Evidence for a low-penetrant extended phenotype of RTPS1 from a kindred with gain of SMARCB1 exon 6

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Abstract

We report on a long-term survivor of an atypical teratoid/rhabdoid tumor (ATRT-TYR) as an index patient, who carries a SMARCB1 exon 6 gain inherited from his father. The father was diagnosed with an unusual sequence of a myxopapillary INI1-negative ependymoma and a relapsing BRAF V600 wild type hairy-cell leukemia. He has two yet healthy sisters aged 33 and 38 years carrying the same variant, from which one had lost an infant to a malignant brain tumor. This family highlights the existence of RTPS1-associated SMARCB1 germline alterations with reduced penetrance and extends the spectrum of involved diseases

Introduction

Pertubations of the SWI/SNF complex are common oncogenic events. The acquired loss of its core subunit member SMARCB1 drives an expanding spectrum of intra- and extracranial tumors, some characterized by rhabdoid features including atypical teratoid/rhabdoid tumor (ATRT), and others without such features, e. g. cribriform neuroepithelial tumor (CRINET), poorly differentiated chordoma, desmoplastic myxoid tumor, sinunasal undifferentiated carcinoma, epithelioid sarcoma, and renal medullary carcinoma. Distinct heterozygous *SMARCB1* germline mutations cause Rhabdoid Tumor Predisposition Syndrome Type 1 (RTPS1), schwannomatosis, or Coffin-Siris syndrome. Although overlap has been reported, a genotype phenotype correlation exists with certain proximal and intronic mutations linked to schwannomatosis, distal missense mutation to Coffin-Siris, and truncating mutations to RTPS1[1].

Methods and results

The index patient **III.1** (Figure 1A) was diagnosed with a non-metastatic posterior fossa brain tumor at the age of 6 years. Histology was consistent with an ATRT. After gross total tumor resection and treatment with radio-chemotherapy according to the EURHAB recommendations, the patient has remained in complete remission for 15 years after diagnosis. His father **II.2** was diagnosed with a*BRAF* V600 wild-type hairy cell

leukemia variant (HCL-v, with co-expression of CD19, CD103, CD11c, CD25) at the age of 47 years. He achieved a complete remission after 5 cycles of cladribin, but suffered from a relapse 9 years later, with a very good partial response to additional cycles of cladribin. With 54 years he underwent surgery for an L2-3 spinal ependymoma. At age 57 a hemi-colectomy for steroid-resistant ulcerative colitis was performed, apparently unrelated to his other malignoma.

Sanger sequencing of *SMARCB1* did not detect any mutations in peripheral blood lymphocytes (PBL) of the index patient **III.1**(Figure 1B)**.** However, a pattern suggesting gain of one copy of exon 6 of *SMARCB1* was found by multiplex ligation-dependent probe amplification (MLPA). MLPA detected an apparently identical*SMARCB1* exon 6 gain in PBL of his father **II.2** and two of his sisters **III.2** , **III.4.** Targeted next generation sequencing (NGS) confirmed the absence of a single nucleotide variant or small indel but could not identify the genomic integration site of the gained exon 6 sequences. *SMARCB1* mutations were absent in PBL of the index patient's grandmother **I.1** , mother **II.1** , and of another sister **III.3** . No material was available from a cousin **IV.1** , who had died from surgical complications of a malignant brain tumor at the age of 8 months. The index patient's grandfather **I.2** had died from non-malignant disease and could not be tested. In the ten children of the grandfather **I.2,** a diagnosis of a malignancy was only known in **II.2** . No detected carrier had any overt intellectual impairment, except for a learning disability in **III.1** attributed to leukencephalopathy after intensive multi-modal treatment at young age. High resolution spinal and lower extremity MRI in **III.1** and **III.2** did not detect dorsal nerve root or peripheral nerve schwannoma.

BAF47 staining was negative in the ATRT of **III.1** and in the myxopapillary ependymoma of **II.1** (Figure 2A-F). Results from the Infinium Methylation EPIC BeadChip DNA methylation analysis for the ATRT and the ependymoma did not assign a molecular diagnosis in the online Heidelberg classifier tool[2]. However,t -SNE plotting associated the ATRT with the clinically favorable subclass of ATRT-TYR (Figure 2G) and the ependymoma with the myxopapillary subclass (Figure 2I). Copy number profiles confirmed a heterozygous loss of Chr 22 in both tumors (Figure 2H, J). In line MLPA detected large deletions of *SMARCB1* in both tumors and indicated, that the allele containing the exon 6 gain was retained in the tumors whereas the wild-type allele was deleted. No additional *SMARCB1*mutation was found in the HCL-v of **II.2** .

