Non-commercial joint-stock company “Semey Medical University”

ANNOTATION

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The theme of the dissertation work:
"Early diagnosis of thyroid cancer at the molecular genetic level"
submitted for the degree of Doctor of Philosophy (PhD)

The specialty: 6D110100 – Medicine
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The Republic of Kazakhstan
Semey, 2019

Relevance. Thyroid cancer occupies a special place in modern oncology for several reasons. First, there is a tendency of its increase in connection with the introduction of a mandatory biopsy in all patients with nodulation more than 1 cm [Dovydenko E.I. 2018] secondly, our territory is unfavorable in terms of background radiation and iodine availability [Essenbetova M.Zh., 2018].

The growth of various forms of thyroid pathology in Kazakhstan is directly related to the effects of natural, environmental factors. According to the world literature, the proportion of thyroid cancer accounts for up to 3% of all malignant tumors, and as you know, the death rate from cancer takes the second place in the world [Dedov II, 2000; Filie, A., 1999, Abdrashitova, A.T., 2018]. According to the World Health Organization (WHO), thyroid pathology occurs in 8 to 18% of the adult population of the globe, which is about 1.5 billion people. The most common pathology of the thyroid gland is nodulation [Atantaeva B.Zh., 2000, Vasilkova O.N., 2019], which is considered as the preclinical collective term that includes a number of various thyroid diseases, accompanied by the formation of nodes is - benign nodules such as nodal colloid, thyroid cysts "pseudoknots" in hypertrophic form of autoimmune thyroiditis (Hashimoto's thyroiditis), thyroid adenoma and malignant tumors of the thyroid gland [Dedov I.I., 2003]. According to the WHO, among the endocrine diseases, the pathology of the thyroid gland ranks second after diabetes mellitus. In the world, more than 665 million people have endemic goiter or other thyroid pathologies, and 1.5 billion people are at risk of developing iodine-deficient diseases. However, according to world statistics, the increase in the number of thyroid diseases in the world is 5% per year. Malignant thyroid tumors make up 1-3% in the structure of oncological pathology [Olshansky V.O., 1996]. According to WHO forecasts, the incidence and mortality from oncological diseases may increase by another 2 times by 2020, which is associated with the late detection of tumors and the lack of guaranteed access to modern methods of cancer diagnosis and treatment. Currently, the diagnosis and prognosis of most oncological diseases are based on data from instrumental methods of research, which does not always allow a correct assessment of the prognosis of the disease and the possibility of treatment, therefore the study of molecular genetic causes of their occurrence is one of the most pressing health problems. According to WHO forecasts, the incidence and mortality from oncological diseases may increase by another 2 times by 2020, which is associated with the late detection of tumors and the lack of guaranteed access to modern methods of cancer diagnosis and treatment. Currently, the diagnosis and prognosis of most oncological diseases are based on data from instrumental methods of research, which does not always allow a correct assessment of the prognosis of the disease and the possibility of treatment, therefore the study of molecular genetic causes of their occurrence is one of the most pressing health problems. Despite significant progress in the identification of genes involved in malignant transformation of cells, there is still no marker system for assessing the risk of developing thyroid cancer in the general population. In recent years, it has been established that the process of malignant cell transformation can occur both as a result of genetic events (deletions, point

and missense mutations, gene rearrangement), and as a result of epigenetic changes; therefore, a comprehensive analysis of the mechanisms of inactivation of genes involved in pathogenesis is important to thyroid cancer. Given the growing prevalence of malignant disease, the young and working age of most patients, it is becoming increasingly necessary to improve the methods and approaches to diagnosis.

Verification of thyroid carcinomas, especially differentiated forms, is complex, due to the heterogeneity of their histological structure and the complex differentiation of tumor cells [Rozhkova E. B., 2007]. WHO recommends using a combination of the morphological method with molecular-biological and genetic with the definition of biomolecular tumor markers as a new approach to the diagnosis of thyroid cancer. The study of biomolecular markers and genetic instability in PTC is important for determining its malignant potential and the choice of therapy.

For comprehensive and integrated study of thyroid cancer, epidemiologists, clinicians, geneticists have created an international consortium for conducting large-scale studies, including genome-wide Association Studies, GWAS, which is one of the modern methods of research related to the search for associations between genomic variants and phenotypic traits.

