Quantification of Thyroid Cancer and Multinodular Goiter Risk in the DICER1 Syndrome: A Family-Based Cohort Study

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Context: The risk of thyroid cancer and multinodular goiter (MNG) in DICER1 syndrome, a rare tumor-predisposition disorder, is unknown.

Objective: To quantify the risk of thyroid cancer and MNG in individuals with DICER1 syndrome.

Design: Family-based cohort study.

Setting: National Institutes of Health (NIH) Clinical Center (CC).

Participants: The National Cancer Institute DICER1 syndrome cohort included 145 individuals with a DICER1 germline mutation and 135 family controls from 48 families.

Interventions: Each individual completed a detailed medical history questionnaire. A subset underwent a 3-day evaluation at the NIH CC.

Main Outcome Measures: The cumulative incidence of MNG (or thyroidectomy) was quantified using the complement of the Kaplan-Meier product limit estimator. We compared the observed number of thyroid cancers in the NCI DICER1 cohort with matched data from the Surveillance, Epidemiology, and End Results (SEER) Program. We performed germline and somatic (thyroid cancer, MNG) DICER1 sequencing.

Results: By the age of 40 years, the cumulative incidence of MNG or thyroidectomy was 75% in women and 17% in men with DICER1 syndrome compared with 8% of control women (P < 0.001) and 0% of control men (P = 0.0096). During 3937 person-years of observation, individuals with DICER1 syndrome had a 16-fold increased risk of thyroid cancer (95% confidence interval, 4.3 to 41;
DICER1 syndrome is an autosomal-dominant, pleiotropic, tumor-predisposition disorder arising from pathogenic germline variants in DICER1, which encodes an endoribonuclease integral to processing microRNAs (1). The prevalence of DICER1 syndrome is unknown; however, the prevalence of loss-of-function DICER1 variants is ~1 of 6800 to 1 of 10,000 in Exome Aggregation Consortium data (Supplemental Table 1). Pleuropulmonary blastoma (PPB), a rare pediatric lung sarcoma, is the hallmark tumor of DICER1 syndrome. Germline loss-of-function mutations of DICER1 are also associated with an increased risk of an unusual constellation of benign and malignant tumors, including PPB, cystic nephroma (CN), ovarian Sertoli-Leydig cell tumor (SLCT), and nasal chondromesenchymal hamartoma (2). Sequence analysis of many DICER1 syndrome conditions has demonstrated missense somatic mutations affecting specific DICER1 “hotspot” amino acids (E1705, D1709, G1809, D1810, and E1813). These mutations result in defective cleavage of a class of mature microRNAs from the precursor microRNA hairpin (3).

Multiple case reports and case series have reported an association between germline mutations in DICER1 and familial multinodular goiter (MNG) (4–8). Eight studies have reported thyroid cancer in 13 people with DICER1 germline mutations or DICER1 syndrome-related tumors (9–16). DICER1-associated thyroid cancer also harbors somatic pathogenic variants in DICER1 hotspot amino acids (Supplemental Table 2). The first five reports of DICER1-associated thyroid cancers described children who had received chemotherapy before the age 10 years for a DICER1-related tumor, prompting speculation of a possible etiologic link between chemotherapeutic agents and an increased risk of thyroid cancer. However, one DICER1 family is notable for multiple members with differentiated thyroid cancer in the absence of chemotherapy or therapeutic radiation exposure (15). In a family-based cohort study, we analyzed epidemiologic, clinical, and genetic data to investigate the risks of thyroid cancer and MNG in association with DICER1 syndrome.

