This chapter describes the genetic characterization of 10 DICER1-related thyroid carcinomas and report on follow-up of affected individuals.

Clinical and Molecular Characteristics May Alter Treatment Strategies of Thyroid Malignancies in DICER1-syndrome

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ABSTRACT

Context

DICER1 syndrome is a rare autosomal-dominantly inherited disorder that predisposes to a variety of cancerous and noncancerous tumors of mostly pediatric and adolescent onset, including differentiated thyroid carcinoma (DTC). DTC has been hypothesized to arise secondarily to the increased prevalence of thyroid hyperplastic nodules in syndromic patients.

Objective

To determine somatic alterations in DICER1-associated DTC and to study patient outcomes.

Design

Retrospective series.

Setting

Tertiary referral centers.

Patients

Ten patients with germline pathogenic *DICER1* variants and early-onset DTC.

Methods

Somatic *DICER1* mutation analysis, extensive somatic DNA variant and gene fusion analyses were performed on all tumors.

Results

Median age at DTC diagnosis was 13.5 years and there was no recurrent or metastatic disease (median follow-up, 8 years). All thyroid specimens showed diffuse nodular hyperplasia with at least one focus suspicious of DTC but without infiltrative growth, extrathyroidal extension, vascular invasion, or lymph node metastasis. Most of the individual nodules (benign and malignant) sampled from the 10 tumors harbored distinct *DICER1* RNase IIIb hotspot mutations, indicating a polyclonal composition of each tumor. Furthermore, nine of 10 DICER1-related DTCs lacked wellknown oncogenic driver DNA variants and gene rearrangements.

Conclusion

On the basis of our clinical, histological, and molecular data, we consider that most DICER1-related DTCs form a low-risk subgroup. These tumors may arise within one of multiple benign monoclonal nodules; thus, hemi-thyroidectomy or, more likely, total thyroidectomy may often be required. However, radioiodine treatment may be unnecessary given the patients' ages and the tumors' low propensity for metastases. (*J Clin Endocrinol Metab 104: 277–284, 2019*)

INTRODUCTION

DICER1 syndrome is a rare autosomal-dominantly inherited disorder that predisposes to a variety of cancerous and noncancerous tumors of mostly pediatric and adolescent onset.[1] The *DICER1* gene encodes a ribonuclease III enzyme involved in cleaving noncoding small RNA precursors to generate mature miRNAs, which in turn, posttranscriptionally regulate expression of many genes.[2]

Pleuropulmonary blastoma (PPB; a rare pediatric lung tumor), cystic nephroma, and ovarian Sertoli-Leydig cell tumor are the hallmark tumors of DICER1 syndrome. The broad tumor spectrum includes rare entities such as botryoid embryonal rhabdomyosarcoma of the uterine cervix, ciliary body medulloepithelioma, pineoblastoma, pituitary blastoma, and nasal chondromesenchymal hamartoma.[3] Furthermore, patients with DICER1 syndrome are at increased risk of developing multinodular goiter (MNG) compared with family controls and differentiated thyroid cancer (DTC) compared with population data from the National Cancer Institute SEER program.[4] It is possible that the increased risk of thyroid malignancy in *DICER1* heterozygotes is secondary to the greatly increased prevalence of benign hyperplastic thyroid nodules (i.e., MNG) in this syndrome. Alterations in *DICER1* are consistent with a two-hit tumor suppressor model, whereby a germline loss-of-function variant is followed by a second somatic mutation. However, in contrast to the typical two-hit model, in the case of *DICER1*, the second hit is most often a missense "hotspot" variant within the sequence encoding the RNase IIIb domain.[5] Studies have shown that somatic *DICER1* hotspot variants are present in benign and malignant thyroid nodules from patients with germline pathogenic *DICER1* variants[4,6,7], as well as those with sporadic adolescent-onset DTC.[8] Furthermore, different somatic *DICER1* variants may be present in distinct thyroid nodules resected from the same individual.[6]

In contrast to sporadic thyroid carcinomas in which point mutations (e.g., of *BRAF* and *RAS* genes), as well as gene fusions (e.g., *RET-PTC 1-12*, *PPARg-PAX8*, *ALK*, and *NTRK*), lead to tumorigenesis and progression through activation of the mitogen-activated protein kinase pathway [9-12], limited data are available on the acquired genetic alterations that induce malignant transformation of DICER1-associated MNG.[13] In this study, we performed genetic characterization of 10 DICER1-related thyroid carcinomas and report on follow-up of the affected persons.

PATIENTS AND METHODS

Study population and design

We studied 10 patients from eight families with germline pathogenic *DICER1* variants who had young-onset nodular thyroid hyperplasia containing at least one reported focus of DTC, diagnosed between 2004 and 2017. Clinical information, pathology reports, and details of medical history were collected from the treating physicians with full patient and/or parental consent. The study was approved by the local ethical committee of the Leiden University Medical Centre (approval no. P14.312).

Histological analysis

The tumors were reviewed by pathologists at the referring institutions and by our central reference pathologist (H.M.).

Molecular analysis

Total nucleic acid (i.e., undivided DNA and RNA) was isolated from formalin-fixed paraffin-embedded tissue cores (0.6-mm diameter and variable length) or microdissected tissue regions

using a fully automated extraction procedure.[14] Broad DNA variant and gene fusion analyses were performed using the following methods. Somatic *DICER1* variant analysis of the RNase IIIa and RNase IIIb domains was performed by conventional Sanger sequencing at either Radboud University Medical Centre or McGill University and Genome Quebec Innovation Centre (primers available on request). Somatic DNA variant analysis was performed using a customized next-generation sequencing AmpliSeq Cancer Hotspot Panel (Thermo Fisher Scientific, Waltham, MA) targeting 50 genes (including *BRAF*, *NRAS*, *HRAS*, *KRAS*, *TP53*, *PTEN*, and *PIK3CA*), as previously described.[15] *TERT* promotor variant (NM_ 198253.2; c.-57A.C, c.-124C.T and c.-146 C.T) analysis was performed by Sanger sequencing.

Gene fusion analysis was performed using the FusionPlex comprehensive thyroid and lung kit, version 2, for Ion Torrent (ArcherDX, Boulder, CO), which captures relevant exons from 34 genes (including *RET*, *NTRK1-3*, and *ALK*) according to the manufacturer's protocol. Data analysis was performed using the online Archer Analysis software, version 5.0 (analysis. archerdx.com). Only "strong-evidence" fusions called by the software were reported. This relatively new method was first validated on 56 formalin-fixed paraffin-embedded DTC samples (data not shown).

