**DICER1 Mutations in Familial Multinodular Goiter With and Without Ovarian Sertoli-Leydig Cell Tumors**

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Marc Tischkowitz, MD, PhD

**Context**  
Nontoxic multinodular goiter (MNG) is frequently observed in the general population, but little is known about the underlying genetic susceptibility to this disease. Familial cases of MNG have been reported, and published reports describe 5 families that also contain at least 1 individual with a Sertoli-Leydig cell tumor of the ovary (SLCT). Germline mutations in DICER1, a gene that codes for an RNase III endoribonuclease, have been identified in families affected by pleuropulmonary blastoma (PPB), some of whom include cases of MNG and gonadal tumors such as SLCTs.

**Objective**  
To determine whether familial MNG with or without SLCT in the absence of PPB was associated with mutations in DICER1.

**Design, Setting, and Patients**  
From September 2009 to September 2010, we screened 53 individuals from 2 MNG and 3 MNG/SLCT families at McGill University for mutations in DICER1. We investigated blood lymphocytes and MNG and SLCT tissue from family members for loss of the wild-type DICER1 allele (loss of heterozygosity), DICER1 expression, and microRNA (miRNA) dysregulation.

**Main Outcome Measure**  
Detection of germline DICER1 gene mutations in familial MNG with and without SLCT.

**Results**  
We identified and characterized germline DICER1 mutations in 37 individuals from 5 families. Two mutations were predicted to be protein truncating, 2 resulted in in-frame deletions, and 1 was a missense mutation. Molecular analysis of the 3 SLCTs showed no loss of heterozygosity of DICER1, and immunohistochemical analysis in 2 samples showed strong expression of DICER1 in Sertoli cells but weak staining of Leydig cells. miRNA profiling of RNA from lymphoblastoid cell lines from both affected and unaffected members of the familial MNG cases revealed miRNA perturbations in DICER1 mutation carriers.

**Conclusions**  
DICER1 mutations are associated with both familial MNG and MNG with SLCT, independent of PPB. These germline DICER1 mutations are associated with dysregulation of miRNA expression patterns.

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in the generation of microRNAs (miRNAs), short, double-stranded, non-coding RNAs that modulate gene expression at the posttranscriptional level.12,13 Relevant to this function are 2 RNase III domains and a PAZ domain, which is a single-stranded RNA binding domain that binds the 3' single-stranded overhang of the precursor miRNA and whose distance from the RNase III domains determines the length of mature miRNAs generated (FIGURE 1).14 We hypothesized that DICER1 mutations could be present in kindred with multiple cases of MNG (with and without SLCTs). We subsequently explored the phenotypic and molecular consequences of the DICER1 mutations observed.

**METHODS**

We used the following criteria to identify cases and families: 3 or more cases of MNG, 2 or more cases of MNG plus 1 case of SLCT, or MNG and SLCT occurring in the same individual. Cases were identified through PubMed searches and through patients referred to the McGill University Cancer Genetics program. Members of 2 MNG/SLCT families described in previous publications15,16 were contacted through the authors (families A and B). Another case of MNG/SLCT (family C) was identified through a local clinician. Histopathological material was reviewed from the MNG cases (V.-H.N., H.R.H., and D.B.-D.S.), the SLCT cases (D.B.-D.S. and J.A.), and the rhabdomyosarcoma case (V.-H.N.). Diagnoses of MNG and SLCT were made by examining tissue slides stained with hematoxylin-eosin. The rhabdomyosarcoma diagnosis was made as previously described.17 Families D and E were described previously as MON236 and MON152, respectively.14,17,18, updated details including newly affected family members are presented in TABLE 1.

The study was approved by the McGill University institutional review board. Each family member provided written informed consent. For minors, the risks and benefits were explained to the parents and they signed on behalf of their children. A larger series of germline DNA from 80 anonymous cases of MNG and differentiated thyroid cancer from centers in the United Kingdom, Italy, and Montreal were screened for DICER1 mutations. Anonymous control DNA samples were used from individuals with no history of cancer who attended the Jewish General Hospital in Montreal between June and September 2009 and who provided written consent. For the immunohistochemical (IHC) analysis, 28 anonymized nonhereditary MNG tissue samples from Hospital Dr. Ohtavía, Salta, Argentina, were used as controls.

