DICER1 screening in 15 paediatric paratesticular sarcomas unveils an unusual DICER1-associated sarcoma

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Abstract

Individuals with DICER1 syndrome, a genetic disorder caused by pathogenic germline variants in DICER1, are at increased risk of developing a wide array of predominantly childhood onset conditions, including genitourinary sarcomas. However, data on DICER1 involvement in paratesticular sarcomas have not been published. Herein, we analyse a series of 15 paediatric paratesticular sarcomas and describe in detail the case of a male infant with a paratesticular myxoid tumour, considered to be a low-grade sarcoma, who also manifested a cystic nephroma, a classic DICER1 syndrome phenotype. He harboured a pathogenic germline DICER1 variant and different somatic hot-spot mutations in each tumour. The paratesticular tumour showed strong and diffuse expression for WT1 and CD10, an unusual immunophenotype in paediatric sarcomas, but typical of tumours of Müllerian origin. The tumour was postulated to arise from the appendix testis, a Müllerian remnant located in the paratestis. Such an origin would be analogous to other DICER1-associated non-epithelial gynaecological tumours, thought to arise from Müllerian derivatives. These findings point towards a key role of DICER1 in Müllerian–derived structures. Supporting this hypothesis is the fact that the other paratesticular sarcomas from the series were either negative or focally positive for WT1 and for CD10, and none had any DICER1 mutations. In summary, we present the first case of a paratesticular sarcoma associated with DICER1 syndrome, emphasising that paratesticular tumours with an unusual histological appearance may suggest an underlying DICER1 mutation, especially in the presence of a personal or family history of DICER1–associated disease. In this context, DICER1 mutation testing could lead to changes in clinical care including implementation of cancer care surveillance strategies.

Keywords: DICER1 syndrome; paratesticular sarcoma; Müllerian origin; genitourinary tract; DICER1 testing

Introduction

The DICER1 gene encodes a ribonuclease essential in the production of microRNAs, which mediate posttranscriptional gene expression [1]. Pathogenic germline variants in DICER1 cause DICER1 syndrome (Online Mendelian Inheritance in Man #606241) [2,3]. Individuals with this syndrome are predisposed to develop benign and malignant tumours such as pleuropulmonary blastoma, cystic nephroma (CN), thyroid tumours and ovarian Sertoli–Leydig cell tumours, amongst others [2–4]. With respect to sarcomas, the subtypes thus far linked to DICER1 mutations are embryonal rhabdomyosarcomas (ERMS), particularly of the...
urogenital tract – including bladder [5], ovary [6], cervix [5,7–9], anaplastic sarcoma of the kidney [10,11] and undifferentiated sarcoma of the ovary [12]. Most recently, somatic mutations in DICER1 have been identified in Mullerian adenosarcomas [13] and in intracranial spindle cell sarcomas [14–16]. The presence of germline and somatic mutations in DICER1 fits the genetic model of DICER1 syndrome [1].

Interestingly, we and others have noted a significant female predominance amongst DICER1 syndrome patients as described in de Kock et al, mainly attributable to the incidence of gynaecological neoplasms and thyroid lesions [2]. Literature review of all published DICER1-associated sarcomas performed by Warren et al showed that 62 of 86 sarcomas occurred in females ($p < 0.0001$, binomial test) [17]. Of these cases, 60% affected the female reproductive system (uterus, cervix and ovary) and 10% the urinary system (kidney and bladder). In contrast, in males, sarcomas were only identified in the brain and urinary system, but none involved male reproductive organs [17].

Paratesticular sarcomas are rare mesenchymal tumours that account for 90% of all malignancies in this location [18]. The term encompasses any tumour arising in paratesticular structures including the epididymis, spermatic cord, tunica vaginalis and various supporting structures. The most common subtypes include liposarcoma, rhabdomyosarcoma (RMS) and leiomyosarcoma; with predominance of ERMS in the first two decades of life [18]. Of note, there are no reported studies of DICER1 involvement in paratesticular tumours.

Herein we describe the pathological and molecular features of an unusual paratesticular tumour in a 10-month-old infant with DICER1 syndrome. Concurrently, the child also manifested a CN, a typical DICER1 syndrome phenotype. The pathology of the paratesticular tumour was of a low-grade myxoid sarcoma, considered to be most likely of Mullerian origin. We also evaluated 14 other paediatric paratesticular sarcomas and none had DICER1 mutations or strongly expressed immunohistochemical markers characteristic of Mullerian-derived tumours.

