmed that the presence of the minor allele C in the genotype increases the risk of PTC 1.5-fold (OR=1.5; 95% CI=1.001–2.288; Fisher's Exact p for genotyping association=0.05 and Exact Armitage p for genotypic additive association=0.03).

Similar results were confirmed by the studies of Jendrzejewski et al. in the Poland population (OR=1.27; 95% CI=1.14–1.42, p = 0.00002) and Mussazhanova et al in the Kazakh population (OR =1.18; 95% CI=0.98–1.28, p=0.07) (24, 25). On the contrary, Hińcza et al indicated no significant difference in the incidence of the TT variant between the DTC patients and the control population (Fisher's exact test, p=0.140) in the Poland population (26). Similar data were published by Wang et al, although this study was conducted in the Chinese population (27).

The SNP rs2439302, at 8p12, is located in the first intron of the NRG1 gene encoding neuregulin 1, which is a human epidermal growth factor receptor 3 (HER3) ligand. NRG1 can activate proliferative and survival mitogen activated protein kinase (MAPK) and AKT signalling pathways under conditions causing HER2/HER3 dimer induction in thyroid cancer cells (28).

The NRG1 gene encodes a membrane glycoprotein that mediates cell-cell signalling and plays a critical role in the growth and development of multiple organ systems. NRG1 is produced in multiple isoforms by alternative splicing and usage of distinct promoters, which allows it to perform a wide variety of functions. Additionally, NRG1 has been demonstrated to be involved in carcinoma development, a more aggressive course of the disease reflected by larger tumour diameter, more advanced N stage at the time of diagnosis, lymph node metastasis (OR=1.24, p=0.016) and a higher multifocality status of the tumour (OR=1.24, p=0.012) (24, 29). However, these associations were only moderate. In our study, we did not confirm the relationship of the rs2439302 variant to susceptibility to PTC.

The results of published studies are also inconsistent in the case of another variant of SNP rs966513 that we were monitoring. SNP rs966513, located about 60 kB upstream and centromeric to FOXE1, was the first and strongest SNP to be consistently reported as a genetic determinant of thyroid cancer susceptibility (30). Its functional significance has only recently been established. The leader rs966513 and several other SNPs on chromosome 9q22.33 were shown. These are in imbalance with rs966513 and modify the activities of long-range enhancers involved in the transcriptional regulation of the FOXE1 and PTCSC2 genes. The rs965513 risk allele has been associated with a decreased expression of FOXE1, untreated PTCSC2, and thyroid stimulating hormone receptor (TSHR) in normal thyroid tissue (31).

In our study, we observed a trend of rs965513A to susceptibility PTC (OR=1.34).

Estrada-Florez et al detected similar significant associations between TC risk and rs965513A (OR=1.41) and also another variants as rs944289T (OR=1.26), rs116909374A (OR=1.96), rs2439302G (OR=1.19), and rs6983267G (OR=1.18) (32).

Tab. 7. Percentage distribution of interactions between SNPs and associated diseases (%).

Marker	All	AH0	AH1	DM0	DM1	IHD 0	IHD 1	OBD 0	OBD 1	OBO 0	OBO 1
DIRC3: t_t	19.74	**12.96**	**36.36**	19.05	23.08	15.94	57.14	22.45	14.81	21.88	8.33
DIRC3: t_c	48.68	**53.70**	**36.36**	50.79	38.46	53.62	0.00	51.02	44.44	48.44	50.00
DIRC3: c_c	31.58	**33.33**	**27.27**	30.16	38.46	30.43	42.86	26.53	40.74	29.69	41.67
NRG1: g_g	19.74	24.07	9.09	20.63	15.38	21.74	0.00	18.37	22.22	18.75	25.00
NRG1: g_c	42.11	37.04	54.55	39.68	53.85	39.13	71.43	42.86	40.74	42.19	41.67
NRG1: c_c	38.16	38.89	36.36	39.68	30.77	39.13	28.57	38.78	37.04	39.06	33.33
PTCSC2: g_g	32.89	33.33	31.82	**26.98**	**61.54**	33.33	28.57	26.53	44.44	31.25	41.67
PTCSC2: g_a	48.68	50.00	45.45	**53.97**	**23.08**	49.28	42.86	48.98	48.15	50.00	41.67
PTCSC2: a_a	18.42	16.67	22.73	**19.05**	**15.38**	17.39	28.57	24.49	7.41	18.75	16.67
14q13_9374: c_c	93.42	94.44	90.91	95.24	84.62	92.75	100.00	**89.80**	**100.00**	92.19	100.00
14q13_9374: c_t	6.58	5.56	9.09	4.76	15.38	7.25	0.00	**10.20**	**0.00**	7.81	0.00
14q13_7424: t_t	43.42	42.59	45.45	42.86	46.15	44.93	28.57	44.90	40.74	42.19	50.00
14q13_7424: t_c	46.05	46.30	45.45	46.03	46.15	46.38	42.86	42.86	51.85	45.31	50.00
14q13_7424: c_c	10.53	11.11	9.09	11.11	7.69	**8.70**	**28.57**	12.24	7.41	12.50	0.00
14q13_4289: t_t	39.47	40.74	36.36	38.10	46.15	40.58	28.57	44.90	29.63	40.63	33.33
14q13_4289: t_c	51.32	50.00	54.55	52.38	46.15	52.17	42.86	42.86	66.67	48.44	66.67
14q13_4289: c_c	9.21	9.26	9.09	9.52	7.69	**7.25**	**28.57**	12.24	3.70	10.94	0.00

AH – arterial hypertension; DM – diabetes mellitus; IHD – ischemic heart diseases; OBD – other benign diseases; OBO – other benign operations

Tab. 8. Percentage distribution of interactions between haplotypes and associated diseases.

