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Evaluation of the genetic alterations landscape of differentiated thyroid cancer in children

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Abstract: Studies investigating the underlying genetic profile in pediatric patients diagnosed with differentiated thyroid cancer (DTC) are quite limited. This study aimed to identify genetic alterations in patients diagnosed with DTC. A total of 20 patients with confirmed DTC were included in the study; however, five were excluded due to the unavailability of thyroid tumor samples. Somatic mutation analysis of the DICER1, BRAF, KRAS, NRAS, and HRAS genes was performed using next-generation sequencing. In addition, gene fusions involving ALK, RET, PTC, ETV6, and NTRK3 were assessed using fluorescence *in situ* hybridization. A family screening was also conducted, and thyroid ultrasonography was performed. Fine-needle aspiration biopsy was carried out for family members when deemed necessary. The mean age at diagnosis was 14.42 ± 2.88 years. Somatic mutations were identified in nine patients (60 %). The detected mutations were as follows: RET/PTC fusion (n=3), BRAF V600E (n=2), NRAS Q61R (n=1), NRAS A59D (n=1), DICER1 E1705K (n=1), and ETV6/NTRK3 fusion (n=1). No statistically significant differences in prognostic factors were observed between patients with and without somatic mutations. As part of the family screening, suspicious thyroid nodules were detected in four parents. One parent underwent hemithyroidectomy, and final pathology revealed papillary thyroid carcinoma. Considering the limited number of similar studies on childhood thyroid cancer, these findings provide a valuable contribution to the existing literature. In particular, the results obtained from family screening may support advancements in early diagnosis, risk stratification, and the development of targeted therapeutic approaches.

Keywords: pediatric differentiated thyroid cancer; NGS; fusion-oncogenes; BRAF; RET-PTC

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Introduction

Thyroid cancer is rare in childhood, accounting for 1.5–3% of cancers diagnosed in children and adolescents [1]. However, according to data from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program, its incidence in individuals under the age of 20 has been increasing by 2 % annually [2, 3].

Differentiated thyroid cancers (DTCs), including papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC), account for 95 % of thyroid cancers in children and adolescents. Among DTCs, 90 % of cases are attributed PTC [4, 5]. Other histological subtypes, such as medullary thyroid cancer, poorly DTC, and anaplastic thyroid cancer, are rarer in childhood [6].

There is evidence suggesting an increasing incidence of DTC in children. Researchers believe that this dramatic rise

is likely due to both an actual increase in thyroid cancer incidence and advancements in diagnostic techniques. According to the SEER report, the incidence of PTC has increased, while no significant change has been observed in the incidence of FTC [2, 3].

In childhood, DTC tends to present with larger tumor size, more extensive regional and nodal spread, and a higher likelihood of distant metastasis compared to adulthood [7, 8]. The prevalence of distant metastasis in children is 20–30 %, whereas in adults, it is significantly lower, ranging from 2 to 5% [9, 10].

The identification of various genetic mutations in children and adolescents has been reported to aid in diagnosis, prognosis determination, and treatment planning [11, 12]. These mutations are predominantly found in the mitogen-activated protein kinase (MAPK) signaling pathway [13]. Studies have shown that the most common mutations in DTC involve BRAF, RAS, RET/PTC, and neurotrophic tyrosine kinase (NTRK) genes [14]. However, only a small proportion of patients included in these studies are under the age of 18, and studies specifically focusing on the pediatric and adolescent population are quite rare. With this study, we aimed to identify the genetic alterations responsible for the development of DTC in children and adolescents.

Materials and methods

Patient selection and review

This study included 20 patients under the age of 18 who underwent thyroidectomy between January 2014 and April 2022 and agreed to participate. Thyroid tumor samples were histologically evaluated. Five patients with insufficient tumor samples were excluded from the study.

During the diagnostic and classification process, the cytological evaluation of patients who underwent fine-needle aspiration biopsy (FNAB) was performed according to the Bethesda system [15]. The pathological diagnosis of DTC was classified based on the World Health Organization (WHO) classification of thyroid carcinoma [16]. Tumor risk stratification was conducted according to the American Thyroid Association (ATA) criteria [17].

The DICER1, BRAF, KRAS, NRAS, and HRAS genes were analyzed using the next-generation sequencing (NGS) method, while the ALK, RET, ETV6, PTC, and NTRK3 genes were analyzed using the fluorescence *in situ* hybridization (FISH) method.

NGS analysis method

Pre-analysis preparation

The DICER1, BRAF, KRAS, NRAS, and HRAS genes included in the DHS-005Z Human Lung Cancer Panel (Qiagen) were analyzed using the NGS method. From the patient's FFPE (Formalin-Fixed, Paraffin-Embedded) tissues, four to five sections of 10 μm thickness were obtained, and DNA isolation was performed using the QIAGEN QIAamp DNA FFPE Advanced Kit. DNA concentration measurements were conducted using the Qubit™ dsDNA HS Assay Kit with the Qubit 4.0 (Thermo Fisher Scientific) device.

Library construction

The initial DNA concentration for library preparation was calculated to be between 40 and 250 ng. Library products were generated following the instructions of the Qiaseq Targeted DNA Custom Panel (Qiagen) kit. In this process, DNA isolated from FFPE samples was sequentially processed into fragmented DNA, followed by the addition of a molecular barcode and primary sample barcode primer, a gene-specific primer with a forward primer, and a universal primer with a secondary sample barcode primer. The quality of the library products was assessed using the QIAGEN Qiaxcel DNA High Resolution Kit, and quantification was performed using the Qubit™ dsDNA HS Assay Kit.