To date, worldwide studies are being conducted aimed at finding the genes responsible for the formation of susceptibility to thyroid cancer. Using GWAS, associations with the susceptibility of thyroid cancer on chromosomes 8q12 (NRG1 gene) [Wang et al., 2013], 9q22 (FOXE1 gene) [Matsuse M et al., 2011], 14q13 (NKX2-1) [Gudmundsson et al., 2009]. These works make a great contribution to understanding the pathogenesis of the disease and play an important role in the selection of treatment tactics. The above makes it possible to take into account the frequency and characteristics of the clinical course of PTC at the early stages and the detectability of the FOXE1 (rs 965513) and NKX2-1 (rs 944289) gene mutations in the indigenous ethnic group of Kazakhstan. It is important to consider that there is a significant originality of the gene pool of the population of the Republic of Kazakhstan, the formation of which has a long and complex history, which should affect the structure of the incidence of oncopathology, including thyroid cancer. Based on the above, we formulated the purpose and objectives of the study.

The above allows us to take into account the frequency and characteristics of the clinical course of PTC in the early stages, the detectability of the FOXE1 (rs 965513) and NKX2-1 (rs 944289) gene mutations in the Kazakh population.

The aime: To identify the relationship of single nucleotide polymorphisms of the FOXE1 (rs 965513) and NKX2-1 (rs 944289) genes in patients with papillary thyroid cancer among the Kazakh population, for use as additional diagnostic markers and the choice of personalized treatment tactics.

Tasks:

1. To conduct an epidemiological assessment of malignant tumors of the thyroid gland in the East Kazakhstan region.

2. To study the frequency of occurrence of polymorphisms of the NKX2-1 (rs944289) and FOXE1 (rs 965513) genes in the studied groups of the Kazakh population.

3. To analyze the association of NKX2-1 (rs944289) and FOXE1 (rs 965513) gene polymorphisms with thyroid papillary carcinoma in the Kazakh population.

4. Develop an algorithm for diagnosing thyroid neoplasm based on molecular genetic studies.

Scientific novelty of the research

For the first time in the Kazakh population, the frequency of occurrence of alleles and genotypes of the FOXE1 (rs 965513) and NKX2-1 (rs944289) genes in patients with papillary carcinoma of the thyroid gland and the control group among the indigenous ethnic group of Kazakhstan was identified.

Practical significance

The results of the study can serve as a basis for determining susceptibility to the development of papillary thyroid cancer, since the polymorphisms of the FOXE1 (rs965513) and NKX2-1 (rs944289) genes are the most significant genetic predictors of thyroid cancer. Polymorphisms of the FOXE1 (rs965513) and NKX2-1 (rs944289) genes can be used as an additional diagnostic marker for determining personalized postoperative treatment tactics.

The main provisions of the dissertation research submitted to the defense:

1. Indicators of the frequency of thyroid cancer in the East Kazakhstan region tend to increase. The average age of the maximum incidence of thyroid cancer was 54 ± 10 years, with women 12 times more often than men.

2. For additional diagnostics of PTS at the molecular genetic level, the carriage of unfavorable alleles of the FOXE1 (rs965513) and NKX2-1 (rs 944289) genes A and T, respectively, serves as confirming criteria.

3. Given the lack of significant population differences according to GWAS data in the FOXE1 (rs965513) and NKX2-1 (rs944289) genes, they can be used as genetic predictors of PTC in the Kazakh population.

4. The algorithm for diagnosing thyroid neoplasm on the basis of molecular genetic studies can become a reliable auxiliary method for predicting PRS in everyday clinical practice.

Personal contribution of the author

The author independently analyzed the scientific literature on the topic of the thesis, as well as the statistics of regional oncological dispensaries of the East Kazakhstan region. The author of the thesis personally carried out the isolation of

DNA and genotyping, summarized the results, performed a statistical calculation of the data obtained. All sections of the thesis goals, objectives and programs of research, collection and processing of material, the development of the basic position of the thesis, conclusions, conclusions and practical recommendations formulated and written by the author independently.

Publications on the dissertation work

There are 14 publications in the study, of which 1 article in the publication indexed in the Scopus database (Journal of the Russian Academy of Medical Sciences) the journal "Medicine" and 2 articles in the journal "Science and Health"); 9 theses in collections of international conferences.

According to this work, a certificate of state registration of rights to the copyright object “Early diagnosis of thyroid cancer at the molecular genetic level” No. Gos. Registration No. 2343 dated July 17, 2018 and two certificates for rationalization proposals.

Approbation of work

The results of the study were reported at international conferences, with the subsequent publication of the thesis in conference proceedings, including materials from international conferences in non-CIS countries “European Public Health Conference and Conference” in Milan, Italy, 2015. , “Japan Endocrine Society Annual Meeting” Tokyo, Japan 2016, The 12 th Asia and Oceania Thyroid Association Congress ”Busan, Korea, 2017, XIV International (XXIII All-Russian) Pirogov Scientific Medical Conference of Students and Young Scientists, Moscow, Russia, 2019; At conferences of the republican level of international importance: XII International Scientific and Practical Conference “Ecology. Radiation. Health", Semey 2016; International Scientific and Practical Conference of Young Scientists "Science and Health", Semey 2016.

