DICER1 Syndrome with an Intronic Germline Variant Causing Splice Alteration

Shutaro Inoue[1], Yoshiko Nakano[1], Mariko Tanaka[1], Yuta Yamada[1], Kentaro Watanabe[1], Moe Hidaka[1], Masahiro Sekiguchi[1], Shota Kato[1], Takeyuki Watadani[1], Jun Fujishiro[1], Haruki Kume[1], Tetsuo Ushiku[1], and Motohiro Kato[1]

[1]Tokyo Daigaku Igakubu Fuzoku Byoin

May 02, 2024

Abstract

Patients with *DICER1* syndrome typically harbor a germline truncating variant in the coding region. Here, we report a case of *DICER1* syndrome caused by an intronic germline variant. The patient was diagnosed with pleuropulmonary blastoma at the 3 years of age, and a somatic p.D1810Y, but not a germline variant in *DICER1* was detected by whole-exome sequencing. After 13 years, he developed urogenital embryonal rhabdomyosarcoma with a somatic p.E1813D in *DICER1*. Further analysis using complementary DNA *DICER1* confirmed that a germline p.I813Ffs*24 and c.2437-15T>G caused the alteration. This report highlights the importance of a germline-dedicated analysis covering introns.

Introduction

DICER1 syndrome is a cancer predisposition disorder characterized by a germline loss-of-function variant in *DICER1* and an increased risk of developing multiple tumors, including pleuropulmonary blastoma (PPB), cystic nephroma, multiple thyroid nodules, and Sertoli–Leydig cell tumors[1,2]. Additionally, extremely rare tumors, including ciliary body medulloepithelioma, nasal chondromesenchymal hamartomas, and cervical embryonal rhabdomyosarcoma (ERMS), are also associated with *DICER1* . Since *DICER1* was revealed as a causative gene of familial PPB in 2009, the number of tumors recognized as *DICER1* -associated has been increasing[3]. In most cases, somatic hotspot mutations in the *DICER1* RNaseIIIb domain (p.E1705, p.D1709, p.D1809, p.D1810, and p.E1813) are detected in tumor tissue as a second hit[1]. Patients with*DICER1* syndrome typically have truncated variants in the exonic regions of *DICER1* [1,4]. *DICER1* germline pathogenic variants are detected in approximately 80% of PPB patients, with the remaining cases considered sporadic[4]. However, *DICER1* syndrome may be underdiagnosed because some cases of genomic alterations are missed by conventional genomic analysis techniques.

Here, we present a case of *DICER1*- related urogenital ERMS that developed in a male 13 years after the PPB diagnosis. The patient was initially diagnosed with sporadic PPB by whole-exome sequencing (WES); however, *DICER1* complementary DNA (cDNA) analysis conducted at 16 years of age revealed a germline intronic variant, which led to the*DICER1* syndrome diagnosis.

Results

A 16-year-old male presented with worsening of hematuria and urinary retention over two weeks. He was initially treated with antibiotics for possible cystitis. He had a history of PPB type II without metastasis at 3 years of age and local recurrences at 4 and 6 years of age, which were treated with complete resection and chemotherapy, including high-dose chemotherapy with hematopoietic cell rescue. During PPB diagnosis, whole-exome sequencing (WES) was performed using tumor tissue and blood samples, which led to the

sporadic PPB diagnosis. A somatic p.D1810Y (c.5428G>T) hotspot mutation in *DICER1* was detected in the primary and recurrent PPB, whereas no pathogenic variant was identified in the germline.[5] Although the patient's mother had thyroid nodules, his other family members do not have any history of *DICER1* -associated disorders (Fig. 1A). A computed tomography scan detected enhanced masses in the bladder and prostate obstructing the urethra (Figs. 1B-C). Further imaging tests revealed multiple nodules in the thyroid gland, which were considered incidentally found benign nodules. No local recurrence of PPB in the lungs or other metastases was observed. The patient underwent a needle biopsy of the prostate lesion. The pathological findings suggested a distant recurrence of PPB or ERMS (Figs. 1D-G). Chemotherapy (topotecan 0.75 mg/m^2 for 5 days plus cyclophosphamide 250 mg/m^2 for 5 days) was started on the patient, and he showed a partial response after four cycles.

DNA panel testing performed using the biopsied sample detected p.E1813D (c.5439G>T) instead of p.D1810Y, which had been found in the first PPB. Although no additional pathogenic variant was detected in the panel testing, the *DICER1* hotspot mutation in the present lesion, the multinodular goiter, and the history of PPB were highly suggestive of *DICER1* syndrome. Therefore, complementary DNA (cDNA) analysis was performed using blood samples, which detected a frameshift pathogenic p.I813Ffs*24 with a 14-base insertion. Subsequent Sanger sequencing revealed an intronic c.2437-15T>G (Fig. 2). Accordingly, the patient was diagnosed with *DICER1* syndrome, and the tumor was diagnosed as ERMS, a second tumor that developed in the context of *DICER1* syndrome. Through cascade testing, the same pathogenic variant was detected in the patient's mother. The patient's other family members do not have any history of *DICER1* -associated disorders.