Materials and methods

Fifteen paediatric cases of paratesticular sarcoma with available formalin-fixed paraaffin-embedded (FFPE) tumour tissue were retrospectively collected through query of the institutional database maintained by the Pathology Department at The Hospital for Sick Children (Toronto, Canada). These included one ectomesenchymoma (5 months old), one unusual ‘polyphenotypic’ sarcoma (10 months old) and 13 ERMS, 4 of which had anaplasia. The median age at diagnosis of the 13 ERMS was 6 years (age range: 10 months to 18 years). No family history of DICER1-associated diseases was recorded in the clinical history. All patients received multimodal therapy. FFPE samples from normal tissue and tumoural tissue from the CN of the index case were retrieved. Two other DICER1-mutated CN cases (resected at 5 and 9 years of age, respectively) were also included as DICER1-mutated controls to evaluate the expression of WT1 and CD10.

DNA was extracted from FFPE samples using the QIAamp DNA FFPE Tissue Kit (Qiagen, Toronto, ON, Canada). Tumour DNA was screened for DICER1 mutations using a custom-design Fluidigm access array (Fluidigm, Markham, ON, Canada) [19]. All variants identified and the sequence coding for DICER1 RNase IIIb domain were PCR-amplified and Sanger sequenced to confirm the findings (primer sequences available upon request). DNA extracted from normal tissue was used to determine the germline origin of the variants. All tumours were reviewed by a paediatric pathologist (PST). Immunohistochemistry results were retrieved from the pathology reports or performed at the time of this study if not available. Additional immunohistochemical studies were performed on 4 μm sections cut from the FFPE blocks. The Dako Omnis Automated System along with the EnVision FLEX visualisation system was used with the following primary antibodies: actin (clone HHF35, undiluted, Dako Agilent #IR700, Santa Clara, CA, USA), desmin (clone D33, undiluted, Dako Agilent #IR606), myogenin (clone F5D, undiluted, Dako Agilent #IR067), WT1 (1:20 dilution, Leica, Concord, Ontario, Canada), CD10 (clone 56C6, undiluted, Dako Agilent #GA648), BCOR (1:200 dilution, Proteintech Group #12107-1-AP, Rosemont, IL, USA) and CD99 (clone 12E7, undiluted, Dako Agilent #IR057). Results for the presence of sarcoma-specific translocations were obtained from the reports. Additional translocation studies were not possible due to poor RNA quality in the paraaffin blocks. The study was approved by the research ethics board of the Hospital for Sick Children (Number 1000060307).

Results

Fifteen patients who had a diagnosis of paratesticular sarcoma were included in the study and DICER1 screening
was performed in tumour DNA. No somatic hot-spot mutations were identified in the sequence encoding the RNase IIIb region of either the ectomesenchymoma or the 13 ERMS. However, tumour DNA from the unusual paratesticular sarcoma was found to harbour two pathogenic mutations in DICER1 (see case report). A summary of the clinical, histopathological and molecular characteristics of the patients is included in Table 1.

Case report

A 10-month-old male infant who presented with a left paratesticular mass was evaluated by the treating oncology team, with additional staging imaging demonstrating a cystic lesion in the left kidney. The surgical approach comprised a left radical orchectomy, hemiscrotectomy and lymph node sampling, combined with a left partial nephrectomy. At the time of resection, the paratesticular mass was diagnosed as a ‘polyphenotypic’ tumour and classified as low risk (embryonal histology, Stage 1, Group II) according to the Children’s Oncology Group Soft Tissue Sarcoma. The renal lesion was confirmed to be a CN (Figure 1A,B). Following surgical management, the child underwent adjuvant chemotherapy with vincristine, doxorubicin, cyclophosphamide alternating with ifosfamide and etoposide. The patient remains well on transition to adult oncology after more than 16 years of follow-up. The family history of the patient reports three relatives who developed adult-onset cancers (breast cancer, oesophageal cancer and cancer of unknown primary site), but there is no mention of DICER1-associated diseases.

