Chapter 6

Somatic mosaic *IDH1* and *IDH2* mutations are associated with enchondroma and spindle cell hemangioma in Ollier disease and Maffucci syndrome

Twinkal C Pansuriya[1], Ronald van Eijk[1], Pio d'Adamo[2], Maayke A J H van Ruler[1], Marieke L Kuijjer[1], Jan Oosting[1], Anne-Marie Cleton-Jansen[1], Jolieke G van Oosterwijk[1], Sofie L J Verbeke[1,3], Daniëlle Meijer[1], Tom van Wezel[1], Karolin H Nord[4], Luca Sangiorgi[5], Berkin Toker[6], Bernadette Liegl-Atzwanger[7], Mikel San-Julian[8], Raf Sciot[9], Nisha Limaye[10], Lars-Gunnar Kindblom[11], Soeren Daugaard[12], Catherine Godfraind[13], Laurence M Boon9,[14], Miikka Vikkula9,[15], Kyle C Kurek[16], Karoly Szuhai[17], Pim J French[18] & Judith V M G Bovée[1]

[1]Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands. [2]Institute for Maternal and Child Health, Instituto di Ricovero e Cura a Carattere Scientifico, Burlo Garofolo, University of Trieste, Trieste, Italy. [3]Department of Pathology, University Hospital Antwerp, Antwerp, Belgium. [4]Department of Clinical Genetics, Lund University Hospital, Lund, Sweden. [5]Department of Medical Genetics, Rizzoli Orthopedic Institute, Bologna, Italy. [6]Istanbul University Medical School, Istanbul, Turkey. [7]Institute of Pathology, Medical University, Graz, Austria. [8]Department of Orthopaedic Surgery and Traumatology, University Clinic of Navarra, Pamplona, Spain. [9]Department of Pathology, University of Leuven, Leuven, Belgium. [10]de Duve Institute, Université catholique de Louvain, Brussels, Belgium. [11]Department of Musculoskeletal Pathology, Royal Orthopaedic Hospital, Birmingham, UK. [12]Department of Pathology, University of Copenhagen, Copenhagen, Denmark. [13]Laboratory of Pathology, Cliniques universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium. [14]Center for Vascular Anomalies, Division of Plastic Surgery, Cliniques universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium. [15]Walloon Excellence in Lifesciences and Biotechnology (WELBIO), Université catholique de Louvain, Brussels, Belgium. [16]Department of Pathology, Children's Hospital Boston, Harvard Medical School, Boston, Massachusetts, USA. [17]Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands. [18]Department of Neurology, Erasmus University Medical Center, Erasmus University, Rotterdam, The Netherlands.

Nat Genet. 2011, doi:10.1038/ng.1004.

Abstract

Ollier disease and Maffucci syndrome are non-hereditary skeletal disorders characterized by multiple enchondromas (Ollier disease) combined with spindle cell hemangiomas (Maffucci syndrome). We report somatic heterozygous mutations in *IDH1* (encoding R132C and R132H substitutions) or *IDH2* (R172S) in 87% of enchondromas (benign cartilage tumors) and in 70% of spindle cell hemangiomas (benign vascular lesions). In total, 35 of 43 (81%) subjects with Ollier disease and 10 of 13 (77%) with Maffucci syndrome carried *IDH1* (98%) or *IDH2* (2%) mutations in their tumors. Fourteen of 16 subjects had identical mutations in separate lesions. Immunohistochemistry to detect mutant IDH1 R132H protein suggested intraneoplastic and somatic mosaicism. *IDH1* mutations in cartilage tumors were associated with hypermethylation and downregulated expression of several genes. Mutations were also found in 40% of solitary central cartilaginous tumors and in four chondrosarcoma cell lines, which will enable functional studies to assess the role of *IDH1* and *IDH2* mutations in tumor formation.

Enchondromas are benign, cartilage-forming tumors within the medullary cavity of the bone[1–3]. Individuals with enchondromatosis syndrome, which encompasses seven major subtypes, develop multiple enchondromas. The most common subtypes are non-hereditary Ollier disease (subtype I) and Maffucci syndrome (subtype II), the latter distinguished by spindle cell hemangiomas that occur in addition to the multiple enchondromas[1,3]. Malignant transformation of enchondromas to chondrosarcomas occurs in >30% of these individuals[3,4].

To date, genome-wide screens have not identified a causative gene for Ollier disease or Maffucci syndrome[5–9]. Individuals with these diseases have an increased incidence of gliomas[3,10] and juvenile granulosa cell tumors[3,11–13]. *IDH1* and, more rarely, *IDH2* mutations in gliomas[14–16] and *GNAS*-activating mutations in juvenile granulosa cell tumors[17] have been reported. Notably, *IDH1* and *IDH2* mutations were recently reported in solitary central and periosteal enchondromas and chondrosarcomas, including in a few tumors from individuals with enchondromatosis[18]. The possibility that *GNAS* mutations are present in enchondromas and chondrosarcomas has not previously been explored.

We therefore assessed whether mutations in *IDH1*, *IDH2* or *GNAS* may cause enchondroma and spindle cell hemangioma formation in Ollier disease and Maffucci syndrome. Sequence analysis of hotspot mutation sites was performed using lesional tissue from 43 individuals with Ollier disease, and this analysis revealed in 33 subjects (78%) the presence of heterozygous mutations in *IDH1* of c.394C>T (encoding an R132C substitution) or c.395G>A (encoding R132H) (NM_005896.2 for both) or in *IDH2* of c.516G>C (encoding R172S) (NM_002168.2) (Supplementary Figure 1a–c). Among the individuals with Maffucci syndrome, 7 of 13 subjects (54%) carried *IDH1* mutations encoding the R132C substitution. Mutations were absent in DNA isolated from the blood, muscle or saliva of the subjects (Supplementary Figure 1b). Mutations in *GNAS* were absent in the tissues examined.

An additional eight tumors had sub-threshold peaks at the position in *IDH1* expected to encode mutations resulting in R132C or R132H substitutions, suggesting that the mutant allele might be present in a small subpopulation of the tumor cells at the limit of or below the level of detection of Sanger sequencing. We therefore performed a hydrolysis probe assay, which is capable of detecting mutant allele frequencies as low as 1%, to look for *IDH1* mutations encoding R132C or R132H[19,20]. Mutations were confirmed in seven of eight tumors (Supplementary Figure 1d–g), and there was insufficient DNA from the eighth tumor for analysis. Thus, in total, 35 of 43 (81%) and 10 of 13 (77%) subjects with Ollier disease and Maffucci syndrome, respectively, had *IDH1* or *IDH2* mutations (Figure 1a, Table 1 and Supplementary Table 1). The frequency of mutations in tumors is shown in Figure 1b.

Other subtypes of enchondromatosis syndromes are known to be caused by mutations in *PTPN11* (metachondromatosis)[21,22] and *ACP5* (spondyloenchondrodysplasia)[23,24] and by *PTHLH* duplication (symmetrical enchondromatosis)[25]. Mutations in *PTH1R*, which encodes a protein involved in enchondral bone formation, are found in ~8% of individuals with Ollier disease but not in those with Maffucci syndrome[5–7]. Previously, an absence of *PTPN11* mutations was shown in the current cohort of individuals[22]. In the current study, we did not detect *PTH1R* mutations in a screen of 35 subjects with Ollier disease or Maffucci syndrome.

Table 1 Results of *IDH1* and *IDH2* mutation analysis

	Total	Gender (M:F) (median age in years)	Number with *IDH1* mutations (%)	Number with *IDH1* R132C (*IDH1* CGT>TGT) (%)	Number with R132H (*IDH1* CGT>CAT) (%)	Number with *IDH2* mutation (%)	Total with *IDH1* or *IDH2* mutation (%)
Ollier disease							
Number of subjects	43	21:21[a] (24)	34 (79)			1 (2)	35 (81)
Enchondroma	25		22 (88)	15(68)	7 (32)	0	22 (88)
Chondrosarcoma grade I	23		20 (87)	18 (90)	2 (10)	0	20 (87)
Chondrosarcoma grade II	8		5 (63)	5 (100)	0	1 (12)	6 (75)
Chondrosarcoma grade III	2		1 (50)	1 (100)	0	1 (50)	2 (100)
Total number of tumors	58		48 (83)	39 (81)	9 (19)	2 (3)	50 (86)
Maffucci syndrome							
Number of subjects	13	5:8 (15)	10 (77)			0	
Enchondroma	5		4 (80)	4 (100)	0	0	
Chondrosarcoma grade I	1		1 (100)	1 (100)	0	0	

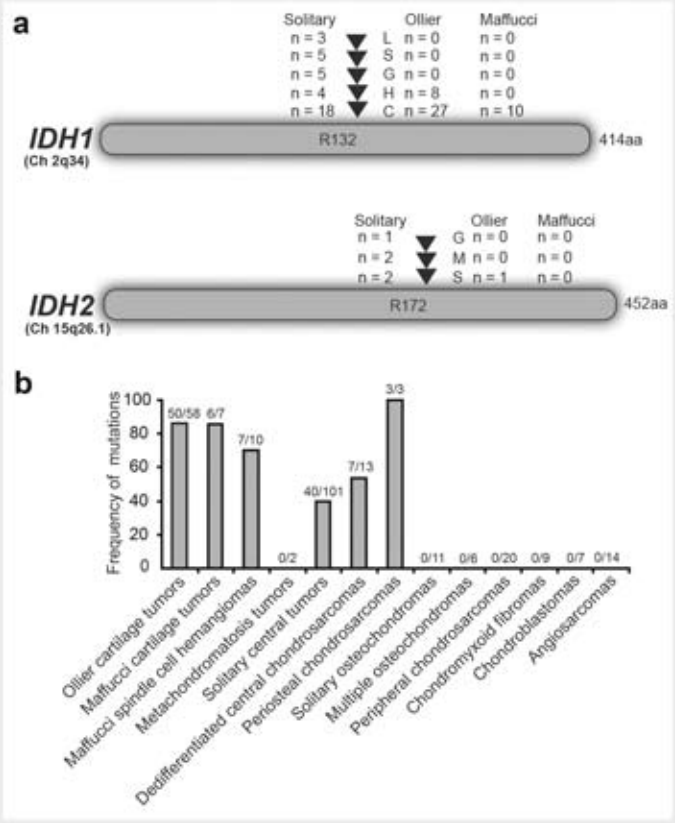
Table 1 (Continue)

	Total	Gender (M:F) (median age in years)	Number with *IDH1* mutations (%)	Number with *IDH1* R132C (*IDH1* CGT>TGT) (%)	Number with R132H (*IDH1* CGT>CAT) (%)	Number with *IDH2* mutation (%)	Total with *IDH1* or *IDH2* mutation (%)
Chondrosarcoma grade II	1		1(100)	1(100)	0	0	
Spindle cell hemangioma	10		7 (70)	7(100)	0	0	
Total number of tumors	17		13 (76)	13 (100)	0	0	
Solitary tumors							
Enchondroma	9		3 (33)	2 (67)	1 (33)	2 (22)	5 (56)
Central chondrosarcoma grade I	20		7[b] (35)	2 (29)	2 (29)	0	7 (35)
Central chondrosarcoma grade II	57		18[b] (32)	9 (50)	1 (6)	3 (5)	21 (37)
Central chondrosarcoma grade III	15		7[b] (47)	5 (71)	0	0	7 (47)
Dedifferentiated chondrosarcoma	13		6[b] (46)	3 (50)	1 (17)	1 (8)	7 (54)
Periosteal chondrosarcoma	3		3 (100)	3 (100)	0	0	3 (100)

[a]Unknown gender for one subject. [b]Other types of mutations present beyond those encoding R132C or R132H.