RESULTS

Clinical characteristics

In total, 10 patients (from eight different families) with DICER1-related thyroid carcinomas were included in this study. Details on six of these cases have been previously published (Table 1). [6,16-19] The mean age (±SD) at DTC diagnosis was 14.7± 6.2 years (range, 7 to 28 years), with a female predominance (70%). Median follow-up after thyroid cancer diagnosis was 8 years (range, 1 to 13 years). All patients in our series underwent total thyroidectomy and eight were treated with adjuvant radioactive iodine according to guidelines or expert opinion at the time. Six patients were diagnosed with at least one other DICER1-related tumor before the DTC diagnosis (Table 1).

Histological characteristics

Each of the 10 thyroid specimens showed diffuse nodular hyperplasia with multiple, discrete, well-circumscribed, and occasionally encapsulated nodules. In seven cases, at least one focus of follicular variant of papillary thyroid carcinoma (FVPTC) was considered during re-evaluation. The diagnosis of thyroid cancer was based primarily on nuclear features such as nuclear enlargement and overlap, irregularly shaped follicles, presence of nuclear clearance, and few mitotic figures. In three of these cases, the lesion was encapsulated or well demarcated without solid features. As such, the diagnosis of noninvasive follicular thyroid neoplasm with papillary-like nuclear features [20,21] was also considered. In the remaining four FVPTC samples (with no clear capsule or demarcation), dominant lesions in the context of hyperplasia was considered given the subtle nuclear characteristics. Poorly differentiated thyroid carcinoma was diagnosed in two patients (patients 4 and 6). No infiltrative growth, vascular invasion, extrathyroidal extension, or lymph node or distant metastasis were identified in any of the cases. On retrospective analyses, the histology of patient 8's right thyroid lobe lesion, which was resected 8 years earlier and which was classified and treated as benign (dominant lesions in the context of hyperplasia), had similar features to the presumed-malignant lesion from the left lobe (Fig. 1). An overview and detailed histology of all tumors is available in Supplemental Fig. 1 and full histological images are available at www.hereditarypathology.com upon request.

Molecular characteristics

We sampled between one and 11 regions from each of 10 thyroid specimens, totaling 35 regions (18 samples were classified as DTC and 17 were classified as hyperplastic nodules). Somatic *DICER1* variants were identified in 15 of 18 previously classified carcinoma samples and in 16 of 17 investigated benign nodules. We found a total of 11 distinct *DICER1* variants affecting five different residues within the RNase IIIb domain (namely, p.Glu1705, p.Asp1709, p. Glu1809, p.Glu1810, and p.Glu1813). Furthermore, loss of heterozygosity of the wild-type allele was present in both lesions from patient 4 who has a predisposing mosaic RNase IIIb hotspot mutation. In patient 8's tumor, we identified the same c.5438A.T somatic *DICER1* variant in the dominant lesion [classified as FVPTC (T1)] and in the surrounding hyperplasic lesion (L10). No additional known thyroid carcinoma diver DNA variants were found in the FVPTC (Fig. 1, II; Table 1).

Remarkably, in 14 of the 15 investigated carcinoma samples, neither common thyroid carcinoma driver DNA variants, nor gene rearrangements were identified. One pathogenic *TP53* variant was identified in a poorly DTC (patient 6). TERT promotor variants, associated with more aggressive carcinoma, were not present in the seven investigated tumors, including both poorly differentiated tumors.

DISCUSSION

In this study, we investigated the clinical, histological, and molecular characteristics of 10 thyroid tumors from young patients with germline/mosaic pathogenic *DICER1* variants. Somatic *DICER1* RNase IIIb hotspot variants were identified in most reported carcinomas and adjacent benign nodules. Secondary somatic *DICER1* variants were therefore not discriminative between benign and malignant disease. However, the identification of these distinct somatic variants in separate presumed-malignant nodules sampled from individual patients' lesions indicates that the tumors are polyclonal lesions, as has been seen in hyperplastic nodules. [4,6] Furthermore, nine of the 10 DICER1-related thyroid carcinomas lacked well-known oncogenic driver DNA variants (e.g,. *BRAF*, *RAS*) and gene rearrangements (e.g., *RET/PTC1-12*, *PPARg-PAX8*, *ALK*, and *NTRK*) that are frequently observed in sporadic thyroid carcinomas. Consistent with our findings, TERT promotor variants have been found to be rare in sporadic pediatric DTC (absent in all 77 tested cases). [22,23] In addition to these molecular findings, occasional ambiguous histological features and lack of extrathyroidal extension, infiltrative growth, vascular invasion, or lymph node or distant metastasis (at a mean follow-up of 8 years), may prompt reconsideration of the diagnosis of carcinoma in a subset of these DICER1-related tumors. Even if these tumors are classified as carcinomas, it appears their malignant potential is limited, and these data lead us to conclude that most DICER1-related DTCs form a low-risk subgroup. Whether this is also the case for DICER1-related poorly differentiated DTC should be determined.

Twelve independent studies (including the current study) have reported thyroid cancer in a total of 31 patients with germline pathogenic *DICER1* variants and/or DICER1 syndrome–related features (Supplemental Table 1). [1,4,7,16-18,24-28] As in previous studies, a subset of our patients (n = 3) had a history of extensive radiation as part of standard PPB diagnosis and treatment. We did not identify gene rearrangements in lesions from these patients despite such alterations being common in thyroid neoplasia from patients with a history of exposure to ionizing radiation through treatment or nuclear power plant accidents. [29,30] Furthermore, research has not suggested that DICER1-associated thyroid cancer is more invasive or less responsive to therapy. [4] On the contrary, recurrent or persistent disease has not been described in any patients reported to date, with a median follow-up of >5 years.