Pathology findings

Histopathological evaluation of the paratesticular lesion demonstrated tumour infiltration into the paratesticular and para-adnexal soft-tissues, sparing the testis (Figure 2A). The tumour was composed of small stellate cells with scanty cytoplasm widely separated by abundant myxoid matrix (Figure 2B). Nuclei were relatively uniform in appearance with small nucleoli (Figure 2C). Mitotic figures were identified (0–1 mitosis per 10 high-power fields, arrow on Figure 2C). No anaplastic features were noted. No epithelial or differentiated mesenchymal components were seen. Immunohistochemical staining demonstrated diffuse positivity for vimentin and focal expression of desmin (Figure 2D) and keratin (Figure 2E) that appeared to involve the same cells (biphenotypic). Staining for actin, myogenin (Figure 2F), S-100 protein, neuron-specific enolase, CD34, CD99 and BCOR was negative. By electron microscopy, there were some paranuclear filamentous aggregates, but no sarcomeric structures or Z-band material and no evidence of neural or epithelial differentiation. The t(11;22) translocation associated with Ewing sarcoma, the t(11;22)

Table 1. Characteristics of the series of patients included in the study

| Case ID | Age     | Follow-up time | Disease outcome | Pathology          | Anaplasia | CD10 expression | WT1 expression | Desmin expression | Myogenin expression | DICER1 status* |
|---------|---------|----------------|-----------------|--------------------|-----------|-----------------|-----------------|-------------------|---------------------|----------------|
| 1       | 15y 5mo | 7y 11mo        | NED             | ERMS               | No        | Foc pos         | Negative        | Positive          | Positive            | No RNase IIIb hot-spot |
| 2       | 4y 10mo | 14y 9mo        | NED             | ERMS               | No        | Foc pos         | Positive        | Positive          | Positive            | No RNase IIIb hot-spot |
| 3       | 6y 2mo  | 12y 3mo        | NED             | ERMS               | No        | Foc pos         | Foc pos         | Positive          | Positive            | No RNase IIIb hot-spot |
| 4       | 5mo     | 26y 3mo        | NED             | Ectomesenchymoma   | No        | Foc pos         | Foc pos         | Positive          | Positive            | No RNase IIIb hot-spot |
| 5       | 5y 10mo | 7y 3mo         | NED             | ERMS               | Yes       | Negative        | Foc pos         | Positive          | Positive            | No RNase IIIb hot-spot |
| 6       | 14y 11mo| 2y 9mo         | NED             | ERMS               | Yes       | Negative        | Foc pos         | Positive          | Positive            | No RNase IIIb hot-spot |
| 7       | 18y     | 12y            | NED             | ERMS               | No        | Negative        | Negative        | Positive          | Positive            | Wild type |
| 8       | 13y 6mo | 7y 2mo         | NED             | ERMS               | Yes       | Negative        | Negative        | Positive          | Positive            | No RNase IIIb hot-spot |
| 9       | 6y      | 11y 6mo        | NED             | ERMS               | No        | Negative        | Negative        | Positive          | Positive            | Wild type |
| 10      | 4y 3mo  | 12y 10mo       | NED             | ERMS               | No        | Negative        | Negative        | Positive          | Positive            | Wild type |
| 11      | 3y 7mo  | 6y 6mo         | NED             | ERMS               | No        | Negative        | Foc pos         | Positive          | Positive            | No RNase IIIb hot-spot |
| 12      | 7y      | 3y             | DOD             | ERMS               | Yes       | Foc pos         | Negative        | Positive          | Positive            | Wild type |
| 13      | 6y 11mo | 3y 6mo         | NED             | ERMS               | Yes       | Negative        | Negative        | Positive          | Positive            | Wild type |
| 14      | 15y 7mo | 1y 11mo        | NED             | ERMS               | No        | Negative        | Foc pos         | Positive          | Positive            | Wild type |

Abbreviations: Diff pos, diffusely positive; DOD, died of disease; ERMS, embryonal rhabdomyosarcoma; Foc pos, focally positive; mo, months; NED, no evidence of disease; y, years.

*DICER1 status: ‘Mutated’, case with a loss-of-function variant and a hot-spot missense mutation in DICER1; ‘No RNase IIIb hot-spot’, case with low Fluidigm target coverage but no hot-spot mutation in RNase IIIb by Sanger sequencing; ‘Wild type’, case with good Fluidigm target coverage that do not harbour mutations in DICER1 coding sequence or splice sites.

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translocation associated with desmoplastic small round cell tumour and the t(2;13) translocation associated with alveolar RMS were not detected by RT-PCR. The tumour lacked sufficient features to qualify as an ERMS.

