Virchows Archiv (2019) 475:625–636
https://doi.org/10.1007/s00428-019-02606-9

ORIGINAL ARTICLE

IDH1 immunohistochemistry reactivity and mosaic *IDH1* or *IDH2* somatic mutations in pediatric sporadic enchondroma and enchondromatosis

Essia Saiji[1] · Fabienne Gumy Pause[2] · Pierre Lascombes[3] · Christelle Cerato Biderbost[1] · Nathalie Lin Marq[1] · Margaret Berczy[1] · Laura Merlini[4] · Anne-Laure Rougemont[1]

Received: 12 March 2019 / Revised: 28 May 2019 / Accepted: 10 June 2019 / Published online: 25 June 2019

Abstract

Mosaic somatic mutations in the isocitrate dehydrogenase 1/2 (*IDH1/2*) genes have been identified in most enchondromas by targeted mutation analysis. Next-generation sequencing (NGS), that may detect even low-level mosaic mutation rates, has not previously been applied to enchondromas. Immunohistochemistry using the H09 clone is routinely used as a surrogate for the common R132H *IDH1* mutation in gliomas. We compared immunohistochemistry and NGS results in a series of 13 enchondromas from 8 pediatric patients. NGS identified a heterozygous *IDH* mutation in all enchondromas, showing identical mutation status in patients with multiple tumors assessed, thereby confirming somatic mosaicism. A majority of the tumors harbored an *IDH1* mutation (p.R132H in 3 tumors; p.R132C in 4 tumors from 2 patients; p.R132L and p.R132G in one tumor each). A p.R172S *IDH2* mutation was identified in 4 enchondromas, but not in the ependymoma from one patient with Ollier disease, who further displayed a heterozygous *STK11* missense mutation. *IDH* mutation rates varied between 14% (indicative of mutations in 28% of the cells and of intratumoral mosaicism) and 45% (tumor content was close to 100%). Cytoplasmic H09 reactivity was observed as expected in tumors with an *IDH1* p.R132H mutation; cross-reactivity was seen with the p.R132L variant. This first NGS study of pediatric enchondromas confirms that *IDH* mutations may occur in a mosaic fashion. *STK11* gene mutations may provide insights in the development of multiple cartilaginous tumors in enchondromatosis, this tumor suppressor gene having been shown in animal models to regulate both chondrocyte maturation and growth plate organization during development.

Keywords IDH mutation · Immunohistochemistry · Next-generation sequencing · NGS · Ollier · FFPE · Children

Introduction

Enchondromas are benign, cartilage-forming tumors, sporadic, and usually solitary. Enchondromas most often are incidental radiographic findings, but may also manifest as a variably painful tumefaction or as a pathological fracture. They most commonly affect the short tubular bones of the hands. The long tubular bones of the limbs are next in frequency, flat bones such as the pelvis representing uncommon localizations. Diagnosis relies on integration of the clinical, radiological, and histological findings [1].

Enchondromatosis, a group of rare skeletal disorders, has in common the occurrence of multiple enchondromas. Ollier disease (OMIM #166000) and Maffucci syndrome (OMIM #614569), both noninherited disorders, represent the most frequent enchondromatoses. In Ollier disease, multiple enchondromas display an asymmetric distribution and may be unilateral. The estimated prevalence of Ollier disease is 1/100,000. Maffucci syndrome is characterized by the further occurrence of cutaneous, soft tissue, or visceral hemangiomas [1].

✉ Anne-Laure Rougemont
anne-laure.rougemont@hcuge.ch

[1] Division of Clinical Pathology, Molecular Pathology Unit, Geneva University Hospitals, Rue Gabrielle-Perret-Gentil 4, 1211 Geneva, Switzerland

[2] Department of Pediatrics, Onco-hematology Unit, Geneva University Hospitals, Rue Gabrielle-Perret-Gentil 4, 1211 Geneva 14, Switzerland

[3] Pediatric Orthopedic Division, Geneva University Hospitals, Rue Gabrielle-Perret-Gentil 4, 1211 Geneva 14, Switzerland

[4] Pediatric Radiology Unit, Geneva University Hospitals, Rue Gabrielle-Perret-Gentil 4, 1211 Geneva 14, Switzerland

Malignant transformation to chondrosarcoma is described in up to 40% of patients with Ollier disease, being lower however (15%) when enchondromas are restricted to the hands and feet. Incidence of malignant transformation is higher (up to 53%) in patients with Maffucci syndrome. In contrast, sporadic enchondromas rarely recur as low-grade chondrosarcoma [1].

The histological classification of well-differentiated cartilaginous tumors of bone is challenging, both in sporadic enchondromas and in enchondromatosis. The highest degree of interobserver variability was found in the distinction between enchondroma and atypical cartilaginous tumor (ATC)/grade I chondrosarcoma. Criteria in favor of malignancy are high cellularity, presence of bone permeation, absence of host bone encasement, open nuclear chromatin, myxoid changes, and age (>45 years). However, in a phalangeal localization, the most important criteria are the presence of cortical destruction, soft tissue extension, or mitotic activity [1, 2].

Isocitrate dehydrogenase (*IDH*) gain-of-function mutations have been described in enchondroma and enchondromatosis. However, identification of *IDH* mutations allows no distinction between benign and malignant tumors [3]. Other types of cartilaginous tumors and mimics, such as chondroblastomas and soft tissue chondromas, do not show *IDH* mutations [4]. *IDH1* codon 132 mutations are frequent in World Health Organization (WHO) grade II and grade III gliomas, occurring in approximately 70% of astrocytomas and oligodendroglial tumors [5]. An IDH1 immunohistochemical antibody specifically reacting with the *IDH1* R132H point mutation has proven useful in both tumor classification and discrimination from reactive gliosis. Only rare publications have addressed the sensitivity and specificity of this antibody in cartilaginous tumors [3, 6, 7].

Somatic mosaic mutations in enchondromas render the determination of the true *IDH* mutation frequency difficult [3, 7, 8]. To increase *IDH* mutation detection rates, we applied the next-generation sequencing (NGS) to our series of tumors. This technology allows detection of even low-level mosaic mutation rates, provides the percentage of mutated cells, and identifies potential additional mutations.

Here, we compare the NGS and immunohistochemical findings in a series of 13 enchondromas, arising either as solitary or multiple tumors, in 8 patients. Analysis was also performed in an ependymoma from a patient with Ollier disease.

Materials and methods

Patient selection

Over a 15-year period (from 2000 to 2015), 8 pediatric cases with available material for immunohistochemistry and NGS were retrieved from the archives of the Division of Clinical Pathology, Geneva University Hospitals. Three male and 5 female patients were aged 4 to 18 years. Five patients had radiologically documented multiple tumors, and material was available from two or more tumors in three patients. Tumors were located in the hands and fingers in 6 patients, being located in the ilium, and femur in the remaining 2 patients.

The youngest patient, aged 4 years at initial surgical resection, was diagnosed at age 2.5 years as having Ollier disease. At the age of 9 years, a large mixed cystic-solid tumor in the fourth ventricle was surgically resected, and histology revealed an ependymoma, WHO grade II that recurred 3 years later as an anaplastic grade III tumor. Enchondromas were located in the distal ulna, hands (metacarpal bones), and fingers. The patient underwent 15 surgical resections in a 6-year period, 4 of which are included in this study.

Clinical findings are summarized in Table 1.