Based on the ERMS diagnosis, the treatment was switched to VAC (4 cycles of vincristine 2 mg, actinomycin D 0.045 mg/kg, and cyclophosphamide 2200 mg/m^2), radiotherapy, and tumor resection, based on standard therapy for intermediate ERMS. At the time of writing, 15 months after treatment completion, the patient remains alive without evidence of ERMS. The thyroid nodules showed no remarkable changes in size since diagnosis.

Discussion

Here, we present a case of urogenital ERMS that developed in a teenager with a history of PPB and a germline intronic pathogenic variant in*DICER1* . During the PPB diagnosis, the tumor was considered sporadic based on WES findings. However, further analysis performed after the patient developed subsequent cancer revealed a genetic susceptibility to *DICER1* -related tumors.

The majority of pathogenic variants in *DICER1* syndrome patients is detected by Sanger sequencing, multigene panel testing, or WES, with all of these focused on coding regions. However, some patients reportedly develop *DICER1* syndrome due to intronic or mosaic pathogenic variants that cannot be detected by conventional sequencing methods or analysis pipelines[4,6–12]. Along with these findings, our case emphasizes the importance of germline-specific analysis, including cDNA analysis. In our case, the analysis of*DICER1* in tumor samples was also critical for confirming the diagnosis. The detection of the hotspot mutation of p.D1810Y in the initial and recurrent PPB tissues and that of p.E1813D in the subsequent tumor demonstrated that different second hits contributed to the patient's tumor pathogenesis. That is, the second tumor was considered a new primary tumor, not a recurrence of PPB.

Although ERMS, particularly female cervical ERMS, is recognized as a manifestation of *DICER1* syndrome, it is infrequently observed in the male urogenital system[13]. At least four bladder ERMS cases have been reported in pediatric patients with *DICER1*syndrome, including three males[14,15]. Additionally, an autopsy case of adult prostatic ERMS with a *DICER1* hotspot mutation with lung and bone metastases and a female case of a bladder tumor with *DICER1* mutations (a hotspot mutation and a truncating mutation) were also reported, although the germline status was not confirmed in these cases[16,17]. Regarding the surveillance protocols for *DICER1* syndrome, regular imaging tests for the early detection of male genitourinary tumors would not be recommended due to their low expected frequency[18,19]. Nevertheless, it is important to consider the possibility of tumors when patients with *DICER1* syndrome show atypical or unexplained symptoms[20].

In summary, the present case highlights the importance of performing germline-specific testing, including cDNA, for the diagnosis of cancer predisposition disorders, Moreover, our report provides evidence that*DICER1* -related ERMS is not exclusively cervical and can develop in men. Transcriptome analysis may be useful in understanding the etiology by supplementing WES and whole-genome sequencing with which cancer predisposition may be underdiagnosed.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

ACKNOWLEDGEMENTS

The germline analysis was performed in a pilot study of pediatric cancer predisposition syndrome conducted by the Japan Children's Cancer Group. The authors thank Dr. Munetoshi Hinata and Mrs. Masayo Matsumura for their technical assistance. This work was partially supported by The Japan Agency for Medical Research and Development under grant numbers JP23ck0106876, JP23ck0106870, and JP23ama221505. This study was also partly supported by a Gold Ribon Network research grant.

AUTHOR CONTRIBUTIONS

MK is the principal investigator and takes primary responsibility for the manuscript content; he designed this study, interpreted the data, wrote the manuscript, and approved its final version. SI and YN evaluated the patient, collected and interpreted the data, and wrote the manuscript. KW, MH, MS, SK, YY, MT, TW, JF, HK, and TU evaluated the patients and collected data. All authors discussed the results and critically reviewed the manuscript.