Sequencing

After quantification of the DNA library products, the appropriate concentrations for each library were calculated according to the sequencing device guidelines. The DNA libraries were then pooled into a single library pool. Sequencing was performed using the Illumina NextSeq550 system.

Data analysis

Following NGS, raw data analysis and variant interpretation steps were performed. The bioinformatic analysis of raw data was conducted using a combination of QIAGEN CLC Genomics Workbench and QIAGEN Clinical Insight Interpret software. Detected variants were interpreted, and clinically significant genetic alterations were listed in the report.

The analysis of somatic data was carried out based on the following criteria: variant read depth (>50×), allele

fraction (>1 %), forward-reverse read balance, read quality (>Q20), presence in homopolymer regions (single nucleotide repeat regions), and minor allele frequency (<1 %). Variants meeting these criteria were classified according to ACMG/AMP guidelines as follows:

- If a variant was associated with an FDA-approved therapy (Level A) or a clinically relevant research-based therapy (Level B) with an impact on treatment response (sensitivity, resistance, or toxicity) and/or prognosis, it was classified as Tier IA/B.
- If a variant was linked to an FDA-approved therapy in a different tumor type, eligibility for clinical trials (Level C), or preclinical study findings without consensus (Level D), it was categorized as Tier IIC/D.
- Variants classified as variants of uncertain significance (VUS), either reported in the literature for the same or different cancer types or not associated with any cancer, were only reported if they were clinically relevant (Tier III). Clinically irrelevant Tier III variants were not included in the report.
- Variants with a minor allele frequency above 1 % in population databases that were not considered common pathogenic variants were classified as Tier IV (benign) and therefore excluded from the report.

For the biological and clinical interpretation of detected variants, the QIAGEN Clinical Insight Interpret 5.6.20200117 software was used. To exclude the possibility of false positives, the NGS analysis was repeated twice for each case.

FISH method

FISH analysis was performed using the ALK (Spec ALK Dual Color Break Apart Probe, Zytovision), RET (Spec RET Dual Color Break Apart Probe, Zytovision), PTC (Spec PTC Dual Color Break Apart Probe, Zytovision), ETV6 (Spec ETV6 Dual Color Break Apart Probe, Zytovision), and NTRK3 (Spec NTRK3 Dual Color Break Apart Probe, Zytovision) probes according to the kit protocol. Tissue sections were pre-processed for FISH using the FISH-Tissue Implementation Kit (Zytolight, Zytovision).

Kit protocol

Sections of 5 µm thickness were obtained from selected FFPE blocks of the cases and mounted onto positively charged slides. The slides were incubated in an oven at 56 °C for 45 min.

- **Deparaffinization:** The sections were incubated in two series of xylene for 20 min. This was followed by sequential immersion in 100 , 85, and 70 % ethanol, each for 2 min, to complete the deparaffinization process.
- **Boiling:** The deparaffinized sections were boiled in preheated citrate solution (98 °C) for 45 min. At the end of the incubation, the sections were washed with distilled water and air-dried.
- **Pepsin treatment:** Once air-dried, a sufficient amount of pepsin solution was applied to the sections. The slides were incubated in a Thermobright device at 37 °C for 20 min. After incubation, the sections were washed in 2× SSC solution and distilled water for 5 min each. They were then sequentially dehydrated in 70 , 85, and 100 % ethanol, each for 2 min, and air-dried.
- **Hybridization:** Each section was treated with individual probes, covered with a coverslip, and sealed with rubber cement to prevent air exposure. The slides were then incubated in a Thermobright device at 75 °C for 10 min for denaturation, followed by overnight hybridization at 37 °C.
- **Post-hybridization washes:** The next day, the sections were washed in three series of preheated wash solutions at 37 °C. After washing, the slides were sequentially dehydrated in 70 , 85, and 100 % ethanol, each for 2 min, and air-dried.
- **Fluorescent staining and analysis:** The preparations were counterstained with DAPI solution and incubated at 4 °C for 20 min. Fluorescence microscopy analysis was then performed, evaluating 100 tumor cells per probe.

Family screening

The parents and, if possible, siblings of the patients included in the study were contacted and enrolled after obtaining their informed consent. Each individual was evaluated by the same endocrinology specialist. Thyroid function tests, thyroid autoantibodies, and thyroid ultrasound (USG) were performed. FNAB was conducted for patients when deemed necessary.

Ethics

Informed consent was obtained from all participants or their legal guardians before inclusion in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki. The study was authorized by the ethics committee of our university (approval date: 03/16/2022, approval no: KAEK-133).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics v23.0 (IBM Corp., Armonk, NY, USA). Categorical variables were presented as frequency (n) and percentage (%). The distribution of the data was assessed using the Kolmogorov–Smirnov (K–S) test. Normally distributed data were expressed as mean ± standard deviation (SD) and compared using the Student's *t*-test. Categorical variables were analyzed using the Chi-square test or Fisher's exact test, as appropriate. A p-value of <0.05 was considered statistically significant.

