



High prevalence of *SMARCB1* constitutional abnormalities including mosaicism in malignant rhabdoid tumors

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Abstract

Intensive analysis of the *SMARCB1* gene in malignant rhabdoid tumors (MRT) revealed eight of 16 patients with constitutional genetic variants. Three patients had mosaicism of deletion/variant of the *SMARCB1* gene, which conventional methods might overlook. The prevalence of cancer predisposition in MRT may thus be higher than previously reported.

Background

Malignant rhabdoid tumor (MRT), including atypical teratoid/rhabdoid tumor (AT/RT), MRT of the kidney, and extracranial MRT, is a rare and highly aggressive malignancy. These three subtypes have a common genetic hallmark, namely, loss-of-function variants or deletions in the *SMARCB1* (also known as the *hSNF5/IN11/BAF47*) gene [1]. Most MRT cells show bi-allelic alteration in

SMARCB1 [1] and are a typical example of Knudson's two-hit hypothesis [2–4].

Variants in the *SMARCB1* gene are not always somatically acquired. Previous studies demonstrated that approximately one-third of patients had a germline *SMARCB1* abnormality resulting in the first hit of Rhabdoid Tumor Predisposition Syndrome (RTPS) [5–7]. Actually, most germline *SMARCB1* abnormalities are presumed to be sporadic, but a few familial cases have been reported [5, 6, 8]. Although some studies reported that individuals with RTPS showed a high rate of multiple tumor development at an early age, the clinical characteristics and prognosis remain controversial [6, 7, 9].

In a study by Acuna-Hidalgo et al., intensive genetic analysis revealed that some variants which were previously

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considered to be de novo actually were post-zygotic mosaic variants with a proportion of 6.5% [10], and it has been suggested that similar mosaic variants may be present in a certain fraction of childhood cancer cases [11]. However, no studies to date have focused on the frequency of post-zygotic mosaicism in MRT. We investigated herein the prevalence of germline variants in the *SMARCB1* gene by intensive analysis, including quantitative assessment of copy numbers and a variant detection assay using droplet digital PCR (ddPCR) capable of detecting low-frequency germline alterations.

Methods

Patients

This study was approved by the ethics committee at the National Center for Child Health and Development (#1035) and written informed consent was obtained from the patients and/or guardians. The enrolled patients received the diagnosis of MRT at the National Center for Child Health and Development, the Shizuoka Children's Hospital or St. Marianna University School of Medicine Hospital between 2006 and 2018. Histological diagnosis was confirmed by the negativity of *SMARCB1* by immunohistochemistry. In total, 16 patients were included. Both tumor-derived DNA and germline DNA were obtained from all patients (Table 1).

DNA isolation and PCR analyses for *SMARCB1*

DNA was extracted using a QIAamp DNA Mini kit or a QIAamp DNA FFPE tissue kit (Quiagen, Hilden, Germany) following the manufacturer's instructions. Standard PCR reactions were carried out using AmpliTaq Gold 360 Master mix (Applied Biosystems, Carlsbad, CA, USA). The primer sequences for each exon of *SMARCB1* are shown in Supplementary Table 1.

First, screening for *SMARCB1* alterations in the tumor specimens was done by direct sequencing and ddPCR in all the samples. SNP array analysis was additionally performed for 12 cases with a sufficient quantity and quality of DNA. Then, analysis of germline DNA samples focusing on the genomic abnormalities detected in the paired tumors in each case was performed.

Droplet digital PCR

The ddPCR analysis was performed using QX-200 (Bio-rad, Hercules, CA, USA) to determine the copy number of each exon of *SMARCB1* (exons are numbered like in NM_003073.3) [12]. Specific primers and probes were designed for each exon (Supplementary Table 2). An *RPP30* gene primer/probe mix (Bio-Rad, Hercules, CA,

USA) was used as an internal control [13]. Sensitivity testing of copy number analysis for exon 4 of *SMARCB1* showed that the detection limit for deletions was as low as 0.75% (Supplementary Fig. 1). Primer/probe sets were also designed for single nucleotide variants and indels detected in tumors by direct sequencing and were used in analyzing for the presence of low-frequency germline abnormalities (Supplementary Table 3).