Analysis performed using a custom-made Agilent tiling array (Supplementary Table 2) did not show evidence of loss or gain of *IDH1, IDH2, PTHLH, PTPN11, PTH1R, EXT1, EXT2* or *ACP5*. Thus, even though individuals with enchondromatosis syndromes have overlapping clinical features, they seem to be genetically discrete entities, with the exception of Ollier disease and Maffucci syndrome, which we have now shown to both contain *IDH1* or *IDH2* mutations.

Figure 1 Frequency of *IDH1* and *IDH2* alterations



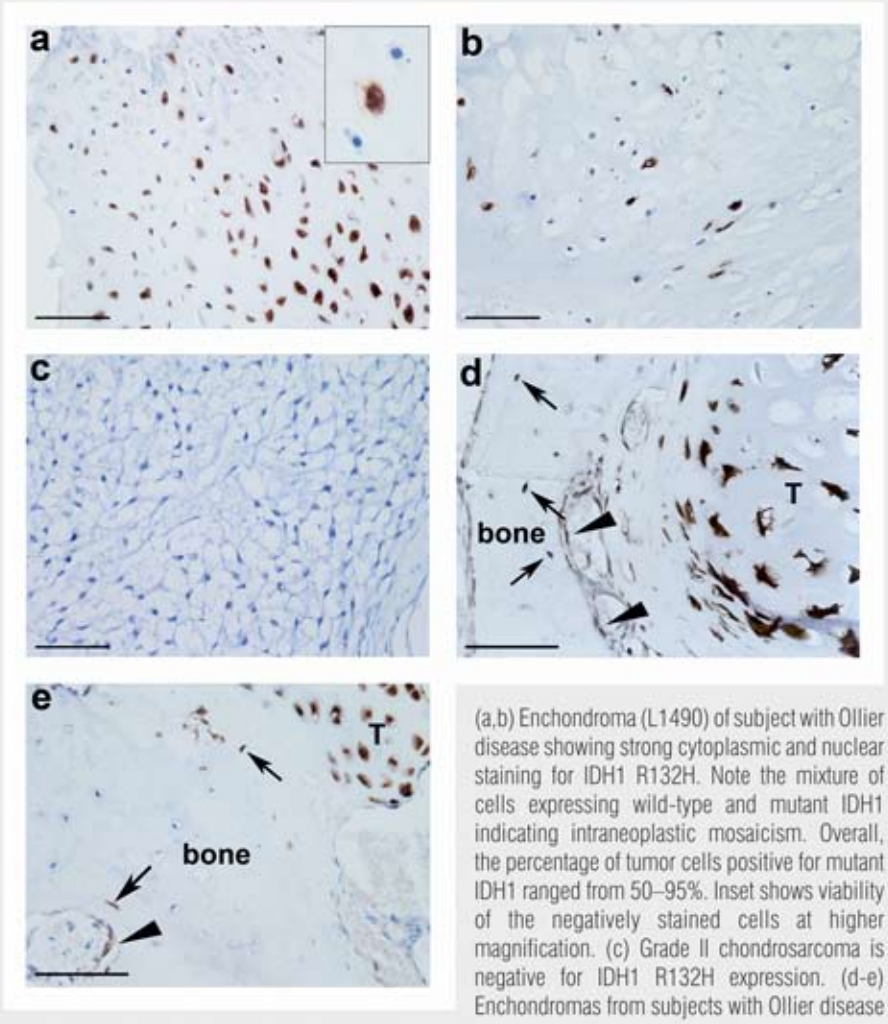
(a) Distribution of the different Arg132 alterations in *IDH1* and Arg172 alterations in *IDH2* among the subjects with Ollier disease, Maffucci syndrome and solitary tumors. (b) Frequency of somatic heterozygous *IDH* (*IDH1* and *IDH2*) mutations in tumors of subjects with Ollier disease or Maffucci syndrome in comparison to different subtypes of solitary cartilaginous tumors and angiosarcomas.

As Ollier disease and Maffucci syndrome are not inherited and enchondromas are often unilateral, we hypothesized that mutations may occur in a somatic mosaic fashion. Fourteen of 16 subjects (88%) possessed identical mutations, including rare variants, in more than one tumor (Supplementary Table 1). We additionally used immunohistochemistry to determine the distribution of the IDH1 R132H mutant protein. Of 68 tumors from subjects with Ollier disease, 17 (25%) showed mutant protein expression, whereas 51 (75%) were negative (Figure 2 and Table 2). Within tumors that were positive for IDH1 R132H staining, we observed a mixture of cells that did and did not express the mutant protein (cells were of the same histologic type and therefore did not include entrapped or supporting elements), a pattern we refer to as intraneoplastic mosaicism (Figure 2a,b). Within these tumors, the percentage of tumor cells staining positive for IDH1 R132H ranged from 50% to 95%. Intraneoplastic mosaicism has also been described for other benign bone tumors. In fibrous dysplasia, experimental evidence showed that both normal cells and those with mutations in *GNAS* were needed to develop fibrous dysplasia–like lesions[26]. Also, in osteochondromas, which are benign cartilaginous tumors arising at the surface of the bone that are caused by mutations in *EXT1* or *EXT2*, a mixture of cells with wild-type *EXT* and cells with mutations in *EXT* was observed[27–30]. The EXT protein is involved in heparan sulfate biosynthesis, and it is hypothesized that cells with mutations in *EXT* that are therefore deficient in heparan sulfate need heparan sulfate from neighboring cells for cellular signaling and survival[31,32].

We additionally studied the surrounding normal tissue of Ollier disease–derived and solitary tumors expressing mutant IDH1 R132H protein and observed a very low frequency (on average $<1\%$) of mutant protein in osteoblasts, osteocytes, adipocytes and fibroblasts (Figure 2d,e). We were able to perform the hydrolysis probe assay on DNA isolated from one normal bone of a subject with Ollier disease, and we did not detect any changes at the *IDH1* locus. Mutant IDH1 R132H protein was absent in 12 bones resected for reasons other than chondrosarcoma removal as well as in normal growth plates and articular cartilage (Table 2). Therefore, our current data support a model of somatic mosaicism, similar to that described for polyostotic fibrous dysplasia in which somatic mosaic mutations in *GNAS* have a causative role[33,34]. Unfortunately, the nature of the samples (decalcified, paraffin-embedded bone) and the occurrence of mutations in single, scattered cells did not allow verification of this theory using other techniques. However, the antibody recognizing IDH1 R132H was shown to be highly reliable for glioma diagnosis[35] and correlated well with sequence analysis in our series.

Twelve tumors were negative for *IDH1* or *IDH2* hotspot mutations. For 5 of these, all exons were sequenced and no mutations were identified. This finding was not surprising, as only *IDH1* mutations affecting Arg132 and *IDH2* mutations affecting Arg140 or Arg172 have been identified in other *IDH*-associated tumors. It is possible that, because of intralesional mosaicism, only a small fraction of tumor cells contains the *IDH1* or *IDH2* hotspot mutations, which may be below the detection level of the techniques used to identify them. Alternatively, mutations in other genes such as *TET2*, in which mutations are mutually exclusive to those in *IDH1* or *IDH2* in acute myeloid leukemia (AML)[36], might be involved[18,37].

Figure 2 Immunostaining for mutant IDH1 R132H protein



(a,b) Enchondroma (L1490) of subject with Ollier disease showing strong cytoplasmic and nuclear staining for IDH1 R132H. Note the mixture of cells expressing wild-type and mutant IDH1 indicating intraneoplastic mosaicism. Overall, the percentage of tumor cells positive for mutant IDH1 ranged from 50–95%. Inset shows viability of the negatively stained cells at higher magnification. (c) Grade II chondrosarcoma is negative for IDH1 R132H expression. (d-e) Enchondromas from subjects with Ollier disease showing occasional cells positive for mutant IDH1 in the surrounding normal bone. Some positively stained osteocytes (arrows) and osteoblasts (arrowheads) are seen. T, tumor tissue (scale bars, 5 μm).

Table 2 Immunohistochemical detection of mutant IDH1 R132H protein

	Total number of tumors	IDH1 R132H–positive tumors/total tumors analyzed (%)
Ollier disease		
Enchondroma	46	14/43[a] (32)
Chondrosarcoma grade I	22	3/17[a] (18)
Chondrosarcoma grade II	10	0/8[a]
Maffucci syndrome		
Enchondroma	9	0/9
Spindle cell hemangioma	14	0/14
Solitary tumors		
Enchondroma	19	4/19 (21)
Central chondrosarcoma grade I	42	4/38[a] (10)
Central chondrosarcoma grade II	36	1/32[a] (3)
Central chondrosarcoma grade III	14	0/11[a]
Central dedifferentiated chondrosarcoma	26	1/24[a] (4)
Periosteal chondrosarcoma	6	1/6 (17)

Table 2 (Continue)

	Total number of tumors	IDH1 R132H–positive tumors/total tumors analyzed (%)
Solitary osteochondroma	20	0/17[a]
Multiple osteochondroma	7	0/7
Peripheral chondrosarcoma	45	0/35[a]
Peripheral dedifferentiated chondrosarcoma	16	0/16
Conventional hemangioma	3	0/3
Hemangioendothelioma	2	0/2
High grade angiosarcoma of bone	44	0/44
High grade angiosarcoma of soft tissue	22	0/22
Controls		
Normal growth plate	3	0/3
Articular cartilage	3	0/3
Normal bone	12	0/12

[a]Not all tumors included were evaluated due to loss of tissue on the tissue microarrays.

Recently, point mutations in *IDH1* or *IDH2* were reported in 56% of solitary central and periosteal cartilaginous tumors[18], and the data within our control group are in concordance with these findings. In total, 40 of 101 (40%) solitary central tumors, 7 of 13 (54%) dedifferentiated chondrosarcomas and 3 of 3 (100%) periosteal chondrosarcomas had *IDH1* or *IDH2* mutations (Figure 1b and Table 1). In six additional tumors, the mutant allele seemed to be present below the detection level of Sanger sequencing. *IDH1* or *IDH2* mutations were absent in other subtypes of cartilaginous tumors, in angiosarcomas (Figure 1b) and in DNA isolated from subjects' blood. Immunostaining for IDH1 R132H protein on tissue microarrays (TMAs) containing cartilaginous and vascular tumor samples confirmed that the expression of mutant IDH1 was restricted to central, dedifferentiated and periosteal cartilage tumors, whereas all other tumors lacked mutant expression (Table 2). Of note, four of eight solitary chondrosarcoma cell lines carried different types of mutations in *IDH1* or *IDH2* (Table 3). To the best of our knowledge, no representative cell lines with *IDH1* or *IDH2* mutations were previously available. *IDH1* or *IDH2* mutations were more frequently found in solitary central tumors located in the hands and feet (11 of 14 tumors) compared to those located in long and flat bones (28 of 84 tumors) ($P = 0.006$, Pearson's $\chi 2$ test), which was also reported previously[18]. This correlation was absent in Ollier disease (20 of 22 tumors from the hands and feet compared to 28 of 34 tumors from long or flat bones, $P = 0.5$, Pearson's $\chi 2$ test). Whereas in gliomas, mutations in *IDH1* or *IDH2* predict a favorable outcome[38], we found no significant prognostic value of these mutations in solitary central cartilaginous tumors using multivariate analysis (Cox regression, P value $= 0.3$).

