Informatics in Medicine Unlocked 25 (2021) 100680

Contents lists available at ScienceDirect

Informatics in Medicine Unlocked

journal homepage: www.elsevier.com/locate/imu

Genes and comorbidities of thyroid cancer

Branimir Ljubic [a,b], Martin Pavlovski [a], Shoumik Roychoudhury [a], Christophe Van Neste [c,d], Adil Salhi [d], Magbubah Essack [d], Vladimir B. Bajic [d], Zoran Obradovic [a,*]

[a] Temple University, Center for Data Analytics and Biomedical Informatics (DABI), Philadelphia, PA, 19122, USA
[b] Rutgers University, the Office of Advanced Research Computing (OARC), Piscataway, NJ, 08854, USA
[c] Center for Medical Genetics (CMGG), Ghent University, Ghent, Belgium
[d] King Abdullah University of Science and Technology (KAUST), Computational Bioscience Research Center (CBRC), Computer, Electrical and Mathematical Science and Engineering (CEMSE) Division, Thuwal, Saudi Arabia

ARTICLE INFO

Keywords:
Thyroid cancer
Comorbidities
Genes
Text mining
Biomedical informatics
DisGeNET

ABSTRACT

Introduction: Thyroid cancer represents 3.1 % of diagnosed cancers in the United States. The objective of this research was to identify comorbidities and discover additional genes potentially related to thyroid cancer and improve current knowledge of genetics and comorbidities associated with this cancer.
Methods: Healthcare Cost and Utilization Project (HCUP) California State Inpatient Database (SID) was used to extract and rank the comorbidities of thyroid cancer. The text mining software - BeFree was utilized to identify and extract genes associated with thyroid cancer and the comorbidities from PubMed abstracts and the DisGeNET expert-curated repositories.
Results: Female patients had 4,485, and male patients 2912 different comorbidities in early stages of thyroid cancer. Females had 3,587, and males 2817 different comorbidities in advanced stages. Through PubMed utilizing the BeFree method, 504 different genes associated with thyroid cancer were discovered, as well as five genes on DisGeNET. The most often genes on PubMed, associated with thyroid cancer were: BRAF, RET, SLC5A5, RAS, and PTEN. Genes found via DisGeNET were BRAF, RET, KRAS, NRAS, and PRKAR1A.
Conclusion: Identified genes and comorbidities, as potential additional risk factors for thyroid cancer, not previously known, could improve the early diagnosis and the survival of patients with thyroid cancer. Genes discovered in this research in association with thyroid cancer could be used to direct decision making for optimal, more personalized treatment of thyroid cancer.

1. Introduction

Thyroid cancer (ThyCa) originates in the thyroid gland, which is located in the front of the neck, just below the larynx. ThyCa represents 3.1 % of newly diagnosed cancers in the United States and occurs predominantly in females [1–3]. There are about 53,990 newly diagnosed ThyCa cases and 2060 deaths each year in the U.S. The incidence of ThyCa is 14.3 per 100,000 people[2,3]. Similar incidence have been observed in many other countries throughout the world. There are four subtypes of ThyCa: Papillary cancer (>85 % of cases), Follicular cancer (5–15 %), Anaplastic cancer (<5 %), and Medullary cancer (5 %) [4].

Patients with comorbidities have a lower quality of life [5]. Comorbidities can negatively contribute to patients' overall health and worsen the prognosis of cancer. ThyCa patients are at high risk of developing cardiac and vascular diseases (CVD) [6]. Observational studies have provided evidence for a potential association between ThyCa and obesity [7]. Severe insulin resistance may be associated with aggressive papillary ThyCa during childhood. Genetic analysis revealed a mutation in the BSCL2 gene [8]. The Q61R mutation of the NRAS gene is one of the most frequent driver mutations of ThyCa [9]. An evaluation of patients with thyroid nodules revealed that 4.6 % of patients had the TERT promoter mutation, and 59.2 % had the BRAF gene mutation [10]. PFKFB2 promoter methylation analysis has potential applicability to better stratify well-differentiated ThyCa patients according to the recurrence risk [11]. Patients with papillary ThyCa have significantly over-expressed genes: SDC1 (syndecan 1), SDC4 (syndecan 4), KLK7, KLK10, SLPI, GDF15, ALOX5, and SFRP2, among others [12]. About 20.5 % of specimens had a mutation in the RAS subfamily (HRAS, KRAS or NRAS), without a big difference between subtypes. Mutations in TSHR, DICER1, EIF1AX, KDM5C, NF1, PTEN, and TP53 were found to be

* Corresponding author. Temple University, SERC 035-02 Center for Data Analytics and Biomedical Informatics, 1925 N 12th St, Philadelphia, PA, 19122, USA.
E-mail address: zoran.obradovic@temple.edu (Z. Obradovic).

https://doi.org/10.1016/j.imu.2021.100680
Received 2 February 2021; Received in revised form 16 July 2021; Accepted 23 July 2021
Available online 26 July 2021

recurrent in patients with ThyCa [13].

The objective of our study was to identify comorbidities and genes potentially associated with ThyCa. We used the HCUP SID California database to extract comorbidities in different stages of ThyCa. The next objective of our study was to analyze associations between ThyCa and comorbidities to genes. We utilized text mining of PubMed to identify genes potentially associated with ThyCa, and comorbidity diseases. DisGeNET (the expert source) [14], and the Cancer Genome Atlas (TCGA) were used to validate the findings of the text mining of PubMed [15]. DisGeNET is one of the largest collections of genes involved in human diseases. It integrates data from expert-curated repositories, the GWAS catalog, animal models, and scientific publications. The overall goal of our research was to analyze comorbidities and identify additional genes potentially related to ThyCa and comorbidities and improve current knowledge of genes and comorbidities associated with ThyCa. Identification of genes and comorbidities, as potential risk factors could improve the early diagnosis of ThyCa and the survival of ThyCa patients.