Table 1. The clinical, histological and molecular characteristics of ten *DICER1* mutation carriers with reported thyroid carcinoma

		Histology (macroscopic/microscopic)			Somatic molecular analysis		
ID	Sex / age at Dx DTC	Thyroid histology# (see suppl. Figure 1)	Multi-focal	Lesion (size, mm)	*DICER1*	Other DNA variant	Gene fusion
3	M/11	PTC	Y	T1	c.5113G>A, p.Glu1705Lys	ND* (no *BRAF/ RAS* variants in FusionPlex)	None identified
4	F/10	PDTC	Y	T1 (4mm)	LOH	None identified	None identified
				T2 (2mm)	LOH	None identified	None identified
5	F/15	FVPTC (or NIFTP)	N	T1 (17mm)	c.5437G>A, p.Glu1813Lys	None identified	None identified
6	F/14	PDTC	Y	T1 (5mm)	c.5437G>C, p.Glu1813Gln	*TP53:* c.1027_1033del 7bp, p.Glu343_ Asn345del fs	None identified
				L1 (12mm)	c.5437G>C, p.Glu1813Gln	ND	ND
7¥	F/23	FVPTC (or DHL)	Y	T1a (3mm)	c.5125G>A, p.Asp1709Asn	ND	ND
				T1b (18mm)	c.5125G>A, p.Asp1709Asn	None identified	ND
				T2 (20mm)	c.5126A>G, p.Asp1709Gly	ND	ND
				T3 (15mm)	c.5437G>A, p.Glu1813Lys	None identified	None identified
		DHL		L1 (20mm)	c.5438A>T, p.Glu1813Val	ND	ND
				L2 (1mm)	c.5126A>G, p.Asp1709Gly	ND	ND
				L3 (2mm)	c.5428G>T, p.Asp1810Tyr	ND	ND
				L4 (4mm)	c.5429A>T, p.Asp1810Val	ND	ND

	Clinical Information					
	hTERT	Personal history (age at Dx)	Follow up DTC	Family history	Germline *DICER1* variant	Reference
	ND	PPB type II (2y), CN (2y), Askin tumour (13y)	5y	PPB, CN, MNG, PitB	c.2379T>G, p.Tyr793*	de Kock *et al*. JCEM, 2014a (case 3) and ANP, 2014b (individual V-1)
	None identified ND	Bilateral renal and lung cysts (2y), Pineoblastoma (7y), bilateral SLCT (13y, 15y), CBME (17y)	12y	None	c.5437G>C, p.Glu1813Gln (mosaic)	de Kock *et al*, JMG 2016 (case 2)
	None identified	Lung cysts	2.5y	MNG	c.3999C>A, p.Cys1333*	Not previously published
	None identified ND	None	12y	MNG, SLCT	c.2256+1G>C, Splice variant	Not previously published
	ND None identified ND None identified ND ND ND ND	None	13y	MNG, PPB and ID 8	c.988G>A, p.Gln330*	Not previously published

Table 1. (continued)

		Histology (macroscopic/microscopic)			Somatic molecular analysis		
ID	Sex / age at Dx DTC	Thyroid histology# (see suppl. Figure 1)	Multi-focal	Lesion (size, mm)	*DICER1*	Other DNA variant	Gene fusion
8¥	F/28	FVPTC (or DHL)	N	T1 (3mm)	c.5438A>T, p.Glu1813Val	None identified	None identified
		DHL (R)*		L1-2 (5-15mm)	c.5113G>A, p.Glu1705Lys	ND	ND
				L3-4 (5mm)	c.5126A>G, p.Asp1709Gly	ND	ND
		DHL (L)		L5 (2mm)	c.5429A>T, p.Asp1810Val	ND	ND
				L6-10 (2-15mm)	c.5438A>T, p.Glu1813Val	ND	ND
9^	F/13	FVPTC (or DHL)	Y	T1 (12mm)	None identified	None identified	None identified
				T2 (5mm)	c.5126A>G, p.Asp1709Gly	ND	ND
				T3 (5mm)	c.5127T>G, p.Asp1709Glu	None identified	None identified
				T4 (6mm)	None identified	None identified	None identified
		DHL		L1 (2mm)	c.5113G>A, p.Glu1705Lys	ND	ND
10^	M/17	FVPTC (or NIFTP)	Y	T1 (2mm)	None identified	None identified	ND
				T2 (4mm)	c.5427_5428delinsTT, p.Asp1810Tyr	None identified	None identified
		DHL		L1 (7mm)	None identified	None identified	ND

Abbreviations: Dx, diagnosis; M, male; F, female; y, year; PPB; pleuropulmonary blastoma; MNG, multinodular goitre; CBME, ciliary body medulloepithelioma; CN, cystic nephroma; SLCT, Sertoli-Leydig cell tumour; PitB, pituitary blastoma; FVPTC, follicular variant papillary thyroid carcinoma; DHL, dominant hyperplastic lesion; NIFTP, non-invasive follicularthyroid

	Clinical Information				
hTERT	Personal history (age at Dx)	Follow up DTC	Family history	Germline *DICER1* variant	Reference
ND	None	1y	See ID 7	c.988G>A, p.Gln330*	Not previously published
ND					
ND					
ND					
ND					
None identified	None	8y	ID 10	c.1363del, p.Val455fs	Diets *et al*. Clin Cancer Res. 2018 (sister ID21)
ND					
None identified					
ND					
ND					
ND	MNG (13)	7y	See ID 9	c.1363del, p.Val455fs	Diets *et al*. Clin Cancer Res. 2018 (ID21)
None identified					
None identified					

neoplasm with papillary-like nuclear features; PTC, papillary thyroid carcinoma; PDTC, poorly differentiated thyroid carcinoma; L, left side; R, right side; LOH, loss of heterozygosity; ND, not done. # All in the context of diffuse nodular hyperplasia with multiple, discrete, well-circumscribed and occasionally encapsulated nodules; ¥Cousins; ^Siblings