**Mutation Analysis**

The 26 coding exons of DICER1 were screened in 80 cases of differentiated thyroid cancer and MNG and probands from 5 families by high-resolution melting analysis using the LightScanner instrument (Idaho Technologies, Salt Lake City, Utah) or sequencing. Primer sequences and the protocol for amplification were adapted from Hill et al10 with some primers being changed to generate smaller amplicons necessary for high-resolution melting (eTable 2 and eMethods). Nontruncating mutations were further investigated by complementary DNA (cDNA) analysis (eMethods).

**RNA Analysis**

Total RNA was extracted from independent cultures of lymphoblastoid cell lines (LCLs) from 9 mutation carrier individuals (family A: II-2; family B: II-1, II-2; family C: III-3; family D: I-5, II-1, III-1; family E: II-4, III-3), 5 noncarriers (3 unrelated spouses from families D and E and 2 unrelated controls) using RNAasy Kit (Qiagen, Valencia, California). Messenger RNA (mRNA) was retrotranscribed using an oligo-dT and Superscript III reverse transcriptase (Invitrogen, Carlsbad, California). Expression of DICER1 mRNA was measured by quantitative real-time polymerase chain reaction. Predesigned TaqMan assays (Applied Biosystems, Foster City, California) were used to specifically amplify cDNA derived from both mutant and wild-type DICER1 and GAPDH mRNAs. Inhibition of nonsense-mediated mRNA decay (NMD) was performed using cycloheximide as previously described.19 NMD is a regulatory process used by the cell to specifically recognize and destroy mRNA transcripts that carry premature termination codons before their translation into truncated and potentially harmful proteins. This process is blocked by protein synthesis inhibitors such as cycloheximide. Full experimental details are provided in the eMethods.

**miRNA Profiling**

miRNA profiling of LCLs from 5 mutation carriers (family D: I-5, II-1, III-1; family E: II-4, III-3) and 5 noncarriers
DICER1 MUTATIONS IN FAMILIAL MULTINODULAR GOITER

(3 unrelated spouses from families D and E, 1 mutation-negative member of family D, II-14, and 1 unrelated control) was performed by the Vancouver Prostate Centre Microarray Facility. Total RNA including the small RNA fraction was isolated and quantified. The quality of the RNA was confirmed prior to fluorescent end-labeling and hybridization to the Unrestricted Human miRNA Microarrays Release 12.0 (design ID 021827) using the Agilent miRNA Microarray System with miRNA Complete Labeling and Hyb Kit version 2 (Agilent Technologies, Santa Clara, California). Following hybridization, the microarrays were scanned, quantified, and analyzed using Agilent system software components (eMethods). Data was normalized by setting values below 0.05 to 0.05 and applying per-chip normalization to a set of positive control genes that were selected with the criteria that raw data were above 20 for all samples. Lists of significantly differentially expressed genes were determined by both a t test

Table 1. Details of Tested Individuals in This Study

<table>
<thead>
<tr>
<th>(Family) Mutation Identified</th>
<th>Individualb</th>
<th>Sex</th>
<th>Disease Status (Age at Diagnosis, y)</th>
<th>Mutation Status</th>
<th>LOH (Tissue)c</th>
<th>IHC (Tissue)c</th>
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(continued)
between the 2 conditions (mutation carrier vs not a mutation carrier) with the P value set at .05 and fold-change filtering with a fold-change value of 2.0. The heatmap was created by Cluster 3.0 and Java gene Treeview software. 20,21

Frozen tissue samples (2 nodules of 1 goiter from a DICER1 mutation carrier) were homogenized in lysis buffer and total RNA was prepared. RNA extracted from normal thyroid tissue (Applied Biosystems/Ambion, Austin, Texas) was used as a control. One microgram of total RNA was reverse transcribed and the cDNA was applied on a TaqMan low-density array, with data acquisition via the Applied Biosystems 7900HT Fast Real-time polymerase chain reaction system. Data analysis was performed using the R analysis software (Applied Biosystems). Individual miRNA expression was validated using TaqMan miRNA Assays (Applied Biosystems), and values were normalized to the endogenous control U6 snRNA (the noncoding small nuclear RNA part of U6 small nuclear ribosomal ribonucleoprotein). Fold change was calculated using the DDCT method. 22 Further experimental details are provided in the eMethods.