2. Background and significance

A population-based observational study was conducted on 378 ThyCa patients, with the goal to analyze the prevalence of comorbidity related to gender, age, histological type, and therapy [16]. This research aimed to show the possible impact of comorbidities on treatment and survival in ThyCa. Another study was conducted to assess the role of comorbidities in overall mortality and causes of death in patients with ThyCa [17]. The group with 3 or more comorbidities had shorter overall survival. ThyCa patients suffering from comorbid diseases experience lower quality of life [18]. Hu and colleagues presented that genetic alterations contribute to ThyCa heterogeneity [19]. ThyCa represents a type of neoplasia in which critical genes are often mutated through two different molecular mechanisms: point mutation (BRAF, RAS, TP53, and CTNNB1) and chromosomal rearrangement (fusion of the RET gene to genes known as RET/PTC rearrangement). Gene therapy is one of the most promising novel therapeutic approaches for ThyCa [20]. Armanious and collaborators reported a gene-expression assay technology that might aid in the differential diagnosis of ThyCa [21]. Various combinations of genes were required to classify specific ThyCas. These and other studies presented the significance of comorbidities and genes in the survival, quality of life, and mortality of ThyCa patients. They also emphasized the importance of genes in modern therapeutic approaches. Published studies described well-known associations between certain genes and ThyCa. Researchers analyzed the contribution of a few of the most frequent comorbidities on quality of life and mortality of ThyCa patients. Our research was conducted on a much bigger dataset (HCUP). We included all possible comorbidities diagnosed in association with ThyCa. Additionally, by text mining of PubMed, we attempted to extract gene-ThyCa-comorbidity associations that were not previously known and that could be potentially used in diagnostics and treatment of ThyCa. The BeFree text mining system, which was specifically designed for text mining of biomedical sources by employing morpho-syntactic information from the text [22], was utilized to extract ThyCa-comorbidity-genes associations from PubMed. BeFree text mining system was previously successfully applied in different text mining searches of PubMed and other biomedical sources [23–25].

3. Methods

We extracted comorbidities associated with ThyCa from the HCUP SID California database (ICD9 format). This database has 35,844,800 inpatient discharge records collected over 9 years (January 2003 to December 2011) from 474 hospitals. We completed analyses separately for patients younger than age 50, and patients older than age 50. In the group of patients older than age 50, the comorbidities associated with ThyCa were downloaded and divided into 2 groups: a) data associated with early stages and b) data associated with advanced stages of ThyCa. We included all patients with ThyCa (ICD9 code 193) without diagnosed metastases into the early stages of cancer and ThyCa patients with already diagnosed metastases (ICD9 codes 196,197,198) into advanced stages. The HCUP database contains only diagnostic code for ThyCa without specifications of types of cancer or stages, which limited our comorbidity analysis to general ThyCa diagnosis without the possibility to analyze comorbidities for each type of ThyCa separately.

In the group of patients age 50 and younger, the number of ThyCa patients identified in the HCUP database, for the available period, was relatively small and we couldn't perform separate analyses for patients with and without metastases. In the group of patients older than age 50, data were analyzed and ranked lists of comorbidities were created by their prevalence, separately for early and late stages of ThyCa for males and females. We calculated the prevalence of development of each of the ThyCa comorbidities according to the following formula:

$$P_i = \frac{n_i}{T} \quad (1)$$

P_i is the prevalence, n_i is the number of patients with comorbidity, and T is the total number of patients with ThyCa in the HCUP database.

Next, we performed text mining of PubMed abstracts to extract genes associated with ThyCa and the comorbidities using the BeFree text mining system. PubMed (MEDLINE) database contains more than 30 million citations and abstracts (as of January 2020). About 20 million of PubMed's records have abstracts. The first step in our text mining of PubMed (MEDLINE) database was to extract all articles about ThyCa, since that was the focus of our study. We identified more than 54,500 PubMed abstracts where the topic was ThyCa. In the next step we identified all genes in these PubMed abstracts, associated with ThyCa. Furthermore, ranked lists of comorbidities were used for the text mining of PubMed abstracts to identify common genes associated with ThyCa and the comorbidities. We also extracted relationships between genes and diseases from expert-curated sources (DisGeNET).

Gene-ThyCa, comorbidity associations discovered in PubMed literature were compared with gene-ThyCa, comorbidity associations collected from DisGeNET. We counted and presented the numbers of abstracts in PubMed, and the curated expert sources, where gene-ThyCa-comorbidity associations were described. We also compared the genetic findings with genes-ThyCa associations contained in TCGA genetic database. Analyses were performed using Python and R programming languages.

As a control, we used Dragon Exploration System (DES) to find genes potentially associated with ThyCa. The workflow of the DES framework has been described in Ref. [26]. We generated the results through the DES system using the query on our local MongoDB that serves as a PubMed and PubMed Central literature repository: (gene genes protein proteins) AND ("thyroid cancer" "thyroid cancers" "thyroid adenoma" "thyroid adenomas" "thyroid papillary adenoma" "thyroid papillary adenomas" "thyroid follicular adenoma" "thyroid follicular adenomas" "thyroid lymphoma" "thyroid lymphomas" "thyroid carcinoma" "thyroid carcinomas" "thyroid tumor" "thyroid tumors" "thyroid neoplasm" "thyroid neoplasms"), that produced 13,818 documents (abstracts and full text articles from PubMed and PubMed Central - no duplications).

4. Results

4.1. ThyCa-related comorbidities

In the group of patients older than age 50, the total number of female patients in the early stages of ThyCa was 12,771 (HCUP data) and in the advanced stages 4725. We found 4485 different comorbidities in the early stages and 3587 in the advanced stages of ThyCa. We constructed ranked lists of the top 50 comorbidities (HCUP data) that female patients in early and advanced stages of ThyCa developed and calculated (formula 1) the prevalence of their occurrence (Table 1).

The most common comorbidity disease in females in both stages was

Table 1
Top 50 comorbidities in early and late stages of ThyCa in female patients older than 50, and prevalence of their development (HCUP data).