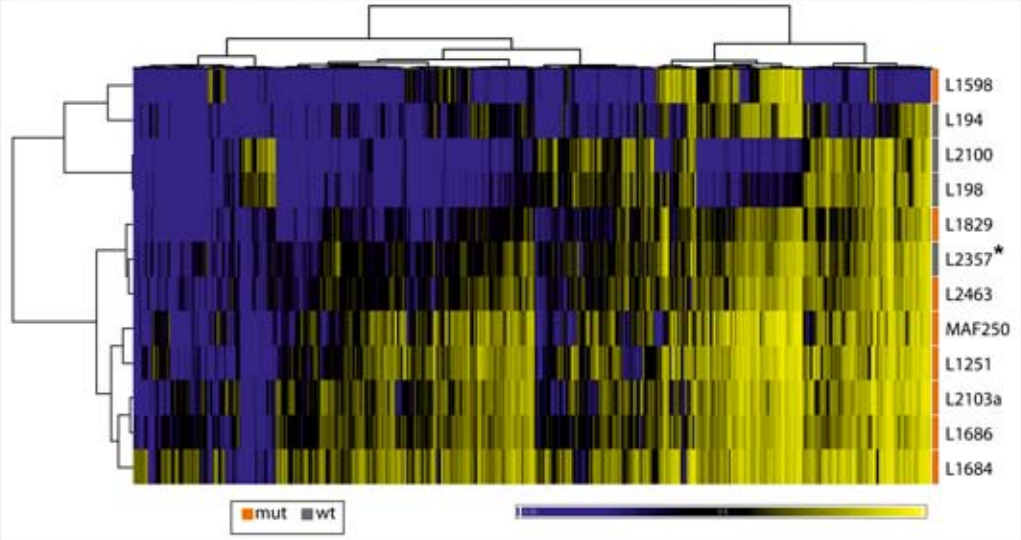
Table 3 *IDH1* or *IDH2* alterations in solitary central chondrosarcoma cell lines and primary tissue culture

Cell line	Tumor type	Tumor grade	Passage	*IDH1* alteration	*IDH2* alteration	Reference
SW1353	Solitary central	CSII	12	WT	R172S	ATCC
JJ012	Solitary central	CSII	15	R132G	WT	51
CH2879	Solitary central	CSIII	16	G105G	WT	52
OUMS27	Solitary central	CSIII	18	WT	WT	53
L835	Solitary central	CSIII	38	R132C	WT	Established by the authors
C3842	Ollier disease	CSII	32	WT	WT	54
L2975	Dedifferentiated CS		31	WT	R172W[a]	Established by the authors
NDCS1	Dedifferentiated CS		12	WT	WT	55

CS, chondrosarcoma; WT, wild type. [a]L2975 had a homozygous mutation in *IDH2* encoding R172W.

IDH1 and *IDH2* mutations have also been reported at lower frequencies in various other cancers, such as AML (8%)[39,40], prostate cancer (2.7%)[40,41], paragangliomas (0.7%)[40,42] and thyroid carcinoma (16%)[43]. The high mutation frequency in enchondromas and the fact that these mutations occur early suggest a causal rather than a bystander role for *IDH1* and *IDH2* mutations in tumorigenesis in Ollier disease and Maffucci syndrome. In gliomas, mutations in *IDH1* and *IDH2* lead to a gain of function, causing the production of 2-hydroxyglutarate (2HG), a structural analog of α-ketoglutarate (α-KG), which thereby reduces α-KG production[44]. In AML, it was demonstrated that mutant IDH expression results in DNA hypermethylation and impairment of hematopoietic differentiation[36], and in gliomas, the presence of an *IDH1* mutation was strongly associated with hypermethylation[45]. Therefore, we used Illumina HumanMethylation27 BeadChips to examine possible differences in methylation between enchondromas with (N = 8) and without (N = 4) *IDH1* mutations, as determined by Sanger sequencing. Unsupervised clustering of the 2,000 most variable CpG methylation sites gave two subgroups (Figure 3).

Figure 3 CpG island methylator phenotype in enchondromas with *IDH1* mutations



Heatmap depicting unsupervised clustering analysis of the 2,000 most variable CpG sites of enchondromas with (orange, N = 8) and without (gray, N = 4) *IDH1* mutation. The level of DNA methylation (beta value) for each probe (columns) in each sample (rows) is represented by color, ranging from 0 (0% methylation, blue) to 1 (100% methylation, yellow). The asterisk indicates sample L2357 in which the mutated allele of *IDH1* encoding R132G was detected in a subpopulation of cells. However, the mutation escaped detection by Sanger sequencing, and therefore the sample is labeled wild type.

One of these subgroups showed an overall higher methylation at the examined CpG sites, a phenotype that is similar to the CpG island methylator phenotype (CIMP) described in colon carcinoma and glioblastoma[45,46]. All but one enchondromas with an *IDH1* mutation were positive for this CIMP. Supervised clustering analysis indicated that 797 CpG sites were differentially methylated by more than 20% (with $P < 0.05$) between enchondromas with and without *IDH1* mutations. Of note, 710 of these differentially methylated CpG sites (89.1%) were methylated in the enchondromas with *IDH1* mutations (Supplementary Table 3). These results are in line with the hypothesis that *IDH1* mutations induce methylation and thus contribute to CIMP occurence[36].

To assess the effect of *IDH1* or *IDH2* mutation on mRNA expression levels in cartilaginous tumors, we performed whole-genome gene expression analysis using Illumina Human-6 v3 arrays. High-quality mRNA was available for only three tumors in which no mutation was found (N = 1) or in which mutations occurred at a frequency below the threshold of detection with Sanger sequencing (thus possibly carrying a low percentage of cells with mutations) (N = 2). Comparison of mRNA expression in these tumors with that from 18 tumors with clearly detectable *IDH1* or *IDH2* mutation using LIMMA analysis revealed 36 differentially expressed probes encoding 33 genes (Supplementary Table 4). Of these 33 genes, 32 were downregulated in the tumor samples with an *IDH1* or *IDH2* mutation. There was no overlap between the affected genes identified by methylation or expression analysis.

One of the most differentially methylated genes was *DLX5*, for which there was a trend of downregulation in the expression data comparing enchondromas from subjects with Ollier disease and controls. However, this difference was not significant (adjusted *P* value = 0.3, Supplementary Figure 2). The controls consisted of two growth plates and four articular or rib cartilage samples. *DLX5* encodes a homeodomain transcription factor that is a cell-autonomous positive regulator of chondrocyte maturation during endochondral ossification, promoting the conversion of immature proliferating chondrocytes into hypertrophic chondrocytes[47,48] The Dlx5 protein also induces expression of Runx2 and osterix, promoting osteogenic differentiation[49,50]. Future studies should reveal whether downregulation of *DLX5* through methylation as a consequence of *IDH1* mutation delays hypertrophic differentiation of chondrocytes and inhibits subsequent osteogenic differentiation, thereby leaving clusters of proliferating chondrocytes behind.

In summary, we report a large multi-institutional series demonstrating somatic heterozygous point mutations in *IDH1* or, rarely, in *IDH2* in tumor tissues of 81% of subjects with Ollier disease and 77% of those with Maffucci syndrome, and we provide evidence for intraneoplastic and somatic mosaicism. Future studies using deep-sequencing approaches should reveal whether the percentage of individuals carrying somatic mosaic mutations in *IDH1* or *IDH2* is even higher than that detected in our series or whether other genes are involved. We show the *IDH1* mutation to be associated with hypermethylation and downregulation of several genes. Future studies will examine whether there is a causal effect, and it will be of great interest to assess how this dysregulation leads to enchondroma and spindle cell hemangioma formation. Finally, this is the first report of four chondrosarcoma cell lines carrying *IDH1* or *IDH2* mutations, thereby providing good *in vitro* models for functional studies to dissect the role of *IDH1* and *IDH2* in Ollier disease and Maffucci syndrome, as well as allowing studies of their function in tumorigenesis in general.

Methods

Subjects and clinical specimens

Fresh-frozen tumor tissues (N = 60) of 44 subjects with multiple cartilage tumors (36 individuals with Ollier disease and 8 with Maffucci syndrome) (Table 1 and Supplementary Table 1) were collected from the EuroBoNeT consortium[8] and the Laboratory of Human Molecular Genetics at the de Duve Institute, Université catholique de Lovain. In addition, paraffin-embedded tumor tissues (N = 15) from 12 subjects were obtained from the archives of the Children's Hospital Boston. Samples were handled according to the ethical guidelines of the host institutions. All samples were coded and the ethical guidelines described in the "Code for Proper Secondary Use of Human Tissue in The Netherlands" (Dutch Federation of Medical Scientific Societies) were followed in all procedures. Chondrosarcoma samples were graded as described[56]. DNA derived from normal saliva, blood or muscle was available from 12 individuals with Ollier disease. The ages of the subjects were documented at the time of operation. Demographic and survival data were obtained from patient records at the host institutions. Written informed consent was obtained for all study participants from whom normal DNA was included. For subjects from whom we only used tumor tissue, the Code for Proper Secondary Use of Human Tissue in the Netherlands guidelines were followed.

For comparison with other cartilage tumors, we included DNA from solitary enchondromas (N = 9), solitary central chondrosarcomas (N = 92), central dedifferentiated chondrosarcomas (N = 13), periosteal chondrosarcomas (N = 3) and 37 peripheral cartilaginous tumors (solitary osteochondroma (N = 11), peripheral chondrosarcomas (N = 20) and multiple osteochondromas (N = 6)), as well as from chondromyxoid fibromas (N = 9), chondroblastomas (N = 7) and osteochondroma-like lesions of metachondromatosis (N = 2). Matching blood-derived DNA was also available from 24 subjects as controls. Additionally, we included DNA from angiosarcomas (N = 14), because individuals with Maffucci syndrome have central cartilage tumors combined with vascular tumors. The angiosarcomas, chondromyxoid fibromas and chondroblastomas were analyzed for *IDH1* mutations only. Thus, in total, we analyzed 261 tumors from 242 subjects.

DNA extraction and mutation analysis

Genomic DNA from frozen tumors containing at least 80% tumor cells, as estimated on haematoxylin and eosin–stained frozen sections, and from blood and saliva was isolated as described previously[8]. DNA from paraffin-embedded tissue was isolated after microdissection as described[8]. For cell lines and primary tissue culture, DNA was isolated from cell pellets using the Wizard Genomic DNA Purification kit (Promega), according to the manufacturer's instructions.

PCR amplification was performed on exon 4 of IDH1 for all the samples. Exon 4 of IDH2 was amplified in samples without *IDH1* mutation, and exon 8 of *GNAS* was studied in samples without *IDH1* or *IDH2* mutation. To correlate with possible *PTH1R* mutations, we also amplified exon 4 of *PTH1R* for mutations encoding G121E and A122T substitutions, exon 5 for mutations encoding R150C and exon 9 for mutations encoding R255H using DNA from 35 subjects with Ollier disease or Maffucci syndrome.

PCR was carried out in a reaction volume of 25 µl, with 10 ng of DNA, 12.5 µl of iQ SYBR green Supermix (Bio-Rad) and 10 pmol M13-tailed primers (sequences provided in Supplementary Table 5). PCR was performed in a CFX 96 Real-Time PCR detection system (Bio-Rad), with an initial denaturation step of 5 min at 95 °C followed by 40 cycles of 10 s at 95 °C, 10 s at 60 °C and 10 s at 72 °C. After a final elongation step of 10 min at 72 °C, a melt curve was obtained to evaluate the quality of the PCR products. PCR products were purified using the Qiagen MinElute 96 UF PCR Purification system and eluted in 25 µl of sterile water. PCR amplicons were sequenced by a commercial entity using standard forward and reverse M13 primers (Macrogen). The sequence trace files were analyzed with Mutation Surveyor DNA Variant Analysis software (version 3.97, SoftGenetics).

To validate the mutations in *IDH1* encoding R132C and R132H, we designed hydrolysis probe assays (probe sequences provided in Supplementary Table 6), using the Custom Taqman Assay Design Tool (Applied Biosystems). Assays were performed on 144 samples, including tumors derived from subjects with Ollier disease and Maffucci syndrome, as well as solitary cartilaginous tumors, chondrosarcoma cell lines and blood from subjects with Ollier disease. Assays were also performed on negative controls (healthy donor DNA) and no template controls. qPCR was carried out in a reaction volume of 10 µl as described previously[57] in a CFX38 Real-Time PCR Detection System (Bio-Rad), with an initial denaturation step of 10 min at 95 °C followed by 40 cycles of 10 s at 92 °C and 30 s at 60 °C. The quantification cycle (Cq) was used for quality assessment and samples with Cq >35 for the wild-type allele were considered as DNA negative. The threshold for the mutant alleles of *IDHI* encoding R132C (c.394C>T) or R132H (c.395G>A) was set after subtracting the highest background signal from the negative controls.