Results

Patient characteristics tumor types, presentation, and initial therapy

A total of 20 patients were included in the study; however, five patients were excluded due to the unavailability of their thyroid tumor samples. The genetic alterations of 15 patients were analyzed. The mean age at diagnosis was 14.42 ± 2.88 years. Eleven patients (73.3 %) were female, while four patients (26.7 %) were male. Hashimoto's thyroiditis was identified in three patients (20 %), and Graves' disease was detected in one patient (6.7 %). Overall, autoimmune thyroiditis was present in four patients (26.7 %). Follicular thyroid carcinoma was diagnosed in two patients (13.3 %), whereas PTC was identified in 13 patients (86.7 %). According to the ATA risk stratification, four patients were classified as low-risk, seven as intermediate-risk, and four as high-risk. The diagnoses, surgical procedures performed, TNM staging, ATA classification, and radioactive iodine (RAI) therapy status of the patients are presented in Table 1.

Molecular genetic analysis, risk stratification (ATA risk classification, TNM staging)

RET fusion was identified in three patients and ETV6/NTRK3 fusion in one patient by FISH analysis. BRAF V600E mutation in two patients, NRAS Q61R mutation in one patient, NRAS A59D mutation in one patient, and DICER E1705K mutation in one patient were detected by NGS analysis. The NGS analysis was repeated twice, yielding consistent results. The allele frequencies of these variants in the respective patients were 31 , 20, 11, 7.7, and 13.5 % (Table 1, Figure 1).

Among the three patients with RET fusion, two underwent hemithyroidectomy, while one underwent total thyroidectomy followed by completion surgery. Two of these patients were classified as low risk according to the ATA risk stratification and had no lymph node metastases, whereas one was classified as high risk with distant lymph node metastases.

Both patients with the BRAF V600E mutation were in the ATA intermediate-risk group and had regional lymph node metastases. Total thyroidectomy was performed in both cases.

The patient with the NRAS Q61R mutation was diagnosed with follicular thyroid carcinoma, classified as ATA intermediate risk, and had regional lymph node involvement.

The patient with the NRAS A59D mutation was classified as high risk, with a tumor stage of 2 and distant lymph node metastases.

The patient with the DICER E1705K mutation was classified as intermediate risk and had distant lymph node metastases.

The patient with the ETV6/NTRK3 fusion was diagnosed at the age of 8. At the time of diagnosis, the tumor was classified as stage 4a, with extensive invasion of neck tissues, laryngeal nerve invasion, and distant lung metastases. Due to laryngeal nerve involvement, surgery was performed in two stages, followed by RAI therapy. No relapse was detected during a three-year follow-up period after diagnosis.

Patient number six had a history of radiotherapy for a previous diagnosis of Ewing sarcoma; however, no mutations were identified in this patient. Due to the limited sample size, statistical analysis to determine associations between genetic alterations and different tumor types could not be performed. The detected mutations, diagnoses, and treatment approaches of the patients are summarized in Table 1. No ALK, KRAS, or HRAS mutations were identified in any patient.

Comparison between tumors with a mutation or fusion versus none

Mutations were identified in nine patients (60 %), whereas no mutations were detected in six patients (40 %). The mean age of patients with mutations was 13.88 ± 3.11 years, while the mean age of those without mutations was 15.23 ± 2.55 years. Among patients with detected mutations, 66.7 % were female and 33.3 % were male. The initial surgical treatment for patients with mutations was total thyroidectomy in 77.8 % of cases, whereas for those without mutations, total thyroidectomy was performed in 33.3 % of cases. N1b nodal invasion was identified in 44.4 % of patients with mutations, compared to 16.7 % of those without mutations. Among patients with mutations, 33.3 % were classified as high risk according to the ATA risk stratification, and 66.7 % received RAI therapy. In contrast, among patients without mutations, 16.7 % were classified as high risk, and 50 % received RAI therapy. No statistically significant difference was observed

Table 1: Patients' diagnosis, treatment, staging, and genetic alterations.

Patient	Mutation/ fusion	Allele frequencies	Thyroid carcinoma types	Thyroidectomy procedure	TNM staging	ATA risk classification	RAİ	Autoimmune thyroiditis
1	–	–	PTC	H & C	T2N1bM0	High	+	–
2	RET/PTC	–	PTC	Hemi	T1aNxM0	Low	–	HT
3	RET/PTC	–	PTC	Hemi	T1aN0aM0	Low	–	HT
4	RET/PTC	–	PTC	TTX & C	T1bN1bM0	High	+	–
5	–	–	PTC	H & C	T3aN1aM0	Intermediate	+	–
6	–	–	PTC	TTX	T1bN1aM0	Intermediate	+	–
7	DICER: E1705K	31 %	PTC	TTX & C	T1bN1bM0	Intermediate	+	–
8	NRAS: Q61R	20 %	FTC	TTX	T1aN1aM0	Intermediate	+	GD
9	BRAF: V600E	11 %	PTC	TTX	T1bN1aM0	Intermediate	+	–
10	BRAF: V600E	7.7 %	PTC	TTX	T1bN1aM0	Intermediate	+	–
11	–	–	FTC	H & C	T1aN0aM0	Low	–	–
12	ETV6/NTRK3	–	PTC	TTX	T4aN1bM1	High	+	–
13	NRAS: A59D	13.5 %	PTC	TTX	T2N1bM0	High	+	–
14	–	–	PTC	TTX	T1aN1aM0	Intermediate	+	HT
15	–	–	PTC	Hemi	T1bNxM0	Low	–	–

PTC, papillary thyroid carcinoma; FTC, follicular thyroid carcinoma; Hemi, hemithyroidectomy; H & C, hemithyroidectomy and completion thyroidectomy; TTX, total thyroidectomy; M1, distant metastases; HT, Hashimoto thyroiditis; GD, Graves disease; ATA, American Thyroid Association.

