INTRODUCTION

**DICER1** (OMIM 606241) is an RNaseIII endonuclease that is crucial for processing pre-miRNA into active mature miRNA. The **DICER1** syndrome is an autosomal dominant cancer predisposition disorder that arises from pathogenic germline variation in **DICER1** and is associated with a variety of benign and malignant tumors, including pleuropulmonary blastoma (PPB), cystic nephroma (CN), Sertoli-Leydig cell tumors (SLCT), multinodular goiter (MNG), thyroid cancer, rhabdomyosarcoma, and pineoblastoma (Doros et al., 2014; Hill et al., 2009). **DICER1**-associated tumors harbor somatic variation at amino acid “hotspot” residues located in the RNaseIIIb metal binding domain (E1705, D1709, G1809, D1810, E1813) is observed in **DICER1**-associated tumors. Previously, we found the prevalence of germline pathogenic **DICER1** variation in the general population is 1:10,600. In this study, we investigated the prevalence of pathogenic **DICER1** germline variation in The Cancer Genome Atlas (TCGA; 32 adult cancer types; 9,173 exomes) and the Therapeutically Applicable Research to Generate Effective Treatment (TARGET; two pediatric cancer types; 175 exomes) cohorts.

**Methods:** All datasets were annotated and binned into four categories: pathogenic, likely pathogenic, variant of unknown significance, or likely benign.

**Results:** The prevalence of **DICER1** pathogenic variants was 1:4,600 in TCGA. A single participant with a uterine corpus endometrial carcinoma harbored two pathogenic germline **DICER1** (hotspot and splice-donor) variants, and a single participant with a rectal adenocarcinoma harbored a germline **DICER1** stop-gained variant. In the smaller TARGET dataset, we observed no pathogenic germline variants.

**Conclusion:** This is the largest comprehensive analysis of **DICER1** pathogenic variation in adult and pediatric cancer populations using publicly available data. The observation of germline **DICER1** variation with uterine corpus endometrial carcinoma merits additional investigation.

**Keywords**
cancer population, **DICER1**, **DICER1** syndrome, prevalence estimate, TARGET, TCGA
G1809, D1810, and E1813), where alteration disrupts enzymatic function (Anglesio et al., 2013; Heravi-Moussavi et al., 2012; Pugh et al., 2014; Seki et al., 2014). An unusual variation on Knudson's two-hit hypothesis is observed in DICER1-associated tumors: typically, the germline copy is a loss-of-function variant and the wild-type copy is disrupted by a somatic hotspot variant that confers some retained function (Brenneman et al., 2015).

In previous work, we developed a scheme to classify germline DICER1 variation modelled after the joint consensus recommendations of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (Kim, Field, Schultz, Hill, & Stewart, 2017). We applied this approach to germline DICER1 variation from a variety of publicly available exome databases. In the largest (the portion of the Exome Aggregation Consortium excluding The Cancer Genome Atlas samples; n = 53,103 exomes), we found the prevalence of DICER1 pathogenic variation to be ~1/10,600, which was more common than expected (Kim et al., 2017). To better understand the neoplasia phenotype associated with DICER1 variation, we now quantify the frequency of DICER1 variation in cancer populations. In this study, we apply our published pathogenicity classification and investigate the prevalence of pathogenic germline (and somatic, when available) DICER1 variants in publicly available genome datasets from cancer cohorts.

2 | MATERIALS AND METHODS

2.1 | The Cancer Genome Atlas (TCGA) datasets

The Cancer Genome Atlas used comprehensive genomic analyses to characterize germline and somatic variants in 32 adult tumor types (total: 9,173; http://portal.gdc.cancer.gov). Germline DICER1 variation was downloaded from the Genomic Data Commons (GDC) application programming interface (API). BAM slicing of every subject with a blood-derived normal sample, excluding acute myeloid leukemia (175 exomes, two cancer types, accessed 12/19/17) was performed.

2.2 | Therapeutically applicable research to generate effective treatments (TARGET) datasets

The TARGET study used comprehensive genomic analyses to characterize germline and somatic variants; data are available for two pediatric tumor types (total: 175). Germline DICER1 variation was extracted from the GDC API. BAM slicing of every subject with a blood-derived normal sample, excluding acute myeloid leukemia (175 exomes, two cancer types, accessed 12/19/17) was performed.