Discussion

RTPS1 is characterized by early-onset and even congenital ATRT, and less commonly extracranial rhabdoid tumors. Synchronous or metachronous tumor sequences occur. Thus, RTPS1 is a negative prognosticator in ATRT. Mutations in RTPS1 are usually truncating mutations in distinction to those in Schwannomatosis or Coffin-Siris syndrome[1]. However, overlap between Schwannomatosis and ATRT has been observed[3]. We here report a family in which a gain of SMARCB1 exon 6 segregates with malignant diseases including ATRT. Though we formally cannot rule out an integration of the gained segment elsewhere in the genome, the combination of germline and somatic analyses strongly suggest this change to be pathogenic to one allele of*SMARCB1,* which is retained in the tumors lacking INI1 staining. Interestingly, *SMARCB1* exon 6 duplications have previously been reported in a pedigree with ATRT and schwannoma[4]. Another pedigree with a CRINET and incomplete penetrance of germline*SMARCB1* exon 6 duplication is on record[5].

Our index patient **III.1** , his sister **III.2** , and his father **II.2** did not present any schwannoma on clinical or MRI examination. One cousin **IV.1** died from a malignant brain tumor in infancy. Her mother **III.4** is an asymptomatic carrier of the*SMARCB1* gain, it is possible that she was also afflicted with an ATRT. Regarding the many siblings and half siblings of the father we could not accrue more information. Thus, the exact incidence of further neoplasm was not ascertained and the founding mutation could not be traced back.

A differential diagnosis of a CRINET was entertained for **III.1** , as CRINETs may be similar to ATRT-TYR on methylation profiling, immunochemistry, and by clinical outcome. However, the absence of any cribriform features and rosettes combined with an abundance of typical rhabdoid cells did not support CRINET as a differential diagnosis. No rhabdoid features were seen in the myxopapillary ependymoma of**II.2** . DNA methylation profiling did neither assign a significant score for the ATRT nor for the myxopapillary ependy-

moma on the Heidelberg classifier. While this is a frequent phenomenon for tumors with germline alterations (unpublished observations), the*t* -SNE suggested the ATRT to belong to the prognostically favorable TYR subgroup, in line with the long-term remission observed in our patient. The spinal tumor showed, both histologically and regarding DNA methylation high similarity to the subgroup of myxopapillary ependymoma, a subgroup that is usually occurring in a sporadic setting.

Apart from the myxopapillary ependymoma, the father suffered from a HCL-v. Classical hairy cell leukemia (HCL-c) is typically associated with oncogenic *BRAF* V600E mutations. Epigenetic driver mutations affecting members of the SWI/SNF complex (e.g. *ARID1A*) have been detected in some of those HCL-c, but at increased frequency in hairy cell leukemia variants (HCL-v) that occur without the *BRAF* V600E mutation.[6,7] However, both ependymoma and HCL-v have so far not been observed in the context of *SMARCB1* mutations.

Together, our report highlights the incomplete penetrance of RTPS1 caused by *SMARCB1* gains and the expanding spectrum of malignancies associated with these mutations.

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Legends

Figure 1: Pedigree of described kindred and summary of molecular results

Individuals with malignant CNS tumors and leukemia are highlighted in**A.** The index patient is marked by an oblique arrow. Confirmed*SMARCB1* exon 6 gain carriers (gain+) and non-carriers (gain-) are identified. Carriers **III.2** and **III.4** are unaffected at age 38 and 33 years. Specimens from individual **IV.1** were not available. Molecular results from available samples are summarized in**B** . MLPA, multiplex ligation-dependent probe amplification;*t* -SNE, *t* -distributed stochastic neighbour embedding

Figure 2: Analysis of CNS tumors from II.2 and III.1

Both ATRT (**A,C**) and ependymoma (**B,E**) display typical histology with rhabdoid features (**C**) or Astra-positive matrix, respectively, (**E**) as hallmark features. Both tumors were negative for SMARCB1 by immunohistochemistry (**D, F**) with arrows indicating non-neoplastic ependymal cells as an internal positive control. Analysis of the DNA methylation profiles revealed a classifier score of 0.48 for the ATRT (0.38 for the TYR-subclass, not shown) and a score < 0.3 for the ependymoma (not shown). Using t -SNE together with > 2500 cases from 82 brain tumor classes from Capper et al.[1], the ATRT clustered with the Tyrosinase subclass (zoom-in shown in **G**) and the ependymoma with the myxopapillary subclass (zoom-in shown in **I**). Copy number plots are shown in (**H**) and (**J**). Scale bar in **A**corresponds to 100 µm in **A, B** . Scale bar in C corresponds to 10 µm in **C-F** .

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