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SHORT COMMUNICATION

Rhabdoid tumor predisposition syndrome caused by SMARCB1 constitutional deletion: prenatal detection of new case of recurrence in siblings due to gonadal mosaicism

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Abstract Rhabdoid tumors are aggressive malignancies that show loss-of-function mutations of *SMARCB1* gene, a member of the SWI/SNF chromatin-remodeling complex controlling gene transcription. One-third of patients affected by rhabdoid tumor harbor a germ-line mutation of *SMARCB1* defining a rhabdoid tumor predisposition syndrome. The occurrence of a second somatic mutation determines the development of neoplasia in a two-hit model. Most germ-line mutations occur de novo, and few cases of recurrence in a sibship have been described. Here we report on a new Italian family with recurrence of *SMARCB1* germ-line deletion in two siblings due to gonadal mosaicism. The deletion was identified in the 9-month-old proband with malignant rhabdoid tumor of the right kidney and disseminated metastases. Testing of both parents confirmed the de novo origin of the mutation, but recurrence was then detected prenatally in a new pregnancy. This is the sixth family with malignant rhabdoid tumor predisposition syndrome with the recurrence of the same germ-line *SMARCB1* mutation in the sibship but not in healthy parents, suggesting that gonadal mosaicism is a less rare event than supposed. The clinical outcome in our patient confirms previous data of poorer outcome in patients with rhabdoid tumor predisposition syndrome.

Keywords Rhabdoid tumor · *SMARCB1* · Gonadal mosaicism · Prenatal diagnosis · 22q deletion

Introduction

Rhabdoid tumors (RTs) are aggressive malignancies of early childhood that may occur in the brain and spinal cord (atypical teratoid/rhabdoid tumor—AT/RTs), kidney (malignant rhabdoid tumor—MRT) and soft tissues (extra-renal malignant rhabdoid tumor—ER-MRT). Both AT/RTs and MRTs show loss of function of *SMARCB1* gene in chromosome band 22q11.2 [1, 2]. *SMARCB1* encodes a subunit of the SWI/SNF chromatin-remodeling complex, which takes part in controlling gene transcription [3]. Germ-line deletions or mutations of *SMARCB1* cause the RT predisposition syndrome (RTPS) [4], and the genetic full inactivation of *SMARCB1*, according to the two-hit Knudson model, is somatically acquired in the tumors. RTPS has an estimated frequency of around 30 % in patients affected by rhabdoid tumor [5, 6]. Most mutations appear to be de novo, and large pedigrees with transmission of the mutation across multiple generations are rare, likely because of the high penetrance and frequent fatal outcome [7]. Rare cases of rhabdoid tumor recurrence in sibship are due to transmission from a healthy carrier parent, even though few cases of gonadal mosaicism of *SMARCB1* mutations have been reported [5–8].

In this report, we describe a new family in which *SMARCB1* germ-line mutation was associated with the development of RT, and the recurrence of the same

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mutation in a second sib indicated parental gonadal mosaicism.

Materials and methods

Case history

The proband is a 9-month-old boy, first child of non-consanguineous healthy parents with unremarkable previous medical history (Fig. 1a). He presented to the emergency department with a 2-month history of persisting vomiting. Physical examination revealed a globus abdomen remarkable for a large mass of the right flank. A total body computed tomography (CT) revealed a mixed density renal mass, measuring 68 × 54 × 48 mm, in the right kidney and multiple pulmonary, and pleural lesions supposed to be metastases. In addition, CT scan discovered cerebral lesions that could be interpreted either as a second primary tumor or as metastases. As referred by pathology unit, the 6 × 4 cm renal mass showed both hemorrhagic and necrotic areas. Histopathological examination revealed an unusual proliferation of cells with eosinophilic cytoplasm, enlarged nuclei, single or multiple nucleoli, and large eosinophilic globular cytoplasmic inclusions. Immunohistochemical stains yielded negative reactions for cytokeratin, myosin, desmin and WT1. Stain for *SMARCB1* showed loss of nuclear expression in the neoplastic cells. The combination of morphologic and immunohistochemical features supported a diagnosis of MRT of the right kidney. The patient underwent right nephrectomy 3 days later followed by a course of chemotherapy (ifosfamide, carboplatin, etoposide) per ICE protocol that failed to reduce extra-renal lesions. He died 4 months later.