2.3 | Cancer variation resource (CanVar) publicly available dataset

CanVar used comprehensive genomic analyses to characterize germline variants in familial early-onset colorectal cancer patients (total: 1,006). Germline DICER1 variation was queried on http://canvar.icr.ac.uk (accessed 2/8/17).

2.4 | Germline variant calling

Germline variants were called jointly by each cancer type using three different germline callers (GATK UnifiedGenotyper, GATK HaplotypeCaller, and FreeBayes), then assembled using bcbio-nextgen (https://github.com/chapmanb/bcbio-nextgen). Variants called with at least two callers were used in the analysis.

2.5 | Variant annotation, filtering, and classification

All exonic and splice-site region (<10 intronic base pairs from intron/exon boundary) variants from the canonical DICER1 transcript (NM_177438.2), including missense, frameshift, nonsense, and synonymous variants, were included. Multi-allelic, deep intronic, and UTR variants were excluded in this analysis to focus on the protein-coding regions. SnpEff (Cingolani et al., 2012) was used to predict pathogenicity in silico, obtain population allele frequencies from different databases, and obtain previously reported variants from ClinVar (2017-01-25 version). Annotation by ClinVar and the Human Gene Mutation Database (HGMD, Qiagen and Institute of Medical Genetics, Cardiff, Wales, UK; version Professional 2017.1) was used to identify previously recognized and interpreted variants.

We used our published scheme to classify variants into pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), and likely benign (LB) categories (Kim et al., 2017). In brief, variants that are loss-of-function (LOF; e.g., nonsense and frameshift), located in the canonical splice site (≤2 intronic basepairs from the intron/exon boundary), missense located in DICER1 hotspots (e.g., E1705, D1709, G1809, D1810, E1813) or credibly reported as pathogenic in at least one publication are classified as P. Classification as LP required a nonsynonymous missense variant to be outside a DICER1 hotspot locus and harbor a bioinformatics pathogenicity prediction of “Deleterious” by metaSVM, a REVEL (Ioannidis et al., 2016) score >0.75, or a CADD (Kircher et al., 2014) score >30 (top 0.01% of the predicted
deleteriousness). In somatic data, we considered loss-of-function and hotspot variation to be pathogenic.

2.6 | Pathology review

Digital slides from tumors in TCGA DICER1 P/LP carriers were subjected to review by an expert pathologist in DICER1-associated tumors (DAH). Hematoxylin- and eosin-stained images were obtained from cbiportal.org Cancer Digital Slide Archive (accessed 9/22/17 from the cbiportal.org tissue resource).

2.7 | Ethical compliance

This study was performed using publicly available, peer-reviewed, published datasets. No additional human-subjects were involved.

3 | RESULTS

3.1 | Classification of unique germline DICER1 variants

We identified 219 (TCGA), 24 (TARGET), and 11 (CanVar) unique exonic and splice-site region DICER1 variants (Tables 1 and Table S2). Most of the variants were classified as VUS and LB. For P/LP variation, 12 unique missense variants, one hotspot missense, one stop-gained, and one splice-donor were found in 17 individuals in TCGA (one person carried two pathogenic variants, p.Asp1810Asn [hotspot] and c.4206+1G>C [splice]). In TARGET, there was one unique missense variant, and one splice-donor variant found in two carriers; no P/LP DICER1 variants were observed in CanVar.

| TABLE 1 | Overview of The Cancer Genome Atlas (TCGA) DICER1 unique variants |
|-----------------|-------------------|-----------------|-------------------|
| TCGA (9,173 exomes) | | | |
| Total unique variants | 219 | | |
| P | 1 hotspot | 1 stop-gained | 1 splice-donor |
| LP | metaSVM | CADD | REVEL |
| | 9 missense | 5 missense | 1 missense |
| VUS | 18 splice regions | 1 in-frame deletion | 1 missense |
| LB | metaSVM | CADD | REVEL |
| | 84 syn | 84 syn | 84 syn |
| | 103 missense | 107 missense | 111 missense |

Note. LB, likely benign; LP, likely pathogenic; P, pathogenic; syn, synonymous; VUS, variant of unknown significance.