SNP array analysis

Genome-wide analysis for copy number alterations and allelic imbalances was performed by single nucleotide polymorphism (SNP) array analysis. DNA extracted from the samples was analyzed using GeneChip Human Mapping 250 K *NspI* array (Affymetrix, Santa Clara, CA, USA). The obtained data were processed using CNAG/AsCNAR software according to the previously described method [14].

Statistical analyses

The difference in the median age between patient groups was analyzed by variant status using the Mann–Whitney *U* test. Overall survival and progression-free survival (PFS) were estimated using the Kaplan–Meier method and compared using the log-rank test. Cox regression models were used to examine the prognostic factors affecting survival time.

Results

The 16 patients included those with AT/RT ($n = 8$), MRT of the kidney ($n = 2$), extracranial MRT ($n = 4$), or both intracranial and extracranial MRT ($n = 2$). The median age at diagnosis was 16.5 months (range, 0–154 months). Bi-allelic alterations of the *SMARCB1* gene were found in the tumor cells of all 16 patients (Table 1). Of the 32 genomic alterations, ten were nonsynonymous nucleotide substitutions and frameshift variants, ten were deletions of one or more exons within *SMARCB1*, and 12 were copy number neutral losses of heterozygosity identified mainly by SNP array analysis. All the detected nonsynonymous variants were nonsense variants or frameshift insertions/deletions leading to the early truncation of the *SMARCB1* protein (Table 1 and Supplementary Fig. 2). No patients had any malformations or neurodevelopmental disorders.

In eight of 16 cases (50%), genomic alterations observed in the tumor-derived DNA were also detected in constitutional DNA. It is worth noting that three patients had germline mosaicism (UPN4, 5 and 8) (Fig. 1 and Supplementary Fig. 3). Two patients had mosaic deletion, including *SMARCB1*, and the average copy number of the deleted region in the *SMARCB1* gene in the germline was 1.60

Table 1 *SMARCB1* alterations in 16 patients with germline and/or somatic abnormalities.

| UPN | Sex | Age at diagnosis (month) | Anatomic site at diagnosis | Germline abnormality ^a | Somatic abnormality | Progressive site | Time to progression (month) | Status | Follow-up period (month) |
|-----|-----|--------------------------|---|--|------------------------------------|--|-----------------------------|--------|--------------------------|
| 1 | F | 0 | Forehead ^b , Neck | c.601C>T, p.(Arg201Ter) | c.(?-207)_(*338_?)del | Multiple CNS | 1 | DOD | 8 |
| 2 | F | 5 | CNS ^b | c.(?-207)_(*338_?)del | CN LOH | CNS | 17 | DOD | 26 |
| 3 | F | 5 | CNS ^b | c.118C>T, p.(Arg40Ter) | c.(?-207)_(*338_?)del | CNS | 25 | alive | 129 |
| 4 | F | 0 | CNS, Kidney ^b , Liver ^b , Lung ^b , Adrenal glands ^b | c.(?-207)_(*1118+1_1119-1)del (mosaic) | CN LOH | | | DOD | 0 |
| 5 | M | 75 | CNS ^b | c.(?-207)_(*338_?)del (mosaic) | CN LOH | CNS | 7 | DOD | 14 |
| 6 | F | 5 | CNS, Liver ^b | c.601C>T, p.(Arg201Ter) | CN LOH | | | DOD | 38 |
| 7 | F | 21 | CNS ^b | c.649_650delAT | CN LOH | | | alive | 25 |
| 8 | M | 46 | CNS ^b | c.157C>T, p.(Arg53Ter) (mosaic) | CN LOH | | | alive | 11 |
| 9 | M | 154 | Liver ^b | | c.(?-207)_(*338_?)del | Multiple liver, Pancreas head, Diaphragm | 10 | DOD | 15 |
| 10 | M | 17 | Kidney ^b | | c.(93+1_94-1)(232+1_233-1)del | Intra-peritoneal, Multiple lung | 17 | DOD | 20 |
| 11 | F | 13 | Kidney ^b , Liver, Lung, Intestine, Lymph node | | c.(?-207)_(*338_?)del (homozygous) | | | DOD | 0 |
| 12 | M | 30 | CNS ^b | | c.881_922del | CN LOH | | alive | 58 |
| 13 | M | 16 | CNS ^b | | c.96delG | CN LOH | | alive | 24 |
| 14 | M | 47 | Neck ^c | | c.553_554insATAGG | c.(232+1_233-1)_(*362+1_363-1)del | 19 | DOD | 25 |
| 15 | M | 83 | Neck ^c | | c.601C>T p.(Arg201Ter) | CN LOH | | alive | 36 |
| 16 | F | 16 | CNS ^c | | c.178_179delAG | CN LOH | | DOD | 5 |