The results of our work were also reported at the Congress of the Association of Endocrinologists EKR Semey, May 2017. XIV International (XXIII All-Russian) Pirogov Scientific Medical Conference of Students and Young Scientists, Moscow, Russia, 2019; At conferences of the republican level of international importance: XII International Scientific and Practical Conference “Ecology. Radiation. Health", Semey 2016; International Scientific and Practical Conference of Young Scientists "Science and Health", Semey 2016.

The results of our work were also reported at the Congress of the Association of Endocrinologists of East Kazakhstan region Semey, May 2017.

Implementation of research results

The materials and the results of the research were introduced into practical public health and the educational process of the Department of Personalized Medicine and Endocrinology of the Non-commercial joint-stock company “Semey Medical University”.

Financing. The research was carried out with the financial support of the Institute for the Study of Disease Caused by Atomic Bombing by Atomic Bombing, Nagasaki University (Nagasaki, Japan)

Materials and methods of research

We have carried out an analytical retrospective case-control study that allows us to retrospectively assess the association of the NKX2-1 (rs944289) and FOXE1 (rs 965513) genes and papillary thyroid cancer in the Kazakh population.

Each of the study participants gave written informed consent to participate in the study, including blood sampling for genetic research. The work protocol was approved by the local Ethical Committee of the Semey State Medical University No. 2 dated March 18, 2015. The research work is performed in accordance with the principles of the Helsinki Declaration.

The object of the study where 1493 persons of Kazakh nationality took part as, of which 485 people with PTCconstituted the main group and 1,008 healthy people, represented the control group. The average age of 44.14 ± 16.76 years. The set of groups, data collection and sampling of biological material for the study was conducted from September 2014 to August 2015. Molecular research began in September 2015 and lasted for two years.

Each of the study participants gave written informed consent to participate in the study, including the collection of venous blood in a volume of 5 ml in vacuum tubes K2 / K3 with EDTA (ethylenediaminetetraacetic acid) for genetic research.

For all the subjects, a comprehensive examination was carried out: palpation of the thyroid gland, ultrasound of the thyroid gland, hormonal examination of the pituitary and thyroid using an enzyme immunoassay.

Formation of a group of cases was carried out among adults of the Kazakh population who are registered with a diagnosis of HF in regional and city oncologic dispensaries (Ust-Kamenogorsk, Semey, Almaty, Astana). The diagnosis of PTC was histologically verified.

The control group consisted of 1008 healthy people of the Kazakh population selected randomly according to the data of the primary health care organizations and living in the above-mentioned cities and regions.

Criteria for inclusion in the control group were the following conditions:

1) Persons of the kazakh nationality

2) Absence of increase in the volume of the thyroid gland according to palpation;

3) Absence of nodular formations and hypoechogenicity of the structure of the thyroid gland according to ultrasound data;

4) The level of thyroid hormones is within normal limits;

5) Absence of pregnancy;

6) Absence of a history of data on the pathology of the thyroid gland and / or on the receipt of iodine preparations, thyroid hormones.

The subject of the study was peripheral blood, serum, DNA to study the prevalence of various variants of the polymorphisms FOXE1 (rs 965513) (genotypes GG, GA and AA) and NKX2-1 (rs944289) (genotypes CC, CT, TT) in

patients with thyroid papillary carcinoma and in the control group of healthy people. To determine the hormonal status used serum obtained from 5 ml of venous blood of the studied.

The analysis was carried out by an enzyme immunoassay on the analyzer "Hoffman Le Roshe" Switzerland, using a set of reagents in the biochemical laboratory of the consultative-diagnostic laboratory "In vitro" Semey.

For typing polymorphisms of the FOXE1 and NKX2-1 genes, DNA preparations of whole blood were used.

The analysis of polymorphism was carried out by the method of polymerase chain reaction (PCR) in the laboratory of molecular genetics of the Institute of Atomic Bombing Diseases, Nagasaki University (Nagasaki, Japan).

DNA isolation was performed using the QIAamp DNA Mini Kit (QIAGEN, Japan) in accordance with the manufacturer's instructions.

Qualitative and quantitative DNA analysis was performed by a NanoDrop 1000 nanodrop (Thermo Scientific, Valtam, USA);

Genotyping was performed by real-time polymerase chain reaction (PCR) (real-time PCR) on a Light Cycler 480 II instrument (Roche, Indianapolis, USA).

