Published in final edited form as:
Nat Genet. ; 43(12): 1256–1261. doi:10.1038/ng.1004.

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

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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 *IDH1* (R132C and R132H) or *IDH2* (R172S) mutations in 87% of enchondromas, benign cartilage tumors, and in 70% of spindle cell

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Authors' contribution: The study was designed, written and reviewed by TCP and JVMGB. Mutation analysis was designed and performed by TCP, MAJHvR, JVMGB, KS, TvW and RvE. Immunohistochemistry was conducted and evaluated by TCP, MAJHvR and JVMGB. TCP, SV, JGvO and DM contributed tissue microarrays. Expression profiling was designed and performed by AMCJ, TCP, JVMGB, JO and analyzed by JO and MK. Methylation profiling was designed by AMCJ, JVMGB and LS, performed by PdA, and PdA and PF analyzed results. KHN, SD, LS, BK, BL, MS, RS, NL, LK, CG, PP, MV, LMB, KCK each contributed frozen or paraffin embedded tissues for multiple patients with Ollier disease or Maffucci syndrome and acquired patient data. The manuscript was approved by all coauthors.

Competing Financial Interests: Authors have no competing financial interests.

hemangiomas, benign vascular lesions. In total, 35 of 43 (81%) patients with Ollier disease and 10 of 13 (77%) patients with Maffucci syndrome carried *IDH1* (98%) or *IDH2* (2%) mutations in their tumors. Fourteen of sixteen patients displayed identical mutations in separate lesions. Immunohistochemistry for mutant R132H *IDH1* protein suggested intraneoplastic and somatic mosaicism. *IDH1* mutations in cartilage tumors are associated with hypermethylation and downregulation of expression of several genes. Mutations were also found in 40% of solitary central cartilaginous tumors and in four chondrosarcoma cell lines, enabling functional studies to assess the role of *IDH1* and *IDH2* mutations in tumor formation.

Enchondroma is a benign cartilage forming tumor within the medullary cavity of the bone [1-3]. Patients with enchondromatosis syndrome, which encompasses seven major subtypes, develop multiple enchondromas. Most common are non-hereditary Ollier disease (subtype I) and Maffucci syndrome (subtype II), the latter distinguished by spindle cell hemangioma in addition to multiple enchondromas [1, 3]. Malignant transformation of enchondromas towards chondrosarcomas occurs in >30% of the patients [3, 4].

Genome-wide screens have not identified a causative gene [5-9]. These patients 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, 15, 16] and *GNAS* activating mutations in juvenile granulosa cell tumors [17] have been reported. Interestingly, *IDH1* and *IDH2* mutations were recently reported in solitary central and periosteal enchondromas and chondrosarcomas, including few tumors from patients with enchondromatosis [18]. The possibility of *GNAS* mutations in enchondromas and chondrosarcomas has not been explored.

We therefore assessed whether *IDH1*, *IDH2,* or *GNAS* mutations may cause enchondroma and spindle cell hemangioma formation in Ollier disease and Maffucci syndrome. Sequence analysis of hotspot positions using lesional tissue from 43 patients with Ollier disease revealed that heterozygous R132C *IDH1* (c.394C>T), R132H *IDH1* (c.395G>A) or R172S *IDH2* (c.516G>C) (Human Genome Variation Society) mutations were present in 33 patients (78%) (Supplementary Fig.1a-c). In Maffucci syndrome, 7 out of 13 patients (54%) carried R132C *IDH1* mutations. Mutations were absent in DNA from patients' blood, muscle or saliva (Supplementary Fig.1b). Mutations in *GNAS* were absent.

An additional 8 tumors demonstrated sub-threshold peaks at the position where R132C or R132H *IDH1* mutations can be expected, suggesting that the mutant allele might be present in a small subpopulation of the tumor cells at the limits or below the detection level of Sanger sequencing. We therefore performed a hydrolysis probes assay, capable of detecting as low as 1% of mutant allele, for the detection of R132C and R132H *IDH1* mutations [19, 20]. Mutations were confirmed in 7 of 8 tumors (Supplementary Fig.1d-g), while from 1 tumor DNA was no longer available. Thus, in total 35 out of 43 (81%) and 10 of 13 (77%) patients with Ollier disease and Maffucci syndrome, respectively, showed *IDH1* or *IDH2* mutations (Fig.1a, Table 1, and Supplementary Table 1). Frequency of mutations in tumors is shown in Fig.1b.

Other subtypes of enchondromatosis syndrome are known to be caused by mutations in *PTPN11* (metachondromatosis) [21, 22], *ACP5* (spondyloenchondrodysplasia) [23, 24] and *PTHLH* duplication (symmetrical enchondromatosis) [25]. Mutations in *PTH1R*, involved in enchondral bone formation, are found in ~ 8% of patients with Ollier disease, but not in patients with Maffucci syndrome [5-7]. Previously, our patients were reported negative for *PTPN11* mutations [22]. Here we did not detect *PTH1R* mutations in a screen of 35 patients. A custom-made Agilent tiling array (Supplementary Table 2) analysis did not find evidence of losses or gains of *IDH1*, *IDH2, PTHLH, PTPN11, PTH1R, EXT1, EXT2* and *ACP5*. Thus, even though patients with enchondromatosis syndromes demonstrate overlapping clinical

features, they appear to be genetically discrete entities, with the exception of Ollier disease and Maffucci syndrome, which we have now shown to contain *IDH1* or *IDH2* mutations.