Differential diagnosis

Given the co-expression of desmin and keratin in the paratesticular sarcoma, we considered desmoplastic small round cell tumour in the differential diagnosis; however, the morphology was atypical for that diagnosis and the expected chromosomal alteration resulting in an EWSR1-WTI fusion was not present [20,21]. It would have been informative to carry out additional molecular genetic testing for sarcoma translocations [20,21] not available at the time of tumour resection but, unfortunately, it was not possible as the quality of the RNA in the paraffin blocks was poor. The diagnosis of primitive myxoid mesenchymal tumour of infancy was also considered but the immunoprofile did not match our case. This tumour expresses BCOR and often CD99 [22], whereas our case was negative for these antigens. Also, the one case we have studied did not possess a DICER1 mutation [9]. Instead, this tumour typically carries an internal tandem duplication of the BCOR gene [22].

Figure 1. Immunohistopathological characterisation of the CN. (A) and (B) The features of a CN consisting of a multi-locular cyst lined by a single layer of hobnailed epithelial cells. The intervening septa are thin and consist of loose fibrovascular tissue containing scattered inflammatory cells but no blastema. There was no solid component to the specimen. (C) Staining for WT1 was negative except for very occasional stromal cells (arrow) and (D) staining for CD10 was negative. Haematoxylin and eosin, original total magnification ×100 (A) and ×200 (B). Immunoperoxidase, original total magnification ×200 (C, D).
Additional immunostaining was performed at the time of this review and showed diffuse, strongly positive expression for WT1 (Figure 2G) and CD10 (Figure 2H). Diffuse and strong expression for both WT1 and CD10 is a distinctly unusual combination in paediatric sarcomas but is typical of tumours of Müllerian origin. While co-expression of WT1 and CD10 can be seen in metanephric stromal tumour of the kidney [23], this case neither possessed the typical morphology for that tumour, nor is there any report of this tumour occurring outside of the kidney. There are rare reports of Wilms tumours occurring in inguinal hernia sacs or in the paratesticular region [24,25] but the tumour in this case was composed of stromal tissue only, lacking the typical appearance of a Wilms tumour. Moreover, while blastema and tubules in a Wilms tumour can be WT1-positive, the stromal component is not. Our

Figure 2. Immunohistopathological characterisation of the paratesticular myxoid sarcoma. The tumour infiltrates the paratesticular soft tissue (A) but does not involve the testis and is composed of small stellate cells with scanty cytoplasm separated by abundant myxoid matrix (B). Nuclei are relatively uniform in appearance with small nucleoli in the larger nuclei (C). Mitotic figures are identified (arrow). Immunohistochemical staining shows cytoplasmic positivity for desmin (D) and keratin (E) whereas staining for myogenin (F) is negative. The tumour also shows strong nuclear expression for WT1 (G) and cell surface staining for CD10 (H). Cases of paratesticular rhabdomyosarcoma were either negative for WT1 or focally positive (I) and, similarly, for CD10 (not illustrated). Haematoxylin and eosin, original total magnification ×40 (A); ×200 (B); ×400 (C). Immunoperoxidase, original total magnification ×200 (E-I).
case of primitive myxoid mesenchymal tumour was negative for WT1.

Müllerian adenosarcomas have similar immunohistochemistry expression profiles and a small percentage has been described to harbour \textit{DICER1} mutations [13,26]. This case has an undifferentiated myxoid morphology that could be viewed as having some similarities to the sarcoma component of adenosarcomas, but

\textbf{Figure 3.} Germline and somatic \textit{DICER1} mutations. Panel (A) shows the c.4308_4311del (p.A1436fs) pathogenic germline variant found in both the paratesticular myxoid sarcoma and the CN. The chromatogram shows the variant in normal tissue, confirming its germline origin. Panel (B) displays the c.5114A > T (p.E1705V) somatic \textit{DICER1} mutation identified in the paratesticular myxoid sarcoma. Panel (C) shows the c.5428G > T (p.D1810Y) somatic \textit{DICER1} mutation identified in the CN. B and C show both the Fluidigm array results (upper image) and Sanger sequencing (chromatogram). The corresponding wild type sequence is included below. In the chromatograms, the germline deletion is indicated with a rectangle and mutations with an asterisk. Abbreviations: Mut allele freq, mutant allele frequency.
the case we report here lacks any epithelial component. While other myxoid tumours, such as myxoma, angioimmxoma, myxoid neurofibroma, myxoid liposarcoma, lipoblastoma and low-grade fibromyxoid sarcoma were considered, these were thought to be unlikely diagnoses in our case on the basis of the patient’s age (infancy), the paratesticular location of the tumour, lack of diagnostic histological features and an immunohistochemical profile which was out of keeping with these other diagnoses. Having reviewed all the possible diagnoses, the tumour was finally classified as a low-grade myxoid sarcoma of likely Mullerian origin.

