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SHORT COMMUNICATION

# Embryonal rhabdomyosarcoma in a patient with a heterozygous frameshift variant in the *DICER1* gene and additional manifestations of the DICER1 syndrome

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**Abstract** Germline mutations in the *DICER1* gene are associated with an inherited cancer predisposition syndrome also known as the DICER1-syndrome, which is implicated in a broad range of tumors including pleuropulmonary blastoma, ovarian Sertoli-Leydig cell tumors, ciliary body medulloepithelioma (CBME), pituitary blastoma, embryonal rhabdomyosarcoma (eRMS), anaplastic renal sarcoma as well as ocular, sinonasal tumors ovarian sex-cord tumors, thyroid neoplasia and cystic nephroma. This study describes a novel, heterozygous frameshift *DICER1* mutation in a patient, who is affected by different tumors of the DICER1-syndrome, including eRMS, CBME and suspected pleuropulmonary blastoma type I. By whole-exome sequencing of germline material using peripheral blood-derived DNA, we identified a single base pair duplication within the *DICER1* gene (c.3405 dupA) that leads to a frameshift and results in a premature stop in exon 21 (p.Gly1136Arg). The metachronous occurrence of two unrelated tumor entities (eRMS and CBME) in a very young child within a short timeframe should have raised the suspicion of an underlying cancer susceptibility syndrome and should be prompt tested for *DICER1*.

**Keywords** DICER1 germline mutation · Frameshift · DICER1-syndrome · Embryonal rhabdomyosarcoma · Medulloepithelioma · Pleuropulmonary blastoma · Renal cysts · Focal nodular hyperplasia

**Abbreviations**

| | |
|---|---|
| Bp | Base pair |
| CBME | Ciliary body medulloepithelioma |
| eRMS | Embryonal rhabdomyosarcoma |
| miRNA | microRNA |
| PPB | Pleuropulmonary blastoma |
| RNAi | RNA interference |
| WES | Whole exome sequencing |

## Introduction

DICER1 is a double-stranded RNA-specific endoribonuclease that plays a major role in the RNA interference (RNAi) and microRNA (miRNA) biogenesis pathway and cleaves precursor miRNAs into active miRNAs. The *DICER1* gene is located on chromosome 14q32.13 and is comprised of 1922 amino acids and 27 exons. The enzyme contains several domains including a DExD/H box helicase domain, PAZ, platform, two RNase III domains, and an RNA-binding domain in the C-terminus [1] (Fig. 1).

Aberrant expression of DICER1 has been identified in various types of cancers caused either by somatic or germline mutations in the *DICER1* gene. Inactivating heterozygous germline mutations in *DICER1* predispose to a pleiotropic tumor syndrome, which leads to a variety of

Arndt Borkhardt and Michaela Kuhlen have contributed equally to this work.