There was sufficient DNA left from 5 of 12 tumors without mutation to perform sequence analysis for all exons of *IDH1* and *IDH2*. One tumor with an *IDH1* mutation was also sequenced. PCR was performed as described above for exon 4, and primer sequences are listed in Supplementary Table 5.

Tiling resolution targeted oligonucleotide arrays

Custom-designed Agilent tiling oligonucleotide array–comparative genomic hybridization analysis consisting of 15,000 probes with a tiling coverage of genes involved in the different types of enchondromatosis syndromes (*IDH1, IDH2, ACP5, PTH1R, PTPN11, EXT1, EXT2* and *PTHLH*) (Supplementary Table 2) was performed to detect possible small intragenic losses and gains in these genes. In total, 16 enchondromas and chondrosarcomas from subjects with Ollier disease or Maffucci syndrome, with (N = 14) and without (N = 2) *IDH1* or *IDH2* mutations were selected. Labeling and hybridization of genomic DNA from freshly frozen tumors and data processing were performed as described[58].

Immunohistochemistry

To examine the protein expression of the IDH1 R132H mutant, immunohistochemistry was performed as described[8] using antibody recognizing IDH1 R132H from Dianova (1:200 dilution in 5% non-fat milk, citrate antigen retrieval and blocking for 30 min with 5% non-fat milk). We used 403 tumors (Table 2) on 19 TMAs, for which details were previously published[8,59-61].

Additional samples from subjects with Ollier disease or Maffucci syndrome were collected through the European Musculo-Skeletal Oncology Society (EMSOS), and clinical details for these individuals are described separately[5]. Glioma tissue with a known *IDH1* mutation was used as a positive control, and primary antibody was omitted as a negative control. Only strong cytoplasmic staining combined with nuclear staining was considered a positive result[35]. To study possible mosaicism in the tumors and in surrounding normal tissues, we selected resection specimens from tumors expressing the mutant IDH1 R132H protein (N = 7) and stained multiple tissue blocks from different areas. All except nine tumors from subjects with Ollier disease that were used for mutation analysis were also included in the TMAs, and results were confirmed.

Statistical analysis for clinical correlation

Follow-up data were available from 83 subjects with solitary tumors (range 2–335 months, mean 115.23). To investigate the relation of *IDH1* or *IDH2* mutation with the clinical features of the subjects, multivariate survival analysis (Cox regression) and cross-tabulations (Pearson's $\chi 2$ test) were performed using SPSS version 16.0. Statistical analysis was not performed for subjects with Ollier disease, because nearly all subjects with available follow-up data had *IDH1* or *IDH2* mutations. All the *P* values reported are two-sided, and $P < 0.05$ was considered to indicate statistical significance.

DNA methylation profiling

A total of 12 samples, which included 8 enchondromas with *IDH1* mutation (4 Ollier enchondromas, 2 Maffucci enchondromas and 2 solitary enchondromas) and 4 enchondromas (1 Ollier enchondroma and 3 solitary enchondromas) without *IDH1* or *IDH2* mutations, were analyzed. Of the 4 enchondromas without *IDH1* mutation, one had cells with mutated *IDH1* encoding the R132G alteration present in a subpopulation, which was below the threshold of detection by Sanger sequencing. Bisulfite treatment was performed using the EZ DNA Methylation kit (Zymo Research). Bisulfite-converted DNA was then hybridized to Illumina HumanMethylation27 BeadChips by following the manufacturer's instructions. Infinium unsupervised clustering analysis was performed using the Ward's clustering algorithm based on Euclidian distance. The 2,000 most variable CpG sites (excluding those on the X and Y chromosomes) were used in the clustering analysis.

Genome-wide gene expression analysis

A total of 21 tumors, including 6 enchondromas and 10 chondrosarcomas (6 grade I and 4 grade II) from subjects with Ollier disease and Maffucci syndrome, as well as 1 solitary enchondroma, 4 solitary chondrosarcomas, grade II, and 6 controls (2 growth plates and 4 normal cartilage), were used. We determined differential expression between tumors with *IDH1* or *IDH2* mutation (N = 18) compared to tumors without detectable *IDH1* or *IDH2* mutation (N = 3) using Sanger sequencing. Two of these samples showed sub-threshold peaks for mutations in *IDH1* encoding R132G and R132C, suggesting that the mutation was present in a minor subpopulation of tumor cells. Expression analysis using Illumina Human-6 v3.0 Expression BeadChips were performed as described previously[8,62,63]. LIMMA analysis[64] was used to determine differential expression between the groups. Probes with Benjamini and Hochberg false discovery rate–adjusted *P* values <0.05 and a log fold change >0.1 were considered to be significantly differentially expressed.

Accession numbers

MIAME-compliant data from the tiling, expression and methylation arrays have been deposited in the GEO database (GSE30844). Sequence data for IDHI and IDH2 has been deposited in GenBank (NM_005896.2 and NM_002168.2).

Acknowledgments

We are grateful to all of the participants and their families for taking part in this study. We would like to thank S. Romeo and C.M.A. Reijnders for providing DNA from cartilage tumors. We are grateful to A.B. Mohseny for help with statistics, to D. van der Geest and T. Krenács for constructing TMAs and to P. Wijers-Koster, D. de Jong, B. van den Akker, R. Duim, M. Winter, I.H. Briaire-de Bruijn and M.E. Bowen for expert technical assistance. C.J.F. Waaijer, P.C.W. Hogendoorn and C.E. de Andrea are acknowledged for fruitful discussion. We would like to acknowledge F. Bertoni, E.L. Staals and P. Bacchini for kindly providing peripheral dedifferentiated chondrosarcomas and vascular tumors, T. Kalinski for the C3842 cell line, M. Namba for the OUMS27 cell line, T. Ariizumi for the NDCS1 cell line and A. Llombart Bosch for the CH2879 cell line. J. Mulliken, J. Upton and S. Fishman kindly provided spindle cell hemangiomas. S.H.M. Verdegaal, A.H.M. Taminiau and M.A.J. van de Sande are acknowledged for contributing patient data. We are thankful to S. Boeuf, R. Forsyth, P. Mainil-Varlet and W. Wuyts, for providing frozen tissue from a single Ollier or Maffucci case or control sample. The continuous support of the Netherlands Committee on Bone Tumors is highly acknowledged. The study was funded by The Netherlands Organization for Scientific Research (917-76-315 to J.V.M.G.B. and T.C.P.), the Liddy Shriver Sarcoma Initiative (to J.V.M.G.B. and J.O.), the Interuniversity Attraction Poles initiated by the Belgian Federal Science Policy, network 6/05, the US National Institutes of Health (AR048564) and the FNRS–Fonds de la Recherche Scientifique (all to M.V.) and the Manton Center for Orphan Disease Research at Children's Hospital Boston (to K.K.). The study was performed within the EuroBoNeT, a European Commission-granted Network of Excellence for studying the pathology and genetics of bone tumors (018814).

Author Contributions

The study was designed, written and reviewed by T.C.P. and J.V.M.G.B. Mutation analysis was designed and performed by T.C.P., M.A.J.H.v.R., J.V.M.G.B., K.S., T.v.W. and R.v.E. Immunohistochemistry was conducted and evaluated by T.C.P., M.A.J.H.v.R. and J.V.M.G.B. T.C.P., S.L.J.V., J.G.v.O. and D.M. contributed tissue microarrays. Expression profiling was designed and performed by A.-M.C.-J., T.C.P., J.V.M.G.B. and J.O. and analyzed by J.O. and M.L.K. Methylation profiling was designed by A.-M.C.-J., J.V.M.G.B. and L.S., performed by P.d.A., and the results analyzed by P.d.A. and P.J.F. K.H.N., S.D., L.S., B.T., B.L.-A., M.S.-J., R.S., N.L., L.-G.K., C.G., M.V., L.M.B. and K.C.K. each contributed frozen or paraffin-embedded tissues for multiple subjects with Ollier disease or Maffucci syndrome and acquired data for these individuals. The manuscript was approved by all authors.Q4Q4Q5Q5

Competing Financial Interests

The authors declare no competing financial interests.