Molecular findings

Given the association of CN with DICER1 syndrome, we screened for DICER1 mutations in both lesions and in normal tissue from the child. Molecular analysis showed that the paratesticular sarcoma harboured a deletion of 4 nucleotides in exon 23 of DICER1 (c.4308_4311del, p.A1436fs, Figure 3A) and a missense mutation modifying one of five ‘hot-spot’ amino acids in the RNase IIIb domain (c.5114A > T, p. E1705V, Figure 3B). The CN revealed two mutations in DICER1: the same pathogenic deletion found in the paratesticular sarcoma (c.4308_4311del, p.A1436fs, Figure 3A) and a different missense hot-spot RNase IIIb mutation (c.5428G > T, p.D1810Y, Figure 3C). The DICER1 deletion was confirmed to be of germline origin through interrogation of DNA extracted from normal tissue. The finding of a germline loss-of-function variant and a somatic hot-spot mutation in DICER1 is characteristic of DICER1 syndrome.

The CN from the patient showed only focal staining for WT1 (Figure 1C) and negative staining for CD10 (Figure 1D), indicating that the DICER1 mutation was not likely inducing the expression of these two antigens. In agreement with this, two other CNs harbouring DICER1 mutations showed only very occasional stromal cells positive for WT1 (both cases) and CD10 (one case). The 14 additional paediatric paratesticular sarcomas (13 ERMS and 1 ectomesenchymoma) screened for DICER1 somatic hot-spot mutations were negative. These cases further served as controls for the immunostaining results (i.e. WT1 and CD10) in the myxoid paratesticular sarcoma. CD10 staining was only focally positive in four cases and WT1 staining was only focally positive in seven cases (Figure 2I). No case showed the diffuse strong expression for WT1 and CD10 seen in the myxoid sarcoma, indicating that this is not a feature of paratesticular sarcomas in general.

Discussion

DICER1 syndrome is a tumour predisposition syndrome that increases the risk of a variety of neoplastic and dysontogenetic lesions such as pleuropulmonary blastoma (PPB), thyroid lesions, CN and genitourinary sarcomas [4]. Here we investigated the potential involvement of DICER1 in a series of 15 paediatric paratesticular sarcomas and describe in detail the case of a child who developed an unusual paratesticular tumour and a typical CN. The child was found to have a germline pathogenic DICER1 variant, coupled with different somatic missense hot-spot mutations affecting metal ion binding residues in the RNase IIIb domain in the paratesticular sarcoma and the CN. The presence of these mutations in DICER1 indicates a causative role for aberrant DICER1 function in the oncogenesis of this paratesticular tumour and includes it as a novel phenotype of DICER1 syndrome.

Paratesticular tumours are rare entities that can arise in any of the paratesticular structures including the epididymis, spermatic cord, tunica vaginalis and supporting structures [18]. A third of all paratesticular masses are malignant, of which 90% are sarcomas [18], with ERMS reported as the most common paediatric malignant subtype [18]. The tumour in this case was not an ERMS but, instead, was considered to be a low-grade myxoid sarcoma. This is not a tumour one expects to find in the paratesticular region and appears to be a rare event. The pathology database at The Hospital for Sick Children, which spans over 30 years, does not contain a similar case. Moreover, the histology of this case does not resemble that of other sarcomas previously associated with DICER1 syndrome [5–10,12,14–16]. As illustrated by Warren and colleagues, these sarcomas typically include foci of different mesenchymal lineages, most commonly rhabdomyoblastic and chondroblastic, as well as large anaplastic cells in addition to undifferentiated small spindle cells. In contrast, this case was composed of a uniform population of small, mildly pleomorphic, stellate cells in a myxoid matrix [17]. Following this description, one might have predicted that the paratesticular ERMS cases with anaplasia would have harboured DICER1 mutations, given that anaplastic sarcoma of the kidney is a known component of the DICER1 syndrome and many of those cases show areas of rhabdomyoblastic differentiation [10,27]. Furthermore, PPB tumours, most of which result from DICER1 mutations, characteristically contain areas with rhabdomyoblastic differentiation, often with anaplasia [28].