For statistical data processing were used Pearson's $\chi 2$ and odds ratios (OR) with 95% confidence intervals (CI). An analysis of the association of the variants of the FOXE1 polymorphisms (GG, GA and AA) and NKX2-1 (CC, CT and TT) with the PTC was performed by calculating the odds ratio (Odds Ratio - OR) and its 95% CI (confidence interval (95% Confidence Interval - CI)). OR = 1 was considered as the absence of association, OR> 1, as a positive association with the risk of developing PTC and OR <1, as a negative association with the risk of developing PTC. The critical level of statistical significance of differences was set at $p < 0.05$. Statistical analysis was performed using IBM SPSS Statistics Version 20 (International Business Machines Corp., Armonk, USA), WINPEPI and SPSS 20.0 (Semey State Medical University). A meta-analysis of genetic risk association studies was performed in the Review Manager program (RevMan 5), Cochrane Collaboration.

Results

Epidemiological evaluation of thyroid malignant tumors in the East Kazakhstan region

To implement the task of the dissertation research on the study of the epidemiological features of thyroid pathologists in the eastern region of Kazakhstan (EKR), we organized a cross-sectional epidemiological study of the incidence and mortality from thyroid cancer among the population of East Kazakhstan region for the period from 2011 to 2015. To perform the work, the accounting and reporting data of the East Kazakhstan Regional Oncology Centre in Ust-Kamenogorsk and the Regional Oncology Centre in Semey city served. The database of cases of morbidity, mortality and differentiation of stages is a territorial cancer registry common for EKR.

We calculated crude morbidity and mortality rates from thyroid cancer for a 5-year observation period per 100 thousand people. In the Republic of Kazakhstan (RK), there is an increase in the incidence of thyroid cancer from 2.5 per 100 thousand population in 2011 to 3.5 per 100 thousand population in 2015, which corresponds to 411 new cases of thyroid cancer in 2011 and 619 - in 2015 in absolute numbers. An analysis of the incidence rates in the East Kazakhstan region (East Kazakhstan) for 5 years revealed the dynamics of an increase in the incidence of thyroid cancer in the region from 2.9 per 100 thousand population in 2011 to 4.2 per 100 thousand population in 2015. Growth trend amounted to +0.39. A similar picture during the study period was also observed in the Republic of Kazakhstan, where there was also an increase in the rough incidence rate of thyroid cancer with a growth trend of +0.25. The crude mortality rate of thyroid cancer in the Republic of Kazakhstan and the East Kazakhstan region for 2011-2015 shows a relatively stable picture with a slight fluctuation of 0.6% 00 to 0.4% 00 in the East Kazakhstan region and at the republican level - 0.5% 00.

An analysis of the incidence of thyroid cancer by sex showed that in 92% of all cases of thyroid cancer, it is found in females.

According to the results of the analysis, the average age of patients with a newly established diagnosis of thyroid cancer was 54 ± 10 years. The largest proportion of the diagnosis of thyroid cancer in the East Kazakhstan region for the period from 2014 to 2015. accounted for a group of 50-59 years and amounted to 29.79%.

Analysis of the association of FOXE1 (rs965513) and NKX2-1 (rs944289) oncogenes with papillary thyroid carcinoma in the Kazakh population

The genetic block of research was directly devoted to the analysis of the association of FOXE1 and NKX 2-1 oncogenes with papillary thyroid cancer in the Kazakh population. First of all, it should be said that NKX2-1 (NK2 homeobox 1) is the first thyroid transcription factor (TTF1-Thyroid Transcription Factor1) and FOXE1 (Forkhead box factor factor E1) is also called the second thyroid transcription factor (TTF2-Thyroid Transcription Factor 2) compelling candidates associated with differentiated malignant tumors of the thyroid gland in various populations because of their role in the genesis of the thyroid gland.

We conducted a molecular genetic study on the polymorphisms of the FOXE1 (rs965513) and NKX2-1 (rs944289) genes in all study participants. For genotyping, DNA from 1493 participants was successfully isolated from blood: in 485 individuals with thyroid cancer and in 1008 conditionally healthy individuals. The general sex and age characteristics of the study are presented in table 1.

Table 1 – Age and sex characteristics of study groups, n = 1493

	Cases (PTC)	Control (healthy)
The number of observations, the absolute number	485	1008
Age range, years	18-87	17-83
Average age	54,8±13,26	39,02±15,84
male, %	9,7	21,3
female, %	90,3	78,7

The ratio of the number of observations in the study groups was approximately 1 case to 2 controls not matched by gender and age. The minimum and maximum age of the subjects was in the equivalent range, from 18 to 87 years in the group of cases and from 17 to 83 years in the group of controls. The average age was 54.8 ± 13.26 years and 39.02 ± 15.84 years, respectively. The prevailing part in the group of patients with PTC were women (90.3%) against the number of males (9.7%). In the control group, most of the observations were also presented by women (78.7%) and less than a third were men (21.3%).