This study was approved by the Commission Cantonale d'Ethique de la Recherche (CCER Review Board, approval number GE 14-229).

IDH1 immunohistochemistry

Immunohistochemistry was conducted on all enchondromas and on the WHO grade II ependymoma from the patient with Ollier disease. Reactivity to the IDH1 R132H (Dianova, mouse monoclonal, clone H09, 0.2 mg/ml; dilution 1:25) primary antibody was identified using the Ventana Discovery XT autostainer. Antigen retrieval was performed by heating slides 52 min in RiboCC buffer (citrate solution pH 6). Slides were incubated with anti-IDH1 R132H antibodies during 30 min at 37 °C, and detection was performed using the DABMap Detection Kit (Ventana, 760-124).

Decalcification was performed in formic acid (methanoic acid) in the tumors from two patients and in ethylenediaminetetraacetic acid (EDTA) in only one patient. The remaining tumors were not subjected to decalcification. External positive and negative immunohistochemistry controls were run in parallel with the clinical cases, in all three conditions (formic acid or EDTA pretreatment vs no decalcification).

Cytoplasmic reactivity was semiquantitatively recorded (0; 1+ = faint staining; 2+ = moderate staining; 3+ = strong staining). An estimation of the percentage of positive tumor cells was made.

Next-generation sequencing

Tumor cell content in enchondromas and the one case of ependymoma was assessed on H&E slides, and the immunohistochemistry techniques were performed prior to DNA extraction from formalin-fixed paraffin-embedded (FFPE) blocks, using the QIAamp DNA FFPE Tissue Kit (QIAGEN). All

Table 1 Clinical, immunohistochemical, and NGS findings

Patient	Sex	Age	Location	Number of lesions	Tumor cells (%)	IHC-C	NGS	Nucleotide	IDH variant	Mut (%)	ACMG	COSMIC
1	F	18 y	Phalanx	Solitary	100	50% 2+/3+	***IDH1***	c.395G>A	p.R132H	39	5	COSM28746
							ATM	c.1810C>T	p.P604S	39	3	COSM22499
2	F	9 y	Femur	Multiple	95	Neg	***IDH1***	c.394C>T	p.R132C	40	5	COSM28747
							ATM	c.998C>T	p.S333F	46	1 or 2	rs28904919
							APC	c.4372C>T	p.P1458S	51	3	rs143796828
			Ilium		95	Neg	***IDH1***	c.394C>T	p.R132C	14	5	COSM28747
							ATM	c.998C>T	p.S333F	42	1 or 2	rs28904919
							APC	c.4372C>T	p.P1458S	51	3	rs143796828
3	F	12 y	Phalanx	Multiple	95	40% 2+	***IDH1***	c.395G>A	p.R132H	33	5	COSM28746
4	F	13 y	Femur	Multiple, monostotic	95	Neg	***IDH1***	c.394C>T	p.R132C	36	5	COSM28747
		8 y	Femur		95	Neg	***IDH1***	c.394C>T	p.R132C	43	5	COSM28747
							MET	c.3029C>T	p.T1010I	47	3	COSM707
							ATM	c.2572T>C	p.F858L	46	3	COSM21826
5	M	10 y	Phalanx	Ollier disease	100	Neg	***IDH2***	c.516G>T	p.R172S	42	5	COSM24090
							STK11	c.1062C>G	p.F354L	51	3	COSM21360
		10 y	Metacarpal		95	Neg	***IDH2***	c.516G>T	p.R172S	35	5	COSM24090
							STK11	c.1062C>G	p.F354L	49	3	COSM21360
		6 y	Hand		100	Neg	***IDH2***	c.516G>T	p.R172S	43	5	COSM24090
							STK11	c.1062C>G	p.F354L	45	3	COSM21360
		4 y	Phalanx		100	Neg	***IDH2***	c.516G>T	p.R172S	31	5	COSM24090
							STK11	c.1062C>G	p.F354L	46	3	COSM21360
		9 y	Ependymoma		60	Neg	*STK11*	c.1062C>G	p.F354L	39	3	COSM21360
			Blood				*STK11*	c.1062C>G	p.F354L	49	3	COSM21360
6	M	7 y	Phalanx	Multiple		30% 1+	***IDH1***	c.395G>T	p.R132L	41	5	COSM28750
7	F	17 y	Phalanx	Solitary	100	Neg	***IDH1***	c.394C>G	p.R132G	43	5	COSM28749
							FGFR3	c.1150T>C	p.F384L	44	1 or 2	COSM724
8	M	7 y	Phalanx	Solitary	100	70% 2+/3+	***IDH1***	c.395G>A	p.R132H	36	5	COSM28746
							KRAS	c.35G>A	p.G12D	3	5	COSM521

F female, *M* male, *y* years, *IHC* immunohistochemistry, *C* cytoplasmic, *NGS* next-generation sequencing, *Mut (mutation) %* the numbers of reads containing a mutant base at the position of interest, *ACMG* American College of Medical Genetics and Genomics

enchondromas contained close to 100% tumor cells. Consecutive slides of the same tissue blocks were used for immunohistochemistry and NGS. DNA was also extracted from peripheral blood lymphocytes in the patient with Ollier disease.

Extracted DNA preparations were quantified by Qubit using the dsDNA HS Assay Kit (Invitrogen, Q32854), and DNA quality was assessed by measuring the ratio OD260/280 on a NanoDrop instrument.

NGS libraries were performed by amplifying 20 ng of DNA with the Ion AmpliSeq™ Cancer Hotspot Panel v2 and the Ion AmpliSeq Library Kit 2.0 (Thermo Fisher). The Ion AmpliSeq™Cancer Hotspot Panel v2 is a single pool of primers designed to amplify 207 amplicons covering approximately 2800 COSMIC mutations from 50 oncogenes and tumor suppressor genes (see https://www.thermofisher.com/order/catalog/product/4475346 for gene list).

Sequencing was performed on Ion Proton, and variants were called using the Torrent Suite Variant Caller software to create VCF files. Reads were then analyzed by loading BAM and VCF files on the NGS GenSearch software (Phenosystems SA). The criteria for evaluation of any variant as reportable were minimum coverage depth of 100× and minimum allelic variant frequency of 5%. Sequence variants complying with these criteria were further assessed by the ClinVar Database (http://www.ncbi.nlm.nih.gov/clinvar/) and the catalog of somatic mutations in cancer (COSMIC database).

OncoScan assay

Briefly, DNA extracted from FFPE blocks was run on the OncoScan® assay according to the manufacturers' instructions [9]. Array fluorescence intensity data (CEL files), generated by Affymetrix® GeneChip® Command Console® (AGCC) software version 4.0, were processed using the OncoScan Console software version 1.1.034 to produce OSCHP files and a set of QC metrics.

STK11 expression analysis

Total RNA extraction from FFPE samples was performed after 3-μm-thick tissue section deparaffinization in xylol and overnight protein digestion using the High Pure miRNA Isolation Kit (Roche).

100 ng of each RNA preparation was used to synthesize the first cDNA strand with the PrimeScript Reverse Transcriptase (RT) Reagent Kit (Takara #RR037A) according to the manufacturer's instructions.

PCR was then performed using one-tenth of the previous reaction and specific primers for *STK11* (forward 5′ CGCAGCATGACTGTGGTG3′ and reverse 5′CGCTGTCC ATTGTGACTGG3′).