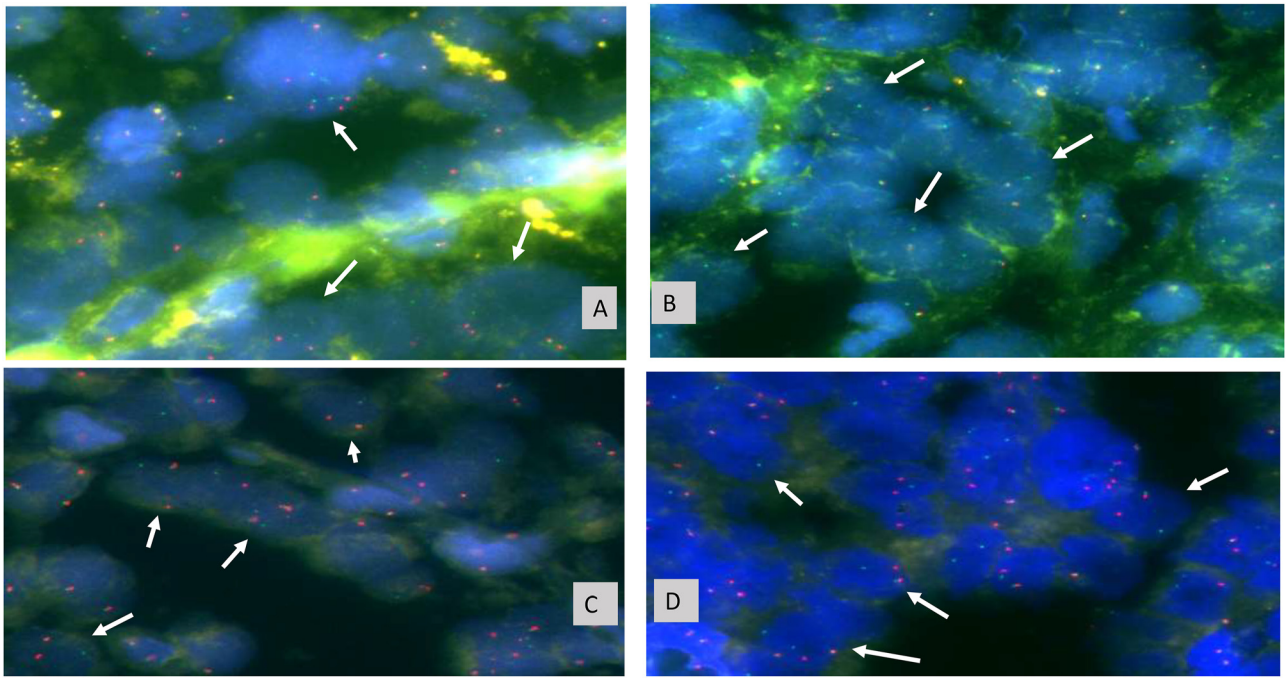


Figure 1: FISH analyses demonstrating rearrangements involving the RET, ETV6, and NTRK3 genes using break-apart probes (ZytoVision, Bremerhaven, Germany). (A) RET break-apart probe showing a classical rearrangement pattern with one fusion (yellow) signal and single red and green signals, indicating separation of 5′ and 3′ regions. (B) RET break-apart probe showing an atypical pattern with one fusion signal and a single green signal, consistent with 3′ (red) signal deletion. (C) ETV6 break-apart probe showing a classical rearrangement pattern with one fusion signal, a single red signal (3′ end), and a single green signal (5′ end). (D) NTRK3 break-apart probe showing a classical rearrangement pattern with one fusion signal, a single red signal (3′ end), and a single green signal (5′ end).

Table 2: Comparison between tumors with a mutation or fusion versus none.

	Mutation/fusion		Overall	p-Value
	Yes (n=9/60 %)	No (n=6/40 %)	n=15	
Age at diagnosis (SD)	13.88 ± 3.11	15.23 ± 2.55	14.42 ± 2.88	0.39[a]
Gender				0.46[b]
Female, n (%)	6 (66.7 %)	5(83.3 %)	11 (73.3 %)	
Male, n (%)	3 (33.3 %)	1 (16.7 %)	4 (26.7 %)	
Initial surgery				0.11[b]
Hemithyroidectomy	2 (22.2 %)	4 (66.7 %)	6 (40 %)	
Total thyroidectomy	7 (77.8 %)	2 (33.3 %)	9 (60 %)	
Tumor type				0.65[b]
PTC	8 (88.9 %)	5 (83.3 %)	13 (86.7 %)	
FTC	1 (11.1 %)	1 (16.7 %)	2 (13.3 %)	
Tumor (T)				0.89[b]
T1	7 (77.8 %)	4 (66.7 %)	11 (73.3 %)	
T2	1 (11.1 %)	1 (16.7 %)	2 (13.3 %)	
T3 + T4	1 (11.1 %)	1 (16.7 %)	2 (13.3 %)	
Nodal spread (N)				0.53[b]
None (0/X)	2 (22.2 %)	2 (33.3 %)	4 (26.7 %)	
N1a	3 (33.3 %)	3 (50.0 %)	6 (40.0 %)	
N1b	4 (44.4 %)	1 (16.7 %)	5 (33.3 %)	
Other thyroid disease				0.46[b]
Hashimoto/Graves	3 (33.3 %)	1 (16.7 %)	4 (26.7 %)	
None	6 (66.7 %)	5 (83.3 %)	11 (73.3 %)	
ATA risk classification				0.75[b]
Low	2 (22.2 %)	2 (33.3 %)	4 (26.7 %)	
Intermediate	4 (44.4 %)	3 (50.0 %)	7 (46.7 %)	
High	3 (33.3 %)	1 (16.7 %)	4 (26./%)	
RAI				0.45[b]
Yes	6 (66.7 %)	3 (50 %)	9 (60 %)	
No	3 (33.3 %)	3 (50 %)	6 (30 %)	

[a]*t*-test, [b]Chi-square test or Fisher's exact test. Except for the age of diagnosis in years, expressed as an average (SD), all other parameters are the number of cases (%). PTC, papillary thyroid carcinoma; FTC, follicular thyroid carcinoma; ATA, American Thyroid Association.

between the groups with and without mutations. The general characteristics, diagnoses, and treatment approaches of patients with and without detected mutations are summarized in Table 2.