3.2 | Prevalence of pathogenic germline DICER1 variants: TCGA

Of 32 cancer types available through TCGA (n = 9,173), we found 10 types (breast invasive carcinoma, bladder urothelial carcinoma, cervical squamous cell carcinoma and endocervical adenocarcinoma, lymphoid neoplasm diffuse large b-cell lymphoma, thyroid carcinoma, head and neck squamous cell carcinoma, lung adenocarcinoma, rectum adenocarcinoma, ovarian serous cystadenocarcinoma and uterine corpus endometrial carcinoma) in 18 subjects that harbored a total of 16 unique (19 total including two in one person) germline P/LP DICER1 variants (Table 2).

Of the 16 P/LP germline DICER1 variants in the TCGA dataset, 13 were unique missense alterations classified as LP, where in silico methods predicted a deleterious effect. Considering the in silico prediction tools separately, there were nine variants with a metaSVM score of “Deleterious,” five variants with a CADD score >30, and one variant with a REVEL score of >0.75. Of these 16 LP DICER1-carriers, we found one patient with a uterine corpus endometrial carcinoma who harbored a p.Ala1578Thr germline DICER1 variant and a somatic DICER1 hotspot variant (p.Asp1709Asn). In addition, another subject with a uterine corpus endometrial carcinoma harbored two germline P DICER1 variants: a splice-donor c.4206+1G>C and an RNase IIIb missense (hotspot) p.Asp1810Asn. Thus in TCGA, the prevalence of DICER1 P/LP was 1:700, 1:1,500, and 1:3,100 by metaSVM, CADD, and REVEL, respectively. Using a more stringent calculation by only considering P variants, the prevalence of DICER1 P was ~1:4,600 (one subject with rectum adenocarcinoma and one subject with uterine corpus endometrial carcinoma; Table 3).

3.3 | Prevalence of pathogenic germline DICER1 variants: TARGET

Of two cancer types (neuroblastoma [142 subjects] and Wilms tumor [33 subjects]) available through TARGET, we found only two subjects harboring LP DICER1 variation, one with neuroblastoma and one with Wilms tumor (Tables S2 and S3), with a metaSVM score of “Deleterious” for two variants and CADD score >30 for one variant. Thus, in the TARGET cohort, the prevalence of DICER1 P/LP was 1:88 by metaSVM and 1:175 CADD; using REVEL, no LP variants were found.

4 | DISCUSSION

In this study, for the first time, we have comprehensively quantified the prevalence of P/LP germline DICER1 variation in the largest publicly available sporadic adult and
| Cancer type (TCGA abbreviation; n) | Gender | Age at diagnosis (years) | Race | DICER1 germline | DICER1 somatic | Frequency of variant in database | Vital status |
|-----------------------------------|--------|-------------------------|------|-----------------|---------------|-----------------------------|-------------|
|                                   |        |                         |      |                 |               | ExAC non-TCGA | ESP | 1,000 genomes |                                      |
| Breast invasive carcinoma (BRCA; 966) | Female | 43                      | White | p.Gly1824Val | None           | None | 7.7 × 10^{-5} | None | Living/disease free |
| Bladder urothelial carcinoma (BLCA; 394) | Female | 82                      | Asian | p.Ser1160Tyr | None           | 4.71 × 10^{-5} | None | None | Living/disease free |
|                                       | Male   | 70                      | White | p.Ala1578Thr  | None           | None | None | None | Living/recurred/progressed Brother colon cancer |
| Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC; 300) | Female | 62                      | White | p.Leu1469Pro  | None           | 1.89 × 10^{-5} | None | 3.99×10^{-4} | Living/disease free |
|                                       | Female | 72                      | Asian | p.Ser1160Tyr | None           | 4.71 × 10^{-5} | None | None | Living/disease free |
| Lymphoid neoplasm diffuse large B-cell lymphoma (DLBCL; 44) | Male | 36                      | White | p.Ile528Thr  | None           | 1.88 × 10^{-4} | 2.0 × 10^{-4} | None | Living/disease free |
| Thyroid carcinoma (THCA; 431) | Female | 64                      | N/A | p.Pro1836Leu | None           | None | None | None | Living/disease free |
| Rectum adenocarcinoma (READ; 153) | Female | 62                      | N/A | p.Glu904*    | None           | None | None | None | Living/disease free |
| Head and neck squamous cell carcinoma (HNSC; 509) | Male | 61                      | White | p.Tyr1835Ser | None           | None | None | None | Living/disease free |
|                                      | Male   | 48                      | AA   | p.Ile528Thr  | None           | 1.88 × 10^{-5} | 2.0 × 10^{-4} | None | Living/disease free |
| Lung adenocarcinoma (LUAD; 441) | Female | 60                      | White | p.Phe1650Cys | None           | None | None | None | Living/recurred/progressed |
|                                    | Male   | 67                      | White | p.Tpr1481Arg | None           | None | None | None | Living/disease free |
|                                      | Male   | 70                      | White | p.Arg201His | None           | None | None | None | Deceased |
| Ovarian serous cystadenocarcinoma (OV; 415) | Female | 39                      | White | p.Asp1390His | None           | None | None | None | Deceased/recurred/progressed |
| Uterine corpus endometrial carcinoma (UCEC; 524) | Female | 57                      | AA   | p.Asp1810Asn <4206+1G>C | None | None | None | None | Living/disease free |
|                                      | Female | 60                      | White | p.Tpr1397Arg | None           | None | None | None | Living/disease free |
|                                      | Female | 69                      | White | p.Ala1578Thr | None           | None | None | None | Living/disease free |