For further sequence analysis, STK11 PCR products from normal and tumor RNA preparations were extracted from a 2% agarose gel, purified using the PeqGold Gel Extraction Kit (PeqLab), and sequenced by Sanger. Sequences were then analyzed using the Sanger GenSearch software (Phenosystems SA).

Results

Radiological findings

Radiology, performed in all cases, showed typical features of enchondroma. Tumors were well marginated and varied from radiolucent to heavily mineralized, with punctuate calcifications. Endosteal erosion or scalloping was seen in a subset of the tumors, whereas expansile lesions were located in the small tubular bones of the hands and fingers. Although a pathological fracture was seen in patient no. 2 within a femoral enchondroma, no other radiological criteria indicative of chondrosarcoma were seen, namely, cortical destruction, permeative osteolysis, periosteal reaction, or edema surrounding the tumor and soft tissue mass [10]. This further highlights the difficulties in the clinical practice in distinguishing enchondroma from well-differentiated chondrosarcoma. Representative radiological findings in patient no. 2 are illustrated in Figs. 1 and 2.

Histological features

Histology showed in all cases a well-differentiated cartilaginous tumor, with a nodular pattern of growth. Chondrocytes, arranged in small clusters in a hyaline matrix, showed small round nuclei and abundant eosinophilic or vacuolated cytoplasm. Bone permeation or cortical destruction was not seen in the rare biopsies sampling bone. Lesions located in the hands and fingers were more cellular and showed mild atypia, with occasional binucleation. Multiple lesions in the same patient showed similar histological features. The cells were stellar, polygonal, or fusiform, with a pale eosinophilic often vacuolated cytoplasm. Nuclei were small, uniform, and round, without significant atypia. No mitotic activity was seen.

Immunohistochemical findings

Four enchondromas from 4 patients displayed cytoplasmic reactivity to the anti-IDH1 R132H antibody, with a variable pattern of staining. Moderate-to-strong cytoplasmic reactivity (2+/3+) was seen in 40 to 70% of the tumor cells in 3 tumors. In the remaining tumor, 30% of the tumor cells showed faint cytoplasmic reactivity (1+). No nuclear reactivity was observed. The remaining 9 enchondromas from 4 patients, as well as the ependymoma, remained entirely negative.

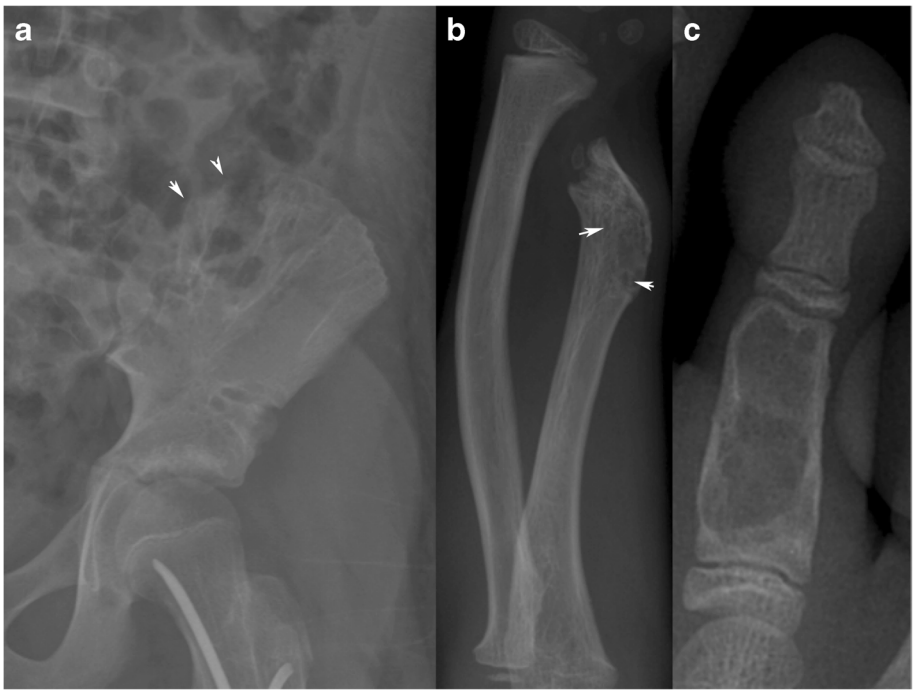


Fig. 1 Representative radiological findings in patient no. 2. X-ray appearance of multiple enchondromas in different locations. **a** Largely erosive with sharply defined scalloped margin enchondroma of the left ileal wing. **b** Eccentric, expansile lytic lesions that contain calcified chondroid matrix in the distal ulna. **c** Lytic expansile lesion in the proximal phalanx, with thinning of the cortex and no appreciable calcified chondroid matrix

Next-generation sequencing findings

NGS identified a heterozygous class 5 ACMG (American College of Medical Genetics and Genomics) *IDH* gene mutation in all the enchondromas. The tumors from all patients but the patient with Ollier disease (patient no. 5) displayed an *IDH1* gene mutation. All 4 enchondromas from the patient with Ollier disease had an *IDH2* mutation. Remarkably, multiple tumors from a same patient showed identical mutational variants. The most frequently observed *IDH1* mutation variant was the p.R132H variant, observed in 3 patients. Then, the p.R132C variant was seen in 2 patients, whereas the p.R132L and p.R132G variants

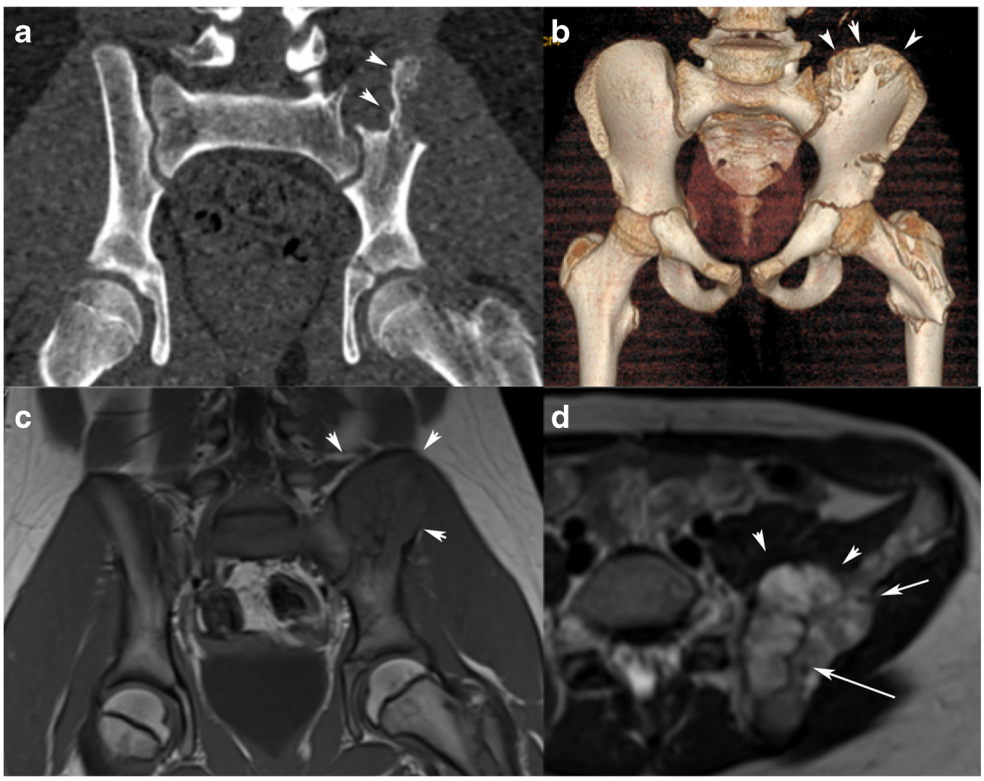


Fig. 2 Representative radiological findings in patient no. 2. Enchondroma of the left ileal wing. Computed tomography on coronal (**a**) and 3D reconstruction (**b**): the lesion is largely erosive, with sharply defined scalloped margins (arrows). MRI coronal T1-weighted MRI (**c**) and axial T2-weighted MRI (**d**) showing the classical appearance of noncalcified hyaline cartilage: intermediate-to-low signal on T1-weighted MRI (**c**) and intense high signal with focal regions of signal drop-out in areas of calcification on T2-weighted MRI (**d**)