UPN, unique patient number, *Dx age*, age of diagnosis, *CN LOH*, copy number neutral loss of heterozygosity, *CNS*, central nervous system, *DOD*, dead of disease, *-207*, the first nucleotide of 5' untranslated region, **338*, the last nucleotide of 3' untranslated region. For descriptions with uncharacterized breakpoints see: <http://amomen.hgvs.org/recommendations/uncertain/>.

^aPeripheral blood was used for germline genetic testing except for UPN9 and 11 using FFPE tissue without pathological tumor infiltration; Reference Sequence: NM_003073.3.

^bFresh frozen diagnosed pathologically was used for tumor genetic testing.

^cFormalin-fixed, paraffin-embedded (FFPE) tissue diagnosed pathologically was used for tumor genetic testing.

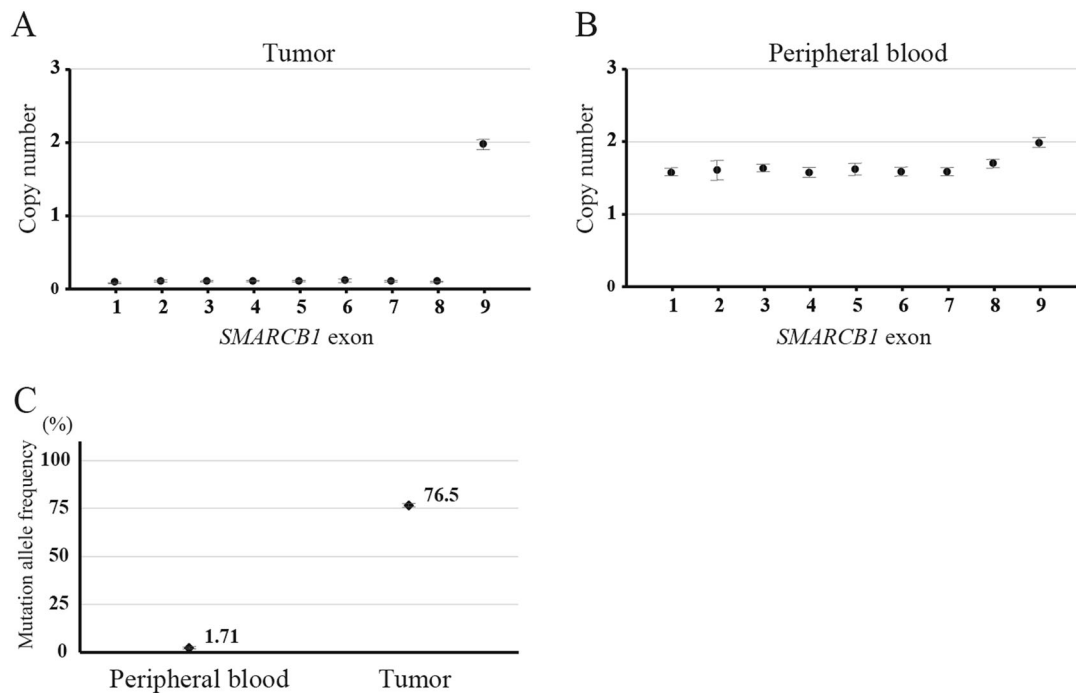


Fig. 1 Representative results of mosaic alterations in *SMARCB1*. **a**, **b** Shown are the copy number analyses for each exon of the *SMARCB1* gene using digital droplet PCR. Homozygous deletion of exons 1–8 was observed in tumor specimens of UPN4 (**a**). The copy number of exons 1–8 was estimated at 1.60, indicating that 40% of cells in

(UPN4) and 1.76 (UPN5), respectively, indicating that 40 and 24% of their cells had heterozygous deletions. For UPN8, the fraction of *SMARCB1* variants in normal cells was 1.7%, indicating that 3.4% of cells had the *SMARCB1* variant.