Note. **Yellow highlights** denote Pathogenic germline variants, **blue highlights** denote hotspot somatic variation. AA, African American; ESP, exome sequencing project; ExAC non-TCGA, portion of the exome aggregation consortium excluding samples from The Cancer Genome Atlas; N/A, not available.
pediatric cancer cohorts. We observed an approximately twofold higher germline prevalence of the most damaging (pathogenic: LOF, splice, hotspot) of such variation in TCGA (9,173 subjects: 1:4,600) than was observed in non-TCGA ExAC (53,103 subjects: 1:10,600) (Kim et al., 2017). In TCGA, we observed germline DICER1 LOF, splice and hotspot variation in individuals with uterine and rectal cancers, which are not known to be germline DICER1-associated. Such variation was not observed, however, in CanVar, a cohort of 1,006 early-onset colorectal cancers. Below we comment on the germline (and when possible, somatic) variation observed in the adult (TCGA) and pediatric (TARGET) datasets.

Of the 32 types of tumors sequenced in the TCGA project with available DICER1 germline data (Table S1), only one (thyroid carcinoma) has genetic and epidemiologic evidence of an association with pathogenic germline DICER1 variation (Khan et al., 2017; Rutter et al., 2016). However, in the TCGA data, we did not observe any pathogenic variation in DICER1 in the germline sequence for thyroid carcinoma; we did observe a LP (nonhotspot missense) variant in one participant with a thyroid carcinoma. One previous report found two TCGA thyroid cancers that harbored DICER1 somatic hotspot variation (Wasserman et al., 2018). The lack of observed germline pathogenic DICER1 variation in TCGA thyroid carcinomas may be secondary to study tissue requirements, which mandated sufficient tumor size with at least 60% tumor nuclei (https://cancergenome.nih.gov/cancersselected/biospeccriteria); this may have biased the study to more severe or aggressive tumors. In addition, the lack of pathogenic germline variation in DICER1 in the TCGA study thyroid carcinoma samples may be attributable to its focus on sporadic (and adult) rather than familial cancers.

Of the 32 TCGA tumors with germline DICER1 variation we analyzed (Table S1), four (testicular, breast, and prostate cancers and melanoma) have been reported in cohorts with germline DICER1 pathogenic variation (DICER1-carriers). A case series of 14 nonseminomatous testicular germ-cell tumors found one germline mutation (Heravi-Moussavi et al., 2012); subsequent work has cast doubt on a true DICER1 association with testicular cancers (Conlon et al., 2015). In an analysis of 209 DICER1-carriers from the International PPB/DICER1 Registry and NCI DICER1 syndrome study, a nonsignificant excess of breast cancer, prostate cancer, and melanoma was observed compared with US cancer registry (SEER) data (Stewart et al., 2019). In the current analysis, we did not observe any germline pathogenic variation in these four tumors, although one woman with breast cancer harbored a p.Gly1824Val nonhotspot missense (LP) variant. Taken together, it is unlikely that germline DICER1 LOF, splice-site, or hotspot variants contribute significantly to the risk of development of these tumors, bearing in mind the caveats of the TCGA study, noted above. The risk conferred by DICER1 nonhotspot missense variation needs additional study.