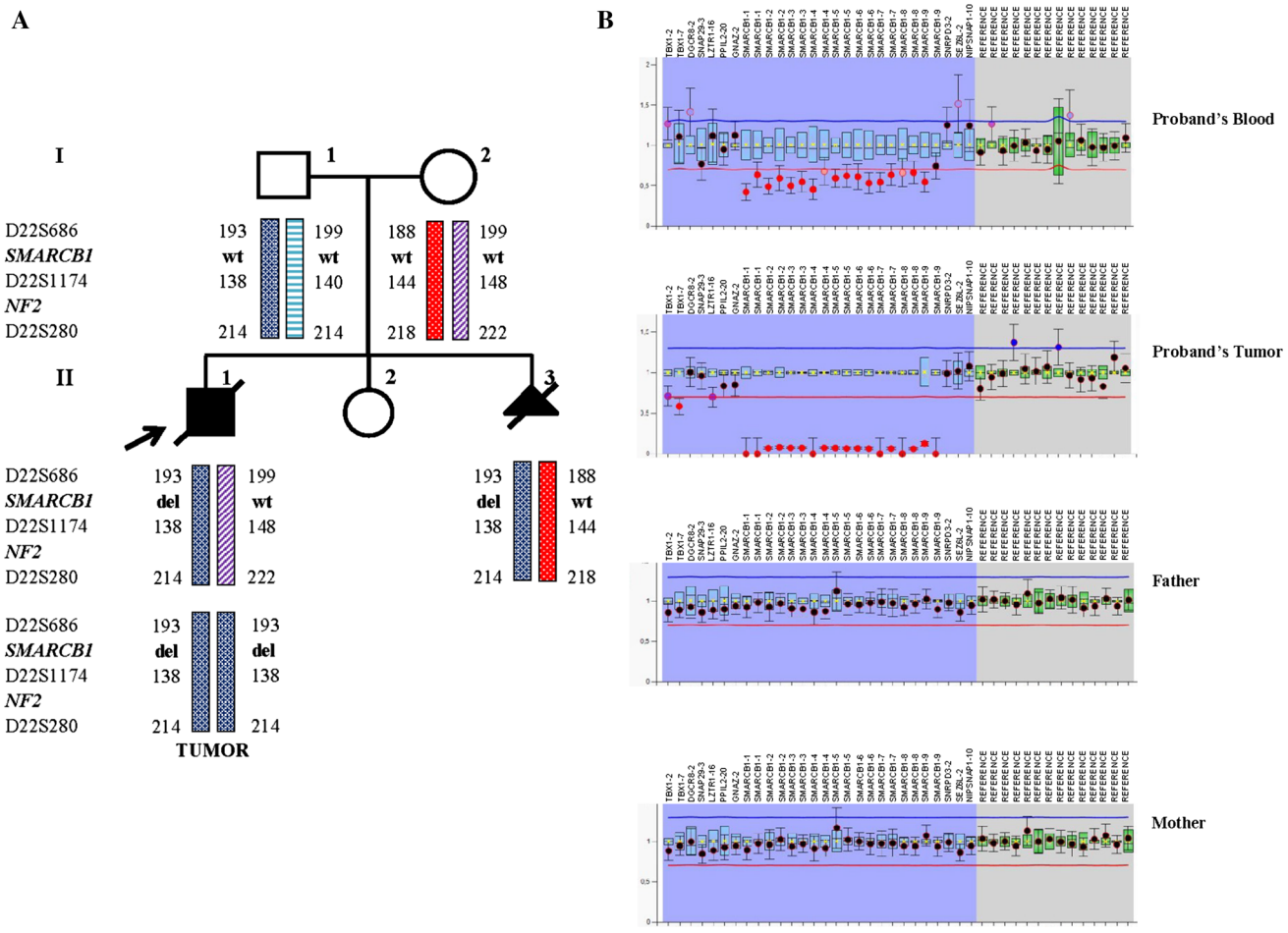

Fig. 1 **a** Microsatellite marker analysis of 22q. Both, the proband and the fetus, inherited the same paternal allele, but two different alleles from the mother. In the tumor sample, loss of heterozygosity along 22q was identified. **b** MLPA analysis of the proband, his tumor and the parents. DNA samples were analyzed by MLPA kit P258-C1. Normalized data from Coffalyzer.net software show a heterozygous deletion of *SMARCB1* in the proband (ratio of 0.5), and a homozygous deletion of the gene in the tumor (ratio of 0), highlighted by the *red dots* that indicate the deleted probes. Both the parents show a normal *SMARCB1* copy number. The results of MLPA and microsatellite analysis in the tumor sample are consistent with mitotic recombination of 22q. (Color figure online)

A new pregnancy occurred after molecular diagnosis in the proband. After prenatal genetic counseling, the couple underwent prenatal diagnosis and amniotic fluid was collected at 17 weeks of gestation.

Microsatellite analysis

Loss of heterozygosity (LOH) on 22q was investigated using microsatellites D22S686, D22S1174 and D22S280. Capillary electrophoresis was performed on 310 Capillary DNA Analyzer (Life Technologies, Carlsbad, CA, USA), and raw data were analyzed using GeneMapper software (Life Technologies, Carlsbad, CA, USA).

Multiplex ligation probe amplification

Copy number changes (deletions or duplications) of *SMARCB1* and flanking genes were analyzed by multiplex ligation-dependent probe amplification (MLPA). *SMARCB1* MLPA test kits P258_C1 (MRC-Holland, Amsterdam, The Netherlands) was used, and electrophoresis data were analyzed using GeneMapper software (Life Technologies, Carlsbad, CA, USA) and normalized by Coffalyzer.net software (MRC-Holland, Amsterdam, the Netherlands).