were observed in one patient each. The numbers of reads containing a mutant base at the position of interest, in all but one specimen, ranged from 33 to 43%. In only one tumor, the percentage of reads with the mutant base was 14%, indicative of a heterozygous mutation in approximately 28% of the tumor cells, since tumor cell content was close to 100%.

The patient with Ollier disease displayed a heterozygous p.R172S *IDH2* mutation in all 4 assessed enchondromas. Both blood and the ependymoma were *IDH1*/*2* wild-type. A germline heterozygous p.F354L mutation, with conflicting interpretations of pathogenicity according to the ClinVar database, was observed in the *STK11* tumor suppressor gene, with no additional second hit gene mutation in the enchondromas or the ependymoma. RT-PCR followed by Sanger sequencing of the PCR products showed expression of both the p.F354L-mutated and wild-type alleles, in blood and in the tumoral specimens, lending no support to gene silencing due to hypermethylation. Furthermore, no loss of heterozygosity (LOH) was seen in the tumor samples by copy number variant (cnv) analysis (data not shown).

Additional mutations of indeterminate significance were seen in all tumors. Distinct *ATM* mutations considered benign or likely benign (ACMG class 1 or 2) according to the ClinVar database and to OncoKB were seen in 3 patients. *FGFR3*, *MET*, and *APC* variants seen in one patient each were considered as benign/likely benign (ACMG class 1 or 2), of conflicting interpretation or of unknown significance (ACMG class 3). An ACMG class 5 p.G12D *KRAS* mutation was seen in a minority of the tumor cells, in one patient. Finally, silent mutations in the *HRAS* (p.H27H) and *PDGFRα* (p.V824V) genes were identified in, respectively, 4 and 5 patients.

Immunohistochemistry results showed some correlation with mutational profile. In accordance with the Dianova datasheet, all 3 tumors with a p.R132H mutation showed moderate-to-strong cytoplasmic reactivity to the IDH1 R132H antibody in a subset (40 to 70%) of the tumor cells. Additionally, faint reactivity was seen in 30% of the tumor cells in the enchondroma displaying a p.R132L *IDH1* variant. As expected, the other two tumors with either p.R132C or p.R132G *IDH1* variants showed no reactivity. Similarly, in the patient with Ollier disease, whose tumors harbored an *IDH2* mutation, neither the enchondromas nor the ependymoma showed IDH1 R132H expression.

Representative histological findings, immunohistochemistry, and NGS results are reported in Table 1 and in Figs. 3 and 4 and 5.

Discussion

Molecular characterization of enchondromas has previously relied on targeted sequence analysis [3, 7, 8, 11, 12]. *IDH* mutations are well characterized in secondary glioblastomas and gliomas [5]. The increased risk of gliomas in Ollier disease and Maffucci syndrome prompted Amary et al. [3] to assess *IDH* mutational status in cartilaginous tumors. In the first description of *IDH1*/*IDH2* mutations in cartilaginous tumors, the authors reported a mutation frequency of 56% in conventional and periosteal chondromas and chondrosarcomas [3]. They stated however that the DNA sequencing method applied may well have underestimated the true frequency of *IDH1*/*IDH2* mutations. Use of a targeted quantitative polymerase chain reaction (qPCR) hydrolysis probes assay, allowing detection of only 1% of mutant allele, confirmed the occurrence in some cartilaginous tumors of mutant alleles below the detection level of Sanger sequencing [7].

To further describe the occurrence of *IDH* mutations in pediatric enchondromas, we used NGS technology, a highly sensitive technique allowing detection of even low-level mutations. In this first report using NGS technology, *IDH* mutations were identified in all 13 pediatric cartilaginous tumors assessed.

Three distinct isocitrate dehydrogenase (IDH) enzymes are encoded by 5 different genes, among which IDH1 and IDH2 are homodimeric enzymes involved in the tricarboxylic acid cycle (TCA cycle, known as the Krebs cycle) (reviewed by Schaap et al. [13]). In solid tumors, *IDH* gain-of-function mutations involve mostly codon 132 of the *IDH1* gene and less frequently codon 172 of the *IDH2* gene, both corresponding to an arginine (R) residue. *IDH* mutations, an early driver event, result in hypermethylation, inducing epigenetic deregulation of chondrocyte differentiation and are sufficient to induce enchondromatosis. In an *Idh1* mutant knock-in mouse model, persistence of hypertrophic chondrocytes and an increase in cell proliferation were observed. Further, a conditional *Idh* mutant mouse model developed enchondroma-like lesions adjacent to the growth plates [11]. Since mutations in the *IDH* genes impair osteogenic differentiation, cartilage differentiation and overgrowth are favored [14]. While *IDH* mutations are essential in the development of benign enchondroma, progression toward high-grade chondrosarcoma relies on additional genetic events [15]. Although *IDH* mutations do not allow for the distinction between enchondromas and chondrosarcomas nor pertain prognostic value [16], the identification of an *IDH* mutation in all assessed cartilaginous tumors of our series opens perspectives in the management of a majority if not all central cartilaginous tumors using novel therapeutic approaches, such as hypomethylating agents [17].

Somatic mosaicism is thought to underlie Ollier disease and Maffucci syndrome [7, 8]. Mosaicism is defined as the coexistence in a single individual of more than one genetically distinct cell lines [18]. The identification of identical *IDH* mutations in both multiple tumors and nontumoral tissue from single individuals lends support to an early postzygotic event resulting in a mosaic pattern of disease distribution [7, 8]. The *IDH1*/*IDH2*-mutant tumors are believed to derive from these