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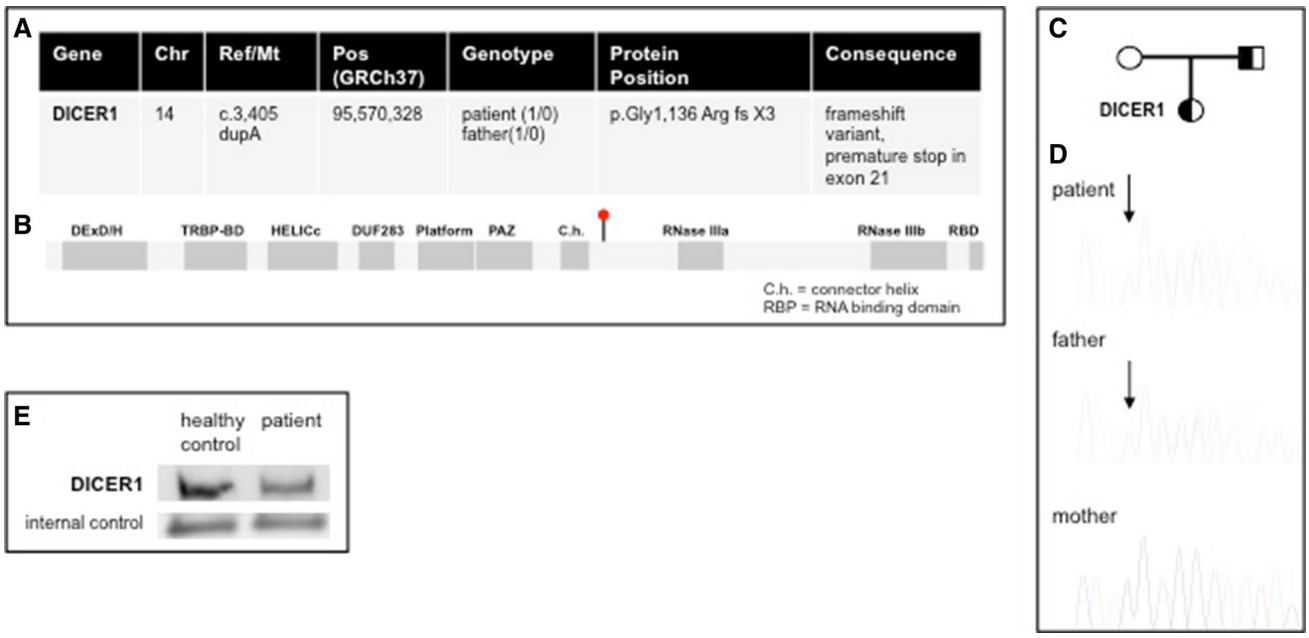


**Fig. 1** **a** Whole-exome sequencing of blood-derived DNA from the patient and its parents revealed a single bp duplication within the DICER1 gene (c.3405 dupA) that leads to a frameshift and a premature stop in exon 21 (p.Gly1136Arg), which is heterozygous in our patient and in the father. **b** The heterozygous frameshift mutation is located at the C-terminus of the RNase IIIa domain in exon 21. **c** Family tree shows the affected patient and the father, who is the carrier of the DICER1 variant, both are marked in *black*. **d** Sanger sequencing confirmed the identified DICER1 frameshift variant being heterozygously in the patient and in the father, while the mother shows the wildtype sequence. **e** Western blot showed reduced protein levels in the patient in comparison to a healthy control sample. Protein was extracted from PBMCs of the patient and a healthy control. For western blot analysis, we used a primary anti-DICER1 antibody in a 1:1000 dilution, while C23 served as an internal control

cancers that are typically diagnosed in early childhood and include both malignant and benign tumors that mostly occur in the lungs, kidneys, ovaries and thyroid. Recent studies by Brenneman et al. proposed that one allele (mostly in germline) harbors a frameshift or a nonsense mutation that cause a complete loss of function (LOF) of *DICER1*, while the other allele harbors a second somatic hotspot mutation within the RNase IIIb domain. However, biallelic LOF mutations have not been described in pleuropulmonary blastoma so far, indicating that retaining several miRNA processing functions might be essential for tumor progression. It was shown that substitutions within these hotspot regions of the RNase IIIb domain cause neomorphic DICER1 function, affecting processing and cleavage of miRNAs, which result in altered expression of several downstream mRNAs that are required for embryogenesis and tumor suppression and can lead to abnormal proliferation [2].

The DICER1-syndrome is characterized by autosomal-dominant inheritance with variable expressivity and seems to have incomplete penetrance, however, detailed penetrance studies have not been published yet and are still under evaluation. It is also known as the pleuropulmonary blastoma (PPB) familial tumor susceptibility syndrome, which is a rare, malignant embryonal tumor of the lung and one of the most common *DICER1* associated tumors [3]. Moreover, patients with familial PPB-tumor predisposition syndrome are prone to a broad range of other tumor entities such as embryonal rhabdomyosarcoma (eRMS) of the uterine cervix, nasal chondromesenchymal hamartoma, cystic nephroma, thyroid gland neoplasia, nephroblastoma (Wilms tumor), and have also been linked to rare tumor entities such as sex-cord stromal tumors (mainly ovarian Sertoli-Leydig tumors), ciliary body medulloepithelioma (CBME), gynandroblastoma, juvenile granulosa cell tumor, and central nervous tumors such as pituitary blastoma and pineoblastoma [4–7].