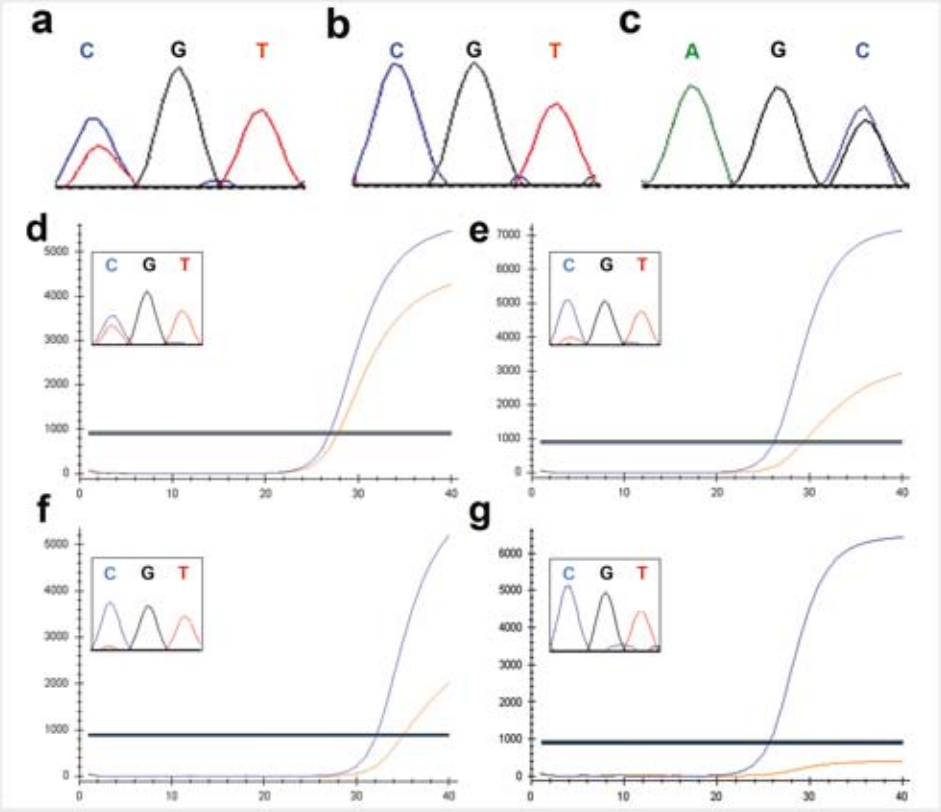
References

1. Spranger, J., Kemperdieck, H., Bakowski, H. & Opitz, J.M. Two peculiar types of enchondromatosis. Pediatr. Radiol. 7, 215–219 (1978).
2. Lucas, D.R. & Bridge, J.A. Chondromas: enchondroma, periosteal chondroma, and enchondromatosis. In World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone (eds. Fletcher, C.D.M., Unni, K.K. & Mertens, F.) 237–240 (IARC Press, Lyon, France, 2002).
3. Pansuriya, T.C., Kroon, H.M. & Bovee, J.V.M.G. Enchondromatosis: insights on the different subtypes. Int. J. Clin. Exp. Pathol. 3, 557–569 (2010).
4. Verdegaal, S.H.M. et al. Incidence, predictive factors and prognosis of chondrosarcoma in patients with Ollier disease and Maffucci syndrome: an international multicenter study of 161 patients. Oncologist (in the press).
5. Hopyan, S. et al. A mutant PTH/PTHrP type I receptor in enchondromatosis. Nat. Genet. 30, 306–310 (2002).
6. Rozeman, L.B. et al. Enchondromatosis (Ollier disease, Maffucci syndrome) is not caused by the PTHR1 mutation p.R150C. Hum. Mutat. 24, 466–473 (2004).
7. Couvineau, A. et al. PTHR1 mutations associated with Ollier disease result in receptor loss of function. Hum. Mol. Genet. 17, 2766–2775 (2008).
8. Pansuriya, T.C. et al. Genome-wide analysis of Ollier disease: is it all in the genes? Orphanet J. Rare Dis. 6, 2 (2011).
9. Pansuriya, T.C. et al. Maffucci syndrome: a genome-wide analysis using high resolution single nucleotide polymorphism and expression arrays on four cases. Genes Chromosom. Cancer 50, 673–679 (2011).
10. Ranger, A. & Szymczak, A. Do intracranial neoplasms differ in Ollier disease and Maffucci syndrome? An in-depth analysis of the literature. Neurosurgery 65, 1106–1113 (2009).
11. Schwartz, H.S. et al. The malignant potential of enchondromatosis. J. Bone Joint Surg. Am. 69, 269–274 (1987).
12. Rietveld, L. et al. First case of juvenile granulosa cell tumor in an adult with Ollier disease. Int. J. Gynecol. Pathol. 28, 464–467 (2009).
13. Leyva-Carmona, M., Vazquez-Lopez, M.A. & Lendinez-Molinos, F. Ovarian juvenile granulosa cell tumors in infants. J. Pediatr. Hematol. Oncol. 31, 304–306 (2009).
14. Yan, H. et al. IDH1 and IDH2 mutations in gliomas. N. Engl. J. Med. 360, 765–773 (2009).
15. Hartmann, C. et al. Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. Acta Neuropathol. 118, 469–474 (2009).
16. Dang, L., Jin, S. & Su, S.M. IDH mutations in glioma and acute myeloid leukemia. Trends Mol. Med. 16, 387–397 (2010).
17. Kalfa, N. et al. Activating mutations of the stimulatory G protein in juvenile ovarian granulosa cell tumors: a new prognostic factor? J. Clin. Endocrinol. Metab. 91, 1842–1847 (2006).
18. Amary, M.F. et al. 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 (2011).
19. van Krieken, J.H. et al. KRAS mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma: proposal for an European quality assurance program. Virchows Arch. 453, 417–431 (2008).
20. Wolff, J.N. & Gemmell, N.J. Combining allele-specific fluorescent probes and restriction assay in real-time PCR to achieve SNP scoring beyond allele ratios of 1:1000. Biotechniques 44, 193–194, 196, 199 (2008).
21. Sobreira, N.L. et al. Whole-genome sequencing of a single proband together with linkage analysis identifies a Mendelian disease gene. PLoS Genet. 6, e1000991 (2010).
22. Bowen, M.E. et al. Loss-of-function mutations in PTPN11 cause metachondromatosis, but not Ollier disease or Maffucci syndrome. PLoS Genet. 7, e1002050 (2011).

23. Lausch, E. et al. Genetic deficiency of tartrate-resistant acid phosphatase associated with skeletal dysplasia, cerebral calcifications and autoimmunity. Nat. Genet. 43, 132–137 (2011).
24. Briggs, T.A. et al. Tartrate-resistant acid phosphatase deficiency causes a bone dysplasia with autoimmunity and a type I interferon expression signature. Nat. Genet. 43, 127–131 (2011).
25. Collinson, M. et al. Symmetrical enchondromatosis is associated with duplication of 12p11.23 to 12p11.22 including PTHLH. Am. J. Med. Genet. A. 152A, 3124–3128 (2010).
26. Bianco, P. et al. Reproduction of human fibrous dysplasia of bone in immunocompromised mice by transplanted mosaics of normal and Gsalpha-mutated skeletal progenitor cells. J. Clin. Invest. 101, 1737–1744 (1998).
27. Jones, K.B. et al. A mouse model of osteochondromagenesis from clonal inactivation of Ext1 in chondrocytes. Proc. Natl. Acad. Sci. USA 107, 2054–2059 (2010).
28. de Andrea, C.E., Prins, F.A., Wiweger, M.I. & Hogendoorn, P.C.W. Growth plate regulation and osteochondroma formation: insights from tracing proteoglycans in zebrafish models and human cartilage. J. Pathol. 224, 160–168 (2011).
29. de Andrea, C.E. et al. Secondary peripheral chondrosarcoma evolving from osteochondroma as a result of the outgrowth of cells with functional EXT. Oncogene 10.1038/onc.2011.311 (1 August 2011).
30. Reijnders, C.M. et al. No haploinsufficiency but loss of heterozygosity for EXT in multiple osteochondromas. Am. J. Pathol. 177, 1946–1957 (2010).
31. Bovée, J.V.M.G. EXTra hit for mouse osteochondroma. Proc. Natl. Acad. Sci. USA 107, 1813–1814 (2010).
32. Clément, A. et al. Regulation of zebrafish skeletogenesis by ext2/dackel and papst1/pinscher. PLoS Genet. 4, e1000136 (2008).
33. Cohen, M.M. Jr. Fibrous dysplasia is a neoplasm. Am. J. Med. Genet. 98, 290–293 (2001).
34. Lietman, S.A., Ding, C. & Levine, M.A. A highly sensitive polymerase chain reaction method detects activating mutations of the GNAS gene in peripheral blood cells in McCune-Albright syndrome or isolated fibrous dysplasia. J. Bone Joint Surg. Am. 87, 2489–2494 (2005).
35. Ikota, H., Nobusawa, S., Tanaka, Y., Yokoo, H. & Nakazato, Y. High-throughput immunohistochemical profiling of primary brain tumors and non-neoplastic systemic organs with a specific antibody against the mutant isocitrate dehydrogenase 1 R132H protein. Brain Tumor Pathol. 28, 107–114 (2011).
36. Figueroa, M.E. et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell 18, 553–567 (2010).
37. Thomas, D.M. Lessons from the deep study of rare tumours. J. Pathol. 224, 306–308 (2011).
38. Parsons, D.W. et al. An integrated genomic analysis of human glioblastoma multiforme. Science 321, 1807–1812 (2008).
39. Mardis, E.R. et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. N. Engl. J. Med. 361, 1058–1066 (2009).
40. Yen, K.E., Bittinger, M.A., Su, S.M. & Fantin, V.R. Cancer-associated IDH mutations: biomarker and therapeutic opportunities. Oncogene 29, 6409–6417 (2010).
41. Kang, M.R. et al. Mutational analysis of IDH1 codon 132 in glioblastomas and other common cancers. Int. J. Cancer 125, 353–355 (2009).
42. Gaal, J. et al. Isocitrate dehydrogenase mutations are rare in pheochromocytomas and paragangliomas. J. Clin. Endocrinol. Metab. 95, 1274–1278 (2010).
43. Hemerly, J.P., Bastos, A.U. & Cerutti, J.M. Identification of several novel non-p.R132 IDH1 variants in thyroid carcinomas. Eur. J. Endocrinol. 163, 747–755 (2010).
44. Dang, L. et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 462, 739–744 (2009).
45. Noushmehr, H. et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 17, 510–522 (2010).

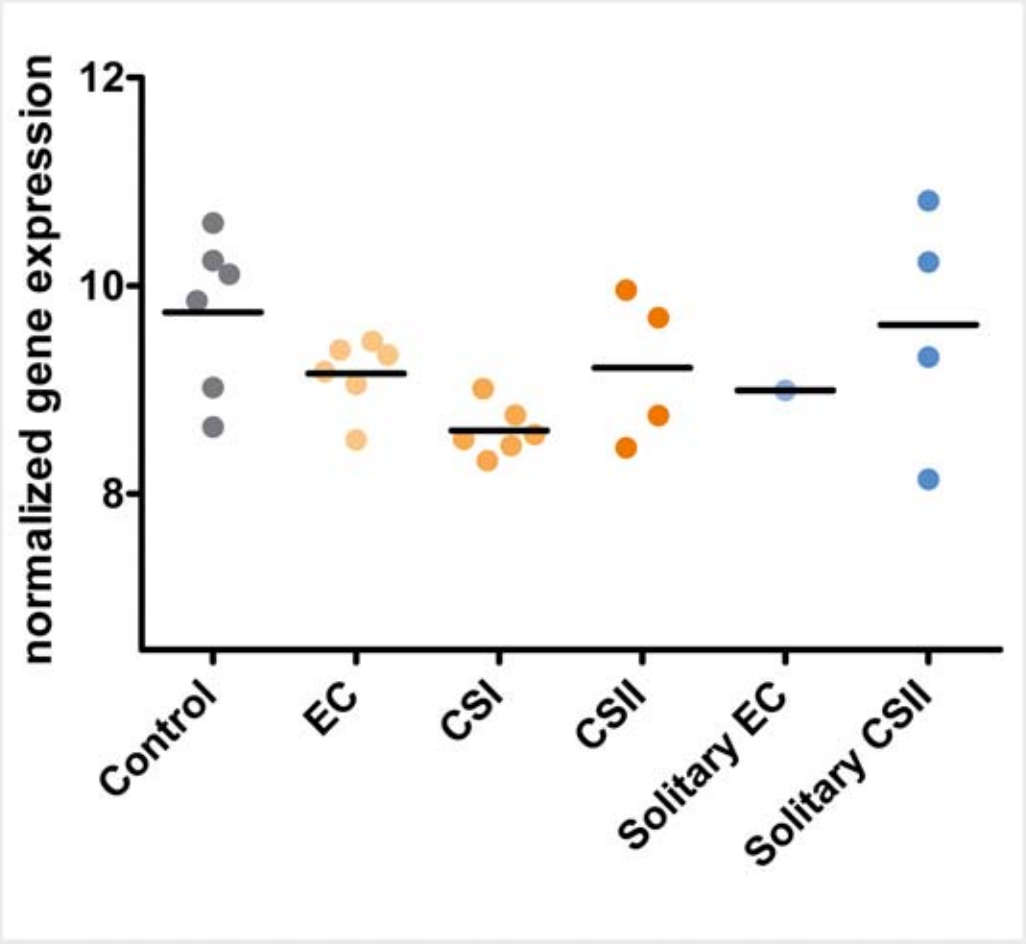
46. Toyota, M. et al. CpG island methylator phenotype in colorectal cancer. Proc. Natl. Acad. Sci. USA 96, 8681–8686 (1999).
47. Chin, H.J. et al. Studies on the role of Dlx5 in regulation of chondrocyte differentiation during endochondral ossification in the developing mouse limb. Dev. Growth Differ. 49, 515–521 (2007).
48. Zhu, H. & Bendall, A.J. Dlx5 is a cell autonomous regulator of chondrocyte hypertrophy in mice and functionally substitutes for Dlx6 during endochondral ossification. PLoS ONE 4, e8097 (2009).
49. Lee, M.H., Kwon, T.G., Park, H.S., Wozney, J.M. & Ryoo, H.M. BMP-2-induced Osterix expression is mediated by Dlx5 but is independent of Runx2. Biochem. Biophys. Res. Commun. 309, 689–694 (2003).
50. Ulsamer, A. et al. BMP-2 induces Osterix expression through up-regulation of Dlx5 and its phosphorylation by p38. J. Biol. Chem. 283, 3816–3826 (2008).
51. Scully, S.P. et al. Marshall Urist Award. Interstitial collagenase gene expression correlates with in vitro invasion in human chondrosarcoma. Clin. Orthop. Relat. Res. 291–303 (2000).
52. Gil-Benso, R. et al. Establishment and characterization of a continuous human chondrosarcoma cell line, ch-2879: comparative histologic and genetic studies with its tumor of origin. Lab. Invest. 83, 877–887 (2003).
53. Kunisada, T. et al. A new human chondrosarcoma cell line (OUMS-27) that maintains chondrocytic differentiation. Int. J. Cancer 77, 854–859 (1998).
54. Kalinski, T. et al. Establishment and characterization of the permanent human cell line C3842 derived from a secondary chondrosarcoma in Ollier's disease. Virchows Arch. 446, 287–299 (2005).
55. Kudo, N. et al. Establishment of novel human dedifferentiated chondrosarcoma cell line with osteoblastic differentiation. Virchows Arch. 451, 691–699 (2007).
56. Evans, H.L., Ayala, A.G. & Romsdahl, M.M. Prognostic factors in chondrosarcoma of bone. A clinicopathologic analysis with emphasis on histologic grading. Cancer 40, 818–831 (1977).
57. van Eijk, R. et al. Rapid KRAS, EGFR, BRAF and PIK3CA mutation analysis of fine needle aspirates from non-small-cell lung cancer using allele-specific qPCR. PLoS ONE 6, e17791 (2011).
58. Szuhai, K. et al. Tiling resolution array-CGH shows that somatic mosaic deletion of the EXT gene is causative in EXT gene mutation negative multiple osteochondromas patients. Hum. Mutat. 32, E2036–E2049 (2011).
59. Verbeke, S.L. et al. Distinct histological features characterize primary angiosarcoma of bone. Histopathology 58, 254–264 (2011).
60. Meijer, D. et al. Expression of aromatase and estrogen receptor alpha in chondrosarcoma, but no beneficial effect of inhibiting estrogen signaling both in vitro and. in vivo. Clin. Sarcoma Res. 1 (2011).
61. Rozeman, L.B. et al. Dedifferentiated peripheral chondrosarcomas: regulation of EXT-downstream molecules and differentiation-related genes. Mod. Pathol. 22, 1489–1498 (2009).
62. Hallor, K.H. et al. Genomic profiling of chondrosarcoma: chromosomal patterns in central and peripheral tumors. Clin. Cancer Res. 15, 2685–2694 (2009).
63. Buddingh, E.P. et al. Tumor-infiltrating macrophages are associated with metastasis suppression in high-grade osteosarcoma: a rationale for treatment with macrophage-activating agents. Clin. Cancer Res. 17, 2110–2119 (2011).
64. Smyth, G.K. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat. Appl. Genet. Mol. Biol. 3, Article3 (2004).