**LOH and IHC Analyses**

DNA was extracted from lymphocytes from the probands and, where available, from macro-dissected, formalin-fixed, paraffin-embedded tumor and goiter tissue. Polymerase chain reaction and evaluation of loss of heterozygosity (LOH) was carried out as previously described. 23 Further details are provided in the eMethods.

IHC analysis was performed on deparaflinized 5-µm tissue sections incubated with anti-DICER antibody ab14601 (1:50 for goiters and the rhabdomyosarcoma, 1:100 for SLCTs) (Abcam, Cambridge, Massachusetts). A total of 28 tissue samples of sporadic MNG, 9 samples of DICER1-related MNG (family B: II-1, II-2; family D: II-1, II-2, III-1, III-5; family E: II-1, II-4, III-3), 2 samples of SLCTs (family B: II-2; family C: III-1), and 1 rhabdomyosarcoma sample (family E: II-3) were analyzed. The sections were then incubated with labeled polymer-HRP antinoum immunoglobulins (Dako Envision + system-HRP; Dako, Glostrup, Denmark) for 30 minutes. Staining was completed by a 10-minute incubation with 3,3′-diaminobenzidine plus substrate-chromogen solution (DAB + ). Counterstain was performed by dipping sections in a bath of hematoxylin for 5 seconds.

**RESULTS**

**DICER1 Mutation Analysis**

We searched for DICER1 mutations in 53 members from the 3 studied families with MNG and/or SLCT and identified, in total, 37 mutation carriers in these families, all but 3 of them being affected by MNG and/or SLCT. Representative electrophoreograms are shown in eFigure 1 and results are summarized in Table 1. Among the 3 MNG/SLCT families, family A was initially described in 1981 as an MNG/SLCT family where the proband (family A: II-2) had MNG at age 16 years and SLCT at age 18 years. 16 The proband and 3 other family members carried a c.871_874delAAAG mutation, which results in an mRNA product that contains a premature termination codon at position p.291. mRNA carrying the mutation cannot be detected because of the action of NMD (eFigure 2A). Family B was a recently reported MNG/SLCT family 21 in whom we identified a c.2457C>T mutation. This new mutation carries a premature termination codon at position p.291. mRNA carrying the mutation cannot be detected because of the action of NMD (eFigure 2B). This new mutation generates a predicted DICER1 protein with a p.Ile813_Tyr819del mutation resulting in an altered PAZ struc-

**Table 1. Details of Tested Individuals in This Study (continued)**

<table>
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<tr>
<th>(Family) Mutation Identified a</th>
<th>Individual b</th>
<th>Sex</th>
<th>Disease Status (Age at Diagnosis, y)</th>
<th>Mutation Status</th>
<th>LOH (Tissue) c</th>
<th>IHC (Tissue) c</th>
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Abbreviations: F, female; IHC, immunohistochemical analysis; LOH, loss of heterozygosity; M, male; MNG, multinodular goiter; RMS, rhabdomyosarcoma; SLCT, Sertoli-Leydig cell tumor of the ovary; U, exact age at diagnosis not known.

a. c is a DNA coding sequence–based nomenclature; numbering commences from the start codon. r. is RNA-based, indicating the effect of the mutation on the messenger RNA.

b. Roman numerals refer to generations; Arabic numerals refer to individuals within a generation (ie, siblings and cousins).

c. No indicates LOH was not present. Absence of text indicates that the tissue was not analyzed either for LOH or for IHC.

d. The proband was untested but is an obligate mutation carrier.

e. We were unable to unequivocally distinguish between the alveolar and embryonal subtypes of RMS.
ture (Figure 2; interactive feature available at http://www.jama.com). This mutation was detected in 3 individuals, including the proband (family B: II-2), who developed an SLCT at age 14 years. In family C, the proband (family C: III-1) had MNG at age 18 years and SLCT at age 32 years, with no family history of cancer or MNG. She and her unaffected mother carried a c.5018_5021delTCAA mutation, which results in an mRNA product that is subject to NMD because it contains a premature termination codon at position p.1672 (eFigure 2A).