ThyCa comorbidities in females				
	Early stages		Advanced stages	
	Comorbidity Disease	Prevalence	Comorbidity Disease	Prevalence
1	Essential hypertension	0.36	Essential hypertension	0.41
2	Acquired hypothyroidism	0.18	History of malignant neo of thyroid	0.35
3	History of malignant neo of thyroid	0.17	Postsurgical hypothyroidism	0.24
4	Hyperlipidemia	0.17	Acquired hypothyroidism	0.23
5	Esophageal reflux	0.17	Hyperlipidemia	0.19
6	Postsurgical hypothyroidism	0.15	Anemia	0.18
7	Diabetes Mellitus type II	0.13	Esophageal reflux	0.18
8	Obesity	0.13	Diabetes Mellitus type II	0.18
9	Delivery, single liveborn	0.11	Hypocalcemia	0.16
10	Asthma	0.10	Personal history of tobacco use	0.14
11	Anemia	0.10	Hypopotassemia	0.13
12	Hypocalcemia	0.10	Obesity	0.13
13	Personal history of tobacco use	0.09	Urinary tract infection	0.12
14	Pure hypercholesterolemia	0.09	Personal history of irradiation	0.12
15	Nontoxic multinodular goiter	0.09	Delivery, single liveborn	0.12
16	Depressive disorder	0.09	Pneumonia	0.10
17	Chronic lymphocytic thyroiditis	0.07	Asthma	0.10
18	Hypopotassemia	0.07	Depressive disorder	0.10
19	Anxiety	0.07	Pure hypercholesterolemia	0.10
20	Urinary tract infection	0.06	Dehydration	0.09
21	Tobacco dependence	0.06	Anxiety	0.09
22	Osteoporosis	0.06	Hypoosmolality/ hyponatremia	0.09
23	Osteoarthrosis	0.06	Acute respiratory failure	0.08
24	Antineoplastic radiation therapy	0.06	Antineoplastic radiation therapy	0.08
25	Nontoxic uni-nodular goiter	0.06	Osteoporosis	0.07
26	Coronary atherosclerosis	0.05	Congestive heart failure	0.07
27	Morbid obesity	0.05	Atrial fibrillation	0.07
28	Congestive heart failure	0.05	Constipation	0.07
29	Dehydration	0.04	Osteoarthrosis	0.07
30	Constipation	0.04	History of breast cancer	0.07
31	History of allergy to penicillin	0.04	Coronary atherosclerosis	0.07
32	History of breast cancer	0.04	Tobacco dependence	0.07
33	Cardiac dysrhythmia	0.04	Cardiac dysrhythmia	0.06
34	Atrial fibrillation	0.04	Acute kidney failure	0.06
35	Chest pain	0.04	Chronic obstructive pulmonary dis.	0.06
36	Post delivery status	0.04	Protein calorie malnutrition	0.06
37	Benign neoplasm of thyroid gland	0.04	Pleural effusion	0.06
38	Pneumonia	0.04	Pulmonary collapse – atelectasis	0.05
39	Hypoosmolality and/ or hyponatremia	0.04	Hypoparathyroidism	0.05
40	Migraine	0.03	Morbid obesity	0.05
41	Chronic obstructive pulmonary dis.	0.03	Acquired hypothyroidism	0.05
42	Acute kidney failure	0.03	History of allergy to penicillin	0.05
43	Long term use of aspirin	0.03	Nausea with vomiting	0.05
44	Thyroid dysfunction in pregnancy	0.03	Hearing loss	0.05
45	Acute posthemorrhagic anemia	0.03	Partial unilat paralysis of vocal cords	0.05
46	Long term use of insulin	0.03	Disorders of magnesium metabolism	0.05
47	Personal history of irradiation	0.03	Chest pain	0.05
48	Acquired hypothyroidism	0.03	Long term use of insulin	0.04
49	Acute respiratory failure	0.02	Acute posthemorrhagic anemia	0.04
50	Protein calorie malnutrition	0.02	Long term use of aspirin	0.04

hypertension, with a lower prevalence in early than in late stages. The next few most frequent comorbidities were different forms of hypothyroidism, history of ThyCa, postsurgical hypothyroidism, as well as other thyroid diseases, which is a specific finding for this population. Hyperlipidemia (obesity), diabetes mellitus type 2, urinary tract infections, cardiovascular diseases, anemia, esophageal reflux, asthma, depressive disorder, history of irradiation are among the most common comorbidities in patients with diagnosed ThyCa and are more prevalent in advanced stages of the diseases. These findings could also have aging as a confounding variable, but the history of ThyCa and thyroid diseases cannot be attributed to aging only, and they are different from expected findings in the general population at the same age.

In the group of patients older than age 50, the total number of male patients with the early stages of ThyCa was 10,425 and with late stages 2389. We discovered 2912 different comorbidities in male patients in the early stages and 2817 different comorbidities in advanced stages. Table 2 shows the top 50 comorbidities as well as the prevalence of their development in the early and late stages of ThyCa.

Male patients had hypertension as the most often comorbidity with higher prevalence in advanced stages. Males had a higher prevalence of a history of tobacco use, Diabetes Mellitus type2, cardiovascular diseases, hyperlipidemia than female patients, while females had a higher prevalence of depressive disorders, and urinary tract infections.

Table 3 shows ranked comorbidities of ThyCa by their prevalence for patients age 50 and younger, for males and females. We identified 1421 male patients and 1547 female patients with ThyCa, younger than age 50. HCUP contains small numbers of patients in this age group, in the studied period, so we analyzed all patients together, regardless of metastases. Males in this age group had a higher prevalence of essential hypertension, tobacco use disorder, hyperlipidemia, and diabetes mellitus than females. Female patients had a higher prevalence of anemia, hypopotassemia, urinary tract infections, obesity, and depressive disorder than male patients.

The entire list of discovered ThyCa comorbidities in the HCUP SID California dataset is provided as Supplementary Table S1. Genes associated with ThyCa and its Comorbidities.

Further, we text mined PubMed abstracts to identify genes associated with ThyCa and its comorbidities. We found 504 different genes associated with ThyCa in PubMed abstracts (see Supplementary Table S2). However, only 154 genes appeared in two or more abstracts (Table 4).

In DisGeNET, we identified five different genes associated with ThyCa. These genes were BRAF (DisGeNET score −0.4), RET (0.4), KRAS

Table 2
Top 50 comorbidities in early and late stages of ThyCa in male patients older than 50, and prevalence of their development (HCUP data).