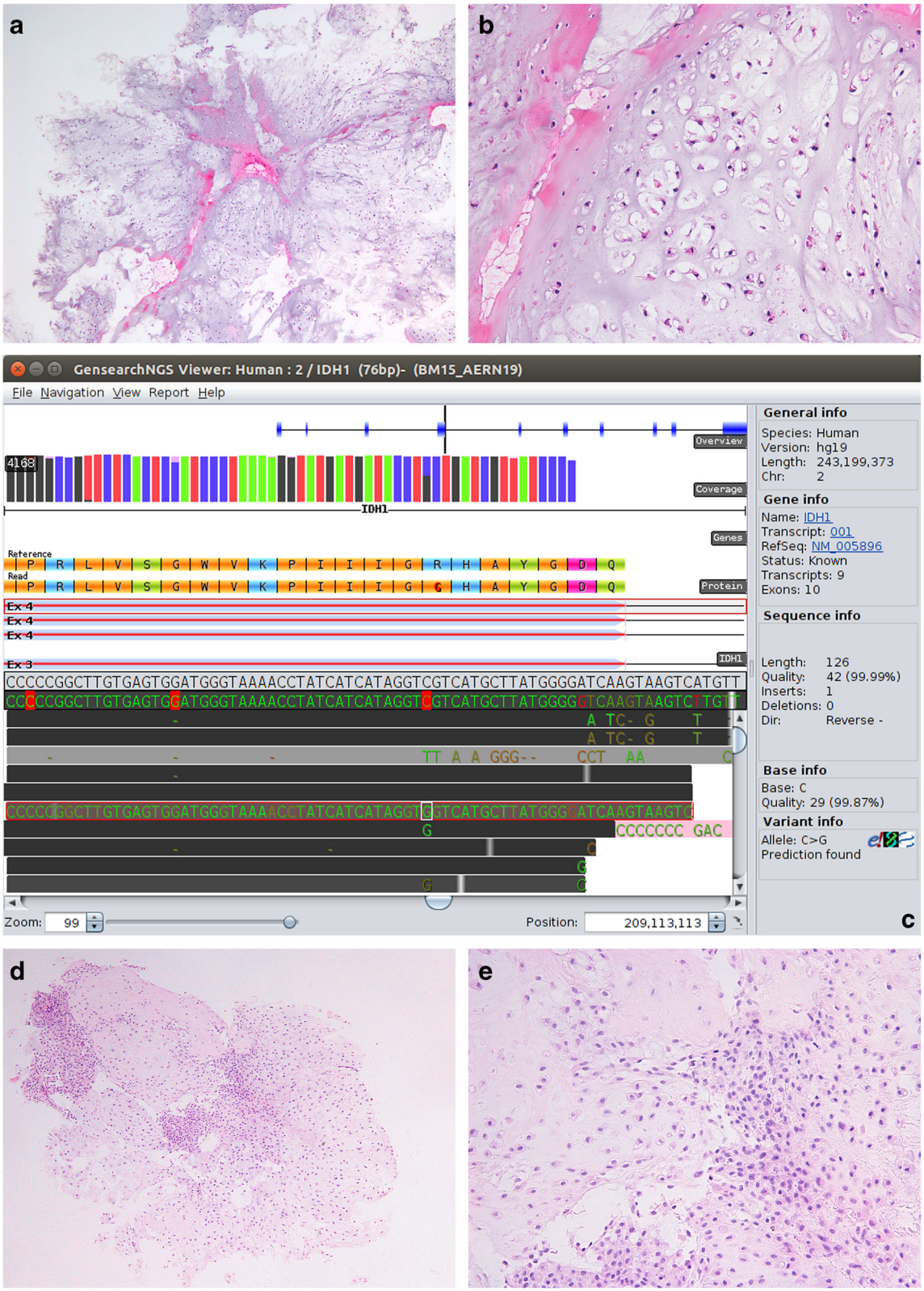
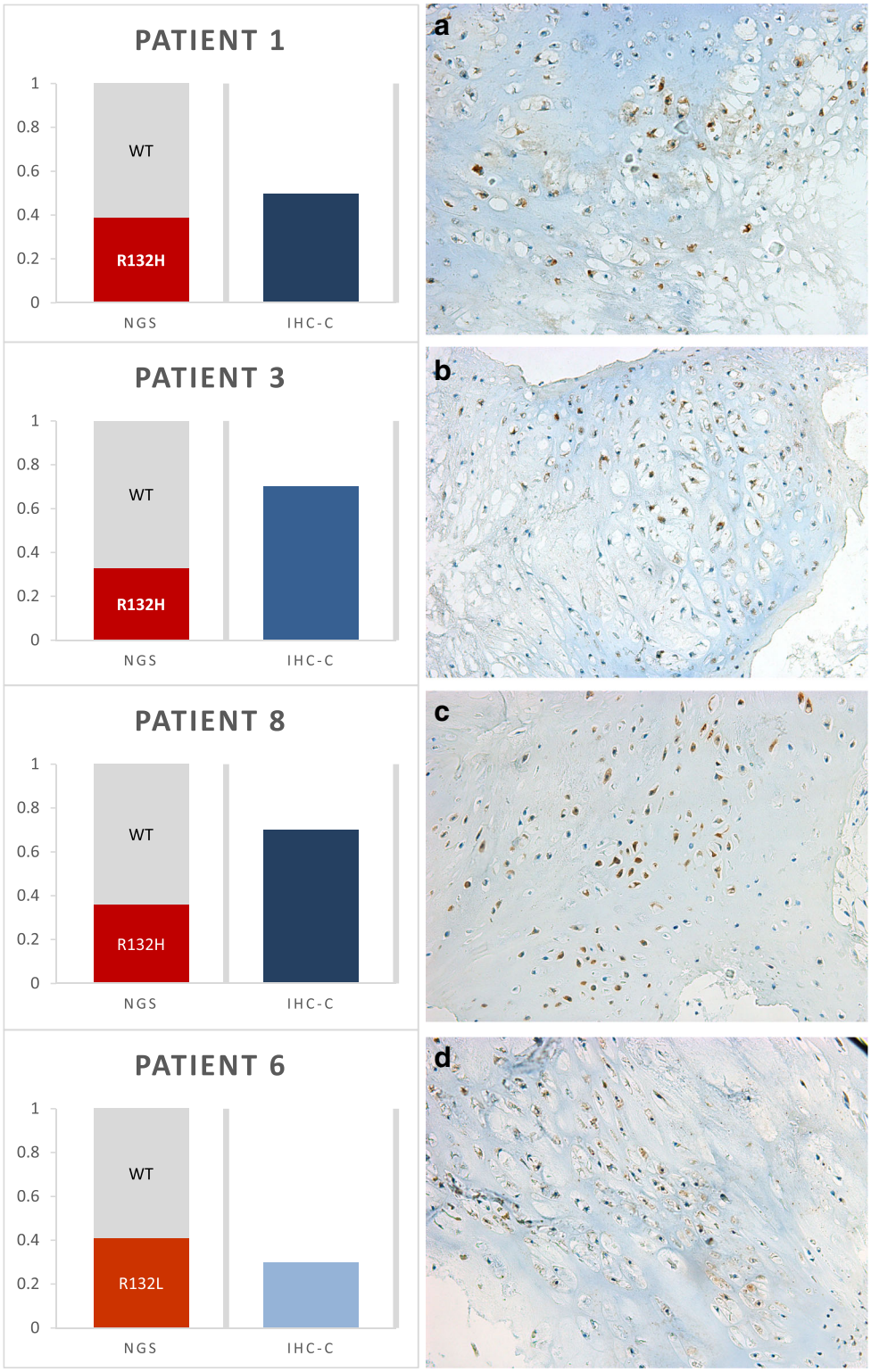


Fig. 3 Representative histological and molecular findings in enchondromas from two patients. Patient no. 7. **a** Classical enchondroma findings. Well-differentiated cartilaginous tumor with an abundant hyaline matrix and a slightly nodular growth pattern (A, hematoxylin and eosin, H&E, original magnification × 40). **b** Chondrocytes in small clusters show nonatypical small nuclei (H&E, × 200). **c** NGS results, showing an *IDH1* p.R132G mutation. Patient no. 5, with Ollier disease. A metacarpal enchondroma shows variable, focally increased cellularity, and mild cytological atypia, at low (**d**) and high power (**e**) (× 40 and × 200, respectively)