DICER1 mutations were also found in both MNG families previously linked to chromosome 14q (families D and E), but not in germline DNA from 71 individuals with differentiated thyroid cancer, 31 of whom also had MNG, and a further 9 individuals with MNG, all of whom had a family history of MNG and differentiated thyroid cancer (Table 2). In family D, we identified a missense DICER1 variant, c.2516C>T, predicted to cause a p.Ser839Phe change in 20 family members, all of whom had MNG; there were no cases of MNG in any of the 10 family members without the variant. Taken together, these observations indicate that p.Ser839Phe is very highly penetrant for MNG. The variant was not present in DNA from 455 anonymous controls with no history of cancer from the Jewish General Hospital in Montreal. On the basis of a prevalence of MNG in the population of 4%,1 and assuming a mutation frequency of 0.005, the probability of segregation of this mutation with MNG in family D purely by chance is approximately $10^{-5}$.

Serine 839 is conserved in DICER1 proteins of higher vertebrates (eFigure 3), and predictive software was used to help establish the overall effect of the p.Ser839Phe mutation present in family D. According to the probabilistic program SIFT (Sorting Intolerant From Tolerant, http://sift.jcvi.org/), which predicts effects of a substitution based on sequences of similar peptides, p.Ser839Phe was assigned a score of 0.05. This is the limit of normal because the SIFT scale designates scores of less than 0.05 as deleterious while scores of 0.05 or greater are predicted to be tolerated.25 Similarly, Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) predicted this mutation was “possibly damaging” (score, 0.442), because the score was between 0.16 and 0.85. The Ser to Phe change was predicted to disrupt an alpha helix in the PAZ domain (Figure 2).

The second MNG kindred (family E) carried a fully segregating splice-site mutation, c.2805-1G>T, resulting in an in-frame deletion of exon 18, r.2805_2987del183 (eFigure 2C), altering the structure of DICER1 by eliminating part of the PAZ domain (eFigure 4). This mutation was not seen in germline DNA from 430 controls.

RNA and Protein Analysis

We analyzed mRNA and protein extracted from LCLs of 7 carriers from families B, D, and E (with nontruncating mutations), 2 carriers from families A and C (with NMD-sensitive muta-

Table 2. Additional MNG and DTC Cases Screened for DICER1 Mutationsa

<table>
<thead>
<tr>
<th>Center Location</th>
<th>No. of Cases</th>
<th>Diagnoses</th>
<th>Median Age (Range), y</th>
<th>Family History</th>
</tr>
</thead>
<tbody>
<tr>
<td>United Kingdom</td>
<td>44</td>
<td>40 DTC only, 4 DTC and MNG</td>
<td>43 (4-60)</td>
<td>17 cases had ≥1 relative with DTC or MNG</td>
</tr>
<tr>
<td>Italy</td>
<td>22</td>
<td>9 MNG only, 13 DTC and MNG</td>
<td>45 (23-66)</td>
<td>All had family history of MNG and DTC; 1-22 cases of MNG per family, 1-4 cases of DTC per family</td>
</tr>
<tr>
<td>Montreal, Canada</td>
<td>14</td>
<td>14 DTC and MNG</td>
<td>50 (23-71)</td>
<td>2 cases had ≥1 relative with DTC or MNG</td>
</tr>
</tbody>
</table>

Abbreviations: DTC, differentiated thyroid cancer; MNG, multinodular goiter.

aAll cases were screened for DICER1 mutations by high-resolution melting.