Results

We performed molecular testing of *SMARCB1* on DNA both from peripheral blood and from the frozen tumor. Multiple ligation-dependent probe amplification (MLPA) demonstrated a heterozygous germ-line deletion of the whole gene in the blood DNA. In malignant tissue, a homozygous deletion of *SMARCB1* was present, while flanking probes showed normal copy number. However, microsatellite marker analysis in 22q demonstrated a loss of heterozygosity (LOH) of the long arm of chromosome 22 (Fig. 1a). These results are consistent with a mitotic recombination of 22q, leading to isodisomy of chromosome 22, typically identified in RTs [9]. The MLPA results from the parents' blood DNA indicated normal *SMARCB1* copy number (Fig. 1b).

A new pregnancy occurred following the identification of a germ-line mutation in the proband (Fig. 1a). After prenatal genetic counseling, the couple carried out prenatal diagnosis to exclude both fetal aneuploidies for advanced maternal age (38 years) and recurrence of *SMARCB1* deletion. MLPA analysis performed on DNA from amniocytes revealed the heterozygous whole gene deletion. After prenatal genetic counseling, the couple opted not to carry on the pregnancy and underwent medical abortion.

We performed 22q microsatellite analysis in the proband's blood and tumor DNAs, the amniotic fluid and the parents DNAs to evaluate the allelic origin of the genomic rearrangement. We demonstrated that the proband and the fetus inherited the same paternal allele (Fig. 1a), retained in the proband's tumor, but two different alleles from the mother. These results are consistent with the paternal origin of the genomic rearrangement.