Methods

Study population, genetic testing, and central pathology review

The National Cancer Institute (NCI) DICER1 syndrome cohort included individuals with a pathogenic germline DICER1 variant (henceforth, “DICER1 carriers”) and family members without a pathogenic germline DICER1 variant (“controls”) enrolled in the DICER1-Related Pleuropulmonary Blastoma Cancer Predisposition Syndrome Natural History Study (protocol nos. 11-C-0034; NCT-01247597) from November 1, 2011 to November 1, 2015. The study recruits individuals with known DICER1-associated tumors (PPB, SLCT, and CN) and their family members. However, neither the families nor the participants were recruited because of thyroid cancer or MNG. After genetic counseling, germline mutation testing of DICER1 was performed on probands using either Sanger or next-generation sequencing assays (1, 17). Blood relatives of probands with pathogenic germline mutations were offered testing for DICER1. Individuals with DICER1-associated tumors but without pathogenic germline DICER1 variants (putative tumor-confined or mosaic DICER1 variation) were excluded from the study. Pathologic materials were obtained whenever possible and were reviewed by two study pathologists (D.A.H., L.P.D.). All participants completed comprehensive questionnaires detailing the cancer diagnoses, noncancer conditions, and medical procedures. Confirmatory medical reports were obtained whenever possible and were data abstracted. The NCI institutional review board approved the study, and all patients (and/or their guardians) provided written informed consent.

Clinical characterization of participants

DICER1 carriers and their family members were evaluated at the National Institutes of Health (NIH) Clinical Center (CC) cohort. Thyroid-stimulating hormone, thyroxine, thyroxine-binding globulin, and serum albumin levels were determined. The participants underwent a complete history and physical examination (including thyroid palpation), received comprehensive evaluations by specialists in endocrinology and otorhinolaryngology, and underwent a detailed thyroid ultrasound examination. A single radiologist (A.L.) systematically reviewed all imaging studies and documented the length and width (in mm), number, location, laterality, and imaging characteristics (e.g., cystic, solid, and partial solid/cystic, and the echogenicity and blood flow for the solid portion of the nodule) for every lesion. MNG was assigned when the gland was both enlarged and contained multiple solid lesions. We excluded from analysis 1 DICER1 carrier and 1 control who self-reported a history of MNG but did not provide their age or date of diagnosis. Two DICER1 carriers who had not reported their age at MNG diagnosis did report their age at thyroidectomy, and we assumed that MNG had occurred at that age.

DICER1 sequencing in MNG and thyroid cancer

Participants NCI-11-02-002 and NCI-77-02-006 underwent thyroidectomies for MNG performed at the CC. The resected thyroid lobes were bivalved, and the matching tissue halves were frozen or embedded in paraffin. DNA was extracted from geographically distinct nodules from the frozen tissue using standard methods. DNA extraction from other paraffin-embedded MNG nodules and thyroid cancer (from thyroidectomy) was

\[ P < 0.05 \] compared with the SEER rates. Of 19 MNG nodules and 3 thyroid cancers, 16 (84%) and 3 (100%), respectively, harbored germline and somatic pathogenic DICER1 mutations.

Conclusions: We propose a model of thyroid carcinogenesis in DICER1 syndrome. Early-onset, familial, or male MNG should prompt consideration of the presence of DICER1 syndrome. (J Clin Endocrinol Metab 102: 1614–1622, 2017)
performed using standard methods. Somatic DICER1 sequencing was performed using next-generation methods (17).

Cases of thyroid cancer in the International PPB Registry

To better understand the natural history of thyroid malignancy in association with DICER1 syndrome, a rare disorder, all available International PPB Registry (IPPBR) patient records (since its founding in 1988) were reviewed. The data for persons referred to the IPPBR with a PPB-associated tumor and/or a DICER1 mutation are accessioned consecutively. The clinical presentation, imaging findings, pathologic materials, family history, treatment, and long-term follow-up data are collected. All pathology materials submitted to the IPPBR, including the thyroid tissue discussed in the present report, were centrally reviewed by IPPBR pathologists (D.A.H., L.P.D.). These data were analyzed separately from the NCI data. The institutional review board at the Children’s Hospitals and Clinics of Minnesota oversees the IPPBR (protocol no. 0909-082), and all participants provided written informed consent.

Statistical analysis

We used the complement of the Kaplan-Meier product limit estimator to quantify the cumulative incidence of a first MNG diagnosis. Participants not evaluated at the CC were considered at risk of MNG from birth until the most recent date at which the individual or a family member had provided updated clinical information. Comprehensive screening of the CC cohort introduced a surveillance bias; thus, these participants were considered at risk from birth until the date of their first clinical evaluation at the NIH. Because partial and full thyroidectomy interferes with the natural history of MNG, these were treated as censoring events if they had occurred before the MNG diagnosis. We estimated the cumulative incidence of both MNG (censoring at thyroidectomy) and thyroidectomy or MNG for the entire cohort and for the CC cohort. As a sensitivity analysis for the latter, we also reviewed the medical histories taken at the CC and updated any missing dates for the MNG diagnosis, thyroidectomy, and previously unreported MNG. Differences in cumulative incidence were assessed using the log-rank test.