DICER1-syndrome should be suspected in children who develop one or more tumors associated with *DICER1* mutations.

## Results

### Case report

We report on a 12-year-old girl with unremarkable family history, who was initially diagnosed with eRMS of the bladder (Fig. 2) at the age of 6 months. As staging investigations additionally revealed a cystic lesion in lung

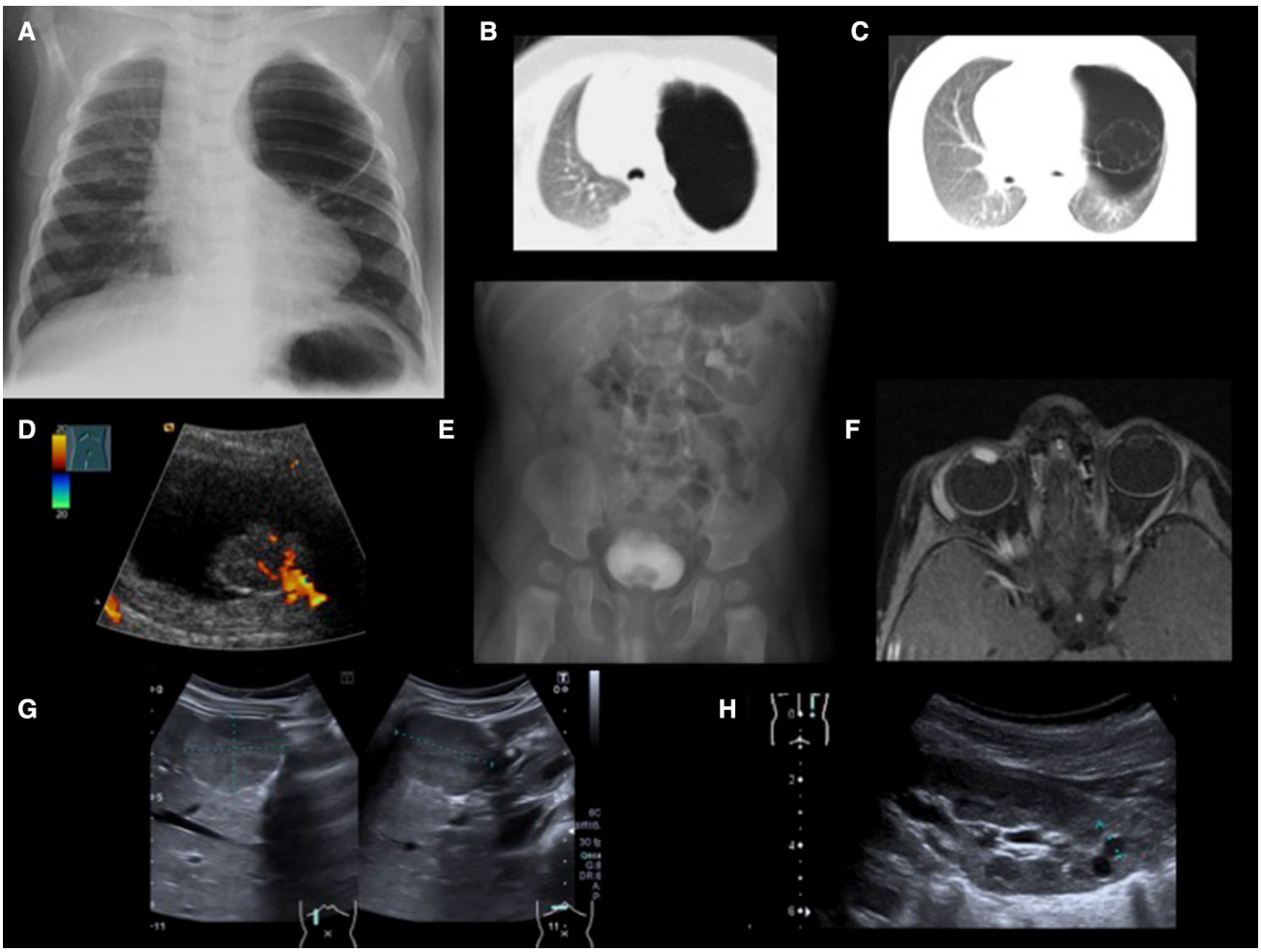


**Fig. 2** **a** X-ray and **b**, **c** CT of the chest: Large cystic lesion of left upper lobe with some cauliflower septs in the basal part. **d** Ultrasound of the bladder: intravesical, solid tumor with pathologic vessels originating from the dorsal bladder wall. **e** Urography 5 min post i.v. contrast: Small lobulated tumor at the base of the bladder. **f** MRI of the orbits, axial T1 weighted, fat saturated image post i.v. contrast: Highly contrast-affine small tumor of the anterior chamber of the right eye with possible invasion of ciliar body and iris. **g** Ultrasound of the liver: Focal nodular hyperplasia in segment IV. **h** Ultrasound of the right kidney: 2 neighbored small cysts in the lower pole

segment I-III, atypical partial resection of the left upper lung lobe was performed three months later. Treatment according to soft tissue sarcoma protocol (CWS 2002-P) with nine courses of multidrug chemotherapy (ifosfamide (subsequently replaced by cyclophosphamide), vincristine and actinomycin D) and seven cycles of maintenance therapy with cyclophosphamide and vinblastine was administered for the bladder tumor as well as complete tumor resection was performed.

Unfortunately, no lung material was kept for later analysis at that time. However, retrospective radiographic evaluation of the pre-operative chest x-ray and CT scans suggests strongly radiographic findings of PPB type I, which is supported by the complex nature of the cyst and by the fact that the patient is DICER1 positive. Treatment recommendations for PPB type I depend on the patient́s age and include chemotherapy in infants and young children. In this case, the multidrug chemotherapy for eRMS included substances, which were also active against PPB.

At the age of $3^{4}/_{12}$ years, ciliary body medulloepithelioma was diagnosed and led to enucleation of the right eye. Now at the age of twelve years, imaging surveillance revealed renal cysts and focal nodular hyperplasia (FNH) of the liver (Fig. 2).