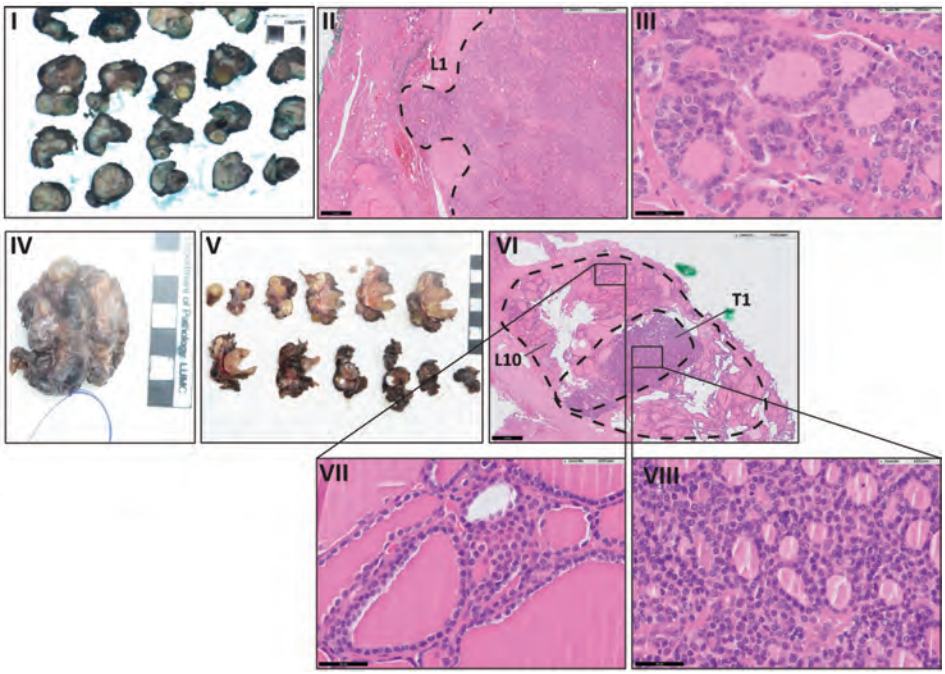

Figure 1. Histology patient ID#8. Panel 1, macroscopy hemi thyroidectomy right; Panel II and III, hematoxylin and eosin stain (HE) (×25 /×200) showing hyperplastic thyroid nodule with a somatic *DICER1* RNase IIIb variant (p.Glu1705Lys), no further DNA variant or gene fusion analysis was performed; Panel IV-V hemi thyroidectomy left, Panel VI, HE (×25) showing 2mm follicular variant papillary thyroid carcinoma (T1) in the context of a non-encapsulated hyperplastic nodule (L10) both with the same somatic *DICER1* RNase IIIb variant (p.Glu1813Val); Panel VII, HE L10 (×200) showing irregularly enlarged colloid-filled follicles, bordered by a flattened epithelium; Panel VIII, HE T1 (×200) showing nuclear features such as nuclear enlargement, indentations and presence of nuclear clearance.

DICER1 is involved in the production of miRNAs, which, in turn, posttranscriptionally regulate gene expression; therefore, we cannot rule out that malignant transformation in DICER1-related thyroid neoplasms may be driven by variations in gene expression without alterations of DNA sequence. Dysregulated miRNAs are associated with cancer initiation and progression in several tumor types .[31] miRNAs can act as both tumor suppressors and oncogenes; each miRNA has multiple mRNA targets, and each mRNA can be the target of multiple miRNAs. More than 100 miRNAs, both upregulated and downregulated, are reported in DTC, but only a few are described in the majority of the studies. Furthermore, some differentially expressed miRNAs have been described as being both upregulated and downregulated in different studies.[32] Recent, small RNA-sequencing studies reported 13 common upregulations (including miR-146b-5p, miR-221-3p, and miR222-3p) and 17 downregulations (including miR-7-3p, miR-204-5p, and miR-1179) in sporadic papillary thyroid carcinoma. The DICER1 RNase IIIb domain is responsible for cleaving the hairpin loop structure from precursor miRNAs to generate mature 5p miRNAs. In DICER1-related PPB and ovarian Sertoli-Leydig cell tumors, the presence of somatic RNase IIIb domain variants, in combination with germline loss of-function *DICER1* mutations, results in a substantial reduction in expression of 5p-derived miRNAs.[33,34] Two *DICER1*-mutated DTCs

from The Cancer Genome Atlas database showed similar overall skewed expression patterns (lower 5p and higher 3p miRNA levels) and no upregulation of commonly upregulated miRNAs in DTC (Supplemental Fig. 2).

Previous observations illustrate the complex role of miRNAs in thyroid tumorigenesis; for example, DICER1 protein levels seem to be higher in sporadic DTC, whereas *DICER1* mRNA expression is lower when compared to matched normal thyroid tissue.[35] Multiple redundant pathways and feedback loops complicate the analysis, as shown by the co-occurrence of decreased expression of *DICER1* and the let-7 miRNA family in one study[35]—*DICER1* mRNA expression is typically inversely related to let-7 levels. Moreover, let-7 was found to reduce RAS levels [36], thus interacting with the mitogen-activated protein kinase pathway, a pathway commonly altered in DTC.

In a mouse model, the arrest of mature miRNA generation in the thyroid induced progressive loss of function and cell dedifferentiation, but the mice did not have increased thyroid size or presence of nodules.[37] Despite the differences observed between human and mouse models, these studies show that *Dicer1* is required for the long-term maintenance of thyroid follicular organization and thyrocyte differentiation.

Childhood DTC is a rare disease, but is the most common endocrine malignancy in children and is the third most common solid tumor, accounting for 0.5% to 3% of all pediatric malignancies. Data from the SEER registry have shown an increased incidence of pediatric DTC, as is the case in adults. Children frequently present with more advanced disease (e.g., lymph node involvement at diagnosis, distant metastases, and multifocal disease) compared with thyroid cancer in adults.[38] Despite the excellent prognosis for pediatric patients with DTC (30-year mortality rate, <5%), morbidity caused by the treatment remains considerable.

Overdiagnosis and thus overtreatment of indolent thyroid tumors is a concern.[39] Diagnostic classification and treatment guidelines are being adapted to address this issue. The term "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" was recently introduced to accommodate certain encapsulated or sharply demarcated lesions with nuclei reminiscent of papillary thyroid carcinoma that were previously classified as noninvasive encapsulated FVPTC.[21]

Until recently, the treatment of pediatric thyroid cancer was predominately based on guidelines for adult patients. In 2015, the American Thyroid Association published the first management guidelines for children with thyroid nodules and DTC.[40] Treatment decisions are guided by the extent of disease and include lobectomy or total thyroidectomy with or without radioactive iodine (RAI) therapy to treat persistent locoregional, nodal disease or distant metastases not amenable to surgery.

All patients in our series underwent total thyroidectomy and eight were treated with adjuvant RAI despite the absence of invasive growth, nodal or distant metastases. This raises concern of unnecessary exposure of a number of these young patients to adverse effects of radiation, which may include the development of second primary cancers (e.g., chronic myeloid neoplasms).[41] Other commonly reported complications of RAI treatment are salivary and lacrimal gland dysfunction, transient gonadal dysfunction, and diastolic dysfunction.[42] Even if the diagnosis of malignancy in patients with DICER1 syndrome is maintained, the behavior of the different, relatively small, distinct lesions (as indicated by the different somatic *DICER1* variants) may be indolent and the risk of recurrent disease and/or metastasis per locus seems low, based on reports published thus far. The American Thyroid Association guidelines do not recommend RAI therapy for pediatric patients with small tumors who do not have persistent locoregional disease, nodal disease, or distant metastases.[40] Furthermore, it is not known whether ionizing radiation may be more harmful in patients with DICER1 syndrome compared with sporadic cases.

In conclusion, on the basis of our clinical, histological, and molecular data, we consider that most DICER1-related DTCs form a low-risk subgroup. Because these tumors may arise from within one of multiple benign monoclonal nodules that constitute a lesion, hemithyroidectomy or total thyroidectomy could often be required, but radioiodine treatment may be unnecessary given the patients ages and the tumors' low propensity for metastases.