The median age at diagnosis with or without germline abnormalities was 5 months (range, 0–75 months) and 23.5 months (range, 13–154 months), for the respective groups, and the age difference was not statistically significant ($p = 0.082$, Supplementary Fig. 4). Two cases with both intracranial and extracranial MRT showed constitutional genomic alterations. Recurrence with or without constitutional abnormalities was observed in four and three patients, respectively, in whom PFS at 2 years was 46.9% and 37.5% ($p = 0.52$, Supplementary Fig. 5).

In three patients (UPN1, 2, and 4) with a constitutional abnormality, genetic analysis of their parents was performed using peripheral blood samples following genetic counseling. None of the parents had any abnormality of the *SMARCB1* gene observed in their offspring.

Discussion

We performed a detailed analysis of the prevalence of *SMARCB1* germline/constitutional abnormalities using the ddPCR assay, and identified three new cases (37.5%) among the eight patients with a germline abnormality with mosaicism

peripheral blood had a heterozygous deletion in this region (**b**). **c** A nonsense variant (c.157C>T) was observed in 76.5% of tumor cells in UPN8 and detected in peripheral blood at a mutant allele frequency of 1.71% indicating a mosaicism rate of 3.42%. Error bars represent 95% confidence intervals.

contributing to the pathogenesis of MRT. These cases had a small proportion of mutated cells indicative of mosaicism and were therefore possibly overlooked by conventional qualitative analyses. Our study revealed a prevalence of RTPS in almost half of the MRT cases exceeding previously reported figures [5–7] and underscoring the importance of constitutional alterations in the etiology of pediatric cancers, as shown in recent, large-scale studies [15, 16].

In our cohort, truncating *SMARCB1* variants, which predispose to RTPS, were observed in all eight patients with constitutional abnormalities including mosaicism [17]. Germline variants in *SMARCB1* are also known as causes of schwannomatosis and Coffin-Siris syndrome, thus some patients may have manifestations of these diseases, as well as of RTPS, although none of our cases had the typical phenotype of schwannomatosis and Coffin-Siris syndrome during follow-up period [12, 17].

Some patients with RTPS show MRT development at an older age. In our cohort, two (UPN5 and 8) of eight patients with RTPS had MRT onset at age 2 years or older, suggesting that genetic screening tests are needed in all patients regardless of age. Interestingly, the two cases showed a low frequency of mosaicism for *SMARCB1* variants.

As in previous reports [5–7], most patients with multiple lesions had germline abnormalities. Bhatt et al. reported MRT development in the soft tissue of a patient with a germline *SMARCB1* variant 8 years after the end of AT/RT

treatment [18]. Germline carriers are presumably at risk of second MRT development, which is difficult to distinguish from a recurrence.

Considering the high frequency of patients with RTPS, genetic counseling for parents is necessary because familial cases have been reported despite their low frequency [5, 6, 8, 9, 19, 20]. Although genetic tests of the parents (UPN1, 2, and 4) were all negative, the possibility of gonadal mosaicism should be considered because there are patients with constitutional mosaicism and also previous reported cases [5, 6, 8, 9, 19, 20].

This study has several limitations. First, due to the small sample size, the precise prevalence of germline variants and the correlation between clinical features and variant origins could not be accurately assessed and require investigation in larger-scale studies. Second, although our ddPCR method was able to detect a frequency of mosaicism as low as 1%, the prevalence of constitutional variants may have been underestimated due to overlooking other, lower frequency mosaicisms.

In conclusion, approximately half the MRT cases in this study had *SMARCB1* constitutional alterations, including low-frequency mosaicism of deletion or nonsynonymous variants. Considering the presence of low-frequency mosaicisms, inherited germline variants in predisposition genes are more important than previously assumed for the pathogenesis of pediatric cancers.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

