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CORRESPONDENCE

Two molecularly distinct atypical teratoid/rhabdoid tumors (or tumor components) occurring in an infant with rhabdoid tumor predisposition syndrome 1

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Atypical teratoid/rhabdoid tumor (ATRT) is a highly malignant brain tumor predominantly arising in young children [1]. Biallelic mutations of the SWI/SNF chromatin remodeling complex members *SMARCB1* or (rarely) *SMARCA4* are the only recurrent genetic alterations [1, 2]. The pathogenesis follows a classical two-hit model and heterozygous germline mutations of *SMARCB1* (associated with rhabdoid tumor predisposition syndrome 1) or *SMARCA4* (rhabdoid tumor predisposition syndrome 2) constitute the first hit in up to 35% of cases [1]. While ATRT is a remarkably homogenous disease on a genetic level, DNA methylation profiles, enhancer landscapes and subgroup-specific transcriptional networks separate ATRT into three distinct molecular subgroups, i.e., ATRT-SHH, ATRT-TYR and ATRT-MYC [3–5]. These subgroups differ with regard to the age of onset, tumor location and imaging features [3–6]. As there may also be differences in response to different therapies and overall survival [7], molecular subgrouping of ATRT is expected to affect patient management in the near future. DNA methylation-based tumor classification has emerged as a robust and reproducible tool for the diagnosis and classification of central nervous system tumors [8]. DNA methylation signatures are supposed to remain stable during the course of the disease [9] and have been successfully employed for molecular subgrouping of ATRT [3–5]. Here we report an infant with rhabdoid tumor predisposition syndrome 1 with a large brain tumor. Supratentorial and infratentorial parts of the tumor demonstrated divergent DNA methylation profiles, suggesting synchronous or metachronous ATRTs of different molecular subgroups and potentially cellular origin.

This 3-month-old boy presented with signs of elevated intracranial pressure. The family history was unremarkable. Initial magnetic resonance imaging revealed hydrocephalus due to a large cystic and contrast-enhancing tumor involving