ThyCa comorbidities in males				
	Early stages		Advanced stages	
	Comorbidity Disease	Prevalence	Comorbidity Disease	Prevalence
1	Essential hypertension	0.36	Essential hypertension	0.53
2	Hyperlipidemia	0.19	History of malignant neo of thyroid	0.37
3	Personal history of tobacco use	0.17	Personal history of tobacco use	0.28
4	History of malignant neo of thyroid	0.16	Hyperlipidemia	0.28
5	Esophageal reflux	0.16	Postsurgical hypothyroidism	0.25
6	Postsurgical hypothyroidism	0.16	Acquired hypothyroidism	0.23
7	Diabetes Mellitus type II	0.16	Anemia	0.21
8	Acquired hypothyroidism	0.15	Diabetes Mellitus type II	0.20
9	Pure hypercholesterolemia	0.15	Esophageal reflux	0.19
10	Coronary atherosclerosis	0.14	Pneumonia	0.15
11	Benign hypertrophy of prostate	0.12	Pure hypercholesterolemia	0.15
12	Obesity	0.12	Coronary atherosclerosis	0.14
13	Tobacco dependence	0.11	Personal history of irradiation	0.14
14	Anemia	0.11	Hypocalcemia	0.13
15	Atrial fibrillation	0.10	Atrial fibrillation	0.13
16	Chronic obstructive pulmonary dis.	0.10	Benign hypertrophy of prostate	0.12
17	Acute kidney failure	0.09	Tobacco dependence	0.12
18	Congestive heart failure	0.08	Acute respiratory failure	0.12
19	Asthma	0.07	Obesity	0.12
20	Pneumonia	0.06	Hypopotassemia	0.11
21	Depressive disorder	0.06	Hypoosmolality/ hyponatremia	0.11
22	Hypopotassemia	0.06	Dehydration	0.11
23	Long term use of aspirin	0.06	Acute kidney failure	0.11
24	Obstructive sleep apnea	0.06	Chronic obstructive pulmonary dis.	0.10
25	Hypocalcemia	0.06	Cardiac dysrhythmia	0.10
26	Dehydration	0.05	Congestive heart failure	0.09
27	Hypertensive chronic kidney disease	0.05	Urinary tract infection	0.08
28	Cardiac dysrhythmia	0.05	Protein calorie malnutrition	0.08
29	History of antineoplastic radiation therapy	0.04	Hearing loss	0.08
30	Long term use of anticoagulants	0.04	Constipation	0.08
31	Nontoxic multi-nodular goiter	0.04	Depressive disorder	0.08
32	Old myocardial infarction	0.04	Antineoplastic radiation therapy	0.08
33	Sleep apnea	0.04	Pleural effusion	0.08
34	Urinary tract infection	0.04	Asthma	0.08
35	Percutaneous coronary angioplasty	0.04	Hypertensive chronic kidney disease	0.08
36	Osteoarthrosis	0.04	Partial unilateral paralysis of vocal cords	0.07
37	Chronic kidney disease	0.04	Long term use of aspirin	0.07
38	Hypoosmolality and/ or hyponatremia	0.04	Old myocardial infarction	0.07
39	Nontoxic uninodular goiter	0.04	Dysphagia	0.07
40	Disorder of kidney and ureter	0.03	Pulmonary collapse. Atelectasis.	0.07
41	Anxiety	0.03	Sepsis	0.07
42	Dysphagia	0.03	Anemia in neoplastic disease	0.06
43	Constipation	0.03	Obstructive sleep apnea	0.06
44	Gout	0.03	Disorder of kidney and ureter	0.05
45	Benign neoplasm of thyroid gland	0.03	Long term use of anticoagulants	0.05
46	Chest pain	0.03	Pneuminitis due to inhalation	0.05
47	Coronary atherosclerosis	0.03	Anxiety	0.05
48	History of malignant neo. of prostate	0.03	Chronic kidney disease	0.04
49	Acute respiratory failure	0.02	Acute posthemorrhagic anemia	0.04
50	Protein calorie malnutrition	0.02	Chest pain	0.04

(0.35), NRAS (0.32) and PRKAR1A (0.32). The DisGeNET score for gene-disease associations considers the number and type of sources (level of curation) and the number of publications supporting the association. The scores range from 0 to 1. We also compared our findings to the filtered genes for thyroid cancer from The Cancer Genome Atlas (TCGA) program. TCGA researchers have been studied papillary ThyCa as the most common type of ThyCa (more than 85 % of total ThyCa). TCGA researchers discovered on the sample of 496 ThyCa patients, that the majority of ThyCa were driven by mutations in genes related to the mitogen-activated protein kinase (MAPK) signaling pathway: BRAF or RAS (NRAS, HRAS, KRAS) genes [15]. TCGA researchers identified novel driver genes: EIF1AX, PPM1D, and CHEK2 [15]. Researchers also observed additional genes (Fig. 1), which are not statistically significant, but they are known to play a role in ThyCa pathogenesis, such as: MLL, ARID1B, PTEN, AKT, PPARG, TP53, RB1, NF, MEN1, TERT, etc. [15] Discoveries of TCGA research reduced the fraction of ThyCa cases with unknown genetic driver, but there are still at least 3.5 % ThyCas that need genetic studies to discover genetic background. Results of our text mining of PubMed using BeFree tool are presented in Supplementary Table S2. We provided the full list of 504 genes associated with ThyCa extracted from PubMed as genes that could potentially contribute to the occurrence of ThyCa.

BRAF gene was found in PubMed in association with 96 different comorbidities. It encodes a B-Raf protein and it's located on a long arm of chromosome 7. In Table 5 we present comorbidities that are associated with the BRAF gene in PubMed abstracts.

Associations between RET gene and comorbidities are presented in Table 6. RET gene is located on chromosome 10 and encodes receptor type tyrosine kinase. The most frequent comorbidity associated with this gene was Multiple endocrine neoplasia [MEN] type IIA (DisGeNET score 0.8), followed by ThyCa and multiple endocrine neoplasia [MEN] type IIB (DisGeNET score 1).

NRAS gene is located on chromosome 1 and encodes NRAS protein. It was described in PubMed abstracts in relationships with 52 different comorbidities. The majority of associations between NRAS and comorbidities were published in a relationship with acute myeloid leukemia (52 abstracts, DisGeNET score – 0.7). The next most frequent diseases in association with the NRAS gene were unspecified leukemias (19 abstracts), malignant melanoma of skin (19), myelodysplastic syndrome (19), acute lymphoid leukemia (10), multiple myeloma (9), etc. KRAS gene is located on chromosome 12 and encodes K-Ras protein. It was predominantly described in relationships with pancreatic (123

Table 3
Ranked comorbidities in ThyCa patients, age 50 and younger: Males (left), and females (right). The prevalence of the occurrence of comorbidities is shown (HCUP data).