We compared the observed number of thyroid cancers in DICER1 carriers in the entire cohort with the expected frequencies from the Surveillance, Epidemiology, and End Results (SEER) Program (SEER 9, 1973 to 2012; November 2014 submission; available at: http://seer.cancer.gov), based on age-, sex-, race-, and birth cohort–specific thyroid cancer incidence data. Two participants were British; we used SEER data for these subjects, because the thyroid cancer incidence and prevalence is roughly comparable between the United Kingdom and the United States (GLOBOCAN 2012, International Agency for Research on Cancer/World Health Organization; available at: globocan.iarc.fr). We calculated the observed-to-expected cancer (O/E) ratio for the cohort, with and without censoring at thyroidectomy (partial or full) for benign MNG or thyroid nodules. We describe the continuous variables using the median and range and tested the differences in continuous variables using the Wilcoxon rank sum test. We assessed the differences in proportions using the \( \chi^2 \) test or an exact test when limited by infrequent observations \( n < 5 \). The analyses were stratified by sex and/or adulthood (age \( \geq 18 \) years), both of which are risk factors for thyroid disease. All statistical tests were two-sided, and \( P \leq 0.05 \) was considered statistically significant. All analyses were conducted using Stata/SE, version 13.1 (StataCorp, College Station, TX). SEER analyses were conducted using SAS, version 9.2, TS2M3 (SAS Institute Inc., Cary, NC) and Surveillance Research Program, National Cancer Institute SEER*Stat software (available at: www.seer.cancer.gov/seerstat), version 8.3.2.

Results

General demographics

The cohort included 145 DICER1 carriers and 135 family controls, of whom 89 DICER1 carriers and 62 family controls were in the CC cohort (Supplemental Table 3). The DICER1 carriers in both cohorts were significantly younger than the family controls. However, no statistically significant differences were found in sex, ethnicity, or race between the cases and controls.

A high risk of MNG in DICER1 carriers compared with family controls

Thirty-two participants in the entire cohort reported a history of MNG. We verified 24 cases (75%) by medical record documentation or pathologic review. Medical reports or pathologic materials were not available for eight cases (25%). The cumulative incidence of MNG in the entire cohort was greater in DICER1 carriers among both females \( (P < 0.001) \) and males \( (P = 0.026) \) compared with family controls [Fig. 1(a)]. The cumulative incidence of MNG in male and female DICER1 carriers and controls at 20, 30, and 40 years is summarized in Table 1. Including both MNG and thyroidectomy as events resulted in similar observed differences in both female \( (P < 0.001) \) and male \( (P = 0.0096) \) DICER1 carriers compared with family controls [Fig. 1(b)]. Exclusion of probands from the analyses did not meaningfully change the results.

Ultrasound identified previously undiagnosed MNG

Before evaluation at the CC, significantly more DICER1 carriers (women and men) had a MNG diagnoses or history of thyroidectomy compared with family controls (Table 2; Supplemental Table 4). CC ultrasonography identified a significantly greater proportion of male DICER1 carriers with MNG compared with male controls \( (5 \text{ of } 12 \text{ vs. } 1 \text{ of } 29; P < 0.01) \). Updating the medical history with clinical data from the CC evaluation resulted in similar estimates of cumulative incidence [Supplemental Fig. 1(a) and 1(b)]. Male DICER1 carriers who did not meet the MNG diagnostic criteria were still more likely to have at least one thyroid lesion detected by ultrasonography \( (P = 0.027) \) and a greater number of thyroid lesions recorded \( (P = 0.001) \) than family controls (Supplemental Table 4). In the CC cohort, 13 DICER1 carriers and 1 control had a history of partial thyroidectomy (Supplemental Table 5). In those with partial...
thyroidectomy, we observed thyroid lesions in 6 of the 13 DICER1 carriers (46%) and 1 control. Of the 13 DICER1 carriers, 4 (30%) underwent multiple thyroid resections. No notable differences were detected in thyroid-stimulating hormone, thyroxine, thyroxine-binding globulin, and serum albumin between the DICER1 carriers and controls.