Abbreviations: NGS, Next Generation Sequencing; WT, wild-type; IHC, immunohistochemistry; C, cytoplasmic; T1 to Tx, tumors 1 to X

IDH1 gene mutation *IDH1 WT*

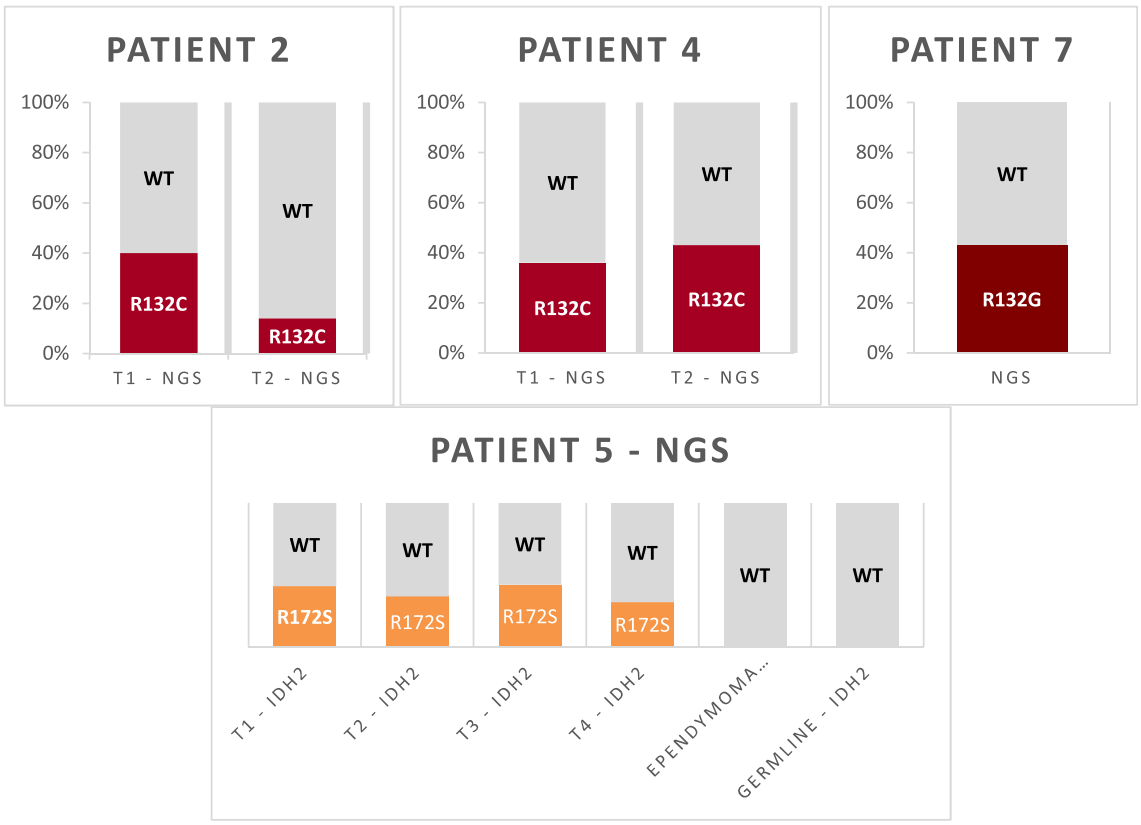
IHC 3+ IHC 2+ IHC 1+

◀ **Fig. 4** NGS findings in tumors with positive IDH H09 clone immunohistochemistry. Moderate-to-marked cytoplasmic IDH1 R132H (H09 clone) reactivity (2+/3+) was seen in the tumors with a R132H *IDH1* variant from patients no. 1 (**a**), no. 3 (**b**), and no. 8 (**c**). Additionally, mild (1+) reactivity was seen in one tumor with a R132L *IDH1* variant, from patient no. 6 (**d**)

IDH-mutated cells. The identification of the same variant in all tumors from the three patients with multiple enchondromas available for analysis highlights somatic mosaicism. A p.R172S *IDH2* mutation was previously reported in all three tumors from a patient with Maffucci syndrome, an enchondroma, one hemangioma, and an anaplastic astrocytoma [12], but *IDH* mutations are usually not a feature of ependymomas [5]. Accordingly, in the patient with Ollier disease, an *IDH2* mutation was identified in all assessed enchondromas, whereas the ependymoma was wild-type.

In the enchondromas, the numbers of reads containing a mutant versus a wild-type base at the position of interest were high, ranging from 33 to 43%, in keeping with driver heterozygous mutations in specimens with a tumor content close to 100%. In one tumor, however, the percentage of reads with the mutant base was 14%, indicative of a heterozygous mutation in approximately 28% of the tumor cells, a finding more in keeping with the previously reported mosaic pattern of *IDH* mutation [3, 7, 8]. This finding, together with the coexistence within a same tumor of both tumor cells positive and negative to the R132H IDH1 mutant protein, lends support to intraneoplastic mosaicism, as defined by Pansuriya et al. [7].

NGS analysis disclosed additional mutations. A heterozygous *STK11* mutation was seen in all tested tumoral and nontumoral tissues from the patient with Ollier disease. Serine/threonine kinase 11 (*STK11*), also known as liver kinase B1 (*LKB1*), first identified as the causal germline mutation in Peutz-Jeghers syndrome (PJS) [19], is a critical tumor suppressor gene showing frequent somatic mutations in a variety of human cancers [20]. *STK11* phosphorylates and regulates the adenosine monophosphate-activated protein kinase (AMPK) and 12 closely related kinases, thereby acting as a regulator of cell polarity, metabolism, adhesion, growth, and survival [21]. The C-terminal noncatalytic region of the LKB1 protein



Abbreviations: NGS, Next Generation Sequencing; WT, wild-type; T1 to Tx, tumors 1 to X

IDH1 gene mutation *IDH2* gene mutation *IDH1/2* WT

Fig. 5 NGS findings in patients with negative IDH immunohistochemistry

encompasses amino acids 309–433. Functional analysis of C-terminal mutations, including the p.F354L mutation, has been shown to impair the LKB1-mediated activation of the AMPK pathway and to result in the loss of cell polarity [22]. Despite these findings, conflicting interpretations of pathogenicity are reported in the ClinVar database. In mice specifically lacking *Stk11* activity within chondrocytes of the endochondral skeleton, mutant chondrocytes retained an immature phenotype; normal function and organization of the growth plate during development was dependent on *Stk11* activity [23]. Further, the loss of *Stk11* action resulted in the development of enchondroma-like tumors throughout the long bones [23]. The development of intestinal hamartomas in PJS requires the biallelic inactivation of the *STK11* tumor suppressor gene [24], and transcriptional silencing by promoter hypermethylation has been suggested as an alternative inactivation mechanism to loss of heterozygosity [25]. We found no second hit event in the tumors from the patient with Ollier disease: the mutation was seen in a heterozygous state, no LOH was observed, and expression of both the p.F354L mutated variant and the wild-type allele was documented by RT-PCR. To our knowledge, *STK11* mutations have not been previously reported in human cartilaginous tumors or in Ollier disease. Additional mechanisms seem to underlie tumorigenesis.