There is one report of an association of colorectal cancer risk with 3′-UTR polymorphisms in DICER1 and other miRNA genes (Cho et al., 2015). In TCGA data, we observed germline pathogenic DICER1 variation in one rectal adenocarcinoma. The rectum adenocarcinoma occurred in a 62-year-old female with a truncating DICER1 variant; we did not observe any somatic P/LP DICER1 variation in the associated tumor. We observed no germline P/LP DICER1 variation in the 410 TCGA participants with colon adenocarcinoma or in the 1,006 individuals with familial early-onset colorectal cancer from the CanVar study. Our analysis suggests that germline DICER1 P/LP variation does not contribute significantly to the risk of development of colorectal cancers.

In one study of 290 endometrial tumors (Chen et al., 2015), six (2%) harbored DICER1 somatic hotspot variation. In the 524 uterine corpus endometrial carcinomas in the TCGA study, one 57-year-old woman harbored both a heterozygous germline hotspot missense variant (p.Asp1810Asn) and a heterozygous germline canonical splice-site variant (c.4206+1G>C); her uterine cancer contained these two germline variants but lacked an additional somatic DICER1 variant. Given that DICER1 is essential for embryogenesis, we hypothesize that these two variants are in cis rather than in trans. To date, DICER1 hotspot variation has been observed in individuals mosaic for such variation, and not constitutionally (Brenneman et al., 2015; Klein et al., 2014). From the available TCGA data, it is not clear if the p.Asp1810Asn variant is constitutional or mosaic. Another possibility is age-related somatic variation, commonly observed in TP53 as clonal hematopoiesis (Genovese et al., 2014). Two women in the TCGA study with a uterine cancer each harbored a germline LP (nonhotspot missense) variant (p.Trp1397Arg; p.Ala1578Thr). In the woman with a germline p.Ala1578Thr variant, her tumor also harbored a known somatic hotspot variant (p.Asp1709Asn). Our

| TABLE 3 | Allele count (AC) of The Cancer Genome Atlas (TCGA) pathogenic (P) and likely pathogenic (LP) DICER1 variation |
|----------|---------------------------------------------------------------------------------------------------------------|
| TCGA 9,173 subjects | # of unique variants | AC |
| Likely pathogenic | 9 missense | 11 |
| Pathogenic | 1 canonical splice variant, 1 hotspot | 1 |
| Pathogenic total AC | 2 |
| P/LP prevalence | 1:706 |
| LOF (P) prevalence | 1:4,600 |

Note. LOF, loss-of-function.
review of the digital pathology available from the uterine corpus endometrial carcinomas from these patients with pathogenic germline variation showed no unusual morphologic features. In summary, 0.6% (3/524) of women in TCGA with a uterine corpus endometrial carcinoma harbored germline P/LP DICER1 variation.

Biallelic DICER1 variation is already known to account for a small percentage of Wilms tumors (Rakheja et al., 2014; Wu et al., 2013). The association of neuroblastoma and DICER1 variation is unsettled. In the modest-sized TARGET (pediatric) germline data available, we observed nonhotspot missense (LP) DICER1 variation in one child with a neuroblastoma (one child with Wilms tumor also harbored a DICER1 Likely Pathogenic variant). In an analysis of 209 DICER1-carriers from the International PPB/DICER1 Registry and NCI DICER1 syndrome study, one case of neuroblastoma was observed, a nonsignificant excess compared with US cancer registry (SEER) data (Stewart et al., 2019). In two large (n = 240; n = 71) somatic sequencing studies of neuroblastoma, no somatic DICER1 variation was observed (Pugh et al., 2013; Sausen et al., 2013). The risk conferred by constitutional bioinformatically predicted severe (LP) nonhotspot missense variation, akin to what we observed in one subject, remains uncertain. Similarly, the frequency of neuroblastoma arising from mosaic DICER1 hotspot missense variation is unknown.

Limitations of this investigation include an inability to detect copy-number changes in DICER1 in the publicly available data. As noted above, the tissue requirements for TCGA mandated tumors with at least 60% tumor nuclei. The TCGA study also focused on sporadic rather than familial cancers. Survival biases may have influenced participant type in both TCGA and TARGET.

We report a range of DICER1 pathogenic variant prevalence in adult and pediatric cancer populations drawn from large publicly available datasets. Compared with the general population prevalence (~1:10,600), the adult cancer cohort (TCGA: ~1:4,600) has trends toward greater frequency of pathogenic DICER1 variation. Our observation of germline P/LP DICER1 variation in 0.6% (3/524) of TCGA uterine corpus endometrial carcinoma merits additional investigation.

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CONFLICT OF INTEREST
The authors declare no relevant conflicts of interest.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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