Risk of thyroid cancer in the entire DICER1 carrier cohort increased compared with the general population

We observed four cases (mean age, 34 years; range, 18.6 to 43 years) of differentiated thyroid cancer (papillary or follicular thyroid carcinoma) among 145 DICER1 carriers (3937 person-years of observation [PYO]) in the entire cohort (Supplemental Table 6). No participants had undergone antecedent therapeutic radiation, and only one participant had received antecedent chemotherapy. The DICER1 carriers in our cohort had a 16-fold (95% confidence interval [CI], 4.3 to 41; \( P = 0.05 \)) increased risk of thyroid cancer compared with the SEER rates. Given the frequency of MNG-associated thyroidectomy in the cohort, censoring at the complete thyroidectomy surgery date resulted in 3722 PYO, yielding an O/E ratio of differentiated thyroid cancer of

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**Figure 1.** Cumulative incidence of MNG for entire DICER1 syndrome cohort. (a) The cumulative incidence of MNG diagnosis censored at thyroidectomy in DICER1 carriers (red) and controls (blue). Shaded areas indicate the 95% pointwise CIs. The cumulative incidence of MNG in women with DICER1 syndrome had reached 50% by age 36 years (95% CI, 25.6 to 60.9). (b) The cumulative incidence for the risk of either MNG diagnosis or thyroidectomy (Table 1).

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**Table 1.** Cumulative Incidence of MNG and MNG and Thyroid Surgery in Male and Female DICER1 Carriers and Controls Stratified by Age

| Age (y) | Female DICER1 Carrier | Female Control | Male DICER1 Carrier | Male Control |
|---------|------------------------|----------------|--------------------|-------------|
| MNG incidence |                         |                |                    |             |
| 20      | 0.23 (0.14–0.38)       | 0              | 0.10 (0.035–0.29)  | 0           |
| 30      | 0.44 (0.30–0.60)       | 0              | 0.14 (0.054–0.33)  | 0           |
| 40      | 0.67 (0.49–0.82)       | 0.061 (0.016–0.23) | 0.14 (0.054–0.33)  | 0           |
| MNG or thyroid surgery incidence |                 |                |                    |             |
| 20      | 0.32 (0.21–0.46)       | 0              | 0.13 (0.52–0.32)   | 0           |
| 30      | 0.52 (0.38–0.66)       | 0.020 (0.0029–0.14) | 0.17 (0.073–0.35)  | 0           |
| 40      | 0.75 (0.59–0.89)       | 0.080 (0.026–0.23) | 0.17 (0.073–0.35)  | 0           |

Data in parentheses are 95% CIs.
18 (95% CI, 4.9 to 46; \(P\), 0.05). Censoring at any thyroidectomy (complete and partial) resulted in 3357 PYO, yielding an O/E ratio of 24 (95% CI, 6.6 to 62; \(P\), 0.05).

Reports of thyroid cancer in the IPPBR

We identified 6 unreported individuals from the IPPBR with a germline DICER1 mutation or DICER1-associated tumor who also had a diagnosis of thyroid cancer and were not a part of the CC cohort (Supplemental Table 6). All were female, with a median age at diagnosis of 10 years (range, 8 to 17). The median latency between the administration of chemotherapy and the development of thyroid cancer was 9 years (range, 3 to 15). Three of the cancers were papillary or multifocal papillary thyroid carcinoma and three were follicular thyroid carcinoma. All six of the patients had received chemotherapy (five for PPB type I or II and one for pineoblastoma) and one patient had also undergone radiotherapy to the thorax for PPB before the thyroid cancer diagnosis.