	Males younger than 50		Females younger than 50	
	Comorbidity Disease	Prevalence	Comorbidity Disease	Prevalence
1	Essential hypertension	0.24	Anemia	0.22
2	Anemia	0.16	History of malignant neo of thyroid	0.15
3	History of malignant neo of thyroid	0.14	Essential hypertension	0.15
4	Postsurgical hypothyroidism	0.12	Hypopotassemia	0.14
5	Tobacco use disorder	0.12	Acquired hypothyroidism	0.13
6	Hypopotassemia	0.11	Urinary tract infection	0.12
7	Dehydration	0.10	Dehydration	0.11
8	Acquired hypothyroidism	0.10	Anemia in neoplastic disease	0.10
9	Hyposmolality and/or hyponatremia	0.09	Tobacco use disorder	0.09
10	Esophageal reflux	0.09	Esophageal reflux	0.08
11	Pure hypercholesterolemia	0.08	Postsurgical hypothyroidism	0.08
12	Diabetes mellitus	0.08	Obesity	0.08
13	Anemia in neoplastic disease	0.08	Pure hypercholesterolemia	0.07
14	Acute kidney failure	0.08	Depressive disorder	0.07
15	Hyperlipidemia	0.08	Personal history of irradiation	0.06
16	Personal history of irradiation	0.07	Delivery, single liveborn	0.06
17	Benign hypertrophy of prostate	0.06	Iron deficiency anemia	0.06
18	Coronary atherosclerosis	0.06	Nausea with vomiting	0.06
19	Atrial fibrillation	0.05	Hyposmolality and/or hyponatremia	0.05
20	Urinary tract infection	0.05	Hypocalcemia	0.05
21	Asthma	0.04	Depressive disorder	0.04
22	Hypertensive chronic kidney disease	0.04	Diabetes mellitus	0.03
23	Iron deficiency anemia	0.03	Chronic lymphocytic thyroiditis	0.03
24	Obesity	0.03	Anxiety	0.03
25	Pneumonia	0.03	Protein-calorie malnutrition.	0.03
26	Hearing loss	0.03	Nontoxic multi-nodular goiter	0.03
27	Depressive disorder	0.02	Hyperlipidemia	0.03
28	Chronic blood loss anemia	0.02	Chronic blood loss anemia.	0.03
29	Nontoxic uninodular goiter	0.02	Nontoxic uninodular goiter	0.02
30	Nontoxic multi-nodular goiter	0.02	Asthma	0.02

abstracts) and colon cancer (103). PRKAR1A gene is located on chromosome 17 and encodes c-AMP dependent protein kinase. It was found in association with 34 diseases in PubMed. The most common relationships were described with Cushing's syndrome (17 abstracts, DisGenet score 0.5), Alzheimer's disease (3), leukemia of unspecified cell type (3), multiple endocrine neoplasia [MEN] type I (3), ThyCa (2), etc.

Twenty genes associated with ThyCa in 10 or more PubMed abstracts, shown in Table 7, had DisGeNET scores of 0.1 or higher, which points to a certain level of significance in associations between these genes and ThyCa. The BRAF gene appeared in association with ThyCa in 112 PubMed abstracts, followed by RET gene (55 abstracts). Both genes have DisGeNET scores of 0.4 and are known to have connections to ThyCa. SLC5A5 gene encodes a member of the sodium-glucose cotransporter family and it's located on chromosome 19. It was mentioned in 41 abstracts and PTEN in 30 abstracts; both genes have DisGeNET scores of 0.1.

Results generated by the use of the DES system are provided in Supplementary Material tables (see Supplementary Table S2).

With genes being risk factors in ThyCa development, genetic testing has increasingly being used to improve diagnosis and optimize the management of patients with ThyCa.

5. Discussion

Comorbidities and genes identified in our study may have potential epidemiologic, diagnostic, therapeutic, and prognostic significance for ThyCa. Kuijpens and colleagues emphasized the importance of comorbidity as a risk factor that affects the treatment and outcome of ThyCa [16]. They performed comorbidity analyses on the dataset of 378 patients provided by the Eindhoven Cancer Registry from the Netherlands. The authors calculated the prevalence for a few frequent comorbidities of ThyCa such as: other malignancy, COPD, cardiovascular and cerebrovascular diseases, hypertension, diabetes mellitus, and tuberculosis. Lee and collaborators studied the role of comorbidities in mortality and causes of death in ThyCa patients [17]. The dataset included 2070 patients from a single institution. They found that the group of patients with comorbidities had shorter overall survival and higher probabilities of death caused by other diseases besides ThyCa. Vissers and co-authors studied the impact of comorbidity on quality of life among ThyCa patients (568 patients) [18]. They analyzed the most prevalent comorbidities and discovered that 44 % of ThyCa patients had 2 or more comorbidities. Thyroid diseases were the most prevalent comorbidity in this group of patients.

The objective of our study was to provide the prevalence of ThyCa comorbidities and show how comorbidities change with the progression of ThyCa. We also wanted to present the variation of the comorbidity prevalence related to genders without focusing on any particular comorbidity disease. Our research included more than 23,000 ThyCa patients from 474 hospitals in California, which is different than other published ThyCa studies which analyzed much smaller datasets from mostly one hospital. This allows us to research the prevalence of ThyCa comorbidities and their evolvement with ThyCa development on a much larger scale of data, which makes our results representative of the entire population of California. The most common comorbidity disease in both genders and both stages was hypertension, followed by few diseases specific to patients who have thyroid gland problems (hypothyroidism, history of ThyCa, etc.). Most of other diseases on ranking lists were similar to findings in the general population, which could be contributed to the aging process.

Pozdeyev and co-workers recently analyzed genetic profiles of 583 advanced differentiated and 196 anaplastic ThyCas [27]. They reported the BRAF (V600E), TERT promoter, and RAS mutations, as well as RET fusions as the most frequent genetic alterations in papillary ThyCa specimens, including TP53, MEN1, NF1, NF2, PIK3CA, PTEN, RBM10, ATM, BRCA2, and BRCA1. In the follicular ThyCa specimens, the most frequent genetic alterations included RAS, TERT promoter, TP53, RBM10, and PTEN. BRAF V600E mutation was less prominent, and a subset of follicular ThyCa specimens had no mutations in BRAF or RAS, but exhibited the co-occurrence of PTEN, TP53, RB1, and MEN1 mutations. Anaplastic ThyCa also exhibited frequent genetic alterations in TP53, TERT, BRAF, and RAS, as well as in tumor suppressor genes, including TP53, RB1, NF2, and NF1, and the PI3K/AKT pathway genes, PIK3CA, and PTEN [27]. The report by Pozdeyev and co-workers shows that four of the genes identified through DisGeNET were frequently genetically altered in ThyCa except for the PRKAR1A gene [27]. However, patients with tumor predisposition Carney complex, caused by the PRKAR1A mutation, often develop papillary or follicular ThyCa. Pringle and co-workers generated mice models with the knockout of PRKAR1A in the ThyCa and reported that 40 % of the animal models developed follicular ThyCa by one year of age [28]. Brose and co-workers

Table 4
Genes (154) described in association with thyroid cancer in 2 or more PubMed abstracts.