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DICER1 mutations in familial multinodular goiter

LOH and IHC Studies

Studies of LOH using DNA extracted from goiters from families B, D, and E, together with constitutional DNA, revealed no evidence of LOH (Table 1, Figure 4). IHC analysis of these goiters using anti-DICER1 antibody ab14601 revealed a mixed picture, with no staining of 2 goiters from family B (II-1 and II-2), but clear cytoplasmic staining of several goiters from families D and E (individual III-1 from family D is shown in Figure 5). To compare these findings with those seen in nonfamilial goiter, we immunostained tissue samples of 28 sporadic MNG from Hospital Dr. A. Oñatibia, Salta, Argentina. Twenty-five of these MNG samples stained with the anti-DICER1 antibody (Figure 5) whereas 3 showed no staining. Therefore, the amount of DICER1 protein did not appear to be associated with DICER1 mutation status, similar to what we observed in LCL RNA and protein.

SLCT tissue from the 3 DICER1 mutation carrier probands in families A through C was analyzed for LOH, and in each case there was no evidence of loss of the wild-type allele (Table 1, Figure 4, and eFigure 5A). IHC analysis of the 2 available SLCTs from DICER1 mutation carriers showed intense expression of DICER1 in Sertoli cells, but the staining was much weaker in Leydig cells (Table 1, Figure 5, and eFigure 5B). By contrast, neither ovarian carcinomas (n = 5, not shown) nor normal ovary (Figure 5) stained with the ab14601 anti-DICER1 antibody.

One DICER1 mutation carrier (family E: II-3) died at age 20 years from a malignant paravertebral tumor characterized at the time as an alveolar rhabdomyosarcoma (molecular reconfirmation of this diagnosis was impossible due to lack of tissue). Alveolar rhabdomyosarcoma has not been observed in DICER1-related PPB families, although unusual sarcomas are characteristic. No LOH was seen in this tumor (eFigure 5A) and IHC analysis revealed diffuse cytoplasmic staining (eFigure 5B).

miRNA Assays

We used RNA extracted from LCLs established from 5 carriers and 4 mutation-negative controls, all from families D and E (plus 1 unrelated control) for the miRNA assays. Global miRNA profiling of a panel of 851 human miRNAs identified 94 miRNAs (11%) that were significantly differentially expressed in the 5 affected DICER1 mutation carriers compared with the 5 unaffected noncarriers (fold change ≥2 and P < .05).

DICER1 expression was measured in lymphoblastoid cell lines derived from carriers and noncarriers from families A through E and from 2 unrelated noncarriers by real-time polymerase chain reaction. There are no significant differences in the level of DICER1 expression when comparing mutation carriers and noncarriers (P = .55) and when comparing carriers of truncating mutations (families A and C) and carriers of nontruncating mutations (families B, D, and E) (P = .49). The identity of each individual carrier is indicated on the x-axis; Con1 through Con5 are noncarrier controls. Error bars indicate 95% confidence intervals.

There is no loss of heterozygosity in Sertoli-Leydig cell tumor (SLCT) and multinodular goiter tissue from the affected proband (individual II-2, family B). A and B were sequenced simultaneously, and C and D were sequenced simultaneously at a different time. gDNA indicates genomic DNA; arrowhead indicates mutation. Nucleotides labeled with N indicate sites where dual peaks are present.
(Figure 6). We then compared miRNA profiles in the RNA extracted from 1 fresh-frozen DICER1-related MNG with normal thyroid tissue to determine miRNAs differentially expressed in the MNG and the normal tissue. Comparing the 2 lists, only 5 miRNAs derived from the same precursors (miR-345, let-7a, miR-99b, miR-133, miR-194) were decreased in RNA from both LCLs and the DICER1-related MNG. Of these, only miR-345 is highly expressed uniquely in the normal thyroid.\(^2^7\) We focused on let-7a and miR-345, and markedly lower levels of both miRNAs were seen in the DICER1-related goiter, when compared with both normal thyroid gland tissue and a follicular thyroid carcinoma (Figure 7).