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SUPPLEMENTAL DATA

Supplemental Table 1. Overview published patients with (suspect) DICER1-related thyroid carcinoma

Reference	ID	Gender/age (at Dx DTC, years)	Reported thyroid histology	Treatment thyroid tumor
de Kock *et al.* JCEM 2014	**Case 1***	**F/9**	**FTC**	**TT+RAI**
	Case 2	**F/7**	**FVPTC**	**TT+RAI**
	Case 3	**M/11**	**Bilateral papillary carcinoma within follicular adenoma**	**TT+RAI**
	Case 4**	F/6	FTC	TT+LND+RAI
	Case 5***	F/16	miFTC associated to a vesicular adenoma	TT
Schultz *et al.* PatholCaseRev 2014		F/8	FVPTC	TT
Puckett *et al.* Journal of Pediatric Sx Case Reports 2015	Mother	F/?	PTC	TT
Rutter *et al.* JCEM 2016	Mother	F/18	DTC	TT
	Patient A	F/12	DTC	TT
	Patient B	F/14	DTC	TT
	Brother	M/?	Multifocal PTC	TT
Durieux *et al.* Virchows Arch 2016	Case 1	F/18	(E)FVPTC	TT+LND
	Case 2	F/12	FTC	TT+LND+RAI
de Kock *et al.* J Med Genet. 2016	**Case 2**	**F/10**	**PTC**	**TT + RAI**
Yoshida *et al.* Hum Pathol. 2017		F/15	FTC	Hemithyroidectomy
Khan *et al.* JCEM 2017	NCI-77-02-004	F/41	PTC	Not specified
	NCI-63-01-001	M/18	FVPTC	Not specified
	NCI-63-02-002	F/43	miFTC	Not specified
	NCI-64-02-00	F/30	Thyroid carcinoma, papillary, macro follicular type	Not specified

Germline *DICER1* variant	Somatic *DICER1* variant thyroid tumor	Other DICER1-related conditions (age at Dx, years)	Treatment history
c.3505dupT	**p.Glu1813Asp**	**Type II PPB(2); relapsed PPB(4)**	**Sx, CTx and PBSCT**
c.3579_3580delCA	**p.Glu1813Gly**	**Type I PPB(1); CBME(6)**	**Sx and CTx**
c.2379T>G	**p.Glu1705Lys**	**Type II PPB and CN(2.7)**	**Sx and CTx**
Not tested	Not tested	PPB(3)	Sx, CTx and BMT
Not tested	Not tested	PPB(3y); cERMS(7); Bladder undifferentiated RMS(12); MNG(16)	Sx and CTx
Yes (not specified)	p.Glu1813Val	Type II PPB(5); NCMH(13.5); SLCT	Sx and CTx
Yes (not specified)	Not tested	SLCT(unknown age)	Sx and CTx
c.5441C>T	Not tested	SLCT(7 and 18)	Unknown
c.5441C>T	p.Asp1709Gly	SLCT(12), CN	None (SLCT after DTC diagnosis)
c.5441C>T	p.Gly1809Arg	None	None
c.5441C>T	p.Asp1709Gly and p.Asp1810His	None	None
Not tested	p.Glu1813Gln	SLCT(17)	Not specified
Not tested	p.Glu1813Gln	SLCT(15)	None (SLCT after DTC diagnosis)
c.5437G>C (mosaic)	**Loss of heterozygosity**	**Bilateral renal and lung csts (2), Pineoblastoma (7), bilateral SLCT (13, 15), CBME (17)**	**Not specified**
c.5426_5442del GGGATATTTTTGAGTCGinsCA	ASK: p.Glu1705Lys; FTC: p.Glu1813Asp	Anaplastic sarcoma of the kidney (ASK)	Sx, CTx, RTx and PBSCT
c.3515_3525del11insA	Not tested	Thyroid nodules(22.6); MNG(26.7)	None
c.3726C>A (p.Tyr1242*)	p.Gly1809Glu	Type II PPB (4)	Sx and CTx
c.3726C>A (p.Tyr1242*)	Not tested	MNG(22); PPB type Ir(39)	None
c.3675C>G (p.Y1225*)	p.Glu1705Lys and p.Asp1709Gly	MNG(16)	None

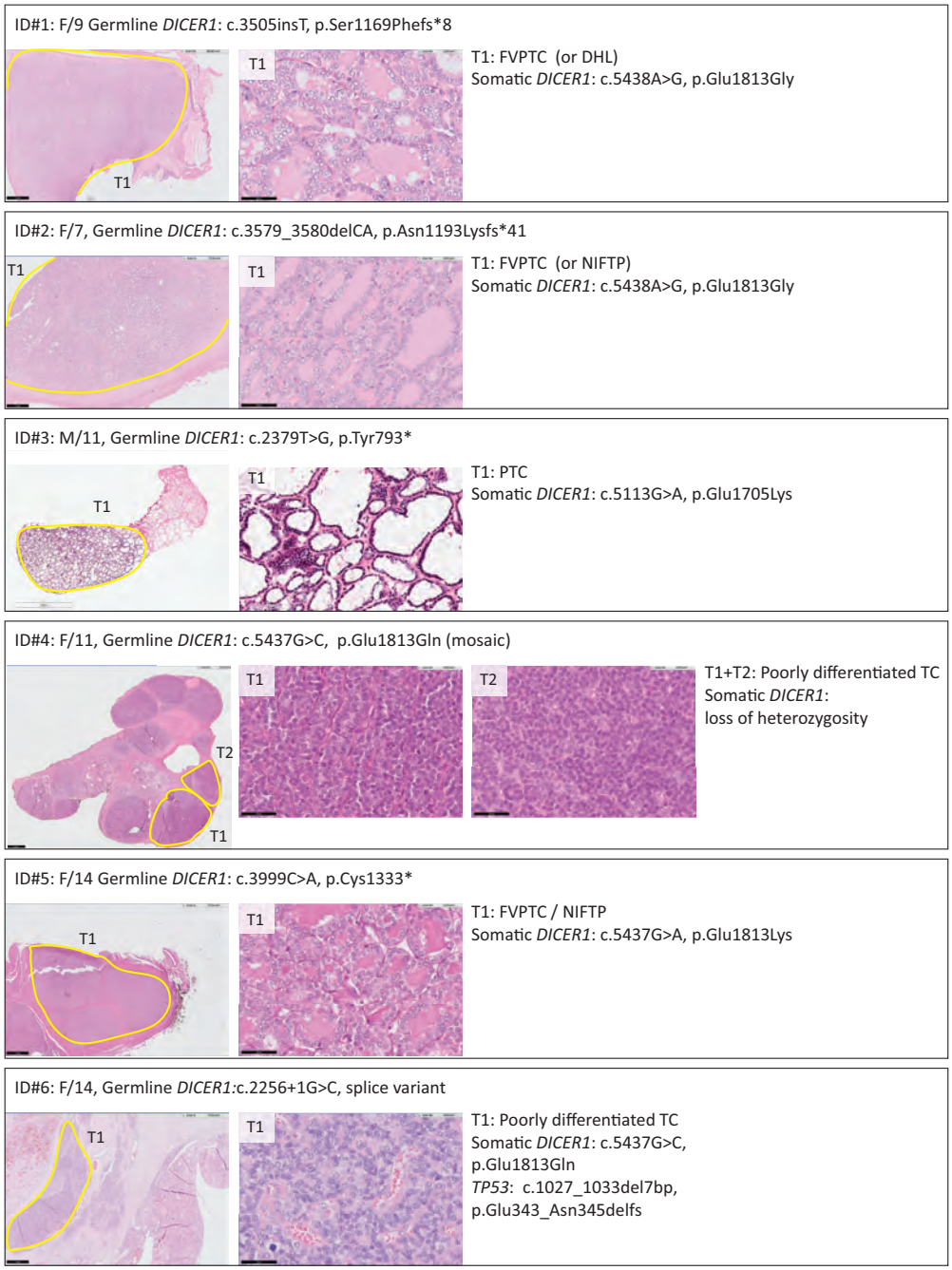
Supplemental Table 1. (continued)