To decision the problem, a comparative analysis of the prevalence of the FOXE1 polymorphism alleles (rs965513) of the GG, GA, AA and NKX2-1 (rs944289) genotypes of CC, CT, TT in patients with PTC and in the control group of healthy people of the Kazakh population was carried out. To compare the level of FOXE1, depending on the genotype, 485 patients with papillary thyroid cancer and 1008 individuals of the control group who were genotyped for single nucleotide polymorphism (rs 965513) of the FOXE1 gene were divided into subgroups depending on which genotypes they are: FOXE1 (rs 965513) AA genotype carrier group; FOXE1 (rs 965513) GG carrier group; and FOXE1 (rs 965513) GA heterozygous group.

Similar subgroups were formed to compare the level of NKX2-1 within the genotypes (NKX2-1 rs944289 CC, TT, TC) from the same groups genotyped for polymorphism (rs 944289) of the NKX2-1 gene.

Determination of the association frequency of SNP FOXE1 rs965513 and NKX2-1 rs944289 with papillary thyroid cancer was carried out by the method of multivariate logistic regression analysis in a multiplicative model of inheritance.

Frequency of allele polymorphism of the gene FOXE1 (rs965513) in the Kazakh population

In various population studies (GWAS SNP), near the FOXE1 rs965513 gene, a relationship with the carriage of the A minor minor allele was found when studying susceptibility to PTC. In our study, the share of allele “A” was revealed leading to an increase in its carriage, both in the homozygous state (AA genotype) and heterozygous (GA genotype).

The frequency distributions of the FOXE1 rs965513 polymorphism in the PTC group and the group of controls represented by healthy individuals were

statistically significantly different ($\chi 2$ = 100.09; D.f. = 2; p = 0.000). In the group of PTC cases, the AA genotype was more than three times more common (17.5%) versus the control group (5.1%). The GA genotype carrier in the group of cases was found in 44.5% of cases and in the control group in 33.5%. Variant GG had a lower frequency of occurrence in the group of individuals with PTC (37.9%) as compared with the control group (61.4%) (table 2).

Table 2 – The frequency of polymorphism FOXE1 rs965513 in study groups, n = 1493

FOXE1 rs965513 polymorphism	PTC, the abs.num. (%)	Control, the abs.num. (%)	χ^2	D.f.	p
GG	184 (37,9)	619 (61,4)	100,09	2	0,000
GA	216 (44,5)	338 (33,5)			
AA	85 (17,5)	51 (5,1)			
Total	485 (100,0)	1008 (100,0)			
Note - $\chi 2$ –Hi-square, D.f. - Degrees of freedom, p - statistical level of significance					

In order to determine the degree of association of the carrier FOXE1 rs965513 with the risk of development of PTC, we calculated the odds ratio (OR) in the groups of PTC and healthy individuals. Thus, the OR for the Kazakh population was 2.367 (95% CI: 2.0044-2.796), which corresponds to an increase in the chances of PTC in the carriers of the A and FOXE1 rs965513 allele 2.367 times. When comparing the carriage association FOXE1 rs965513 in other populations (Japanese, Icelanders, Germans), we did not find significant population differences, since in each of the studies this gene acted as a risk factor for the development of PTC (OR> 1) (table 3).

Table 3 – The association rs965513 (9q22.33, FOXE1) with PTC in Kazakh and other populations

Populations	The frequency of the allele "A" FOXE1 rs965513		OR	95% CI		p
	PTC	Control		top	lower	
Kazakhs	0,3979	0,2182	2,367	2,0044	2,796	3,26E-23
Japanese	0,0899	0,0555	1,6829	1,3254	2,1369	2,17E-05
Islanders	0,490	0,352	1,77	1,57	2,0	6,8E-20
Germans	0,454	0,356	1,51	1,16	1,97	0,003
Note - OR - odds ratio, 95% CI - 95% confidence interval, p - statistical significance level						

Frequency of alleles of the NKX2-1 polymorphism (rs 944289) in the Kazakh population

For the NKX2-1 gene (rs944289), the identification of the minor T allele was considered to be a link with PTC.

The distributions of alleles and genotypes of the polymorphic marker NKX2-1 (rs 944289) were significantly different in the compared groups ($\chi 2 = 100.09$; D.f. = 2; p = 0.000). In the group of cases of PTC, the TT genotype was more than 1.5 times more common (30.5%) versus the control group (20.7%). The CT genotype carrier in the group of cases was found in 49.7% of cases and in the control group in 50.4%. Variant CC had a lower frequency of occurrence in the group of individuals with PTC (19.8%) as compared with the control group (28.9%) (table 4).