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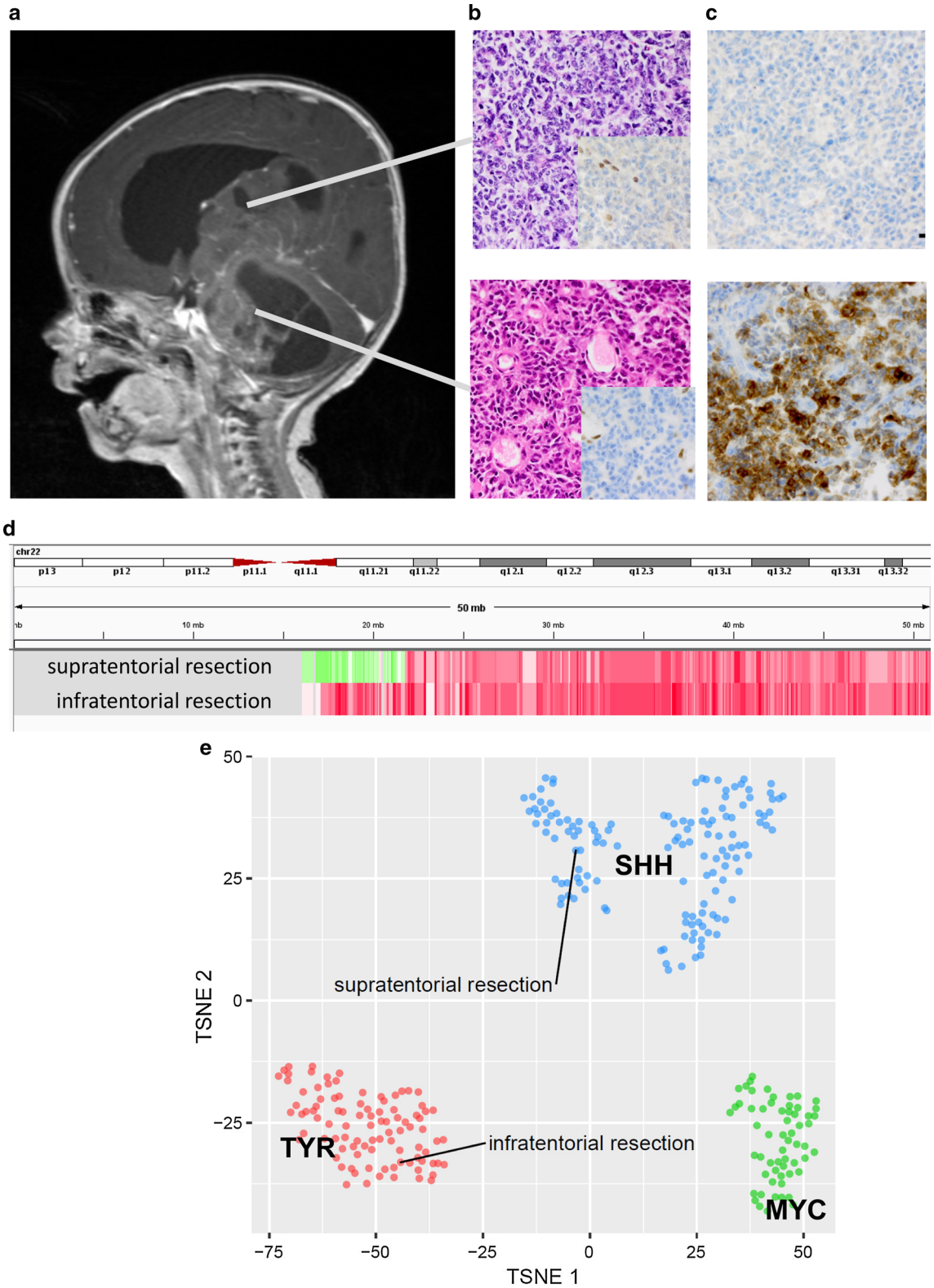

supra- and infratentorial structures. A supratentorial biopsy yielded the diagnosis of ATRT. Two months later, supra- and infratentorial portions of the tumor had increased in size (Fig. 1a). The supratentorial tumor portions were resected first, followed 1 week later by resection of the infratentorial tumor portions. Samples obtained from the resection of the supratentorial tumor portions as well as the resection of the infratentorial tumor portions were examined. Both

◂**Fig. 1** Magnetic resonance imaging, histopathology and molecular profiling. Magnetic resonance imaging showed a large tumor with supratentorial and infratentorial components and cystic appearance (**a**). On histopathological examination of a supratentorial and infratentorial sample, malignant rhabdoid tumors displaying loss of SMARCB1/INI1 protein expression (insets) were encountered (**b**). Tyrosinase staining was negative in the supratentorial sample, but positive in the infratentorial sample (**c**). DNA copy-number profiles showed a heterozygous deletion (red) retaining two copies of the centromeric part of 22q (green) in the supratentorial sample, but a heterozygous deletion of the complete 22q arm in the infratentorial sample (**d**). DNA methylation profiles of the supratentorial and infratentorial sample were divergent and clustered with ATRT-SHH and ATRT-TYR, respectively, of a reference cohort of 289 ATRT on t-SNE plot (**e**)