- Versteeg I, Sevenet N, Lange J, Rousseau-Merck MF, Ambros P, Handgretinger R, et al. Truncating mutations of hSNF5/INI1 in aggressive paediatric cancer. *Nature*. 1998;394:203–6.
- Reisman D, Glaros S, Thompson EA. The SWI/SNF complex and cancer. *Oncogene*. 2009;28:1653–68.
- Roberts CW, Biegel JA. The role of SMARCB1/INI1 in development of rhabdoid tumor. *Cancer Biol Ther*. 2009;8:412–6.
- Knudson AG Jr. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci USA*. 1971;68:820–3.
- Bourdeaut F, Lequin D, Brugieres L, Reynaud S, Dufour C, Doz F, et al. Frequent hSNF5/INI1 germline mutations in patients with rhabdoid tumor. *Clin Cancer Res*. 2011;17:31–8.
- Eaton KW, Tooke LS, Wainwright LM, Judkins AR, Biegel JA. Spectrum of SMARCB1/INI1 mutations in familial and sporadic rhabdoid tumors. *Pediatr Blood Cancer*. 2011;56:7–15.
- Kordes U, Gesk S, Fruhwald MC, Graf N, Leuschner I, Hasselblatt M, et al. Clinical and molecular features in patients with atypical teratoid rhabdoid tumor or malignant rhabdoid tumor. *Genes Chromosomes Cancer*. 2010;49:176–81.
- Sevenet N, Sheridan E, Amram D, Schneider P, Handgretinger R, Delattre O. Constitutional mutations of the hSNF5/INI1 gene predispose to a variety of cancers. *Am J Hum Genet*. 1999;65:1342–8.
- Bruggers CS, Bleyl SB, Pysher T, Barnette P, Afify Z, Walker M, et al. Clinicopathologic comparison of familial versus sporadic atypical teratoid/rhabdoid tumors (AT/RT) of the central nervous system. *Pediatr Blood Cancer*. 2011;56:1026–31.
- Acuna-Hidalgo R, Bo T, Kwint MP, van de Vorst M, Pinelli M, Veltman JA, et al. Post-zygotic point mutations are an under-recognized source of de novo genomic variation. *Am J Hum Genet*. 2015;97:67–74.
- Renaux-Petel M, Charbonnier F, They JC, Fermey P, Lienard G, Bou J, et al. Contribution of de novo and mosaic TP53 mutations to Li-Fraumeni syndrome. *J Med Genet*. 2018;55:173–80.
- Holsten T, Bens S, Oyen F, Nemes K, Hasselblatt M, Kordes U, et al. Germline variants in SMARCB1 and other members of the BAF chromatin-remodeling complex across human disease entities: a meta-analysis. *Eur J Hum Genet*. 2018;26:1083–93.
- Kluwe L. Digital PCR for discriminating mosaic deletions and for determining proportion of tumor cells in specimen. *Eur J Hum Genet*. 2016;24:1644.
- Yamamoto G, Nannya Y, Kato M, Sanada M, Levine RL, Kawamata N, et al. Highly sensitive method for genomewide detection of allelic composition in nonpaired, primary tumor specimens by use of affymetrix single-nucleotide-polymorphism genotyping microarrays. *Am J Hum Genet*. 2007;81:114–26.
- Zhang J, Walsh MF, Wu G, Edmonson MN, Gruber TA, Easton J, et al. Germline mutations in predisposition genes in pediatric cancer. *N Engl J Med*. 2015;373:2336–46.
- Gröbner SN, Worst BC, Weischenfeldt J, Buchhalter I, Kleinheinz K, Rudneva VA, et al. The landscape of genomic alterations across childhood cancers. *Nature*. 2018;555:321.
- Smith MJ, Wallace AJ, Bowers NL, Eaton H, Evans DGR. SMARCB1 mutations in schwannomatosis and genotype correlations with rhabdoid tumors. *Cancer Genet*. 2014;207:373–8.
- Bhatt MD, Al-Karmi S, Druker H, Gupta A, Lu M, Malkin D, et al. Second rhabdoid tumor 8 years after treatment of atypical teratoid/rhabdoid tumor in a child with germline SMARCB1 mutation. *Pediatr Blood Cancer*. 2019;66:e27546.
- Janson K, Nedzi LA, David O, Schorin M, Walsh JW, Bhattacharjee M, et al. Predisposition to atypical teratoid/rhabdoid tumor due to an inherited INI1 mutation. *Pediatr Blood Cancer*. 2006;47:279–84.
- Gigante L, Paganini I, Frontali M, Ciabattini S, Sangiuolo FC, Papi L. Rhabdoid tumor predisposition syndrome caused by SMARCB1 constitutional deletion: prenatal detection of new case of recurrence in siblings due to gonadal mosaicism. *Fam Cancer*. 2016;15:123–6.