Molecular genetic analysis and family screening

The parents and, when possible, the siblings of the patients undergoing genetic alteration analysis were evaluated by an endocrinology and metabolism specialist, and thyroid ultrasonography (USG) was performed. Hashimoto's thyroiditis was detected in six patients' mothers and one patient's father (23.3 %). In the family screening, suspicious thyroid nodules were identified in two patients' mothers and two patients' fathers. One parent declined FNAB. Among the family members who underwent FNAB, one was diagnosed with a benign lesion, while another had an acellular specimen and is currently under follow-up. In one family member, the initial biopsy revealed atypia of undetermined significance (AUS), while a second biopsy indicated a follicular lesion of undetermined significance (FLUS). This individual subsequently underwent hemithyroidectomy, and the final pathology confirmed papillary thyroid carcinoma. No mutations were detected in this patient's son.

The father of the patient with the ETV6/NTRK3 fusion and widespread metastases at diagnosis was found to have multiple hypoechoic nodules on thyroid USG. Two FNAB procedures performed on this family member revealed a colloidal nodule and an acellular specimen. The patient's follow-up is ongoing.

Due to the limited number of cases, statistical correlation between the detected mutations in patients and the findings from family screening could not be established. The characteristics and findings of family members are presented in Table 3.

Table 3: Parental screening results.

	Mother							Father				
Patient	**Mutation/ fusion**	**Hypothyroidism/ hyperthyroidism**	**Autoimmune thyroiditis**	**Elevated thyroglobulin**	**Thyroid USG**	**İİAB**	**Thyroidectomy procedure/thyroid carcinoma types**	**Hypothyroidism/ hyperthyroidism**	**Autoimmune thyroiditis**	**Elevated thyroglobulin**	**Thyroid USG**	**İİAB**
1	–	–	HT	–	Hashimoto thyroiditis	N/E		–	–	–	N	N/E
2	RET/PTC	–	–	–	N	N/E		–	–	–	N	N/E
3	RET/PTC	–	–	–	N	N/E		–	–	–	N	N/E
4	RET/PTC	–	–	–	N	N/E		–	–	–	N	N/E
5	–	–	–	–	Right lobe 13 × 11 × 10 mm, well-defined margins, hypoechoic nodule (+)	1-Atypia of undetermined significance 2-Follicular lesion of undetermined significance	Hemi/PTC	–	–	–	N	N/E
6	–	–	–	–	N	N/E		–	–	–	N	N/E
7	DICER: E1705K	–	–	–	N	N/E		–	–	–	N	N/E
8	NRAS: Q61R	–	HT	–	N	N/E		–	HT	–	Right lobe 20 × 16 × 17 mm hypoechoic nodul	Benign
9	BRAF: V600E	–	–	–	Right lobe, 10 × 5.5 mm hypoechoic nodule (+)	Did not give approval		–	–	–	N	N/E
10	BRAF: V600E	–	HT	–	Hashimoto thyroiditis	N/E		–	–	–	N	N/E
11	–	–	HT	–	Hashimoto thyroiditis	N/E		–	–	–	N	N/E
12	ETV6/ NTRK3	–	HT	–	Hashimoto thyroiditis	N/E		–	–	+	Right lobe 8 × 13 × 12 mm, left lobe 9 × 7 × 9 mm, multiple hypoechoic nodules with solid and cystic areas	1-Colloid nodule/ 2acellular specimen
13	NRAS: A59D	–	–	–	N	N/E		–	–	–	N	N/E
14	–	–	HT	–	Hashimoto thyroiditis	N/E		–	–	–	N	N/E
15	–	–	–	–	N	N/E		–	–	–	N	N/E

N/E, not evaluated; N, normal; PTC, papillary thyroid carcinom; Hemi, hemithyroidectomy; HT, Hashimoto thyroiditis.

Discussion

The prevalence of genetic alterations in DTC among children and adolescents remains controversial, and their role as prognostic factors is still unclear. This uncertainty arises from the small sample sizes of pediatric cohorts and the heterogeneity of the studied populations. Through this study, we contribute to a field where significant knowledge gaps persist.

In our study, we detected genetic mutations in 60 % of cases. Newfield et al. [18] identified genetic mutations in 82.8 % of cases in their study utilizing NGS analysis. Other pediatric studies have reported mutation detection rates ranging from 69.7 to 96.3 % [19–21]. In our study, the mean age at diagnosis was 14.42 ± 2.88 years. However, the mean age of patients with detected mutations was 13.88 ± 3.11 years, whereas for those without mutations, it was 15.23 ± 2.55 years. Previous studies have also reported similar mean ages at diagnosis (14.9 and 14.5 years) [20, 21].