Table 4 – Frequency of polymorphism NKX2-1 (rs944289) in study groups

NKX2-1 (rs944289) polymorphism	PTC, the abs.num. (%)	Control, the abs.num. (%)	χ^2	D.f.	p
CC	96 (19,8)	291 (28,9)	100,09	2	0,000
CT	241 (49,7)	508 (50,4)			
TT	148 (30,5)	209 (20,7)			
Total	485 (100,0)	1008 (100,0)			
Note - $\chi 2$ –Hi-square, D.f. - Degrees of freedom, p - statistical level of significance					

To determine the degree of association of NKX2-1 carriage (rs944289) with susceptibility to PTC, the OR was also calculated in groups of PTC and healthy individuals. Thus, OR for the Kazakh population was 1.46 (95% CI: 1.2515-1, 7027), which corresponds to an increase in the chances of PTC in the carriers of the NKX2-1 «T» allele (rs944289) 1.46 times. When comparing the carriage association NKX2-1 (rs944289) in other populations (Japanese, Icelanders, Germans), we also did not find significant population differences, since in each of the studies this gene acted as a risk factor for the development of PTC (OR> 1) (table 5).

Table 5 – Association rs944289 (14q13.3 NKX2-1) with PTC in Kazakh and other populations

Populations	The frequency of the allele "T" NKX2-1 (rs944289)		OR	95% CI		p
	PTC	Control		top	lower	
Kazakhs	0,5536	0,4593	1,46	1,2515	1,7027	1,33E-06
Japanese	0,4653	0,4109	1,2479	1,0909	1,4279	0,0014
Islanders	0,644	0,558	1,44	1,26	1,63	2,5E-08
Germans	0,411	0,411	1,00	0,77	1,30	0,95
Note - OR - odds ratio, 95% CI - 95% confidence interval, p - statistical significance level						

In a paired analysis of the interaction between the two SNP risks (rs965513 A and rs944289T) associated with the PTC method of logistic regression analysis in a multiplicative model with correction for age and gender, we found that a combination of two risk alleles (rs965513A and rs944289T: OR = 3.13) directly proportional to the risk of developing PTC in comparison with the absence of risk (rs965513G and rs944289T: OR = 1.91) and heterozygous carriage (rs965513GA and rs944289T: OR= 2.33). The interaction between rs965513 (9q22, located next to FOXE1) and rs944289 (14q13.3 NKX2-1, NK2 homeobox 1 neighborhood) is shown in table 6.

Table 6 – Analysis of the risk of development of PTC associated with various combinations of genotypes (diplotypes) in rs965513 and rs944289, n = 485

		frequency (%)			
rs965513	rs944289	Cases	Control	OR (95% CI)	*p-evaluation
GG	CC	38 (20,7)	184 (29,7)	Reference group	
	CT	89 (48,4)	293 (47,3)	1,46 (0,95-2,23)	0,84
	TT	57 (31,0)	142 (22,9)	1,91 (1,19-3,05)	0,007
GA	CC	39 (18,1)	88 (26,0)	Reference group	
	CT	115 (53,2)	192 (56,8)	1,32 (0,85-2,06)	0,22
	TT	62 (28,7)	58 (17,2)	2,33 (1,38-3,93)	0,002
AA	CC	19 (22,4)	19 (37,3)	Reference group	
	CT	37 (43,5)	23 (45,1)	1,64 (0,71-3,79)	0,25
	TT	29 (34,1)	9 (17,6)	3,13 (1,16-8,47)	0,025
* Logistic regression analysis in a multiplicative model with correction for age and gender. Risk alleles are rs965513A and rs944289T. The rs965513G and rs944289C allele is a reference category (minimal risk)					

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A higher prevalence of the marker among people with a certain pathology as compared to healthy ones indicates a predisposition (increased risk), and a lower one - to resistance to the disease (reduced risk). The study of genetic susceptibility to pathology at the population level using polymorphic markers of various candidate genes is to test the assumption that the presence of a marker is associated with early development and / or rapid progression of the disease.

On the basis of the data obtained, we concluded that carriers of the AA and GA FOXE1 genotypes have an increased risk of developing PTC 2,367 (95% CI: 2,0044-2,796). The estimated risk of homozygous carriers of rs965513 and rs944289 in individuals with a double homozygous state with risk alleles is more than twice the risk compared with individuals who carry one risk allele in one locus (4, 8-fold and 3.2-fold).

General conclusion

The results of our molecular genetic study proved that for papillary thyroid cancer among the Kazakh population such factors as genetic polymorphic markers exist. In any case, the obtained results allow to consider the polymorphisms of the

FOXE1 rs965513 and NKX2-1 rs944289 genes as molecular genetic predictors of carcinogenesis of PTC. The study of polymorphic loci of candidate genes, as well as the identification of genotypes and alleles associated with the disease, allows us to assess the genetic risks of developing PTC. The FOXE1 (rs965513) and NKX2-1 (rs944289) genes function as genetic risk factors for PTC despite their ethnicity, due to the absence of significant population differences.

In the end, the results obtained in the thesis work made it possible to develop an algorithm for diagnosing thyroid cancer in the Kazakh population.