## Identification of a novel, heterozygous frameshift variant in the *DICER1* gene

We performed whole-exome sequencing (WES) analysis of peripheral blood-derived DNA from the patient and the parents with a mean coverage of 96% at 30× or better. The patient showed a mean coverage (overall bases/size of capture targets) of 304-fold, while the father and the

mother had a mean coverage of 314-fold and 264-fold, respectively. We identified a frameshift variant of one bp insertion, according to the sequence we refer to this variant as a duplication (c.3405 dupA). The variant was further confirmed by conventional Sanger sequencing (Fig. 1). Sequencing analyses revealed that the *DICER1* variant occurs heterozygous within the patient and father, while the mother harbors the wildtype sequence. The frameshift mutation (p.Gly1136Arg) introduces a premature stop codon three amino acids downstream of the mutation in exon 21, producing a truncated *DICER1* protein. Since frameshift mutations are mainly deleterious and are expected to disrupt gene function due to a complete absence of the gene by lack of transcription or nonsense-mediated decay [8], the identified protein-truncating frameshift mutation is interpreted as a disease-causing variant.

Additionally, we observed low DICER1 protein levels in the patient's PBMCs compared to a healthy control sample (Fig. 1e).

## Discussion

In this study, we successfully identified a novel heterozygous, frameshift germline mutation in *DICER1* in a patient affected by different tumor types of the DICER1-syndrome spectrum including eRMS, CBME, and suspected PPB type I. The child additionally presented with focal nodular hyperplasia of the liver, which yet was not described in DICER1-syndrome. In general, FNH is a nonmalignant neoplasia of the liver that is usually very uncommon (0.02%) and rarely described in the pediatric population. However, it has been reported more frequently in individuals that were treated with high doses of chemotherapy or hematopoietic stem cell transplantation [9]. Therefore, it has to be considered that the FNH of the liver in our patient could also result from multidrug chemotherapy.

Interestingly, the father of our patient who harbors the same mutation was always in good medical condition indicating that the disease penetrance of this heterozygous germline variant appears to be low. This is in line with previously published data on other mutations causing the DICER1-syndrome showing variable penetrance and clinical presentation [5, 7]. However, the penetrance for any specific *DICER1*-related disorder is not yet known and studies are currently under analyses.