The most commonly reported mutation in pediatric DTC is the RET/PTC fusion. Various studies have reported its prevalence ranging from 15 to 77 % [22–24]. In our study, RET/PTC fusion was also the most frequently detected genetic alteration, identified in three patients (20 %). Among these, two patients were classified as low risk and one as high risk according to the ATA risk stratification. Previous studies evaluating its role as a prognostic factor have not demonstrated a significant association [24–26].

The second most frequently detected mutation in children and adolescents is BRAF V600E, accounting for 28 % of cases [27–29]. In our study, BRAF V600E was identified in two patients (13.3 %). While some studies have found no association between BRAF V600E mutation and prognosis, Alzahrani et al. [30] reported higher rates of persistence and recurrence in patients harboring this mutation. Additionally, Franko et al. [31] demonstrated an increased frequency of metastasis to the lateral cervical lymph nodes. In our study, both patients with BRAF V600E had local lymph node involvement and were classified as ATA intermediate-risk. Following total thyroidectomy and RAI therapy, no recurrence was observed.

Mutations in RAS genes can occur in HRAS, KRAS, or NRAS proteins. Compared to the adult population, these mutations are rarer in pediatric cases, and no association with prognosis has been reported [24, 26]. In our cohort, NRAS mutations were identified in two patients (13.3 %). The patient with the NRAS A59D mutation was classified as ATA high-risk, had a tumor stage of 2, and presented with distant lymph node metastases. In contrast to our findings, previous studies have reported that adult patients with RAS mutations rarely present with lymph node or distant metastases [32]. Similarly, Franko et al. [31] observed less metastatic behavior in pediatric patients with RAS-mutant tumors. However, due to the low number of RAS-mutant cases in their cohort, they were unable to reach statistically significant conclusions.

The ETV6-NTRK3 fusion has recently gained attention among researchers. Its prevalence is high in pediatric and adolescent DTC, making it the third most frequently detected mutation [19, 26, 33]. In our study, a single patient with this fusion was diagnosed at 8 years of age. At the time of diagnosis, the tumor was classified as stage 4a, with extensive local invasion and distant metastases. However, the patient's prognosis was favorable with appropriate treatment. Consistent with previous studies, NTRK3 fusions have been associated with poor clinical features, widespread disease, and metastases [19, 23, 26, 33]. In a study comparing it to the BRAF mutation, ETV6-NTRK3 fusions were found to be associated with larger tumor sizes and increased lymphovascular invasion at presentation [19].

Previous studies have demonstrated that, compared to adult patients, fusion oncogenes are three times more prevalent in pediatric PTC [18, 34]. In general, RET fusions are the most commonly observed, followed by ETV6/NTRK3 fusions [19–21, 33, 35]. Consistent with the literature, we identified RET/PTC fusion in three patients and ETV6/NTRK3 fusion in one patient. Some studies have suggested an association between radiation exposure and an increased frequency of RET/PTC3 fusions [36]. In our cohort, one patient had a history of radiation exposure; however, no mutation was detected in this patient. ALK fusions have not been reported in previous studies on the pediatric population, and we similarly did not detect ALK mutations in our study.

Although some differences were observed between genetic anomalies or mutation types and tumor size as well as lymph node metastasis, no significant association was identified [19]. However, in a European cohort study, fusion oncogenes were associated with larger tumor size [20]. Larger cohorts are needed to draw more definitive conclusions on this subject. Similarly, in our cohort, no statistically significant difference was found between patients with and without genetic anomalies.

In our study, autoimmune thyroiditis was identified in 26.7 % of patients. According to United States (U.S.) data, the prevalence of autoimmune thyroiditis in adolescents in the general population is 4.8 % [37]. Newfield et al. [18] reported a prevalence of 43.5 % in their cohort and found that PTC was more frequently detected (75 %) in patients with Hashimoto's thyroiditis. Among our four patients with autoimmune thyroiditis, three had detectable genetic alterations (two with RET/PTC fusions and one with an NRAS Q61R mutation). In the same study, genetic alterations were detected in 100 % of patients with Hashimoto's thyroiditis (HT), compared to 61.5 % of those without HT. The distribution of HT varied depending on

the type of genetic alteration. Specifically, among patients with BRAFV600E mutations, 63.6 % had HT and 36.4 % did not. In those with PAX8-PPARγ rearrangements, 25 % had HT and 75 % did not. For RET-PTC fusions, 75 % of patients had HT and 25 % did not, while among patients with other fusion types, 60 % had HT and 40 % did not [18].

Familial thyroid cancers have been established as hereditary malignancies in previous epidemiological studies. These studies have demonstrated a significantly increased risk among affected individuals' family members [38]. However, no associated germline mutations or somatic mutations commonly detected in PTC (such as RET/PTC, BRAF, and RAS) have been identified [39]. In a study by Rios et al. [40] comparing the relatives of patients diagnosed with PTC to the healthy population, the incidence of thyroid cancer was reported as 5.5 % in the study group and 1.3 % in the control group. Additionally, 50 % of patients were found to have benign thyroid disease. To contribute to the existing literature on this topic, we conducted a family screening in our study. Suspicious thyroid nodules were detected in four parents, and further evaluation was planned. One of these parents was diagnosed with PTC and underwent hemithyroidectomy. However, no somatic mutations were detected in their child. Furthermore, in the parent of the patient with ETV6/NTRK3 fusion and advanced-stage disease, multiple hypoechoic nodules were identified. Although no definitive genetic mutation has yet been linked to familial PTC, we believe that family screening may be beneficial for early diagnosis. If genetic mutations are identified in the future, personalized and targeted diagnostic and therapeutic approaches can be implemented. Previous studies and systematic reviews have also supported this perspective [39, 40].