**COMMENT**

Here we report germline DICER1 mutations in familial MNG and MNG/SLCT families. In the 2 larger families with MNG, the DICER1 mutations were highly penetrant for goiter. MNG is prevalent in the general population and also occurs within the wide spectrum of conditions occurring in kindred with PPB.\(^1^0,2^8\) Germline DICER1 mutations are found in more than half of all children with PPB and most of the mutations identified are predicted to result in truncated proteins.\(^9,2^9\) The penetrance of the reported DICER1 mutations for PPB (and the other major clinical characteristics of the syndrome) is low, with many gene carriers remaining unaffected into adulthood. By contrast, the 3 nontruncating mutations reported here (eFigure 1) were highly penetrant for MNG. Moreover, the absence of all other known features of the PPB-FTDS in the large family D kindred with 4 generations of affected individuals suggests that the functional effect of the p.Ser839Phe mutation is qualitatively different from that associated with truncating mutations. Similarly, the mutation in family E resulted in a DICER1 protein that lacks most of the PAZ domain (eFigure 4) but was otherwise normal. In family B, in which 3 individuals developed early-onset MNG, the PAZ domain was significantly altered by the in-frame deletion of 7 amino acids.

Two of the 3 DICER1 mutations seen in the MNG/SLCT families were truncating and distributed throughout the gene with no clear genotype-phenotype correlation differentiating them from those PPB-FTDS families with reported DICER1 mutations\(^9\) (Figure 6). The median diagnosis age of 3 DICER1-related SLCTs in this study and 6 reported PPB-associated SLCTs\(^1^1\) was 13 years, considerably younger than median age at onset of 19 years in sporadic SLCTs\(^7\) (P =.009, Mann-Whitney test).

The disease spectrum associated with PPB is broad,\(^1^0\) and we have confirmed by molecular means that SLCT belongs in the PPB-FTDS but can occur without PPB. It is likely that most of the reported MNG/SLCT cases (eTable 1) harbor DICER1 mutations and that our findings explain the observation first made by Jensen et al.\(^3^0\) Familial SLCT cases have also been reported, but whether this results from DICER1 mutations is not answered by our study because the families described here had only 1 case of SLCT per kindred. We did screen an affected familial SLCT proband\(^3^1\) but did not identify a DICER1 mutation; it remains possible that DICER1 mutations will be implicated in other familial SLCT.

**Figure 6. Immunohistochemistry of Multinodular Goiter and Sertoli-Leydig Cell Tumors**

A MNG (sporadic case)  
B MNG (III-1, family D)  
C Normal ovary  
D SLCT (III-2, family C)

Representative staining of DICER1 (A) in multinodular goiter (MNG) from the sporadic cases (Hospital Dr A. Oñativia, Salta, Argentina) and (B) from a mutation carrier (individual III-1, family D). Images are at 20× magnification. Tissue sections were incubated with anti-DICER antibody (1:50), labeled with polymer-HRP antiflourescein, stained with DAB++, and counterstained with hematoxylin. DICER1 staining (reddish brown) is detected in cells bordering the vesicles and is heterogeneous, being partly dependent on the amount of cytoplasm. No difference in staining is evident when comparing hereditary and sporadic cases of MNG. Representative staining of DICER1 in normal ovary (C) compared with the Sertoli-Leydig cell tumors (SLCTs) (D) from individual III-2, family C (see also eFigure 5 for the DICER1 immunohistochemical study of the SLCTs from individual II-2, family B). The tumor is composed of prominent cords and tubules of immature Sertoli cells with discrete clusters of Leydig cells. The Leydig cells contained a round nucleus with prominent nucleolus and an abundant pale and eosinophilic cytoplasm. The immature Sertoli cells have small round nuclei and scanty cytoplasm. Cytoplasm of Sertoli cells was strongly stained and Leydig cells show very weak staining. No staining was detected in normal ovary.
or that there are other genes responsible for familial SLCT with genes in the miRNA processing pathway, such as PASHA/DGCR8 or DROSHA, being reasonable candidates.

We also analyzed DICER1 in germline DNA from probands from large series of differentiated thyroid cancer cases with and without MNG, and no potentially disease-causing variants were identified. Although in general differentiated thyroid cancer cases appear not to be associated with DICER1 mutations, differentiated thyroid cancer cases in the context of SLCT or PPB may harbor DICER1 mutations.