AKT1	EZH2	ID1	PIK3CG	TERT	PAX8	MMRN1
ALK	F9	IGF1	PPARG	TG	CXCR4	SIRT1
FAS	FGF2	IGF1R	PRKAR1A	TGFB1	CCDC6	CBX7
ATM	FGFR2	IL4	MAPK1	THBS1	NCOA4	HPGDS
CCND1	FHIT	CXCL8	MAP2K1	THRA	RASAL1	SGSM3
BRAF	FOXE1	IL10	MAP2K7	THRB	MADD	TPCN1
PTTG1IP	FOXO3	KDR	TAS2R38	TIMP1	TP63	ZNF395
CAV1	FN1	KRAS	PTCH1	NKX2-1	SOCS1	TMPRSS4
RUNX2	MTOR	LGALS3	PTEN	SEC62	TNFSF10	NDRG2
CD40	FUCA1	SMAD4	NECTIN1	TNF	INPP4B	SEMA6A
CD44	GABPA	MMP2	RAF1	TP53	PROM1	ARHGAP24
CDK5	GOT2	MUC1	RAP1GAP	TP73	PTTG1	HT
CDKN1A	GSTM1	MYC	RARB	TPO	SLIT2	PRIMA1
CDKN2A	GSTM2	NFE2L2	RET	TSHR	BAG3	CCDC80
CCR6	GSTP1	NFKB1	RPE65	TWIST1	NR1D1	DACT2
CTNNB1	GSTT1	NOTCH1	SDHD	UVRAG	YAP1	MIR126
CYP19A1	HABP2	NRAS	SHH	VDR	TXNRD2	MIR146A
DUSP6	HGF	NTRK1	SLC2A1	VEGFA	TXNIP	MIR197
ECM1	HIF1A	SLC26A4	SLC5A5	VEGFC	WDR3	MIR21
EGF	HMGA1	PIK3CA	SLC22A3	VIM	CKAP4	MIR25
EGFR	HRAS	PIK3CB	STAT3	XRCC1	RASSF1	MIR146B
EPHB2	TNC	PIK3CD	TAF1	XRCC3	ZHX2	KLLN

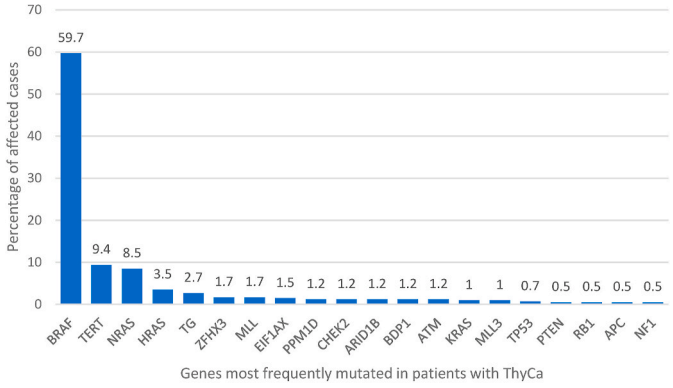

Fig. 1. Twenty genes most frequently mutated in patients with ThyCa – TCGA data.

Table 5
Comorbidities of ThyCa that are associated with BRAF gene in four or more PubMed abstracts. The number of abstracts where the association between BRAF and comorbidities is described is presented, as well as the DisGeNET score for each of the comorbidities.

Comorbidity	No. of abstracts	DisGeNET score
Malignant neoplasm of thyroid gland	112	0.4
Malignant neoplasm of colon	75	0.4
Malignant melanoma of skin	59	0.5
Secondary malignant neoplasm of lymph nodes	51	0.1
Leukemic reticuloendotheliosis	50	0.6
Endocardial cushion defects	13	0.2
Malignant neoplasm of ovary	13	0.6
Chronic lymphocytic thyroiditis	11	0.1
Brain neoplasm	10	0.5
Secondary malignant neoplasm of lung	10	0.1
Leukemia of unspecified cell type	9	0.09
Multiple myeloma	9	0.49
Neurofibromatosis, type 1 [von Recklinghausen's disease]	8	0.38
Malignant neoplasm of liver, secondary	7	0.07
Malignant neoplasm of pancreas	6	0.06
Malignant neoplasm of stomach	5	0.35
Chromosomal anomalies	4	0.04
Rash and other nonspecific skin eruption	4	0.04
Malignant neoplasm of prostate	4	0.34

Table 6
RET gene and association with comorbidities of ThyCa in three or more PubMed abstracts. The number of abstracts where the association between RET and comorbidities is described is presented, as well as DisGeNET score for each of the comorbidities.

Comorbidity	No. of abstracts	DisGeNET score
Multiple endocrine neoplasia [MEN] type IIA	334	0.8
Malignant neoplasm of thyroid gland	55	0.4
Multiple endocrine neoplasia [MEN] type IIB	43	1
Hyperparathyroidism	16	0.2
Secondary malignant neoplasm of lymph nodes	13	0.1
Neurofibromatosis, type 1 [von Recklinghausen's disease]	11	0.1
Neuroendocrine tumors	8	0.08
Myeloid leukemia, acute	7	0.07
Endocrine disorder	6	0.06
Multiple endocrine neoplasia [MEN] type I	6	0.16
Primary hyperparathyroidism	6	0.07
Chronic lymphocytic thyroiditis	6	0.07
Parkinson's disease	5	0.05
Disorders of thyroid gland	5	0.05
Malignant neoplasm of pancreas	5	0.05
Rheumatoid arthritis	3	0.03
Chromosomal anomalies	3	0.03
Leukemia of unspecified cell type	3	0.03
Nontoxic nodular goiter	3	0.02

demonstrated that a specific inhibitor of BRAF V600E, Vemurafenib, induces antitumor activity in patients with progressive, BRAF (V600E)-positive papillary ThyCa [29]. Carr and co-workers showed that the continuous administration of Sunitinib, a tyrosine kinase inhibitor that targets VEGFR, PDGFR, c-KIT, FLT3, and RET, was effective in patients with iodine-refractory well-differentiated ThyCa and medullary ThyCa [30]. The RET gene fusion, CCDC6-RET, is frequently identified in thyroid and lung carcinomas.