Reference	ID	Gender/age (at Dx DTC, years)	Reported thyroid histology	Treatment thyroid tumor
	IPPBR 5501	F/17	FTC	Not specified
	IPPBR 5502	F/15	FVPTC	Not specified
	IPPBR 5503	F/10	Follicular thyroid carcinoma, follicular with areas of papillary	Not specified
	IPPBR 5504	F/8	PTC	Not specified
	IPPBR 5505	F/9	FVPTC	Not specified
	IPPBR 5507	F/10	FVPTC	Not specified
Gullo *et al.* Am J Clin Pathol 2018		F/12	DTC	TT
Diets *et al.* Clin Cancer Res. 2018	**ID 21**	**M/17**	**PTC**	**TT + RAI**
	Sister ID 21	**F/13**	**FTC**	**TT + RAI**

Abbreviations: Dx, diagnosis; M, male; F, female; FVPTC, follicular variant papillary thyroid carcinoma; PTC, papillary thyroid carcinoma; DTC, differentiated thyroid carcinoma; FTC, follicular thyroid carcinoma; miFTC, minimal invasive FTC, TT, total thyroidectomy; RAI, radioactive iodine treatment; PPB, pleuropulmonary blastoma; MNG, multinodular goitre; CBME, ciliary body medulloepithelioma; CN, cystic nephroma; SLCT, Sertoli-Leydig cell tumour;

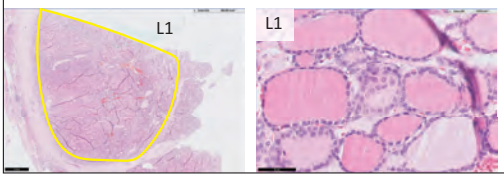
Germline *DICER1* variant	Somatic *DICER1* variant thyroid tumor	Other DICER1-related conditions (age at Dx, years)	Treatment history
Yes (not specified)	Not tested	Type II PPB (2)	Sx and CTx
not tested	Not tested	Type II PPB (3)	Sx, CTx and RTx
not tested	Not tested	Type II PPB (1)	Sx and CTx
Yes (not specified)	Not tested	Type I PPB (1)	Sx and CTx
Yes (not specified)	Not tested	Type II PPB (5)	Sx and CTx
not tested	Not tested	Pineoblastoma(?); PPB type Ir(17)	CTx and RTx
(p.Arg1060Ilefs*7)	p.Glu1813Gly and p.Asp1810Asn	Cervix ERMS(7)	Sx and CTx
c.1363del, (p.Val455fs)	**p.Asp1810Tyr**	**MNG(13)**	**None**
c.1363del (p.Val455fs)	**p.Asp1709Gly and p.Asp1709Glu**	**None**	**None**

Sx, surgery; CTx; chemotherapy, RTx; radiotherapy; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation
In bold, patients reported in this manuscript. * also Shin *et al*. Thyroid 2012; ** also Oue *et al*. PediatrBlood Cancer 2007; *** also Rome *et al*. PediatrBlood Cancer 2008



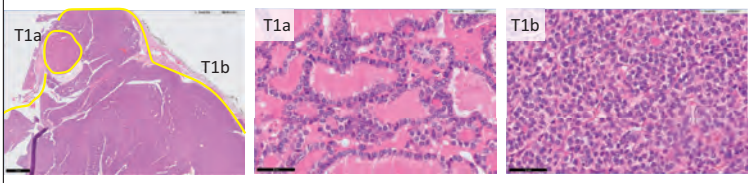
Supplemental Figure 1. Histology DICER1-associated thyroid carcinoma. Left panels overview tumor / lesion in 5 or 10x magnification, right panel(s) details 200x magnification. M; male, F; female, FVPTC; follicular variant papillary thyroid carcinoma, PTC; papillary thyroid carcinoma, TC; thyroid carcinoma; NIFTP; noninvasive follicular thyroid neoplasm with papillary-like nuclear features, T; tumor, L; lesion

ID#6: F/14, Germline *DICER1*:c.2256+1G>C, splic variant

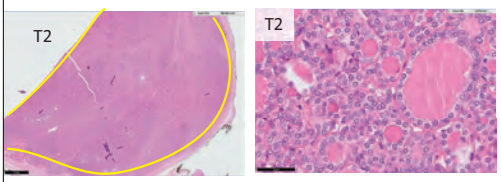


L1: Hyperplastic nodule:
Somatic *DICER1*: c.5437 G>C, p.Glu1813Gln

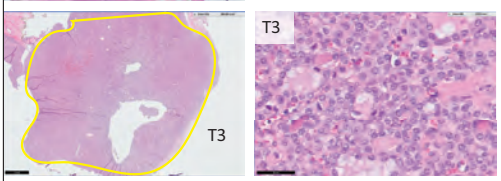
ID#7: F/23 Germline *DICER1*: c.988G>A, p.Gln330*