Benign thyroid nodules and thyroid cancer in DICER1 carriers harbored somatic second hits in DICER1 hotspot codons

Pathologic material was available from 23 thyroid lesions from 16 DICER1 carriers (Table 3; Supplemental Table 7). We observed somatic mutations in DICER1 hotspot amino acids in three of three malignant nodules (100%) from two individuals (Table 3). In 16 of 19 nodules (84%) from 13 participants with MNG (including 2 participants with concomitant Hashimoto’s thyroiditis), we found somatic mutations in DICER1 hotspot amino acids, in addition to the germline DICER1 mutation. No pathogenic somatic DICER1 mutations (including hotspot lesions) were found in the remaining 3 of the 19 nodules. In three DICER1 carriers for whom multiple nodules were tested, different nodules harbored distinct mutations in hotspot amino acids, suggesting that the nodules had arisen from distinct clonal events (Table 3).

Discussion

To the best of our knowledge, this is the first report of a cohort of DICER1 carriers that has quantified the excess risk of MNG and thyroid cancer. Our data support a significantly increased risk of MNG compared with family controls and a significantly elevated risk of thyroid cancer compared with the population data from the NCI-SEER program. Thyroid lesion mutation analysis revealed somatic DICER1 hotspot mutations in both benign and malignant thyroid nodules from DICER1 carriers, including multiple distinct somatic DICER1 mutations from different thyroid nodules resected from the same individual.

In our study, DICER1 carriers were more likely to report a history of diagnosed MNG than were family controls. Our data show that three of four women and one of six men with DICER1 syndrome will develop MNG or undergo thyroidectomy by age 40 years. Moreover, in those undergoing partial thyroidectomy, most will develop MNG in residual thyroid tissues or will require additional thyroid surgery. Clinically, nodular thyroid disease might be the most common phenotypic abnormality in individuals with germline DICER1 mutations (in particular, in females), with higher penetrance than previously estimated (13).

Early-onset, familial, or male MNG should prompt a careful personal and family history focused on DICER1-associated tumors (especially PPB, CN, SLCT). The head
circumference should also be measured, because macrocephaly is also associated with DICER1 syndrome (18). If found, DICER1 testing is indicated. In the absence of these findings (or signs or symptoms of other genetic disorders such as the PTEN hamartoma tumor syndrome), DICER1 testing should still be considered, especially if young children are at risk in the pedigree. We acknowledge that the frequency of germline DICER1 mutations in patients referred for MNG evaluation is unknown. However, although the diagnosis and management of MNG is important in its own right, the thyroid phenotype might be most useful as an entry point for genetic testing and, ultimately, screening of younger family members for PPB, a potentially lethal tumor that is most curable in its earliest, cystic form. It has been suggested that DICER1 carriers aged <8 years should undergo chest computed tomography to evaluate for PPB. Children aged <4 years should undergo renal ultrasonography to monitor for the development of CN. Girls and young women should be educated about the signs and symptoms of SLCT and semiannual or annual pelvic ultrasound examinations should be considered. In males and females with germline DICER1 mutations, a baseline thyroid ultrasound examination should be performed in late childhood.

We have established that DICER1 carriers have a statistically significant, 16- to 18-fold increased risk of developing differentiated thyroid carcinoma. This risk increased to 24-fold after censoring of complete and partial thyroidectomies. Our experience and data do not suggest that DICER1-associated thyroid cancer is more invasive or less responsive to therapy; however, further investigation is needed from prospective studies. DICER1 carriers with a thyroid nodule should receive standard management before consideration for thyroid resection, including thyroid and neck ultrasonography, to assess for evidence of bilateral thyroid disease and metastasis to cervical lymph nodes. More data are needed to develop evidence-based guidelines for the screening of MNG and thyroid cancer in association with DICER1 syndrome. Fine needle aspiration of suspicious thyroid nodules and lymph nodes should be performed to detect possible occult malignancy and to optimize the surgical outcome (19, 20). No data are yet available that support prophylactic thyroidectomy in those with DICER1 syndrome. Multiple endocrine neoplasia type 2 is the only familial tumor predisposition syndrome for which prophylactic thyroidectomy is indicated (21). The prevalence and natural history of patients with small foci of malignancy (e.g., our patient in NCI-77-02-004; Supplemental Table 6) needs more study.