Silent, synonymous *HRAS* and *PDGFRA* mutations were observed in the tumors from a majority of the patients. Oncogenes are enriched not only for missense mutations but also for synonymous mutations [26]. Although the sequence of the encoded protein remains by definition unmodified, synonymous mutation target exonic splicing motifs tend to create exonic splicing enhancers in oncogenes and may affect alternative splicing. Synonymous mutations can also alter protein folding [26].

Immunohistochemistry using the H09 clone is used as a surrogate for the common R132H *IDH1* mutation, reported in 88% of gliomas. In cartilaginous tumors, the p.R132C has been reported as the most frequent variant [3, 6, 8], a finding that may render immunohistochemistry less useful in this setting. In our small series of patients, the *IDH1* R132H variant was the most frequently observed (3/7 patients with an *IDH1* mutation), whereas the R132C variant was identified in 2 patients. Although comparative studies between IHC and genetic testing report 98–100% sensitivity and 100% specificity of immunohistochemistry in detecting *IDH1* R132H mutations in glioma [27–29], the H09 clone recognizing the R132H mutation has been described by Agarwal et al. [30] as showing cross-reactivity with other *IDH1* mutation variants. In a series of 50 gliomas, 3 of 4 tumors with a variant R132L type of mutation were focally immunoreactive with H09. Accordingly, in the one patient with a R132L *IDH1* variant, we observed faint cross-reactivity to the H09 clone.

In conclusion, NGS allowed identification of *IDH* mutations in all assessed enchondromas, suggesting that a majority if not all central cartilaginous tumors may be managed using novel therapeutic approaches targeting *IDH* mutations. Immunohistochemistry using the IDH1 R132H antibody, although of limited sensitivity since enchondromas show a wide variety of *IDH1/2* variants, confirmed together with NGS the presence of intratumoral mosaicism. The multiplex PCR panel used, assessing 50 major oncogenes and tumor suppressor genes, identified few additional mutations. *Stk11* loss in animal models is sufficient to induce enchondroma-like tumor formation. Although a germline mutation in the *STK11* tumor suppressor gene was identified in the patient with Ollier disease, we failed to document a second hit alteration in the enchondromas. Further descriptions are needed before inferring causality in human enchondromatosis.

Acknowledgments The authors thank Mrs. P. Bordignon for the immunohistochemistry techniques and Dr. L. Ho and Dr. S. Clement-Leboube for the OncoScan analysis.

Authors' contributions
- Have substantially contributed to the conception or design of the work (Anne-Laure Rougemont and Essia Saiji) or the acquisition, analysis, or interpretation of data for the work (Essia Saiji, Fabienne Gumy Pause, Pierre Lascombes, Christelle Cerato Biderbost, Nathalie Lin Marq, Margaret Berczy, Laura Merlini, and Anne-Laure Rougemont).
- Have drafted the work (Anne-Laure Rougemont and Essia Saiji) or revised it critically for important intellectual content (Fabienne Gumy Pause, Pierre Lascombes, Christelle Cerato Biderbost, Margaret Berczy, Nathalie Lin Marq, and Laura Merlini).
- Gave final approval of the version to be published (Essia Saiji, Fabienne Gumy Pause, Pierre Lascombes, Christelle Cerato Biderbost, Nathalie Lin Marq, Margaret Berczy, Laura Merlini, and Anne-Laure Rougemont).
- Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved (Essia Saiji, Fabienne Gumy Pause, Pierre Lascombes, Christelle Cerato Biderbost, Nathalie Lin Marq, Margaret Berczy, Laura Merlini, and Anne-Laure Rougemont).

Compliance with ethical standards

The authors/coauthors declare that the study has complied with the ethical standards. This study was approved by the Commission Cantonale d'Ethique de la Recherche (CCER Review Board, approval number GE 14-229).

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

References

1. (2012) World Health Organization Classification of Tumors. Pathology and genetics. IARC Press, Lyon
2. Eefting D, Schrage YM, Geirnaerdt MJ, Le Cessie S, Taminiau AH, Bovee JV, Hogendoorn PC, Tc EBN (2009) Assessment of interobserver variability and histologic parameters to improve reliability in classification and grading of central cartilaginous tumors. Am J Surg Pathol 33:50–57. https://doi.org/10.1097/PAS.0b013e31817eec2b