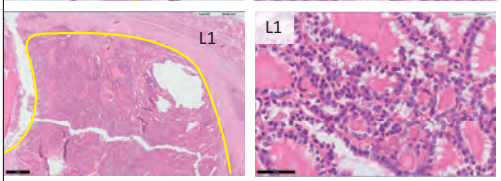
T2a+b: FVPTC (or DHL)
Somatic *DICER1*:
c.5125G>A, p.Asp1709Asn



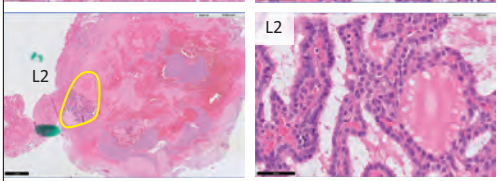
T2: FVPTC (or DHL)
Somatic *DICER1*: c.5126A>G, p.Asp1709Gly



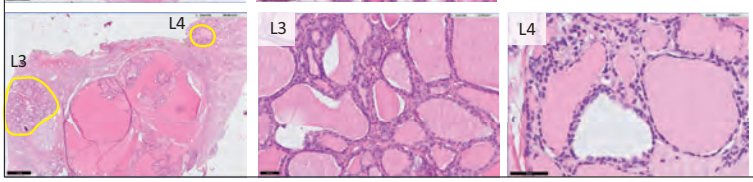
T3: FVPTC (or DHL)
Somatic *DICER1*: c.5437G>A, p.Glu1813Lys



L1: Hyperplastic nodule:
Somatic *DICER1*: c.5438A>T, p.Glu1813Val



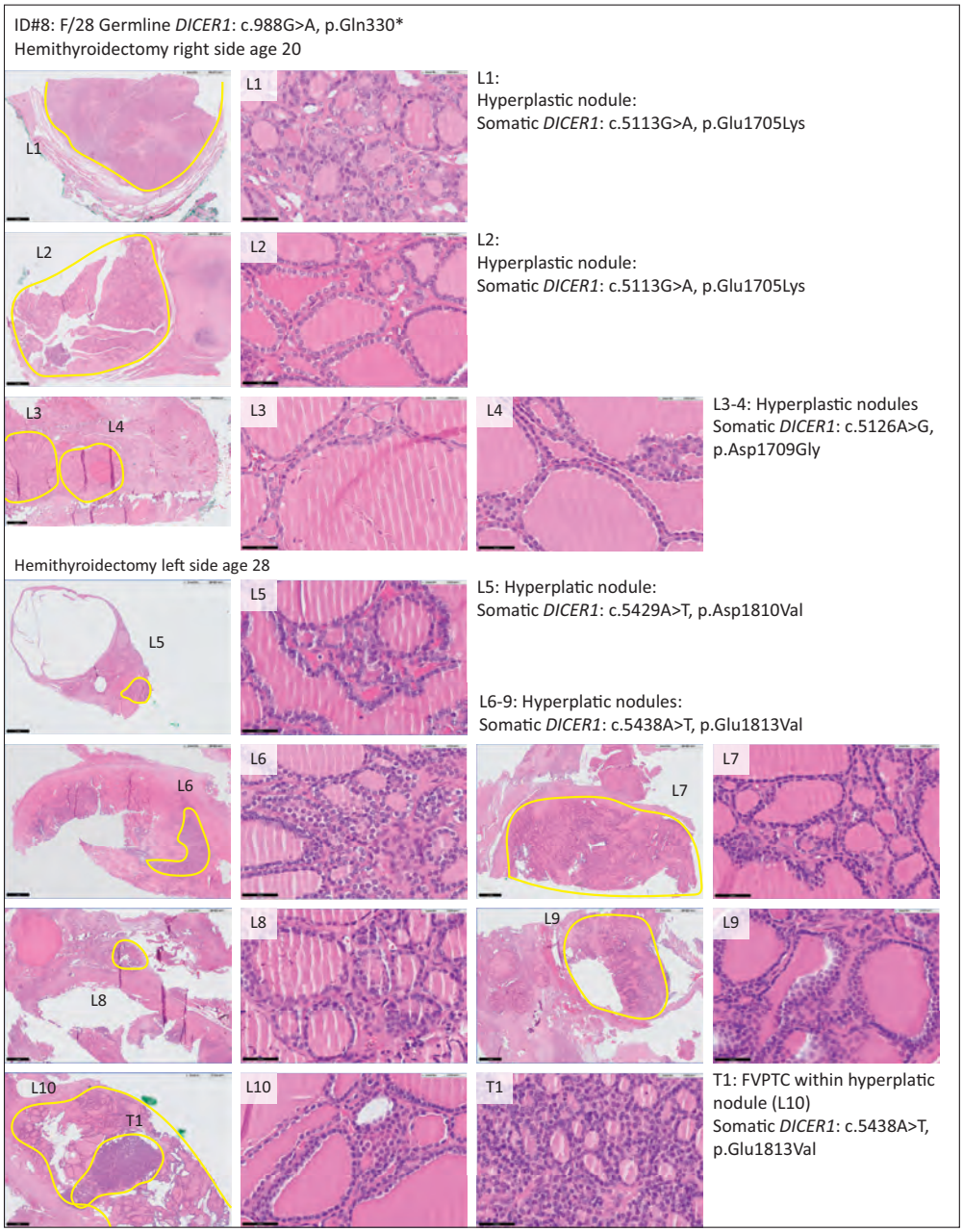
L2: Hyperplastic nodule:
Somatic *DICER1*: c.5426A>G, p.Asp1709Gly