REFERENCES

1. de Kock L, Wu MK, Foulkes WD. Ten years of DICER1 mutations: provenance, distribution, and associated phenotypes. Hum Mutat 2019;40:1939-1953. 2. Stewart DR, Best AF, Williams GM, et al. Neoplasm risk among individuals with a pathogenic germline variant in DICER1. J Clin Oncol 2019;37:668-676. 3. Hill DA, Ivanovich J, Priest JR, et al. DICER1 mutations in familial pleuropulmonary blastoma. Science 2009;325:965. 4. Brenneman M, Field A, Yang J, et al. Temporal order of RNase IIIb and loss-of-function mutations during development determines phenotype in pleuropulmonary blastoma / DICER1 syndrome: a unique variant of the two-hit tumor suppression model. F1000Res 2015;4:214. 5. Seki M, Yoshida K, Shiraishi Y, et al. Biallelic DICER1 mutations in sporadic pleuropulmonary blastoma. Cancer Res 2014;74:2742-2749. 6. Schultz KA, Stewart DR, Kamihara J, et al. DICER1 tumor predisposition. In: Adam MP, Feldman J, Mirzaa GM, et al. (eds.). GeneReviews®. Seattle: University of Washington; 2014. 7. Schultz KAP, Harris A, Messinger Y, et al. Ovarian tumors related to intronic mutations in DICER1: a report from the international ovarian and testicular stromal tumor registry. Fam Cancer 2016;15:105-110. 8. Verrier F, Dubois d'Enghien C, Gauthier-Villars M, et al.Mutiple DICER1-related lesions associated with a germline deep intronic mutation. Pediatr Blood Cancer 2018;65:e27005. 9. Fraire CR, Mallinger PR, Hatton JN, et al. Intronic germline DICER1 variants in patients with Sertoli-Leydig cell tumor. JCO Precis Oncol 2023;7:e2300189. 10. Apellaniz-Ruiz M, Sabbaghian N, Chong AL, et al.Reclassification of two germline DICER1 splicing variants leads to DICER1 syndrome diagnosis. Fam Cancer 2023;22:487-493. 11. de Kock L, Wang YC, Revil T, et al. High-sensitivity sequencing reveals multi-organ somatic mosaicism causing DICER1 syndrome. J Med Genet 2016;53:43-52. 12. Terraf P, Pareja F, Brown DN, et al. Comprehensive assessment of germline pathogenic variant detection in tumor-only sequencing. Ann Oncol 2022;33:426-433. 13. Kommoss FKF, Stichel D, Mora J, et al. Clinicopathologic and molecular analysis of embryonal rhabdomyosarcoma of the genitourinary tract: evidence for a distinct DICER1-associated subgroup. Mod Pathol 2021;34:1558-1569. 14. Cross SF, Arbuckle S, Priest JR, Marshall G, Charles A, Dalla Pozza L. Familial pleuropulmonary blastoma in Australia. Pediatr Blood Cancer 2010;55:1417-1419. 15. Doros L, Yang J, Dehner L, et al. DICER1 mutations in embryonal rhabdomyosarcomas from children with and without familial PPB-tumor predisposition syndrome. Pediatr Blood Cancer 2012;59:558-560. 16. Miyama Y, Makise N, Miyakawa J, Kume H, Fukayama M, Ushiku T.An autopsy case of prostatic rhabdomyosarcoma with

DICER1 hotspot mutation. Pathol Int 2021;71:102-108. 17. Eckstein M, Agaimy A, Woenckhaus J, et al. DICER1 mutation-positive giant botryoid fibroepithelial polyp of the urinary bladder mimicking embryonal rhabdomyosarcoma. Hum Pathol 2019;84:1-7. 18. Schultz KAP, Williams GM, Kamihara J, et al. DICER1 and associated conditions: identification of at-risk individuals and recommended surveillance strategies. Clin Cancer Res 2018;24:2251-2261. 19. Bakhuizen JJ, Hanson H, van der Tuin K, et al. Surveillance recommendations for DICER1 pathogenic variant carriers: a report from the SIOPE Host Genome Working Group and CanGene-CanVar Clinical Guideline Working Group. Fam Cancer 2021;20:337-348. 20. Faure A, Atkinson J, Bouty A, et al. DICER1 pleuropulmonary blastoma familial tumour predisposition syndrome: what the paediatric urologist needs to know. J Pediatr Urol 2016;12:5-10.

LEGENDS

FIGURE 1. The patient's clinical features

A: Family pedigree. The black symbols represent patients with*DICER1* syndrome. Our patient is represented by the red arrow. PPB, pleuropulmonary blastoma; ERMS, embryonal rhabdomyosarcoma; MNG, multinodular goiter. **B–C:** Pelvic magnetic resonance image of the prostate tumor indicated by arrowheads. T1-weighted images with gadolinium enhancement show a solid mass in the prostate gland that constricts the urethra (**B**) and a tumor at the trigone protruding into the intraluminal space of the bladder (**C**).**D–E:** Histology of the prostate tumor unveils two distinct patterns: The first one is characterized by hypocellular proliferation of polygonal cells with eccentric nuclei and eosinophilic cytoplasm with an edematous stroma (**D**), whereas the other exhibits a solid proliferation of short spindle cells with nuclear enlargement and hyperchromasia (**E**). **F** –**G** : Histology of the primary pleuropulmonary blastoma (**F**) and the first relapse (**G**) similar to that of the prostate tumor.

FIGURE 2. Results of the genomic analysis of *DICER1*

A: Genomic localization and sequence of the intronic mutation of c.2437-15T>G located in intron 15. The intronic sequence is depicted in lowercase letters, whereas the succeeding sequence of exon 16 is in uppercase letters. **B:** Sanger sequencing of*DICER1* complementary DNA synthesized from mRNA obtained from the peripheral blood samples of the case with (upper) or without puromycin (lower). The splicing variant is more prominent in the sample that received puromycin treatment, denoting a truncating effect. **C:**Sanger sequencing of *DICER1* DNA in an exon–intron boundary extracted from peripheral blood samples.