We hypothesize that the increased risk of thyroid malignancy in DICER1 carriers is secondary to the greatly increased prevalence of premalignant lesions (i.e., thyroid nodules) in DICER1-associated MNG. We thus propose a stepwise model (Fig. 2) in which benign

| ID/Sex/Age at Thyroid Surgery (y) | Pathologic Diagnosis | Tissue Diagnosis on Block Selected for Sequencing | Lesion No. | Germline DICER1 Mutation | Amino Acid Change | Allele Frequency of Germline DICER1 Mutation Measured in Tissue Sequenced (%) | Somatic RNase IIIb DICER1 Mutation/ Amino Acid Change | Somatic RNase IIIb Mutant Allele Frequency (%) | Lesional Tissue Material Sequenced (%) |
|----------------------------------|----------------------|-----------------------------------------------|-----------|------------------------|------------------|--------------------------------------------------------------------------------|----------------------------------------|-----------------------------------------|-----------------------------------|
| NCI-11-02-002/F/41.6            | MNG                  | Nodular hyperplasia                           | 1         | c.1870C>T; p.Arg624*   | 47.3 (542/1146) | c.5126A>G; p.Asp1709Gly 48.1 (13/27) | 50                                    |                                        |                                   |
|                                  |                      |                                               | 2         | c.1870C>T; p.Arg624*   | 51.1 (1021/1986) | c.5429A>T; p.Asp1709Val 38.8 (184/474) | 50                                    |                                        |                                   |
|                                  |                      |                                               | 3         | c.1870C>T; p.Arg624*   | 48.7 (974/1988) | c.5126A>G; p.Asp1709Gly 49.4 (44/89) | 60                                    |                                        |                                   |
|                                  |                      |                                               | 4         | c.1870C>T; p.Arg624*   | 49.8 (995/2000) | c.5437G>C; p.Asp1713Gln 5.6 (45/804) | 20                                    |                                        |                                   |
| NCI-77-02-006/F/32.4            | Hyperplastic gland with adenomatoid nodule | Nodular hyperplasia with Hashimoto’s thyroiditis | 1         | c.3515_3525del11insA   | 60.5 (259/428) | c.5126A>G; p.Asp1709Gly 47.6 (10/21) | 50                                    |                                        |                                   |
|                                  |                      |                                               | 2         | c.3515_3525del11insA   | 53.7 (605/1127) | c.5126A>G; p.Asp1709Gly 47.6 (10/21) | 50                                    |                                        |                                   |
|                                  |                      |                                               | 3         | c.3515_3525del11insA   | 47.6 (609/1279) | c.5126A>G; p.Asp1709Gly 22.5 (94/409) | 50                                    |                                        |                                   |
| NCI-63-01-001/M/18.6            | Thyroid cancer       | Thyroid carcinoma, papillary, follicular variant | 1         | c.3515_3525del11insA   | 59.4 (609/1025) | c.5126A>G; p.Asp1709Gly 25.9 (94/375) | 50                                    |                                        |                                   |
|                                  |                      |                                               | 2         | c.3515_3525del11insA   | 31 (100/323)    | c.5126A>G; p.Asp1709Gly 4.9 (4/81) | 10                                    |                                        |                                   |
| NCI-64-02-002/F/30.6            | MNG                  | Thyroid carcinoma, papillary, macrofollicular type | 1         | c.3675C>G; p.Tyr1225*  | 48.9 (976/1995) | c.5131G>A; p.Glu1705Val 5.2 (15/287) | 10                                    |                                        |                                   |
|                                  |                      |                                               | 2         | c.3675C>G; p.Tyr1225*  | 51.8 (1034/1996) | c.5131G>A; p.Glu1705Val 11.3 (30/266) | 15                                    |                                        |                                   |
nODULES develop secondary to biallelic mutations in DICER1, and, over time, additional genetic mutations are acquired that prompt malignant transformation of these nodules. At present, the identity and relative importance of the other genes involved in DICER1-associated thyroid carcinogenesis are unknown. We observed a DICER1 germline mutation and a second, somatic hotspot DICER1 mutation in 16 of 19 sequenced benign thyroid nodules (84%). We performed sequence analysis on whole tissue sections without microdissection or cores of the lesional tissue (nodule), which is suboptimal for assessment of clonality. Comparing crude visual estimates of the percentage of lesional cells on a slide with mutant allele frequency, we estimated that some, but not all lesions, were clonal. We hypothesize that those thyroid epithelial cells with biallelic DICER1 mutations have a growth advantage and with time will continue to proliferate, forming adenomatous cellular nodules. Other groups have also observed somatic hotspot DICER1 mutations in thyroid nodules at mutant allele frequencies consistent with a clonal etiology for each nodule (22). Other studies, including those from our group (11–13, 15, 16, 22), have reported biallelic DICER1 mutations at a high somatic mutant allele frequency in DICER1-associated thyroid cancer. Because biallelic DICER1 mutations are a feature of both DICER1-associated thyroid nodules and DICER1-associated thyroid cancer, this suggests that mutations in other genes are required for thyroid cancer pathogenesis in DICER1 syndrome. This is akin to the classic model of cancer development in familial adenomatous polyposis caused by mutations in APC (23, 24). Given a sufficient number of benign premalignant lesions, malignant transformation of one or several of these lesions over time will elevate the risk of cancer dramatically. The specific factors (or exposures) that increase the risk of DICER1-associated thyroid cancer are unknown. However, as our data have shown, over time, many DICER1 carriers will undergo total or partial thyroidectomy to remediate symptomatic goiter, which would reduce the risk of malignant transformation in the thyroid. If correct, this model predicts that DICER1 carriers will account for a decreasing percentage of thyroid cancer cases as the population ages. Sequencing studies have supported this prediction. In one study of 88 people with thyroid cancer, none had germline DICER1 mutations (25). Only two pathogenic somatic DICER1 mutations were found in 496 papillary thyroid carcinomas sequenced by The Cancer Genome Atlas (13, 26).