The limitations of our study include its retrospective design and the small cohort size. However, this single-center sample contributes valuable data to a field that requires extensive research and clarification. Due to the small sample size and the diversity of genetic anomalies, statistical analyses were limited. Another limitation is that DTC typically has an indolent course, and long-term follow-up is challenging, particularly as some patients transition to adult care. Consequently, it was not possible to establish an association between genetic anomalies and relapse rates.

Conclusions

In conclusion, this study provides important findings on the genetic alterations observed in pediatric and adolescent patients with DTC, with somatic mutations detected in 60 % of the cases. Although no significant associations were found between genetic mutations and prognostic factors in our study, the mutations identified, particularly in genes such as RET/PTC, BRAF, NRAS, and ETV6/NTRK3, may offer valuable insights into the molecular mechanisms underlying pediatric DTC. Additionally, the results from family screening, including the detection of suspicious thyroid nodules in some first-degree relatives and a case of familial papillary thyroid carcinoma, suggest a possible genetic predisposition and emphasize the importance of regular follow-up for at-risk family members. Larger, multi-center, and long-term follow-up studies are needed to fully elucidate the prognostic impact of these genetic mutations. Nevertheless, our study provides a foundation for future research and supports the potential role of genetic screening in early diagnosis, risk stratification, and the development of targeted therapeutic approaches for pediatric thyroid cancer.