In addition to molecular screening, methylation profiling has been useful in identifying distinct subtypes of DICER1 syndrome.
of tumours, such is in the case of sarcomas of the central nervous system [29]. Indeed, a group of predominantly paediatric intracranial sarcomas with DICER1 mutations was found to have a distinct methylation signature [14]. In the case of embryonal tumours with multi-layered rosettes (ETMRs, another paediatric brain tumour), although a different methylation pattern has been identified, the cluster includes both DICER1-mutated and DICER1-wild type ETMRs [30]. Therefore, studying and comparing the methylation status of the case presented here, of DICER1-associated sarcomas of the urogenital tract and of DICER1-wild type tumours would be interesting to check if the clustering observed is mainly dictated, as expected, by the tissue of origin or if, instead, DICER1 status is the main determinant.

After developing a differential diagnosis, we considered this lesion to be a low-grade sarcoma of Müllerian origin as shown by the diffuse and strong expression for both WT1 and CD10, a feature typical of tumours of Müllerian origin and highly restricted to this group of tumours [31–33]. A Müllerian origin for this tumour would be concordant with other DICER1-associated non-epithelial gynaecological tumours (e.g. cervical embryonal RMS [5,7–9] or Müllerian adenosarcomas [13,26]), which arise from Müllerian tissues. Indeed, a female preponderance has been observed in patients with DICER1 syndrome, as a result of the high incidence of gynaecological tumours and frequent thyroid disease [2,17]. In the setting of DICER1 mutations, over 70% of all sarcomas occur in females, the majority manifesting in female reproductive tract organs [17]. However, no sarcoma in male reproductive organs has previously been identified with characteristic DICER1 molecular changes [17].

Müllerian ducts differentiate and form the fallopian tubes, uterus, cervix and upper portion of the vagina in female embryos; whereas in males, they regress shortly after their formation under the influence of the anti-Müllerian-hormone produced by the Sertoli cells of the testes, except for some remnants [34]. Taking into account the location of the sarcoma in our case, it could have arisen from the appendix testis, a vestigial remnant of the Müllerian duct in the male. Cases of tumours arising from the appendix testis are very rare and seem mainly to be epithelial [35–37]. There is one report of a stromal paratesticular tumour also postulated to be Müllerian in origin based on the immunophenotype (including strong CD10 expression) and its resemblance to endometrial stromal sarcoma of the uterus [38]. This case differs from the current report in that the patient was of adult age, the histology was different and the tumour was located in the tail of the epididymis (i.e. not the site of the appendix testis). Conversely, gynaecological tumours of probable Wolffian origin have not been found to harbour DICER1 mutations (as seen by Mirkovic et al [39] and by our group, unpublished data, n = 2). This is interesting because Wolffian ducts are the embryonic structures that form the male internal genitalia and typically regress in females with the exception of some remnants [34].

Nagaraja et al studied mice with inactivated Dicer1 in Müllerian duct mesenchyme-derived tissues, revealing the down-regulation of specific miRNAs predicted to regulate Wit and Hox genes, important for Müllerian duct differentiation and mesenchyme-derived structures [40]. We postulate that microRNA alterations as a result of DICER1 mutations could result in persistence of Müllerian remnants, through microRNA-mediated inhibition of differentiation, which could predispose to tumour formation in Müllerian-derived structures.

Herein, we reported the first case of a paratesticular sarcoma associated to DICER1 syndrome. In addition, our findings highlight that the identification of unusual tumours in Müllerian-derived tissues, including the paratestis, should raise suspicion for DICER1 syndrome, especially if there is a personal or familial history of DICER1-associated diseases. In these cases, DICER1 testing may be recommended, as the identification of a pathogenic germline variant in DICER1 could lead to changes in clinical care including implementation of cancer surveillance strategies [4].

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Author contributions statement

MA-R conceived and performed molecular analyses, analysed genetic data, carried out the literature search, generated figures and drafted the manuscript. NC, RG, PM, CG collected and interpreted pathological and clinical data. JRP interpreted clinical data and provided critical review of the data and manuscript. PST
was the expert pathologist for this study. PST and WDF contributed to the study design, data interpretation, literature research and writing of the manuscript. WDF supervised the study. All authors reviewed the paper and had final approval of the submitted version.

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