Figure S1 Output of sequencing and hydrolysis probes assay



a, b) Example of Sanger sequencing results showing that R132C *IDH1* mutation was present in enchondroma and absent in corresponding blood DNA of a patient with Ollier disease. c) Example of Sanger sequencing results showing R172S *IDH2* mutation in a single patient with Ollier disease. d-g) Relative Fluorescent Units (RFU) are plotted against the quantification cycle (Cq). The horizontal line at 950 RFU indicates the threshold level for allele calling. All samples show a positive signal for both wild type (blue) and the *IDH1* c.395C>T, p.R132C mutant allele (orange). d) L1684 carrying the R132C *IDH1* mutation on Sanger sequencing (inset) confirmed with hydrolysis probes assay. e) L1980 and f) L204 have minor *IDH1* positive cell populations. The R132C *IDH1* mutant allele is barely visible after Sanger sequencing (inset) but it clearly presents as mutant using hydrolysis probes assay. g) L172 is negative for the mutation, in Sanger sequencing (inset) as well as in hydrolysis probes assay.

Figure S2 Normalized expression levels of *DLX5*



EC: enchondroma, CS : chondrosarcoma

Table S1 Clinical information of patients with Ollier disease and Maffucci syndrome

Patient ID	Sample ID	*IDH1* mutation	*IDH2* mutation	Gender	Age	Disease	Tumor	Tumor location
01	L1083	R132C		male	48	Ollier	CSI	metacarpal
	L2218	R132C		male	49	Ollier	CSI	digit V
02	L172[3,6]	no mutation	no mutation	male	40	Ollier	CSII	scapula
03	L271	R132C		female	26	Ollier	CSI	distal femur
	L286 T	R132C		female	23	Ollier	CSII	femur
04[1]	L149 T3	R132C		male	34	Ollier	CSI	unknown
	L204 II[2,3]	R132C		male	26	Ollier	CSI	femur
	L253 T3	R132C		male	26	Ollier	CSI	tibia
05	L206	R132C		female	25	Ollier	EC	hand
06	L816-1	no mutation	R172S	male	68	Ollier	CSIII	humerus
	L813 T	no mutation	R172S	male	68	Ollier	CSII	femur
07[1]	L898[6]	no mutation	no mutation	male	18	Ollier	CSI	femur
08[1]	L1251	R132H		male	15	Ollier	EC	hand
	L2220	R132H		male	14	Ollier	EC	digit
09	L1974	R132C		male	48	Ollier	CSII	scapula
010[1]	L1975	R132C		male	31	Ollier	CSII	femur
011	L1976	R132C		male	41	Ollier	CSII	tibia
012[1]	L1977	R132C		male	41	Ollier	CSI	tibia
	L1978	R132C		male	38	Ollier	EC	foot
	L1979	R132C		male	41	Ollier	CSI	tibia
	L3363	R132C		male	36	Ollier	EC	toe
013	L1980[2]	R132C		female	63	Ollier	CSII	knee
014	L1685	R132C		female	23	Ollier	CSI	pubic bone

Table S1 (Continue)

Patient ID	Sample ID	*IDH1* mutation	*IDH2* mutation	Gender	Age	Disease	Tumor	Tumor location
O15	L1686	R132C		male	18	Ollier	EC	phalanx
	L1687	R132C		male	18	Ollier	CSI	phalanx
O16	L2386	R132H		female	13	Ollier	CSI	digit III
O17[1]	L2463	R132C		female	12	Ollier	EC	tibia
O18	L1629	R132C		male	36	Ollier	EC	unknown
	L1630	R132C		male	36	Ollier	CSI	iliac bone
O19	L2095	R132C		female	23	Ollier	EC	distal tibia
O20[5]	L2590[2]	R132H		female	36	Ollier	CSI	metatarsal I
	L2761	no mutation	no mutation	female	37	Ollier	CSI	tibia
O21	L2098[3]	no mutation	no mutation	female	15	Ollier	CSII	humerus
O22	L2099	R132C		female	49	Ollier	CSI	prox humerus
O23	L2100	no mutation	no mutation	male	27	Ollier	EC	femur
O24	L2103a	R132C		male	39	Ollier	EC	phalanx
	L2103b	R132C		male	39	Ollier	CSI	distal phalanx
O25	L2104a	R132C		male	36	Ollier	CSIII	prox tibia
O26[1]	L2221	R132C		female	42	Ollier	CSI	distal femur
O27[1]	L1513	R132C		female	23	Ollier	CSI	distal femur
O28[1]	L1490	R132H		female	12	Ollier	EC	phalanx
O29	L2640	R132C		female	34	Ollier	EC	phalanx
	L2641	R132C		female	34	Ollier	EC	thumb
O30	L2205	R132C		male	6	Ollier	EC	illum
O31	L1683	R132C		unknown	29	Ollier	CSI	metacarpal
O32[1]	L2280	R132C		female	24	Ollier	CSI	left acromion

Table S1 (Continue)

Patient ID	Sample ID	*IDH1* mutation	*IDH2* mutation	Gender	Age	Disease	Tumor	Tumor location
O33	L2513[3]	no mutation	no mutation	male	33	Ollier	CSI	pelvis
O34	L2746	R132C		female	58	Ollier	CSI	digit II
O35[1]	L3325A	R132H		male	6	Ollier	EC	hand
O36[1]	L3362A	R132C		female	6	Ollier	EC	proximal tibia
	L3362C	R132C		female	6	Ollier	EC	left distal femur
O37	OLR30[4]	R132C		male	8	Ollier	EC	right leg
O38	S-03-3802[2,4]	R132H		female	16	Ollier	EC	hand
O39	S-05-4941[4]	R132H		male	13	Ollier	EC	hand
O40	S-05-6625[4]	R132C		female	11	Ollier	EC	hand
O41	S-08-3234[4]	R132H		female	4	Ollier	EC	hand
O42	S-08-7943[4]	no mutation	no mutation	male	12	Ollier	EC	hand
O43	S-08-9181[4]	no mutation	no mutation	female	12	Ollier	EC	hand
M1	L1684	R132C		female	37	Maffucci	EC	phalanx
M2	L2097b	R132C		female	19	Maffucci	EC	toe
	L2097a	R132C		female	19	Maffucci	EC	prox tibia
M3	L2102	R132C		male	29	Maffucci	CSII	distal femur
M4	MAF100[3]	R132C		male	birth	Maffucci	CSI	hand
M5	MAF200	R132C		female	4	Maffucci	SCH	right hand
M6	MAF210[3]	no mutation	no mutation	female	-	Maffucci	SCH	-
M7	MAF230	R132C		female	2	Maffucci	SCH	right foot
M8	MAF250[3]	no mutation	no mutation	female	3	Maffucci	EC	right hand
M9	S08-0010959 4A[4]	no mutation	no mutation	male	15	Maffucci	SCH	digit
	S08-0010959 5A[4]	no mutation	no mutation	male	15	Maffucci	SCH	forearm

Table S1 (Continue)

M10	S08-0007382[2,4]	R132C		male	9	Maffucci	SCH	hand
M11	S05-0006227[2,4]	R132C		male	10	Maffucci	SCH	digit
M12	S97-0002538 1A[4]	R132C		female	30	Maffucci	SCH	foot
	S97-0002538 3A[4]	R132C		female	30	Maffucci	SCH	first web space
M13	S03-0001121[4]	R132C		female	23	Maffucci	EC	digit
	S97-0004447 4B[2,4]	R132C		female	17	Maffucci	SCH	lower back

All patients were diagnosed as having Ollier disease or Maffucci syndrome based on the radiographical features and/or presence of more than two cartilaginous tumors (Ollier disease) in combination with hemangioma (Maffucci syndrome). [1] indicates DNA from normal tissue was also tested, [2] indicates cases negative in Sanger sequencing but positive in hydrolysis probes assay, [3] indicates cases used for sequencing of all exons of IDH1 and IDH2, [4] indicates DNA was isolated from paraffin embedded tissue, [5]indicates patient with multiple tumors in which one tumor showed clearly mutation in the sequencing and another tumor was negative for the mutation. DNA was unavailable to perform hydrolysis probe assay. [6]Two chondrosarcomas of patients with Ollier disease were negative at the mutation analysis, while other tumors of the same patients demonstrated positive cells at R132H IDH1 immunohistochemistry, suggesting that the percentage of patients carrying mutations in IDH1 or IDH2 is even higher than we report in this study. EC: enchondroma, CS: chondrosarcoma, SCH: spindle cell hemangioma.