3. Amary MF, Bacsi K, Maggiani F, Damato S, Halai D, Berisha F, Pollock R, O'Donnell P, Grigoriadis A, Diss T, Eskandarpour M, Presneau N, Hogendoorn PC, Futreal A, Tirabosco R, Flanagan AM (2011) IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumours. J Pathol 224:334–343. https://doi.org/10.1002/path.2913
4. Damato S, Alorjani M, Bonar F, McCarthy SW, Cannon SR, O'Donnell P, Tirabosco R, Amary MF, Flanagan AM (2012) IDH1 mutations are not found in cartilaginous tumours other than central and periosteal chondrosarcomas and enchondromas. Histopathology 60:363–365. https://doi.org/10.1111/j.1365-2559.2011.04010.x
5. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B, Bigner DD (2009) IDH1 and IDH2 mutations in gliomas. N Engl J Med 360:765–773. https://doi.org/10.1056/NEJMoa0808710
6. Arai M, Nobusawa S, Ikota H, Takemura S, Nakazato Y (2012) Frequent IDH1/2 mutations in intracranial chondrosarcoma: a possible diagnostic clue for its differentiation from chordoma. Brain Tumor Pathol 29:201–206. https://doi.org/10.1007/s10014-012-0085-1
7. Pansuriya TC, van Eijk R, d'Adamo P, van Ruler MA, Kuijjer ML, Oosting J, Cleton-Jansen AM, van Oosterwijk JG, Verbeke SL, Meijer D, van Wezel T, Nord KH, Sangiorgi L, Toker B, Liegl-Atzwanger B, San-Julian M, Sciot R, Limaye N, Kindblom LG, Daugaard S, Godfraind C, Boon LM, Vikkula M, Kurek KC, Szuhai K, French PJ, Bovee JV (2011) Somatic mosaic IDH1 and IDH2 mutations are associated with enchondroma and spindle cell hemangioma in Ollier disease and Maffucci syndrome. Nat Genet 43:1256–1261. https://doi.org/10.1038/ng.1004
8. Amary MF, Damato S, Halai D, Eskandarpour M, Berisha F, Bonar F, McCarthy S, Fantin VR, Straley KS, Lobo S, Aston W, Green CL, Gale RE, Tirabosco R, Futreal A, Campbell P, Presneau N, Flanagan AM (2011) Ollier disease and Maffucci syndrome are caused by somatic mosaic mutations of IDH1 and IDH2. Nat Genet 43:1262–1265. https://doi.org/10.1038/ng.994
9. Foster JM, Oumie A, Togneri FS, Vasques FR, Hau D, Taylor M, Tinkler-Hundal E, Southward K, Medlow P, McGreeghan-Crosby K, Halfpenny I, McMullan DJ, Quirke P, Keating KE, Griffiths M, Spink KG, Brew F (2015) Cross-laboratory validation of the OncoScan(R) FFPE assay, a multiplex tool for whole genome tumour profiling. BMC Med Genet 8:5. https://doi.org/10.1186/s12920-015-0079-z
10. Herget GW, Strohm P, Rottenburger C, Kontny U, Krauss T, Bohm J, Sudkamp N, Uhl M (2014) Insights into enchondroma, enchondromatosis and the risk of secondary chondrosarcoma. Review of the literature with an emphasis on the clinical behaviour, radiology, malignant transformation and the follow up. Neoplasma 61:365–378. https://doi.org/10.4149/neo_2014_046
11. Hirata M, Sasaki M, Cairns RA, Inoue S, Puviindran V, Li WY, Snow BE, Jones LD, Wei Q, Sato S, Tang YJ, Nadesan P, Rockel J, Whetstone H, Poon R, Weng A, Gross S, Straley K, Gliser C, Xu Y, Wunder J, Mak TW, Alman BA (2015) Mutant IDH is sufficient to initiate enchondromatosis in mice. Proc Natl Acad Sci U S A 112:2829–2834. https://doi.org/10.1073/pnas.1424400112
12. Moriya K, Kaneko MK, Liu X, Hosaka M, Fujishima F, Sakuma J, Ogasawara S, Watanabe M, Sasahara Y, Kure S, Kato Y (2014) IDH2 and TP53 mutations are correlated with gliomagenesis in a patient with Maffucci syndrome. Cancer Sci 105:359–362. https://doi.org/10.1111/cas.12337
13. Schaap FG, French PJ, Bovee JV (2013) Mutations in the isocitrate dehydrogenase genes IDH1 and IDH2 in tumors. Adv Anat Pathol 20:32–38. https://doi.org/10.1097/PAP.0b013e31827b654d
14. Suijker J, Baelde HJ, Roelofs H, Cleton-Jansen AM, Bovee JV (2015) The oncometabolite D-2-hydroxyglutarate induced by mutant IDH1 or -2 blocks osteoblast differentiation in vitro and in vivo. Oncotarget 6:14832–14842. https://doi.org/10.18632/oncotarget.4024
15. Suijker J, Oosting J, Koornneef A, Struys EA, Salomons GS, Schaap FG, Waaijer CJ, Wijers-Koster PM, Briaire-de Bruijn IH, Haazen L, Riester SM, Dudakovic A, Danen E, Cleton-Jansen AM, van Wijnen AJ, Bovee JV (2015) Inhibition of mutant IDH1 decreases D-2-HG levels without affecting tumorigenic properties of chondrosarcoma cell lines. Oncotarget 6:12505–12519. https://doi.org/10.18632/oncotarget.3723
16. Cleven AHG, Suijker J, Agrogiannis G, Briaire-de Bruijn IH, Frizzell N, Hoekstra AS, Wijers-Koster PM, Cleton-Jansen AM, Bovee J (2017) IDH1 or -2 mutations do not predict outcome and do not cause loss of 5-hydroxymethylcytosine or altered histone modifications in central chondrosarcomas. Clin Sarcoma Res 7:8. https://doi.org/10.1186/s13569-017-0074-6
17. Mondesir J, Willekens C, Touat M, de Botton S (2016) IDH1 and IDH2 mutations as novel therapeutic targets: current perspectives. J Blood Med 7:171–180. https://doi.org/10.2147/JBM.S70716
18. Youssoufian H, Pyeritz RE (2002) Mechanisms and consequences of somatic mosaicism in humans. Nat Rev Genet 3:748–758. https://doi.org/10.1038/nrg906
19. Hemminki A, Markie D, Tomlinson I, Avizienyte E, Roth S, Loukola A, Bignell G, Warren W, Aminoff M, Hoglund P, Jarvinen H, Kristo P, Pelin K, Ridanpaa M, Salovaara R, Toro T, Bodmer W, Olschwang S, Olsen AS, Stratton MR, de la Chapelle A, Aaltonen LA (1998) A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature 391:184–187. https://doi.org/10.1038/34432
20. Herrmann JL, Byekova Y, Elmets CA, Athar M (2011) Liver kinase B1 (LKB1) in the pathogenesis of epithelial cancers. Cancer Lett 306:1–9. https://doi.org/10.1016/j.canlet.2011.01.014
21. Shackelford DB, Shaw RJ (2009) The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. Nat Rev Cancer 9:563–575. https://doi.org/10.1038/nrc2676
22. Forcet C, Etienne-Manneville S, Gaude H, Fournier L, Debilly S, Salmi M, Baas A, Olschwang S, Clevers H, Billaud M (2005) Functional analysis of Peutz-Jeghers mutations reveals that the LKB1 C-terminal region exerts a crucial role in regulating both the AMPK pathway and the cell polarity. Hum Mol Genet 14:1283–1292. https://doi.org/10.1093/hmg/ddi139
23. Lai LP, Lilley BN, Sanes JR, McMahon AP (2013) Lkb1/Stk11 regulation of mTOR signaling controls the transition of chondrocyte fates and suppresses skeletal tumor formation. Proc Natl Acad Sci U S A 110:19450–19455. https://doi.org/10.1073/pnas.1309001110
24. Hemminki A, Tomlinson I, Markie D, Jarvinen H, Sistonen P, Bjorkqvist AM, Knuutila S, Salovaara R, Bodmer W, Shibata D, de la Chapelle A, Aaltonen LA (1997) Localization of a susceptibility locus for Peutz-Jeghers syndrome to 19p using comparative genomic hybridization and targeted linkage analysis. Nat Genet 15:87–90. https://doi.org/10.1038/ng0197-87
25. Esteller M, Avizienyte E, Corn PG, Lothe RA, Baylin SB, Aaltonen LA, Herman JG (2000) Epigenetic inactivation of LKB1 in primary tumors associated with the Peutz-Jeghers syndrome. Oncogene 19:164–168. https://doi.org/10.1038/sj.onc.1203227
26. Supek F, Minana B, Valcarcel J, Gabaldon T, Lehner B (2014) Synonymous mutations frequently act as driver mutations in human cancers. Cell 156:1324–1335. https://doi.org/10.1016/j.cell.2014.01.051
27. Capper D, Weissert S, Balss J, Habel A, Meyer J, Jager D, Ackermann U, Tessmer C, Korshunov A, Zentgraf H, Hartmann C, von Deimling A (2010) Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. Brain Pathol 20:245–254. https://doi.org/10.1111/j.1750-3639.2009.00352.x

28. Jabbar KJ, Luthra R, Patel KP, Singh RR, Goswami R, Aldape KD, Medeiros LJ, Routbort MJ (2015) Comparison of next-generation sequencing mutation profiling with BRAF and IDH1 mutation-specific immunohistochemistry. Am J Surg Pathol 39:454–461. https://doi.org/10.1097/PAS.0000000000000325
29. Preusser M, Wohrer A, Stary S, Hoftberger R, Streubel B, Hainfellner JA (2011) Value and limitations of immunohistochemistry and gene sequencing for detection of the IDH1-R132H mutation in diffuse glioma biopsy specimens. J Neuropathol Exp Neurol 70:715–723. https://doi.org/10.1097/NEN.0b013e31822713f0
30. Agarwal S, Sharma MC, Jha P, Pathak P, Suri V, Sarkar C, Chosdol K, Suri A, Kale SS, Mahapatra AK (2013) Comparative study of IDH1 mutations in gliomas by immunohistochemistry and DNA sequencing. Neuro-Oncology 15:718–726. https://doi.org/10.1093/neuonc/not015