	All	AH0	AH1	DM0	DM1	IHD 0	IHD 1	OBD 0	OBD 1	OBO 0	OBO 1
14q13_haplo: ttc	61.84%	62.96%	59.09%	61.90%	61.54%	63.04%	50.00%	63.27%	59.26%	60.94%	66.67%
14q13_haplo: ccc	29.61%	30.56%	27.27%	30.95%	23.08%	27.54%	50.00%	29.59%	29.63%	30.47%	25.00%
14q13_haplo: tcc	3.29%	1.85%	6.82%	3.17%	3.85%	3.62%	0.00%	**1.02%**	**7.41%**	2.34%	8.33%
14q13_haplo: ttt	1.32%	0.93%	2.27%	0.79%	3.85%	1.45%	0.00%	2.04%	0.00%	1.56%	0.00%
14q13_haplo: cct	1.97%	1.85%	2.27%	1.59%	3.85%	2.17%	0.00%	3.06%	0.00%	2.34%	0.00%
14q13_haplo: ctc	1.97%	1.85%	2.27%	1.59%	3.85%	2.17%	0.00%	1.02%	3.70%	2.34%	0.00%
haplo_group: 1	61.84%	62.96%	59.09%	61.90%	61.54%	63.04%	50.00%	63.27%	59.26%	60.94%	66.67%
haplo_group: 2	29.61%	30.56%	27.27%	30.95%	23.08%	27.54%	50.00%	29.59%	29.63%	30.47%	25.00%
haplo_group: 3	8.55%	**6.48%**	**13.64%**	**7.14%**	**15.38%**	9.42%	0.00%	7.14%	11.11%	8.59%	8.33%

AH – arterial hypertension; DM – diabetes mellitus; IHD – ischemic heart diseases; OBD – other benign diseases; OBO – other benign operations

Literary sources state that the 14q13 chromosome locus is likely to contain more than one genetic variant predisposing to thyroid cancer (22, 33, 34, 35). Therefore, the other three SNPs variants rs1537424, rs944289 and rs116909374 we observed are located on this chromosome 14. In the functional study, rs944289 at 14q13.3 was shown to regulate expression of PTCSC3, a lincRNA gene with tumour suppressor properties in thyroid cancer cell lines (36). Restoration of PTCSC3 expression in cell lines inhibited cell growth and affected the expression of genes corresponding to (a) DNA replication, recombination and repair, gene expression, amino acid metabolism; (b) cellular movement, tumour morphology, cell death; and (c) cellular assembly and organization, cellular function and tissue morphology networks. PTCSC3 expression was significantly downregulated in PTC as compared to normal thyroid in the study of PTC from Japan (35), in line with the mentioned work. Of note, rs944289 (PTCSC3) and rs2439302 (NRG1) were associated not only with thyroid cancer, but also with follicular adenoma (35). This indicates that the mechanisms mediated by PTCSC3 and NRG1 are likely to play roles not only in carcinogenesis, but more broadly in thyroid tumorigenesis.

The SNP rs116909374 reported in the association with serum thyroid stimulating hormone level and thyroid malignancy (33) may point at the MAP3K12 binding inhibitory protein 1 (MBIP) gene whose product regulates JNK pathway involved in intracellular signalling of many types of human cancers including thyroid malignancy.

Our study did not confirm the association between SNPs rs944289 and rs116909374 and PTC.

The SNP rs1537424 reported in association with Hashimoto's Thyroiditis (37). The results of our study suggest that the presence of a minor allele T in the genotype tends to have a protective effect on the susceptibility to PTC.

For three SNPs variants located at the 14q13 chromosome locus (rs1537424, rs944289 and rs116909374), we also performed a haplotype analysis, suggesting that TTC haplotype carriers could have an increased susceptibility to PTC (OR = 1.54).

In the next part of our study, we performed formal tests of interaction between all SNPs and associated diseases. We evaluated the associations with the following diseases: arterial hypertension (AH), diabetes mellitus (DM), ischemic heart diseases (IHD), disorder of lipid metabolism (DLM), other benign diseases (OBD), musculoskeletal disorders (MD), other benign diseases (OBD) and other malignant diseases (OMD).

From all monitored interactions, we evaluated the following, which could be further monitored:

rs966423 and AH and IHD (p=0.06 and 0.009 respectively); rs1537424 and DLM (p=0.08); rs116909374 and OBD (p=0.08); rs965513 and DM (0.04); 14q13 haplotype and MD (p=0.05).

In the last years, many steps forward have been made in the genetic characterization of TC, providing molecular markers for diagnosis, risk stratification, and treatment targets. However, many other steps need to be done in order to diagnose TCs with aggressive behaviour, to tailor the most appropriate target therapy, and to monitor the response to the therapies using new molecular approaches.

Access to personalized disease risk prediction and prognosis requires that further studies include minor and rare SNPs, non-SNP genetic information, gene-gene interactions, ethnicity, non-genetic and environmental factors, and the development of more advanced computational algorithms.

Study limitations

There are limitations to this study that could be addressed in future research. The study did not have the power to detect small differences in the estimated haplotype frequencies and haplotype interactions between the groups, the sample size was too small to perform meaningful subanalyses. The results of the study are the starting point for focusing further research on interesting interactions, whether in genotypic, haplotype analysis or interactions of risk factors with PTC. The results should be interpreted in a careful manner.

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