samples displayed rhabdoid tumor cells and loss of tumoral SMARCB1/INI1 expression (Fig. 1b) and the diagnosis of ATRT was confirmed. Parents had given informed consent for participation in the European Rhabdoid Tumor Registry (EU-RHAB) as well as the Molecular Neuropathology 2.0 (MNP 2.0) study. A sample from the supratentorial resection was submitted to the Department of Neuropathology Heidelberg within the MNP 2.0 study, whereas a sample from the infratentorial resection was submitted to the Institute of Neuropathology Münster, reference center of EU-RHAB. Subsequently, molecular profiling of both samples was independently performed in the context of the two studies using the Methylation EPIC BeadChip array (850 k, Illumina, San Diego, CA).Subsequently, molecular profiling of both samples was independently performed in the context of the two studies using the Methylation EPIC BeadChip array (850 k, Illumina, San Diego, CA). Interestingly, DNA methylation-based classification using the Heidelberg Brain Tumor Classifier (version v11b4) [8] classified the supratentorial sample as ATRT-SHH (score: 0.99), whereas the infratentorial sample was classified as ATRT-TYR (score 0.99). In addition, immunohistochemical stainings for OTX2 and tyrosinase, known to be highly expressed in ATRT-TYR (but not in ATRT-SHH) [4, 5, 10] were positive in the infratentorial sample, but negative in the supratentorial sample (Fig. 1c). Furthermore, DNA copy-number profiles derived from intensity values of 850 k data showed a 22q deletion retaining two copies of the centromeric part in the supratentorial sample, but a heterozygous deletion of the complete 22q arm in the infratentorial sample (Fig. 1d). The discrepant DNA methylation-based classifier results were further confirmed in the unsupervised t-SNE analysis of the methylation profiles of the two samples together with published [4, 5] and unpublished reference ATRT samples ($N=289$, Fig. 1e). To exclude the possibility of sample mix-up, genotype analyses of the 850 k data sets were performed. Comparison of intensity signatures of CG sites targeting SNPs showed high correlation of rs-loci (> 99.6%) and autosomal CpG loci (93.9%), suggesting a high probability that both samples were derived from the same patient. For germline analyses, fluorescence in situ hybridization (FISH) of the *SMARCB1* locus, *SMARCB1* sequencing and multiplex ligation-dependent probe amplification (MLPA) were performed as described previously [2, 11]. While no germline alterations could be detected using FISH and sequencing, MLPA demonstrated a *SMARCB1* duplication affecting exons 2–5 (Supplementary Fig. 1, Online Resource 1). Both parents showed normal MLPA results, suggesting a de novo *SMARCB1* germline alteration in our patient. Adjuvant chemotherapy was administered and the supratentorial tumor component initially responded well to therapy. Despite all efforts, however, the patient succumbed to disease 9 months after the diagnosis had been established.

The finding of divergent DNA methylation signatures in infratentorial and supratentorial portions of a large ATRT is of biological interest, but also has diagnostic implications and clinical relevance. Children with rhabdoid tumor predisposition syndrome often develop synchronous or metachronous tumors, a fact contributing substantially to the dismal outcome in this patient group [11]. In this setting, ATRTs characteristically occur together with rhabdoid tumors of the kidney or other non-CNS malignant rhabdoid tumors. Recently, molecular profiling of paired samples of ATRT and non-CNS malignant rhabdoid tumors revealed divergent copy-number alterations and DNA methylation signatures, suggesting a non-clonal origin [12]. Similarly, in the present case, infratentorial and supratentorial portions of the tumor also showed divergent copy-number alterations and DNA methylation signatures. Even though the possibility of clonal evolution from one single tumor cannot be excluded, in light of the different DNA methylation signatures, our findings are compatible with independent development of two ATRTs from different cells of origin harboring different alterations of the *SMARCB1* locus. Prospective sampling and molecular profiling of different tumor regions over the course of the disease are expected to contribute to a better understanding of intratumoral heterogeneity in ATRT and might also address the question if molecular heterogeneity is due to the development of independent synchronous/metachronous tumors or clonal evolution. Finally, the current case highlights the importance of a close collaboration between study centers in case multiple patient samples are being processed in the context of several clinical studies.

In conclusion, our findings suggest that molecularly distinct atypical teratoid/rhabdoid tumors (or tumor components) of different molecular subgroups may occur in infants with rhabdoid tumor predisposition syndrome. This possibility needs to be taken into account to avoid molecular misclassification.

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