Table S2 Tiling Array design

Gene	Number of probes
IDH1	312
IDH2	208
PTHLH	338
PTPN11	413
PTH1R	104
EXT1	833
EXT2	334
ACP5	93

Table S3(L) Different methylated sites

Index	TargetID	ProbeID_A	ProbeID_B	L1684. AVG_Beta	L1829. AVG_Beta	L1598. AVG_Beta	MAF250. AVG_Beta	L2103a. AVG_Beta	L1251. AVG_Beta	L1686. AVG_Beta
22413	cg22442090	2070324	2070008	0.1848371	0.2185057	0	0	0.5814463	0	0.3329082
8399	cg08450982	3800139	2350523	0.1100114	0.2611653	0.09177592	0	0.1041835	0.03501094	0.1062992
6684	cg06690548	4480167	3360008	0.170406	0.8494078	0.2249152	0.02788536	0.2761823	0.1059524	0.316043
7884	cg07922606	7320719	7320398	0.2281141	0.3192771	0.1360397	0.07273802	0.2583545	0.166755	0.1644749
6407	cg06434428	7160086	4670435	0.1261764	0.3005146	0.1435832	0.01117799	0.2098659	0.03615886	0.1997608
7782	cg07823492	1430132	1430491	0.2043384	0.366	0.2075359	0.08771437	0.2065943	0.1085657	0.1974618
21663	cg21663431	830441	5910075	0.155234	0.4232825	0.291686	0	0.1709022	0.04581732	0.1790756
1336	cg01324261	1240243	1780161	0.1456849	0.256	0.1069736	0.07170542	0.1258803	0.07535322	0.0931624
21433	cg21453309	1050390	4900377	0.08465447	0.2001105	0.07835243	0.006024096	0.07591522	0.04675159	0.07536232
4175	cg04126335	4120110	2350491	0.07277453	0.08045325	0.02733485	0	0	0.03635243	0.0203749
17852	cg17826679	7200400	2900390	0.09147287	0.2878049	0.1851064	0.004569839	0.1399118	0.05752961	0.1170739
26566	cg26608667	3390538	6660161	0.2009503	0.2532403	0.1254081	0.184076	0.2259414	0.2004545	0.1914157
12016	cg12069042	2030600	2490600	0.1988473	0.4683586	0.190639	0.1105858	0.2567744	0.1410658	0.2619452
8523	cg08578641	6250376	5570446	0.09503632	0.2448716	0.1318403	0.01808682	0.0778626	0.04301759	0.08563194
22547	cg22580512	7560017	1450017	0.07238949	0.1943258	0.1190033	0.01297968	0.07011494	0.05758963	0.06808074
635	cg00616135	2940546	2940437	0.0608931	0.2368263	0.09176788	0	0.07741386	0.1147471	0.06829489
21396	cg21416237	2230022	5360022	0.2673218	0.4347915	0.283985	0.04835473	0.2963827	0.2016468	0.2902088
597	cg00573606	7560440	1450438	0.1443299	0.1787749	0.1125917	0.125	0.114158	0.1400481	0.130849
24384	cg24433189	4860484	6270520	0.08097929	0.1744723	0.07089874	0.002761954	0.08934221	0.019631	0.05668151
9754	cg09837648	780474	5340474	0.3633727	0.7797911	0.3566205	0.319695	0.5624712	0.354095	0.4788733
14470	cg14467840	2850021	6760370	0.2900697	0.5274104	0.2131003	0.03192365	0.3517469	0.1923225	0.3182451
19878	cg19884658	10364	2680280	0.008781978	0.1063014	0	0.009250399	0.05360395	0	0.02420091
21088	cg21120063	4860500	6270315	0.1170727	0.2642019	0.1594053	0.0177548	0.1404155	0.09257835	0.1353896
17261	cg17233506	4070075	6900433	0.06682252	0.1921083	0.04269211	0.03136435	0.06766749	0.04909448	0.06471622
4146	cg04099420	4810170	5720474	0.06028369	0.1963658	0.07692308	0.1207865	0.01805416	0	0.1229682

Table S3(R) Different methylated sites

L2463. AVG_Beta	L2100. AVG_Beta	L198. AVG_Beta	L194. AVG_Beta	L2357. AVG_Beta	Gene. Symbol	ttest	Meth change	FC mut v wt	FC mut v normal	present in TCGA GBM set
0.2210636	0.7407407	0.6489918	0.4687225	0.5575524	*GIMAP5*	0.004	-0.412	0.028	0.150	
0.09696969	0.8642069	0.7077352	0.05559368	0.2513404	*NUMBL*	0.020	-0.369	-0.039	0.112	
0.4333574	0.8141414	0.778991	0.4648357	0.6074498	*SLC7A11*	0.027	-0.366	-0.033	0.053	390
0.2048392	0.7948602	0.8080082	0.2226377	0.4000449	*HIST1H3E*	0.006	-0.363	#N/A	#N/A	464
0.1782178	0.8043478	0.7143432	0.1621918	0.3712297	*HAPLN1*	0.009	-0.362	0.786	0.576	
0.36	0.8855434	0.820531	0.1263489	0.387401	*HOXB1*	0.028	-0.338	-0.035	0.012	
0.1839364	0.7947037	0.7532467	0.1120901	0.3646728	*SLC44A2*	0.030	-0.325	1,404	0.711	
0.08252427	0.7004992	0.6179664	0.1820128	0.2545721	*SCRG1*	0.006	-0.319	1,764	-0.361	
0.1275912	0.7261189	0.6586753	0.05085223	0.1736886	*FAM101A*	0.023	-0.315	-0.079	0.106	
0.05769231	0.7164502	0.5984252	0.02212052	0.07015858	*ITGA10*	0.026	-0.315	1,916	-0.420	
0.1122292	0.7545383	0.6392597	0.07336745	0.2717073	*SLC44A2*	0.022	-0.310	1,404	0.711	
0.5584695	0.7240678	0.7241541	0.1895579	0.5725678	*MGC11257*	0.017	-0.310	0.498	0.257	
0.4331599	0.7592163	0.758442	0.2721696	0.4795041	*PLXNB1*	0.014	-0.310	0.568	-0.301	
0.1280915	0.7596288	0.6122671	0.07479157	0.2008523	*DNAI1*	0.023	-0.309	0.030	-0.084	
0.07233369	0.7394772	0.6367751	0.05336951	0.1303279	*NCOR2*	0.029	-0.307	-0.282	-0.036	
0.1190832	0.714386	0.6241342	0.09505542	0.1650683	*LACTB*	0.021	-0.304	-0.706	0.034	
0.2993279	0.8938702	0.783901	0.2034191	0.3889681	*FKBP10*	0.033	-0.302	0.247	0.497	
0.1179525	0.8153057	0.6043462	0.1489976	0.1714141	*COL6A3*	0.021	-0.302	-0.679	1,504	
0.06192122	0.7015101	0.579423	0.04418118	0.1459529	*SSTR5*	0.023	-0.298	0.056	-0.024	
0.7733009	0.9437935	0.9032156	0.6508114	0.6881803	*PLXNB1*	0.021	-0.298	0.568	-0.301	
0.549842	0.8075926	0.7791875	0.2329037	0.6083576	*S100A1*	0.039	-0.298	1,191	-0.133	
0.1129032	0.5730909	0.5045181	0	0.2651962	*KLHL21*	0.008	-0.296	-0.009	-0.342	1091
0.1823814	0.6842263	0.5601755	0.1337752	0.3406341	*UNQ830*	0.009	-0.291	2,885	0.309	
0.07642701	0.6301561	0.6038228	0.0558343	0.1407421	*HOXB1*	0.022	-0.284	-0.035	0.012	
0.1092095	0.6932516	0.5830303	0.08073654	0.1232877	*RIPK1*	0.028	-0.282	-0.181	-0.052	

Table S3(L) (Continue)

Index	TargetID	ProbeID_A	ProbeID_B	L1684. AVG_Beta	L1829. AVG_Beta	L1598. AVG_Beta	MAF250. AVG_Beta	L2103a. AVG_Beta	L1251. AVG_Beta	L1686. AVG_Beta
20794	cg20847746	6650356	2750356	0.1929653	0.3885039	0.231499	0.07259647	0.2080692	0.1544944	0.1847996
3567	cg03547924	3190041	3190037	0.02684124	0.1699452	0	0	0	0	0
6847	cg06848073	7150059	7380719	0.124525	0.231997	0.07859922	0.02826087	0.142126	0.06252853	0.1259131
9966	cg10052840	7050681	7050435	0.1203767	0.3130285	0.1096991	0.1614029	0.2367575	0.1123471	0.1593329
9959	cg10045881	1010270	6280041	0.1494812	0.3503103	0.05745342	0.02035351	0.1898605	0.1281764	0.168134
13743	cg13795840	6980129	4280187	0.1100332	0.2652093	0.06920471	0.1749946	0.167054	0.1975574	0.3542843
12975	cg13047596	150082	6450301	0.5389208	0.7248635	0.4978155	0.1450187	0.671567	0.3976912	0.5655454
15475	cg15475323	2100747	2940692	0.1287273	0.08169014	0	0	0	0	0.04441454
1430	cg01420388	1190561	1710524	0.2410425	0.3628534	0.2367991	0.06038838	0.2407344	0.1060032	0.2303674
15705	cg15679095	4760008	1300192	0.1538889	0.295082	0.159322	0.130039	0.1450151	0.103276	0.1687284
3408	cg03386869	1170044	6250615	0.1460108	0.3103026	0.1372227	0.3778547	0.197478	0.2128764	0.1907164
19932	cg19948393	1740091	4760091	0.2464419	0.4473319	0.4261934	0.1096902	0.6353386	0.1225178	0.4469705
16256	cg16191009	2570731	5550731	0.1770948	0.2913216	0.4751006	0.2666419	0.2000456	0.1570743	0.2180338
9574	cg09632136	3420343	5130220	0.02897416	0.1118048	0.02324037	0	0.07220497	0.0457097	0.02737851
25148	cg25152942	3400520	1340242	0.07860489	0.1571952	0.05012531	0	0.04748284	0.00886918	0.05239521
876	cg00850538	6550022	6220451	0.2188414	0.2772898	0.1610817	0.01903367	0.1445154	0.1327635	0.1514966
10849	cg10942056	7050553	6130553	0.1651434	0.2745672	0.1806854	0.1381476	0.1513778	0.1097837	0.1929419
5950	cg05955301	5560358	4920010	0.1525364	0.296394	0.1417554	0.09851447	0.1450467	0.1120531	0.1575179
25348	cg25370441	2570301	5550301	0.07834359	0.1119465	0.04806346	0.1100413	0.3050847	0.06473517	0.2055716
23528	cg23579062	4860037	6270056	0.2410155	0.3861935	0.3878559	0.03389142	0.2333427	0.1234737	0.1953359
9822	cg09893305	3310121	540706	0.1382676	0.1964353	0.08	0.05917553	0.1050864	0.04451583	0.06615315
8090	cg08124030	3990278	3170762	0.2457971	0.2063718	0.05837563	0.0362117	0.05248807	0.07717042	0.05683356
2997	cg02989257	450240	6250450	0.2070794	0.3285059	0.2397534	0.02399166	0.2522295	0.1088371	0.2612613
3562	cg03544320	110487	6100066	0	0	0.07678617	0	0	0	0.02673267
26750	cg26782833	3360343	5220431	0.1487889	0.1715006	0	0	0.1806495	0.08852802	0.1208927

Table S3(R) (Continue)