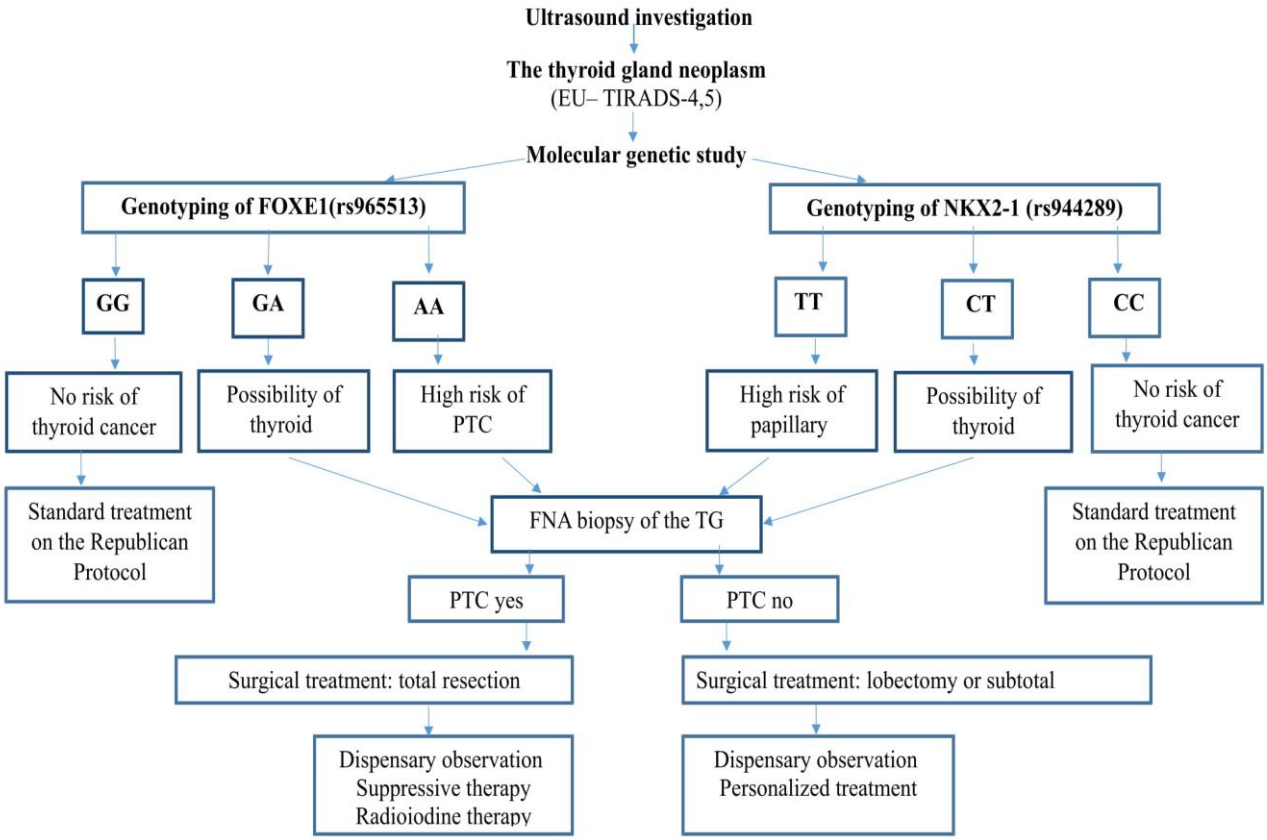
Taking into account the high frequency of propagation of the FOXE1 (rs965513) and NKX2-1 (rs944289) genes in PTC, screening for the presence of tumor markers in the Kazakh population can be an effective method for early diagnosis at the molecular level. In ecologically unfavorable conditions, in places of endemia and the consequences of radiation exposure, the study of predisposing oncogenes, such as FOXE1 (rs965513) and NKX2-1 (rs944289), should be carried out in the form of screening studies of risk groups. This circumstance makes it necessary to revise the existing clinical protocol on thyropathology.

Algorithm for diagnosis of thyroid neoplasm based on molecular genetic studies

For the timely diagnosis of thyroid cancer, any nodal formation in this organ should be considered as a potential tumor. To verify the diagnosis, the diagnostic complex should include a compulsory ultrasound, morphological and molecular genetic study with the determination of single nucleotide polymorphisms rs965513 of the FOXE1 gene and rs944289 of the NKX2-1 gene.

Multistep diagnostics makes it possible in the overwhelming majority of observations to establish an accurate diagnosis and, in a series of observations, to identify the initial forms of cancer and to predict the propensity for the development of PTC in the indigenous ethnic group of Kazakhstan. We have developed and introduced into practice an algorithm to identify the initial forms of thyroid cancer in the majority of patients.