None of the DICER1-related SLCTs showed evidence of LOH, and even though the numbers are small, these findings are consistent with the notion from animal models that DICER1 does not function as a classic tumor suppressor gene but that instead tumors develop as a result of miRNA dysregulation through a possible haploinsufficiency effect.32 The lack of correlation, however, between DICER1 mutation status and both mRNA and protein levels of DICER1 we observed here (Figure 3) suggests that mecha-

**Figure 6.** miRNA Microarray Study in DICER1 Mutation Carriers and Noncarriers

Microarray study showing differentially expressed microRNAs (miRNAs) between carriers and noncarriers of mutations in DICER1 from families D and E. From left to right, carriers of mutation are family E, individuals III-3 and II-4, and family D, individuals I-5, II-1, and III-1. Noncarriers are family D, individual II-15, and 3 unrelated (married-in) controls from families D and E. Unsupervised hierarchical clustering showed clustering of the samples according their mutation status. Cluster 3.0 software was used, wherein miRNA expression values were median-centered, normalized, and clustered using Pearson uncentered correlation distance and average linkage. The heatmap was divided in 3 consecutive panels from left to right. In those cases where a miRNA hairpin precursor gives rise to 2 miRNAs, the less predominant miRNA is indicated by an asterisk. The color scale indicates the magnitude of miRNA expression in log2, with green indicating low expression and red high expression.
We observed that selective DICER1 cut the pre-miRNAs to their determining where the RNase domains of TTF1 twin between PAZ domain function and the disruption rather than a true haploins-phenotype is the result of specific PAZ disruptions do not represent hypomorphic protein, is associated with familial nuclear ribosomal ribonucleoprotein.

miR-345 and let-7a are down-regulated in goiter tissue from individual III-3 from family E compared with normal thyroid and follicular thyroid cancer (FTC) tissues from noncarriers of DICER1 mutations. Error bars indicate 95% confidence intervals; U6 snRNA indicates the noncoding small nuclear RNA part of U6 small nuclear ribosomal ribonucleoprotein.

miRNAs have a crucial role in human DICER1 mutation carriers may be complex and may differ between tissues. DICER1 has several highly conserved domains, including the PAZ domain (Figure 1, Figure 2, and eFigure 4), which appears to be critical for DICER1 function: a purified C-terminal fragment of DICER1 containing both RNase domains and double-strand RNA binding domain showed no double-strand RNA cleavage activity.33 PAZ acts as a molecular ruler, determining where the RNase domains of DICER1 cut the pre-miRNAs to their final size.34 We observed that selective disruption of the PAZ domain, in a setting of an otherwise normal DICER1 protein, is associated with familial MNG. It is possible that these mutations do not represent hypomorphic DICER1 alleles, but instead the goiter phenotype is the result of specific PAZ disruption rather than a true haploinsufficiency. This might indicate the presence of a close relationship between PAZ domain function and the regulation of thyroid development. In a similar vein, a single missense mutation in the thyroid transcription factor TTF1 results only in thyroid disease,35 whereas loss of an entire allele, in both humans and in animal models, results in much more serious phenotypes.3

In summary, we report DICER1 mutations to be associated with both autosomal-dominant susceptibility to MNG and SLCT occurring with MNG. This latter association was first observed more than 30 years ago by Jensen and colleagues.30 Our study confirms clinical observations10,11 and definitively extends the tumor spectrum of DICER1 mutation beyond PPB, cystic nephroma, embryonal rhabdomyosarcoma, and lung cysts.9,41 Further, mutations in other genes in the miRNA processing pathway may explain some of these syndromic disease combinations. Unlike SLCT, MNG is a very common condition worldwide1 and determining the role of dysregulated miRNA processing in the development of sporadic MNG could be an important avenue for future research.

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Figure 7. Quantification of Expression Levels of MicroRNAs let-7a and miR-345 by Real-Time Polymerase Chain Reaction in Frozen Tissues

miR-345 and let-7a are down-regulated in goiter tissue from individual III-3 from family E compared with normal thyroid and follicular thyroid cancer (FTC) tissues from noncarriers of DICER1 mutations. Error bars indicate 95% confidence intervals; U6 snRNA indicates the noncoding small nuclear RNA part of U6 small nuclear ribosomal ribonucleoprotein.
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