Knowledge of related comorbidities and genes is important for the treatment regimens for ThyCa. However, the choice of therapy is determined based on the presence of specific mutations, and other factors, of specific genes such as BRAF, VEGF receptors, PET, and RET/PTC, KDR, KIT, and PDGFRA in individual patients [31]. The list of considered genes should be expanded, which would hopefully increase preventive and treatment options and improve outcomes.

We discovered 504 different genes in PubMed, and 5 different genes associated with ThyCa in DisGeNET. If we consider gene-ThyCa

Table 7
Genes association with ThyCa in 10 or more PubMed abstracts and their DisGeNET scores.

	Gene	No. of PubMed abstracts	DisGeNET score
1	BRAF	112	0.4
2	RET	55	0.4
3	SLC5A5	41	0.1
4	PTEN	30	0.1
5	TG	27	0.1
6	TP53	26	0.1
7	TSHR	17	0.1
8	PIK3CA	16	0.1
9	PTCH1	16	0.1
10	PPARG	13	0.1
11	TERT	13	0.1
12	VEGFA	13	0.1
13	CCDC6	13	0.1
14	AKT1	12	0.1
15	PIK3CB	12	0.1
16	PIK3CD	12	0.1
17	PIK3CG	12	0.1
18	F9	11	0.1
19	TAS2R38	11	0.1
20	PAX8	11	0.1

associations described in 2 or more abstracts, there are 149 more gene-thyroid cancer associations discovered on PubMed than on curated sources. If a certain gene has been mentioned in multiple abstracts where ThyCa and comorbidities have been described, that could indicate a potential association between ThyCa or comorbidity and that gene. Genes discovered in associations with ThyCa in PubMed abstracts need further evaluation by genetic experts in order to confirm their relevance. Genetic findings could be used to identify more individuals who would benefit from genetics consultations. This would result in a better quality of guidelines for genetic testing and early discovery of patients that are potentially at increased risk to develop ThyCa.

Limitations of the study: We performed text mining on PubMed abstracts. Full articles contain more data and it's possible to uncover more genes in them. Sometimes, well-known genes associated with certain cancers are not mentioned in abstracts and analyzing only abstracts could lead to findings that omit some of the genes that are listed on expert-curated sources. In our research, we opted to use BeFree text mining tool, which is specifically designed to extract relations between diseases, genes, and drugs from biomedical text. Other specialized text mining software for biomedical data, such as BioBERT could produce slightly different results. Since our text mining project is a robust time-consuming process, we did not attempt to use different text mining tools, and we cannot conclude how would results of text mining be different in scenarios of different text mining tools. Any results obtained by the text mining technique would require expert verification to confirm the quality of extracted findings. Another limitation is in the selection of the database that we used as the source of data for our research. HCUP SID California database contains specific population and diseases influenced by risk factors, climate, and environmental factors present in California. The population in other geographic areas could have different rankings of the most often comorbidities. Since we included all possible comorbidities registered in the dataset, variations in total numbers of comorbidities would likely be small between different geographical regions, but rankings of the most often comorbidities could be different between different populations.

Furthermore, we had the HCUP dataset that contains data for the period of 9 years (2003–2011) available for this research. We didn't have full histories of patients available, so we could not set any constraints on the timeline of comorbidities because we couldn't know at what point were patients diagnosed with ThyCa, and did comorbidities start before or after the diagnosis of ThyCa. If we obtain additional years of this dataset in the future, we could analyze the timeline of comorbidities in relation to ThyCa development.

6. Conclusion

Cancer diseases are complex and require further investigations of comorbidities and genes, as risk factors. Text mining of big data accumulated in PubMed (a rich resource of medical scientific publications) extracted associations between genes and ThyCa and comorbidity diseases. Results achieved in our study could contribute to a better understanding of risk factors (genes, comorbidities) of ThyCa. Prevention, early discovery, and treatment of comorbidities could lead to better outcomes of ThyCa. A better understanding of the genetic changes that lead to ThyCa can be used to direct decision making for optimal, more personalized management of ThyCa. Genes associated with ThyCa found only in PubMed abstracts should be evaluated as potential risk or predictive factors for the development of this cancer. There is a need for more genetic and comorbidity studies of ThyCa in the future.

Authors' contributions

BL, MP, ZO, SR, AS, ME, VB, CVN designed the project. BL, MP, AS, ME, VB wrote the manuscript. BL, MP, SR searched and downloaded data from HCUP. CVN, VB, AS, ME text mined PubMed and DisGeNet. BL performed comorbidity network analysis. BL, SR, VB, AS, ME and CVN completed genes-diseases associations analysis. ZO performed writing-review and editing. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors gratefully acknowledge the support of King Abdullah University of Science and Technology (KAUST), Office of Sponsored Reaserch (OSR) Awards no. OSR-2018-CPF-3657-03 and FCC/1/1976-24-01, and KAUST Baseline Research Fund (BAS/1/1606-01-01). Healthcare Cost and Utilization Project (HCUP), Agency for Healthcare Research and Quality provided data used in this study.

We did not need the approval of the Ethics Committee for this study, since we used the publicly available dataset (HCUP) and we followed guidance for the use of that particular database.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.imu.2021.100680.

References

[1] McDow AD, Pitt SC. MPHS. Extent of surgery for low-risk differentiated thyroid cancer. Surg Clin 2019;99(4):599–610.
[2] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018;68(1):7–30.
[3] Henley SJ, Ward EM, Scott S, et al. Annual report to the nation on the status of cancer, part I: National cancer statistics. Cancer 2020;126:2225–49. https://doi.org/10.1002/cncr.32802.
[4] Kumar V, Abbas A, Fausto N, et al. Robbins and cotran pathologic basis of diseases. ninth ed. 2014.
[5] Goldfarb M, Casillas J. Thyroid cancer-specific quality of life and health-related quality of life in young adult thyroid cancer survivors. Thyroid 2016;26(7):923–32. https://doi.org/10.1089/thy.2015.0589.
[6] Park J, Blackburn BE, Ganz PA, et al. Risk factors for cardiovascular disease among thyroid cancer survivors: findings from the Utah cancer survivors study. J Clin Endocrinol Metab 2018;103(7):2468–77. https://doi.org/10.1210/jc.2017-02629.
[7] Pazaitou-Panayiotou K, Polyzos SA, Mantzoros CS. Obesity and Thyroid Cancer: epidemiologic associations and underlying mechanisms. Obes Rev 2013;14(12):1006–22. https://doi.org/10.1111/obr.12070.
[8] Lima GEDCP, Fernandes VO, Montenegro APDR, et al. Aggressive papillary thyroid carcinoma in a child with type 2 congenital generalized lipodystrophy. Arch