Discussion

In this report, we describe a new case of RT associated with a recurrent germ-line mutation of *SMARCB1* gene in offspring of non-carrier parents. Even though *SMARCB1* loss-of-function mutations may be found in the majority of RTs, some clinical features may help in identifying people at risk for RTPS, i.e., carrying a constitutional mutation in *SMARCB1* predisposing to RTs [5]. In our patient, the young age at presentation and the presence of synchronous lesions in different sites were strongly suggestive of an underlying germ-line alteration of *SMARCB1*. Unfortunately, we were not able to confirm independent origin of the lesions searching for different second hit (somatic mutations on the normal allele) in extra-renal lesions as surgery was not performed and parents denied autopsy after demise. A severe course and a poorer prognosis, as in our case, have been reported for patients with RTPS due to germ-line mutations [5, 10, 11], compared to cases with sporadic RTs and constitutional wild genotype. According to Bourdeat [5], the impact of a germ-line mutation on prognosis would mainly be due to the low age at diagnosis that determines a less intensive treatment administered to avoid short- and long-term complication. However, Kordes et al. [10, 11] have recently studied the outcome of RTPS children and concluded that: (a) RTPS is not inevitably associated with detrimental outcome, (b) intensive treatment with curative intent is recommended, and (c) factors like healthy carrier of the mutation in the family, non-metastatic disease and older age at presentation represent favorable prognostic factors.

In our family, the recurrence of the same mutation is consistent with gonadal mosaicism in the father. Five families with documented gonadal mosaicism for *SMARCB1* mutation leading to recurrence of RT and/or other malignancies in kindred have been described so far (summarized in Table 1). We have described the sixth family in which recurrence of *SMARCB1* mutation is explained by gonadal mosaicism. In all these patients, as well as in our proband, the outcome was very poor as expected in case of RTPS.

Recurrence of *SMARCB1* mutation in siblings of a patient with RTPS, even when parents do not carry the mutation in the blood DNA, should not be excluded because there is a low but concrete risk of recurrence, due

Table 1 Clinical and molecular features of pedigrees reported with *SMARCB1* gonadal mosaicism

References	Sex	Dx age (months)	Type of malignancy	Anatomic site	*SMARCB1* germ-line abnormality	*SMARCB1* somatic abnormality	Inheritance
Sevenet et al. 1999 (pedigree 2)	M	na	Medulloblastoma	CNS	na	na	De novo
	M	na	MRT	Extra-renal	c.472C > T	c.472C > T	
Sevenet et al. 1999 (pedigree 3)	M	4	CPC	CNS	c.591delC	Deletion	De novo
	F	2	AT/RT	CNS	c.591delC	Deletion	
	M	12	AT/RT	CNS	c.591delC	c.591delC	
Eaton et al. 2010 (family 1)	F	5	Sarcoma	Bladder	c.20_43delinsT	Deletion	De novo
	F	2	AT/RT	CNS	c.20_43delinsT	Deletion	
Bruggers et al. (2011)	M	At birth	na	na	na	na	De novo
	F	6	na	na	Duplication E4-5	na	
	M	3 weeks	na	na	Duplication E4-5	na	
Bourdeaut et al. (2010)	M	3	AT/RT	CNS	c.472C > T	na	De novo
	F	8	AT/RT	CNS	c.472C > T	na	
Present report	M	9	MRT and AT/RT	CNS, renal	Deletion	Deletion	De novo
	F	Prenatal (17 weeks)	–	–	Deletion	na	

CPC choroid plexus carcinoma, *MRT* malignant rhabdoid tumor, *AT/RT* atypical teratoid rhabdoid tumor, *CNS* central nervous system, *na* not available

to gonadal mosaicism. Recurrence of the mutation in a new pregnancy represents a challenging issue in prenatal genetic counseling, because the a priori risk of developing RT in the setting of a germ-line mutation is not yet known. Anyway, earlier onset of neoplasia in germ-line mutation carrier and the absence of *SMARCB1* mutation in sibs or parents of affected individuals (with the exception of rare healthy carrier) suggest a high penetrance for RTPS. Finally, the outcome for children with RTs due to RTPS is not yet delineated and perspectives on treatment option are in the beginning.

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Compliance with ethical standards

Conflict of interest None declared.

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