L2463. AVG_Beta	L2100. AVG_Beta	L198. AVG_Beta	L194. AVG_Beta	L2357. AVG_Beta	Gene. Symbol	ttest	Meth change	FC mut v wt	FC mut v normal	present in TCGA GBM set
0.3505025	0.6944162	0.6570533	0.2225519	0.4454353	*S100A1*	0.011	-0.282	1,191	-0.133	
0.001570681	0.5249376	0.6315151	0	0.06845966	*GDF5*	0.030	-0.281	-0.126	-0.015	
0.1495913	0.655163	0.5972655	0.06163643	0.2781244	*FBXO44*	0.018	-0.280	0.021	-0.116	
0.3349209	0.7983117	0.5258636	0.3274615	0.2133758	*SEMA6B*	0.019	-0.273	0.050	0.023	
0.327693	0.5857295	0.540077	0.1012312	0.5483743	*CHI3L2*	0.020	-0.270	0.354	0.794	572
0.1755696	0.7014493	0.6546378	0.07059923	0.4035294	*C19orf24*	0.031	-0.268	-0.017	0.072	
0.6243036	0.9550463	0.9281293	0.5156818	0.7552499	*UNQ830*	0.043	-0.268	2,885	0.309	
0.04527448	0.5131129	0.5662505	0	0.139446	*FLJ36070*	0.020	-0.267	-0.085	0.039	
0.2469696	0.7782214	0.5771658	0.1644304	0.4109969	*FBXO2*	0.023	-0.267	1,632	0.267	76
0.2614504	0.7508929	0.6610615	0.1117647	0.2444969	*KIAA0240*	0.037	-0.265	0.263	0.034	
0.170475	0.7734748	0.7174081	0.1164253	0.3232704	*ITGBL1*	0.043	-0.265	-0.024	0.149	
0.2643803	0.6773944	0.5769199	0.7040836	0.4487841	*ANKRD33*	0.026	-0.264	0.087	1,089	
0.2490185	0.6727494	0.593568	0.4537455	0.3487101	*CPNE9*	0.004	-0.263	-0.008	0.070	
0.0221843	0.6050724	0.4538116	0.09945256	0.05256327	*NNMT*	0.018	-0.261	-0.062	0.602	
0.06869689	0.6067747	0.4844106	0.01828411	0.1466346	*MIA*	0.024	-0.256	1,372	0.860	
0.1840491	0.6743674	0.6218376	0.1169231	0.2503329	*CRIM1*	0.029	-0.255	-0.061	0.199	
0.2329791	0.7069409	0.6260623	0.1986153	0.2034768	*DISP1*	0.023	-0.253	0.299	0.204	
0.1770082	0.6899129	0.6175333	0.1202698	0.2225815	*PRELP*	0.030	-0.252	0.380	-0.987	
0.06458333	0.6749688	0.5190797	0.04010184	0.2499024	*FLJ20184*	0.040	-0.247	0.002	0.014	
0.2228435	0.7401806	0.6223531	0.1797537	0.3574482	*DNAI1*	0.040	-0.247	0.030	-0.084	
0.1334746	0.6442155	0.4569626	0.09595714	0.1984501	*HAPLN1*	0.018	-0.246	0.786	0.576	
0	0.4266539	0.638139	0.08256881	0.1989589	*TM4SF1*	0.026	-0.245	-0.190	0.468	
0.337653	0.722585	0.6732684	0.1561091	0.3039578	*COL16A1*	0.047	-0.244	1,119	1,727	
0.001634958	0.4430544	0.3302785	0.2155054	0.03828045	*CRMP1*	0.002	-0.244	0.037	0.025	194
0.1074561	0.5517241	0.5608496	0.1124418	0.1504986	*MGC4268*	0.023	-0.242	0.086	0.166	

For more detail please see *Nat Genet.* 2011, doi:10.1038/ng.1004.

Table S4 Differentially expressed genes between tumors with and without *IDH1* or *IDH2* mutations at Sanger sequencing

Probe ID	Target ID	logFC	adj.PVal
6280168	*SERPINA3*	3.24	0.04267
4220431	*EXT1*	-0.73	0.03714
4210750	*STARD7*	-0.73	0.04397
2690541	*C18ORF10*	-0.57	0.04310
5050608	*TIMM23*	-0.49	0.03737
7320386	*TTL*	-0.42	0.04267
780021	*OPN3*	-0.37	0.04293
7380709	*YWHAB*	-0.35	0.02844
7040600	*ARSB*	-0.35	0.04267
2060112	*CCNYL1*	-0.29	0.02035
4480341	*DHCR24*	-0.29	0.03737
6270148	*AK5*	-0.29	0.02711
1410398	*CCNYL1*	-0.29	0.04152
6380193	*DLX3*	-0.27	0.02844
70270	*MGC39900*	-0.27	0.03099
4200070	*MGC39900*	-0.27	0.03313
6100390	*CD276*	-0.24	0.04267
6480333	*TCIRG1*	-0.23	0.04901

Table S4 (Continue)

Probe ID	Target ID	logFC	adj.PVal
2810022	*C10RF163*	-0.21	0.03174
5290358	*CPT1A*	-0.21	0.04293
3840750	*15E1.2*	-0.20	0.04267
4570242	*LARGE*	-0.19	0.03455
1050278	*SRD5A1*	-0.19	0.04293
6840753	*SPTLC2*	-0.18	0.03737
1570064	*KIAA1522*	-0.18	0.03737
6900309	*ARSB*	-0.18	0.04901
3440451	*ADAMTS7*	-0.17	0.04293
2450202	*KIF3C*	-0.17	0.00149
4920382	*VAC14*	-0.16	0.03737
360463	*SRR*	-0.16	0.03737
4180376	*PI4KII*	-0.14	0.04267
1450451	*DOPEY2*	-0.11	0.04293
1580397	*ISCA2*	-0.11	0.02844
2000020	*CAMKK2*	-0.11	0.03737
3170102	*C12ORF49*	-0.11	0.02844
10440	*MARS2*	-0.10	0.03778

Table S5 Primers used for Sanger sequencing

Gene	Direction	Exon	Tissue type	Primer sequence (5' to 3')
IDH1	Forward	4	Frozen	TGTAAAACGACGGCCAGTCCATCACTGCAGTTGTAGGTT
IDH1	Reverse	4	Frozen	CAGGAAACAGCTATGACCCACATACAAGTTGGAAATTTCTGG
IDH1	Forward	4	Paraffin	TGTAAAACGACGGCCAGTCGGTCTTCAGAGAAGCCATT
IDH1	Reverse	4	Paraffin	CAGGAAACAGCTATGACCGCCAACATGACTTACTTGATCC
IDH1	Forward	2	Frozen	TGTAAAACGACGGCCAGTGGGCTGTCTGGCAGGTACTA
IDH1	Reverse	2	Frozen	CAGGAAACAGCTATGACCTGTTGGAATTCGTTGTTGGA
IDH1	Forward	3	Frozen	TGTAAAACGACGGCCAGTACCGCGTGTGAAACATAACA
IDH1	Reverse	3	Frozen	CAGGAAACAGCTATGACCGTTTGCTACACGGAGGGGTA
IDH1	Forward	5	Frozen	TGTAAAACGACGGCCAGTTCCTTACAATTCCTGCTAGGG
IDH1	Reverse	5	Frozen	CAGGAAACAGCTATGACCTTGTGCCTTTATTCATGCCA
IDH1	Forward	6	Frozen	TGTAAAACGACGGCCAGTTGGTGGGTGATTTTAGCCTT
IDH1	Reverse	6	Frozen	CAGGAAACAGCTATGACCTGGTTTTGTTTCACTCCTGCT
IDH1	Forward	7	Frozen	TGTAAAACGACGGCCAGTTGTTTGGGACAAGCAGATGA
IDH1	Reverse	7	Frozen	CAGGAAACAGCTATGACCCAAAACTCCCCTTCCCAAAT
IDH1	Forward	8	Frozen	TGTAAAACGACGGCCAGTTGCTCTTCATGCAGTTGGAC
IDH1	Reverse	8	Frozen	CAGGAAACAGCTATGACCTGCACACAAAACACTGAGCA
IDH1	Forward	9	Frozen	TGTAAAACGACGGCCAGTCCATGCCATGAAAATGTGTT
IDH1	Reverse	9	Frozen	CAGGAAACAGCTATGACCGATGCTCTGAGCCCAGTGAG
IDH1	Forward	10	Frozen	TGTAAAACGACGGCCAGTGGACTTTACCACTACCTGCTACC
IDH1	Reverse	10	Frozen	CAGGAAACAGCTATGACCTGGCCTGAGCTAGTTTGATCT
IDH2	Forward	4	Frozen	TGTAAAACGACGGCCAGTTTGTTGCTTGGGGTTCAAAT
IDH2	Reverse	4	Frozen	CAGGAAACAGCTATGACCCTGCAGAGACAAGAGGATGG
IDH2	Forward	4	Paraffin	TGTAAAACGACGGCCAGTAACATCCACGCCTAGTCC
IDH2	Reverse	4	Paraffin	CAGGAAACAGCTATGACCCAGTGGATCCCCTCTCCAC
IDH2	Forward	1	Frozen	TGTAAAACGACGGCCAGTCTCGTTCGCTCTCCAGCTT

Table S5 (Continue)

Gene	Direction	Exon	Tissue type	Primer sequence (5' to 3')
IDH2	Reverse	1	Frozen	CAGGAAACAGCTATGACCGCCACCGTCCCTCAAGTC
IDH2	Forward	2	Frozen	TGTAAAACGACGGCCAGTATGATGCGCTGTGTGTCC
IDH2	Reverse	2	Frozen	CAGGAAACAGCTATGACCGGGACAGAACAATCCCTGG
IDH2	Forward	3	Frozen	TGTAAAACGACGGCCAGTGTCCCTGAGTCACTGGGGT
IDH2	Reverse	3	Frozen	CAGGAAACAGCTATGACCCCTGTGACCCTCCCTGG
IDH2	Forward	5	Frozen	TGTAAAACGACGGCCAGTAGCTCCTCGCCTAGCCAT
IDH2	Reverse	5	Frozen	CAGGAAACAGCTATGACCTGAAGAGACAAGCTGGGAGA
IDH2	Forward	6	Frozen	TGTAAAACGACGGCCAGTCCAGGCTAGGGCACCAC
IDH2	Reverse	6	Frozen	CAGGAAACAGCTATGACCGGGAAGAAAGGCCACAGAGT
IDH2	Forward	7	Frozen	TGTAAAACGACGGCCAGTCCTCTCCCCATAACAGACCTT
IDH2	Reverse	7	Frozen	CAGGAAACAGCTATGACCAGAAGACCAACAGTCCACCC
IDH2	Forward	8	Frozen	TGTAAAACGACGGCCAGTAGGCCCTGAGAGAAAGGCT
IDH2	Reverse	8	Frozen	CAGGAAACAGCTATGACCGGTAGAGGGGCATTGTGAGG
IDH2	Forward	9	Frozen	TGTAAAACGACGGCCAGTGCTCTTGATCTCCCTGCAAC
IDH2	Reverse	9	Frozen	CAGGAAACAGCTATGACCGGACCCAGAGCCTGTCCT
IDH2	Forward	10	Frozen	TGTAAAACGACGGCCAGTGCACAGATGGGGTCTCATTC
IDH2	Reverse	10	Frozen	CAGGAAACAGCTATGACCAGGGTCTGCCTACCACCC
PTH1R	Forward	4	Frozen	CCTGTCTGCCGGAATGG
PTH1R	Reverse	4	Frozen	TGATTGAAGTCATAAATGTAGTCCG
PTH1R	Forward	5	Frozen	TTGGAGCTAGGGGTTCAGTG
PTH1R	Reverse	5	Frozen	GTAGTTGGCCCACGTCCTGT
PTH1R	Forward	9	Frozen	ATCCACATGCACCTGTTCCT
PTH1R	Reverse	9	Frozen	GGCAGAGGGGTACTCACGTA
GNAS	Forward	8	Frozen	TGTAAAACGACGGCCAGTTCGGTTGGCTTTGGTGAGATCCAT
GNAS	Reverse	8	Frozen	CAGGAAACAGCTATGACCTGACTTTGTCCACCTGGAACTTGGT

Table S6 Probes used in hydrolysis probe assays for *IDH1*

Name	Direction	Sequence	Dye	Remark
R132C *IDH1*	Forward	CTTGTGAGTGGATGGGTAAAACCTA	-	-
R132H *IDH1*	Forward	CTTGTGAGTGGATGGGTAAAACCTA	-	-
R132C *IDH1*	Reverse	CACATTATTGCCAACATGACTTACTTGAT	-	-
R132H *IDH1*	Reverse	CCAACATGACTTACTTGATCCCCATA	-	-
R132C *IDH1*_V	-	AAGCATGACGACCTATG	VIC	Reporter 1
R132H *IDH1*_V	-	CATCATAGGTCGTCATGC	VIC	Reporter 1
R132C *IDH1*_M	-	AAGCATGACAACCTATG	FAM	Reporter 2
R132H *IDH1*_M	-	ATCATAGGTCATCATGC	FAM	Reporter 2