The effect of antecedent chemotherapy on the prevalence and pathogenesis of DICER1-associated thyroid cancer is uncertain. Of the reported and unreported individuals (Supplemental Tables 2 and 6) with DICER1-associated thyroid cancer, the majority had received alkylating agents to treat the primary DICER1-associated tumor. The epidemiologic evidence showing that alkylating agents, known mutagens, increase the risk of thyroid cancer is mixed (27, 28). These clinical observations are in accordance with our model, in which the mutagenic effects of an alkylating agent might accelerate the stepwise progression of a pre-existing MNG toward carcinogenesis. However, caution is needed when interpreting data from these published and unpublished case reports, which are subject to publication and referral biases. We note that, unlike the case report data, only a few (one of four) of the thyroid cancer cases in the NCI cohort had received an antecedent alkylating agent (Supplemental Table 6). The role of chemotherapy in malignant transformation within the thyroid in those with DICER1 syndrome remains to be determined and merits additional investigation.

We acknowledge several study limitations, including a relatively small sample and potential biases in both ascertainment and selection. The invitation of families to the CC was not randomized, and the participants who enrolled in the study might differ from those with DICER1 mutations in the general population. A subset of MNG diagnoses and thyroidectomy history was unverified, despite our best efforts. The diagnostic criteria from different institutions for MNG likely varied in the United States. Although statistically significant, the total number of people with thyroid cancer was small.

**Conclusions**

To the best of our knowledge, we have quantified, for the first time, the substantial excess of MNG in individuals with DICER1 syndrome and report a 16- to 24-fold increase in the risk of thyroid cancer compared with the expected incidence in the SEER data. MNG might be the most penetrant phenotypic feature of DICER1 syndrome, especially in women. Early-onset, familial, or
male MNG should prompt consideration of the presence of DICER1 syndrome, especially in the case of a family history of other DICER1-associated cancers. The role of antecedent chemotherapy in the prevalence and pathogenesis of DICER1-associated thyroid cancer remains to be determined, as do other risk factors. We do not recommend prophylactic thyroidectomy for DICER1 carriers. For DICER1 carriers undergoing elective thyroidectomy secondary to symptoms or cosmesis, we recommend that worrisome thyroid nodules undergo fine needle aspiration to detect occult malignancy and optimize surgical planning. Our genetic and epidemiologic data suggest a model of thyroid carcinogenesis in which benign thyroid nodules could be considered premalignant lesions in a stepwise evolution toward thyroid cancer.

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