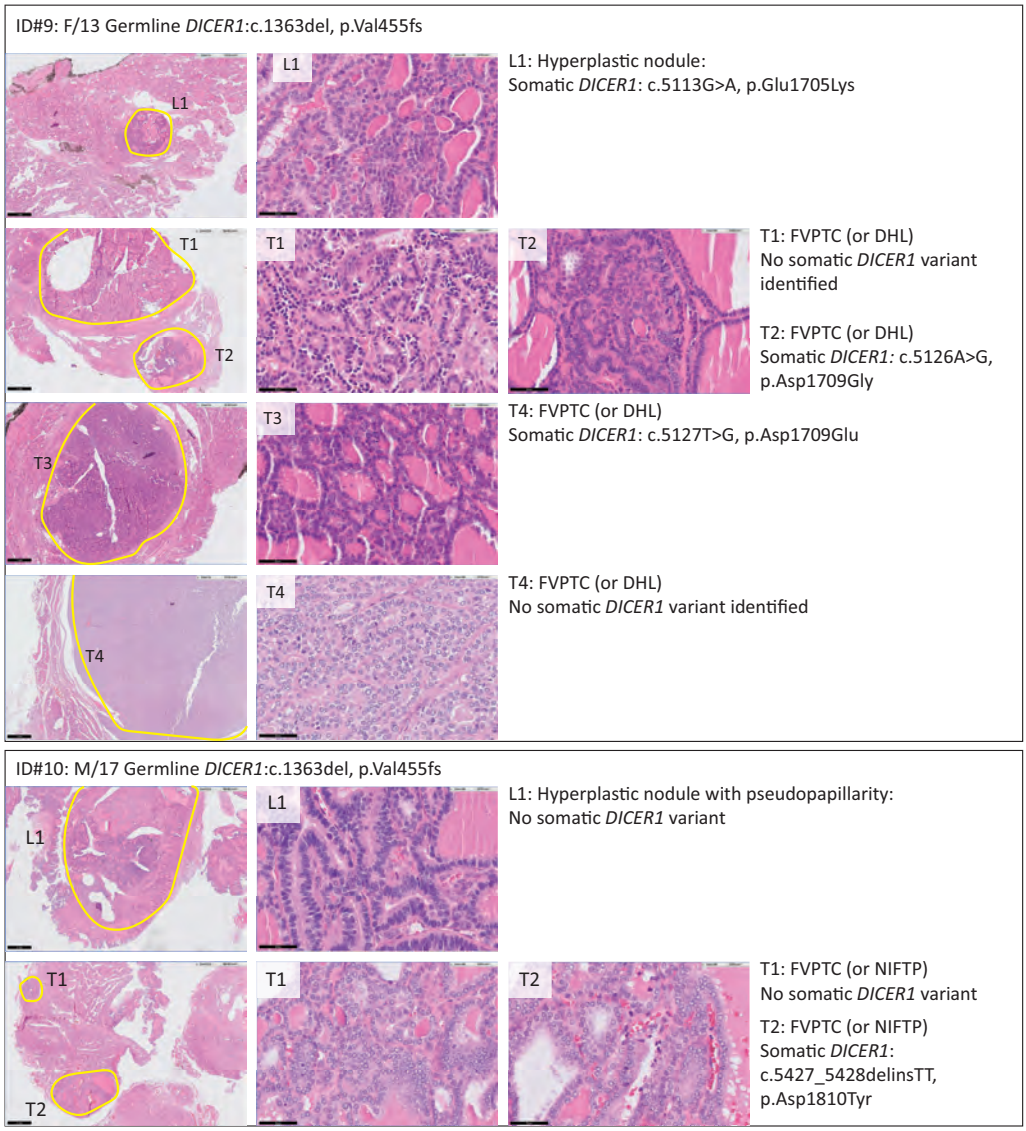
L3: Hyperplastic nodule:
Somatic *DICER1*:
c.5428G>T, p.Asp1810Tyr

L4: Hyperplastic nodule:
Somatic *DICER1*:
c.5429A>T, p.Asp1810Val

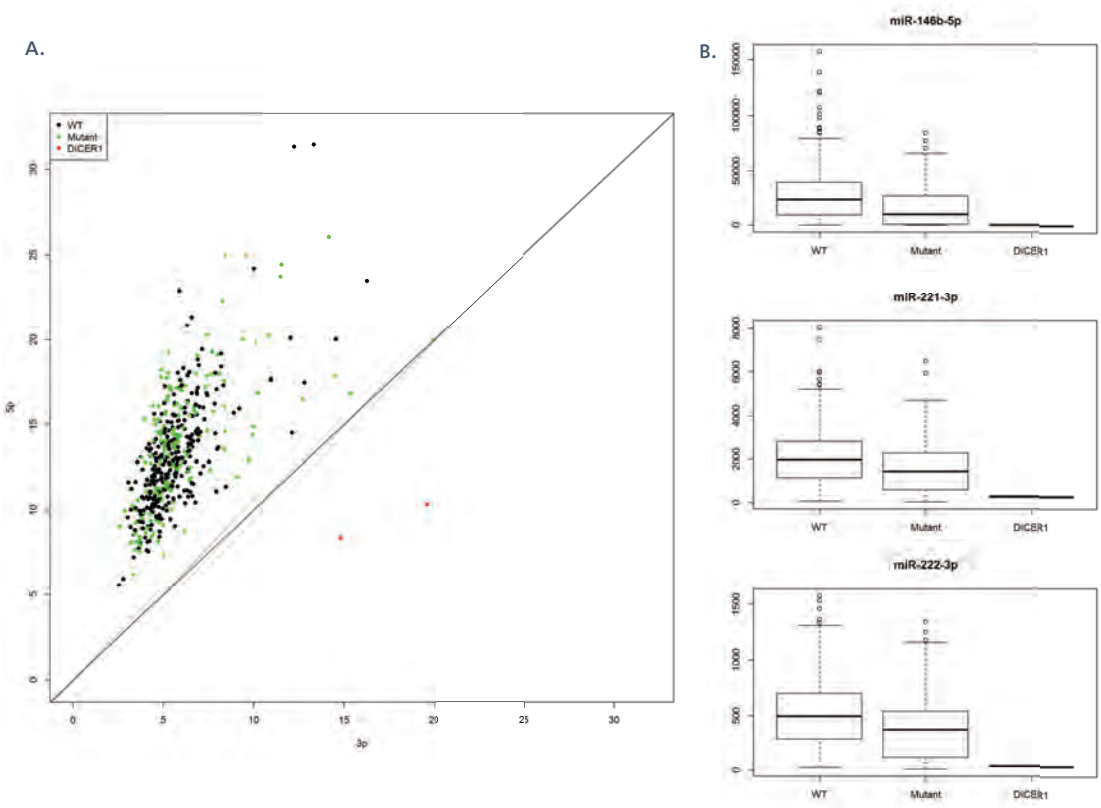
Supplemental Figure 1. (continued)



Supplemental Figure 1. (continued)



Supplemental Figure 1. (continued)



Supplemental Figure 2. miRNA expression DTC TCGA database. **A.** Lower overall normalized median expression of 5p miRNAs compared to non-*DICER1* mutated differentiated thyroid carcinoma (reads per million). **B.** miRNA that are commonly upregulated in differentiated thyroid carcinoma (miR-146b-5p, miR-221-3p and miR-222-3p) seems to be lower in DICER1-related thyroid carcinoma (reads per million). miRNA data obtained from http://firebrowse.org, analysis with R version 3.4.3. WT= no *BRAF*, *RAS*, *EIF1AX*, or *DICER1* mutation; Mutant = *BRAF*, *HRAS*, *NRAS*, *KRAS* or *EIF1AX*; *DICER1* = one loss of function DICER1 mutations and one *DICER1* hotspot RNaseIIIb domain mutation.