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References

1. Enomoto Y, Enomoto K, Uchino S, Shibuya H, Watanabe S, Noguchi S. Clinical features, treatment, and long-term outcome of papillary thyroid cancer in children and adolescents without radiation exposure. World J Surg 2012;36:1241–6.
2. Holmes LJ, Hossain J, Opara F, Rakha A, Warenius HM. Pediatric thyroid carcinoma incidence and temporal trends in the USA (1973–2007): race or shifting diagnostic paradigm? Int Sch Res Not 2012; 2012:1–10.
3. Golpanian S, Perez EA, Tashiro J, Lew JI, Sola JE, Hogan AR. Pediatric papillary thyroid carcinoma: outcomes and survival predictors in 2504 surgical patients. Pediatr Surg Int 2016;32:201–8.
4. Vaisman F, Corbo R, Vaisman M. Thyroid carcinoma in children and adolescents – systematic review of the literature. J Thyroid Res 2011; 2011:845362.
5. Park S, Jeong JS, Ryu HR, Lee CR, Park JH, Kang SW, et al. Differentiated thyroid carcinoma of children and adolescents: 27-year experience in the Yonsei University Health System. J Kor Med Sci 2013;28:693–9.
6. Paulson VA, Rudzinski ER, Hawkins DS. Thyroid cancer in the pediatric population. Genes (Basel) 2019;10:723.
7. Mazzaferri EL, Kloos RT. Current approaches to primary therapy for papillary and follicular thyroid cancer. J Clin Endocrinol Metab 2001;86: 1447–63.
8. Sugg SL, Ezzat S, Rosen IB, Freeman JL, Asa SL. Distinct multiple RET/PTC gene rearrangements in multifocal papillary thyroid neoplasia. J Clin Endocrinol Metab 1998;83:4116–22.
9. Dinauer CA, Tuttle RM, Robie DK, McClellan DR, Svec RL, Adair C, et al. Clinical features associated with metastasis and recurrence of differentiated thyroid cancer in children, adolescents and young adults. Clin Endocrinol (Oxf) 1998;49:619–28.
10. Zaydfudim V, Feurer ID, Griffin MR, Phay JE. The impact of lymph node involvement on survival in patients with papillary and follicular thyroid carcinoma. Surgery 2008;144:1070–7.
11. Xing M, Alzahrani A, Carson K, Viola D, Elisei R, Bendlova B, et al. Association between BRAF V600E mutation and mortality in patients with papillary thyroid cancer. JAMA 2013;309:1493–501.
12. Tufano RP, Teixeira GV, Bishop J, Carson KA, Xing M. BRAF mutation in papillary thyroid cancer and its value in tailoring initial treatment: a systematic review and meta-analysis. Medicine (Baltim) 2012;91: 274–86.
13. Hsiao SJ, Nikiforov YE. Molecular approaches to thyroid cancer diagnosis. Endocr Relat Cancer 2014;21:T301–13.
14. Agrawal N, Akbani R, Aksoy BA, Ally A, Arachchi SL, Asa SL, et al. Integrated genomic characterization of papillary thyroid carcinoma. Cell 2014;159:676–90.
15. Cibas ES, Ali SZ. The 2017 Bethesda system for reporting thyroid cytopathology. Thyroid 2017;27:1341–6.
16. Lloyd RV, Osamura YR, Kloppel G, Rosai J. WHO classification of tumours of endocrine organs. Lyon: WHO Press; 2017.
17. Francis GL, Waguespack SG, Bauer AJ, Angelos P, Benvenga S, Cerutti JM, et al. Management guidelines for children with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association guidelines task force on pediatric thyroid. Thyroid 2015;25: 716–59.
18. Newfield RS, Jiang W, Cheng SM, Hantash FM, McPhaul MJ, Nikita ME, et al. Mutational analysis using next generation sequencing in pediatric thyroid cancer reveals BRAF and fusion oncogenes are common. Diagn Mol Pathol 2022;31:67–75.
19. Prasad ML, Vyas M, Horne MJ, Virk RK, Morotti R, Liu Z, et al. NTRK fusion oncogenes in pediatric papillary thyroid carcinoma in northeast United States. Cancer 2016;122:1097–107.
20. Alzahrani AS, Alswailem MM, Al-Hindi HN, Goljan E, Alsudairy N, Abouelhoda M, et al. Genetic alterations in pediatric thyroid cancer using a comprehensive childhood cancer gene panel. J Clin Endocrinol Metab 2020;105:3324–34.
21. Pekova B, Sykorova V, Dvorakova S, Vaclavikova E, Moravcova J, Katra R, et al. RET, NTRK, ALK, BRAF, and MET fusions in a large cohort of pediatric papillary thyroid carcinomas. Thyroid 2020;30:1771–80.
22. Sassolas G, Hafdi-Nejjari Z, Ferraro A, Decaussin-Petrucci M, Rousset B, Borson-Chazot F, et al. Oncogenic alterations in papillary thyroid cancers of young patients. Thyroid 2012;22:17–26.
23. Ricarte-Filho JC, Li S, Garcia-Rendueles MER, Montero-Conde C, Voza F, Knauf JA, et al. Identification of kinase fusion oncogenes in post-chernobyl radiation-induced thyroid cancers. J Clin Investig 2013;123: 4935–44.
24. Nikita ME, Jiang W, Cheng SM, Hantash FM, McPhaul MJ, Newbury RO, et al. Mutational analysis in pediatric thyroid cancer and correlations with age, ethnicity, and clinical presentation. Thyroid 2016;26:227–34.
25. Fenton CL, Lukes Y, Nicholson D, Dinauer CA, Francis GL, Tuttle RM. The ret/PTC mutations are common in sporadic papillary thyroid carcinoma of children and young adults. J Clin Endocrinol Metab 2000;85:1170–5.
26. Picarsic JL, Buryk MA, Ozolek J, Ranganathan S, Monaco SE, Simons JP, et al. Molecular characterization of sporadic pediatric thyroid carcinoma with the DNA/RNA ThyroSeq v2 next-generation sequencing assay. Dev Pathol 2016;19:115–22.
27. Geng J, Wang H, Liu Y, Tai J, Jin Y, Zhang J, et al. Correlation between BRAF V600E mutation and clinicopathological features in pediatric papillary thyroid carcinoma. Sci China Life Sci 2017;60:729–38.
28. Poyrazoğlu Ş, Bundak R, Baş F, Yeğen G, Şanlı Y, Darendeliler F. Clinicopathological characteristics of papillary thyroid cancer in children with emphasis on pubertal status and association with BRAFV600E mutation. J Clin Res Pediatr Endocrinol 2017;9:48–55.
29. Hardee S, Prasad ML, Hui P, Dinauer CA, Morotti RA. Pathologic characteristics, natural history, and prognostic implications of BRAFV600E mutation in pediatric papillary thyroid carcinoma. Pediatr Dev Pathol 2017;20:206–12.
30. Alzahrani AS, Qasem E, Murugan AK, Al-Hindi HN, Alkhafaji D, Almohanna M, et al. Uncommon TERT promoter mutations in pediatric thyroid cancer. Thyroid 2016;26:235–41.
31. Franco AT, Ricarte-Filho JC, Isaza A, Jones Z, Jain N, Mostoufi-Moab S, et al. Fusion oncogenes are associated with increased metastatic capacity and persistent disease in pediatric thyroid cancers. J Clin Oncol 2022;40:1081–90.
32. Kakarmath S, Heller HT, Alexander CA, Cibas ES, Krane JF, Barletta JA, et al. Clinical, sonographic, and pathological characteristics of RAS-positive versus BRAF-positive thyroid carcinoma. J Clin Endocrinol Metab 2016;101:4938–44.
33. Cordioli MICV, Moraes L, Bastos AU, Besson P, Alves MTDS, Delcelo R, et al. Fusion oncogenes are the main genetic events found in sporadic papillary thyroid carcinomas from children. Thyroid 2017;27:182–8.
34. Vanden Borre P, Schrock AB, Anderson PM, Morris JC 3rd, Heilmann AM, Holmes O, et al. Pediatric, adolescent, and young adult thyroid carcinoma harbors frequent and diverse targetable genomic alterations, including kinase fusions. Oncologist 2017;22:255–63.
35. Pekova B, Dvorakova S, Sykorova V, Vacinova G, Vaclavikova E, Moravcova J, et al. Somatic genetic alterations in a large cohort of pediatric thyroid nodules. Endocr Connect 2019;8:1552–63.

36. Thomas GA, Bunnell H, Cook HA, Williams ED, Nerovnya A, Cherstvoy ED, et al. High prevalence of RET/PTC rearrangements in Ukrainian and Belarusian post-chernobyl thyroid papillary carcinomas: a strong correlation between RET. J Clin Endocrinol Metab 1999;84: 4232–8.
37. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 2002;87: 489–99.
38. Frich L, Glattre E, Akslen LA. Familial occurrence of nonmedullary thyroid cancer: a population-based study of 5673 first-degree relatives of thyroid cancer patients from Norway. Cancer Epidemiol Biomarkers Prev 2001;10:113–7.
39. Capezzone M, Robenshtok E, Cantara S, Castagna MG. Familial non-medullary thyroid cancer: a critical review. J Endocrinol Investig 2021; 44:943–50.
40. Ríos A, Rodríguez JM, Navas D, Cepero A, Torregrosa NM, Balsalobre MD, et al. Family screening in familial papillary carcinoma: the early detection of thyroid disease. Ann Surg Oncol 2016;23:2564–70.