As part of this algorithm, the patient's route with suspected thyroid pathology (according to complaints, palpation) or with a nodal neoplasm of the thyroid gland with a diameter (D) => 1 identified during a screening examination begins with a molecular genetic tests to determine the risk markers for PTC polymorphisms of the FOXE1 rs965513 and the NKX2-1 rs944289 genes. If pathological alleles are detected in the homozygous and heterozygous state – FOXE1 rs965513: AA (the risk is 2.37 times higher than the other genotypes), GA (increased risk, there is a likelihood of development of PTC) and NKX2-1 rs944289 – TT (risk is higher 1.46 times compared with the other genotypes), CT (increased risk, there is a likelihood of development of PTC), the patient is regarded as a potential candidate for more detailed diagnostics for detecting PTC.



Algorithm for diagnosis of thyroid neoplasm based on molecular genetic studies

Algorithm for diagnosis of thyroid neoplasm based on molecular genetic studies

In this case, it is prescribed to conduct a fine-needle aspiration biopsy of a neoplasm of 8-10 points. When a diagnosis of thyroid cancer is confirmed, a patient should receive prompt treatment after a biopsy. In the case of homozygous carriage of the risk allele FOXE1 rs965513: AA and NKX2-1 rs944289 - TT, it is recommended to perform a total resection of the thyroid gland with the subsequent prescription of suppressive therapy and radioiodine therapy. In the case of the heterozygous genotypes FOXE1 rs965513: GA and NKX2-1 rs944289 – CT, it is recommended to perform a lobectomy of the affected thyroid lobe followed by taking it for life dispensary observation from the appropriate specialist (oncologist, endocrinologist) and personalized therapy.

In patients with thyroid neoplasms detected by ultrasound diagnostics according to complaints or screening and negative markers rs965513 GG and NKX2-1 rs944289 CC recommend standard treatment according to the protocol of Respublican Center for Health Development. Thus, the introduction of an algorithm for diagnosing a thyroid neoplasm using molecular genetic studies in clinical practice is a direct factor in reducing the hereditary risk of developing thyroid cancer.

Practical recommendations

1. The study of the association of the polymorphism of the FOXE1 (rs965513) and NKX2-1 (rs944289) genes in patients with PTC makes it possible to predict susceptibility to the development of the disease in individuals of the Kazakh population. Screening for the carrier of polymorphisms of the FOXE1 rs965513 and NKX2-1 genes (rs944289) can be a method of early diagnosis, taking into account their distribution and the presence of associations with cases of papillary thyroid cancer in the Kazakh population.

2. Identification of polymorphisms of the FOXE1 genes (rs965513) genotype A and NKX2-1 (rs944289) genotype T can be used as an additional diagnostic marker and in determining the personalized tactics of postoperative treatment.

3. The introduction of the algorithm for diagnosing a thyroid neoplasm using molecular genetic studies in clinical practice can be a method for the early diagnosis of thyroid cancer.

Conclusions

1. Epidemiological indicators of the frequency of thyroid cancer in the East Kazakhstan region and according to republic-wide data of the Republic of Kazakhstan tend to increase. This trend can be traced in absolute numbers and in the change of the intensive indicator. More often, thyroid cancer is found in people aged 50 years (54 ± 10 years), and in women it is 12 times more common than in men. Analysis of the incidence rates for 5 years revealed the growth rate of the thyroid cancer in the East Kazakhstan region 2.9% 00 in 2011. up to 4.2% 00 in 2015.

2. Analysis of the prevalence of polymorphisms of the NKX2-1 (rs944289) and FOXE1 (rs965513) genes showed that the occurrence of alleles in the Kazakh population differs from the European and East Asian populations and has an intermediate value.

3. a) A link has been established between the polymorphism (rs944289) of the NKX2-1 gene and papillary thyroid cancer. The homozygous carriage of the allele "T" - TT genotype contributes to the development of PTC (OR = 1.46; 95% CI = 1.2515-1.7027), the carriage of CC and CT genotypes hinders the development of PTC.

b) As a result of the analysis of the distribution of the allele “A” of the polymorphism (rs965513) of the FOXE1 gene, a higher frequency was found in the PTC group (39.8%) relative to the control group (21.8%). The content of the G allele in the PTC group was relatively lower - 60.2% versus 78.2% in the control group. Carriers of the AA and GA FOXE1 genotypes have an increased risk of developing PTC (OR = 2,367; 95% CI: 2,0044-2,796).

4. An algorithm was developed for diagnosing thyroid neoplasms using molecular genetic studies, where it was recommended that when detecting tumor markers of FOXE1 rs965513 genes (genotypes GA and AA) and NKX2-1 rs944289 (genotypes CT and TT), a personalized approach is shown in patients with thyroid nodulation in treatment, determining the need for surgery and the use of radioiodine therapy.

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