The metachronous occurrence of two unrelated tumor entities such as eRMS and CBME in a very young child within a short period of time should raise the suspicion of an underlying cancer susceptibility syndrome and should have prompted further investigations. This holds particularly true, as due to modest penetrance, variable phenotype, and de novo mutations the family history may not be helpful [10]. However, our patient first presented in 2004 before the first DICER1 tumor predisposition syndrome was described in 2009 by Hill et al. [3].

## Conclusion

Infants and young children presenting with a malignancy within the spectrum of DICER1-syndrome should be considered for genetic testing as they are at increased risk of developing additional tumors within the first two decades of their life. However, comprehensive surveillance protocols for DICER1-syndrome still have to be established and to prove efficiency in terms of superior survival.

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**Author's contribution** JF carried out the Sanger-sequencing and the Western Blot analysis. JF and MK drafted the manuscript, SB and TB obtained informed consent, asked for the family history, and cared for the patient. JS performed and interpreted the radiological imaging. AB and MK designed and supervised the project. AB critically revised the manuscript for important intellectual content. All authors approved the final manuscript as submitted.

**Compliance with ethical standards**

**Conflict of interest** The authors declare no conflict of interest.

**Ethical approval** The study was approved by the ethics committee of the Heinrich-Heine-University Duesseldorf, Germany (reference number 4886).

**Informed consent** Written informed consent was obtained from both parents.

## References

1. Foulkes WD, Priest JR, Duchaine TF (2014) DICER1: mutations, microRNAs and mechanisms. Nat Rev Cancer 14(10):662–672. doi:10.1038/nrc3802
2. Brenneman M, Field A, Yang J, Williams G, Doros L, Rossi C et al (2015) Temporal order of RNase IIIb and loss-of-function mutations during development determines phenotype in DICER1 syndrome: a unique variant of the two-hit tumor suppression model. F1000Research 4:214. doi:10.12688/f1000research.6746.1
3. Hill DA, Ivanovich J, Priest JR, Gurnett CA, Dehner LP, Desruisseau D et al (2009) DICER1 mutations in familial pleuropulmonary blastoma. Science 325(5943):965. doi:10.1126/science.1174334
4. de Kock L, Druker H, Weber E, Hamel N, Traubici J, Malkin D et al (2015) Ovarian embryonal rhabdomyosarcoma is a rare

manifestation of the DICER1 syndrome. Hum Pathol 46(6):917–922. doi:10.1016/j.humpath.2015.02.008
5. Doros L, Yang J, Dehner L, Rossi CT, Skiver K, Jarzembowski J et al (2012) DICER1 mutations in embryonal rhabdomyosarcomas from children with and without familial PPB-tumor predisposition syndrome. Pediatr Blood Cancer 59(3):558–560. doi:10.1002/pbc.24020
6. Priest JR, Williams GM, Manera R, Jenkinson H, Brundler MA, Davis S et al (2011) Ciliary body medulloepithelioma: four cases associated with pleuropulmonary blastoma—a report from the International Pleuropulmonary Blastoma Registry. Br J Ophthalmol 95(7):1001–1005. doi:10.1136/bjo.2010.189779
7. Slade I, Bacchelli C, Davies H, Murray A, Abbaszadeh F, Hanks S et al (2011) DICER1 syndrome: clarifying the diagnosis, clinical features and management implications of a pleiotropic tumour predisposition syndrome. J Med Genet 48(4):273–278. doi:10.1136/jmg.2010.083790
8. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17(5):405–424. doi:10.1038/gim.2015.30
9. Masetti R, Biagi C, Kleinschmidt K, Prete A, Baronio F, Colecchia A et al (2011) Focal nodular hyperplasia of the liver after intensive treatment for pediatric cancer: is hematopoietic stem cell transplantation a risk factor? Eur J Pediatr 170(6):807–812. doi:10.1007/s00431-010-1388-z
10. Kuhlen M, Borkhardt A (2015) Cancer susceptibility syndromes in children in the area of broad clinical use of massive parallel sequencing. Eur J Pediatr 174(8):987–997. doi:10.1007/s00431-015-2565-x