Endocrinol Metab 2019;63(1):79–83. https://doi.org/10.20945/2359-3997000000096.
[9] Demin DE, Afanasyeva MA, Uvarova AN, et al. Constitutive expression of NRAS with Q61R driver mutation activates processes of epithelial-mesenchymal transition and leads to substantial transcriptome change of Nthy-ori 3-1 thyroid epithelial cells. Biochemistry (Mosc) 2019;84(4):416–25. https://doi.org/10.1134/S0006297919040096.
[10] Censi S, Barollo S, Grespan E, et al. Prognostic significance of TERT promoter and BRAF mutations in TIR-4 and TIR-5 thyroid cytology. Eur J Endocrinol 2019;181(1):1–11. https://doi.org/10.1530/EJE-19-0073.
[11] Camargo Barros-Filho M, Barreto Menezes de Lima L, Bisarro Dos Reis M, et al. *PFKFB2* promoter hypomethylation as recurrence predictive marker in well-differentiated thyroid carcinomas. Int J Mol Sci 2019;20(6):E1334. https://doi.org/10.3390/ijms20061334.
[12] Reyes I, Reyes N, Suriano R, et al. Gene expression profiling identifies potential molecular markers of papillary thyroid carcinoma. Canc Biomarkers 2019;24(1):71–83. https://doi.org/10.3233/CBM-181758.
[13] Nicolson NG, Murtha TD, Dong W, et al. Comprehensive genetic analysis of follicular thyroid carcinoma predicts prognosis independent of histology. J Clin Endocrinol Metab 2018;103(7):2640–50. https://doi.org/10.1210/jc.2018-00277.
[14] Pinero J, Bravo A, Queralt-Rosinach N, et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. Nucleic Acids Res 2017;45:D833–9. https://doi.org/10.1093/nar/gkw943.
[15] The Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell 2014;159(3):676–90. https://doi.org/10.1016/j.cell.2014.09.050.
[16] Kuijpens JL, Janssen-Heijnen ML, Lemmens VE, et al. Comorbidity in newly diagnosed thyroid cancer patients: a population-based study on prevalence and the impact on treatment and survival. Clin Endocrinol 2006;64(4):450–5. https://doi.org/10.1111/j.1365-2265.2006.02492.x.
[17] Lee YK, Hong N, Park SH, et al. The relationship of comorbidities to mortality and cause of death in patients with differentiated thyroid carcinoma. Sci Rep 2019;9(1):11435. https://doi.org/10.1038/s41598-019-47898-8.
[18] Vissers PA, Thong MS, Pouwer F, et al. The impact of comorbidity on Health-Related Quality of Life among cancer survivors: analyses of data from the PROFILES registry. J Canc Surviv 2013;7(4):602–13. https://doi.org/10.1007/s11764-013-0299-1.
[19] Hu J, Yuan IJ, Mirshahidi S, et al. Thyroid carcinoma: phenotypic features, underlying biology and potential relevance for targeting therapy. Int J Mol Sci 2021;22(4):1950. https://doi.org/10.3390/ijms22041950.
[20] Al-Humadi H, Zarros A, Al-Saigh R, et al. Genetic basis and gene therapy trials for thyroid cancer. CANCER GENOMICS PROTEOMICS 2010;7(1):31–49.
[21] Armanious H, Adam B, Meunier D, et al. Digital gene expression analysis might aid in the diagnosis of thyroid cancer. Curr Oncol 2020;27(2):e93–9. https://doi.org/10.3747/co.27.5533.
[22] Bravo A, Piñero J, Queralt-Rosinach N, Rautschka M, Furlong LI. Extraction of relations between genes and diseases from text and large-scale data analysis: implications for translational research. BMC Bioinf 2015;16:55. https://doi.org/10.1186/s12859-015-0472-9.
[23] À Bravo, Li TS, Su AI, et al. Combining machine learning, crowdsourcing and expert knowledge to detect chemical-induced diseases in text. Database 2016;2016:baw094. https://doi.org/10.1093/database/baw094.
[24] Ljubic B, Pavlovski M, Alshehri J, et al. Comorbidity network analysis and genetics of colorectal cancer. In: Informatics in Medicine Unlocked. 21; 2020. p. 100492. https://doi.org/10.1016/j.imu.2020.100492.
[25] Wu Y, Luo R, Leung HC, et al. Renet: a deep learning approach for extracting gene-disease associations from literature. In: International Conference on Research in Computational Molecular Biology; 2019 May 5. p. 272–84 [Springer, Cham].
[26] Kordopati V, Salhi A, Razali R, et al. DES-mutation: system for exploring links of mutations and diseases. Sci Rep 2018;8. https://doi.org/10.1038/s41598-018-31439-w. 1, article 13359.
[27] Pozdeyev N, Gay LM, Sokol ES, et al. Genetic analysis of 779 advanced differentiated and anaplastic thyroid cancers. Clin Canc Res 2018;24(13):3059–68. https://doi.org/10.1158/1078-0432.CCR-18-0373.
[28] Pringle DR, Yin Z, Lee AA, et al. Thyroid-specific ablation of the Carney complex gene, PRKAR1A, results in hyperthyroidism and follicular thyroid cancer. Endocr Relat Canc 2012;19(3):435–46. https://doi.org/10.1530/ERC-11-0306.
[29] Brose MS, Cabanillas ME, Cohen EE, et al. Vemurafenib in patients with BRAF (V600E)-positive metastatic or unresectable papillary thyroid cancer refractory to radioactive iodine: a non-randomized, multicentre, open-label, phase 2 trial. Lancet Oncol 2016;17(9):1272–82. https://doi.org/10.1016/S1470-2045(16)30166-8.
[30] Carr LL, Mankoff DA, Goulart BH, et al. Phase II study of daily sunitinib in FDG-PET-positive, iodine-refractory differentiated thyroid cancer and metastatic medullary carcinoma of the thyroid with functional imaging correlation. Clin Canc Res 2010;16(21):5260–8. https://doi.org/10.1158/1078-0432.CCR-10-0994.
[31] Capp C, Wajner SM, Siqueira DR, et al. Increased expression of vascular endothelial growth factor and its receptors, VEGFR-1 and VEGFR-2, in medullary thyroid carcinoma. Thyroid 2010;20(8):863–71. https://doi.org/10.1089/thy.2009.0417.