Since these disorders are not inherited and the enchondromas are often unilateral, we further hypothesized that mutations may occur in a somatic mosaic fashion. Fourteen of sixteen patients (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 R132H IDH1 mutant protein. Of 68 tumors from patients with Ollier disease, 17 tumors (25%) showed mutant protein expression while 51 (75%) tumors were negative (Table 2, Fig.2). We observed a mixture of cells without (wild-type) and with expression of R132H IDH1 mutant protein (of the same histologic type, i.e., not including entrapped elements and supporting elements), which we refer to as intraneoplastic mosaicism (Fig.2a and b). The percentage of positive tumor cells ranged from 50% to 95%. Intraneoplastic mosaicism is also described for other benign bone tumors. In fibrous dysplasia, experimental evidence showed that both normal and *GNAS* mutated cells were needed to develop fibrous dysplasia-like lesions [26]. Also, in osteochondromas, benign cartilaginous tumors arising at the surface of the bone that are caused by mutations in *EXT1* or *EXT2*, a mixture of *EXT* wild-type and *EXT* mutated cells was observed [27-30]. EXT is involved in heparan sulphate biosynthesis, and it is hypothesized that *EXT* mutated cells that are deficient in heparan sulphate, need heparan sulphate from neighboring cells for cellular signaling and survival [31, 32].

We additionally studied the surrounding normal tissue of Ollier related and solitary mutated tumors and surprisingly, a very low frequency (on average <1%) of mutant protein was observed in osteoblasts, osteocytes, adipocytes and fibroblasts (Fig. 2d and e). Hydrolysis probes assay could be performed on DNA isolated from one normal bone of patient with Ollier disease, which was negative. Mutant R132H IDH1 protein was absent in 12 bones resected for reasons other than chondrosarcoma, normal growth plates and articular cartilage (Table 2). Therefore, our current data support somatic mosaicism, similar to somatic mosaic *GNAS* mutations causing polyostotic fibrous dysplasia [33, 34]. Unfortunately, the nature of the material (decalcified paraffin-embedded bone tissue) and the occurrence of the mutation in single scattered cells do not allow verification using other techniques. However, the R132H IDH1 antibody was shown to be highly reliable in 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 was not surprising because only R132 *IDH1* and R172 *IDH2* mutations have been identified in other *IDH*-associated tumors. It is possible that because of intralesional mosaicism, only small sub-fraction of tumor cells contain the *IDH1* or *IDH2* hotspot mutations, which may be below the detection level of the techniques used. Alternatively, mutations in other genes such as *TET2,* which is mutually exclusive with *IDH1* or *IDH2* mutations in cases of acute myeloid leukemia [36], might be involved [18, 37].

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 periosteal chondrosarcomas displayed *IDH1* or *IDH2* mutations (Fig.1b, Table 1). In 6 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 (Fig.1b) and in patients' blood. Immunohistochemistry for the R132H IDH1 mutant protein on tissue microarrays (TMA) containing cartilaginous and vascular tumors confirmed that mutant protein

expression was restricted to central, dedifferentiated and periosteal cartilage tumors, while all other tumors were negative (Table 2). Interestingly, four of eight solitary chondrosarcoma cell lines carry different types of mutations in *IDH1* or *IDH2* (Table 3). To the best of our knowledge, no cell lines with *IDH1* or *IDH2* mutations are currently available. *IDH1* or *IDH2* mutations were more frequently found in solitary central tumors located in hands and feet (11 out of 14) versus those located in long and flat bones (28 out of 84) (p=0.006, Pearson Chi-Square test), which was also reported previously [18]. This correlation was absent in Ollier disease (20 out of 22 versus 28 out of 34, p=0.5, Pearson Chi-Square test). While 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).

IDH1 or *IDH2* mutations have also been reported at lower frequencies in various other tumors such as acute myeloid leukemia (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 they are early events suggest a causal rather than a bystander role for *IDH1* or *IDH2* mutations in tumorigenesis in Ollier disease and Maffucci syndrome. In gliomas, mutant *IDH1* or *IDH2* leads to gain of function by producing 2-hydroxyglutarate (2HG), a structural analogue of α-KG, and by ultimately reducing α-KG production [44]. In AML, it was demonstrated that mutant IDH protein results in DNA hypermethylation and impairment of hematopoietic differentiation [36], and in gliomas the presence of an *IDH1* mutation is strongly associated with hypermethylation [45]. Therefore, we used Illumina HumanMethylation27 BeadChip (Illumina Inc., CA) to assess a possible difference in methylation between enchondromas with (n = 8) and without (n = 4) *IDH1* mutations detectable at Sanger sequencing. Unsupervised clustering based on the 2000 most variable CpG methylation sites resulted in 2 subgroups (Fig.3). One of these subgroups showed an overall higher methylation at the examined CpG sites and is therefore similar to the CpG island methylator phenotype (CIMP) as described in colon carcinoma and glioblastoma [45, 46]. All but one enchondroma with an *IDH1* mutation were CIMP+. Supervised clustering analysis indicated that 797 CpG sites are differentially methylated by more than 20% (at $p<0.05$) between enchondromas with and without *IDH1* mutations. Interestingly 710 (89.1%) of these differentially methylated CpG sites 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 the CIMP phenotype [36].

To assess the effect of *IDH1* or *IDH2* mutations on mRNA expression levels in cartilaginous tumors, we performed whole-genome gene expression analysis using Illumina Human-6 v3 array (Illumina Inc., CA). High quality mRNA was available for only three tumors in which mutation was negative (n=1) or below the threshold of Sanger sequencing (thus possibly carrying a low percentage of mutated cells)(n=2). Comparison with 18 tumors with clearly detectable *IDH1* or *IDH2* mutations using *LIMMA* analysis showed 36 differentially expressed probes encoding for 33 genes (Supplementary Table 4). 32 of 33 genes were down regulated in tumor samples with an *IDH1* or *IDH2* mutation. There was no overlap between the affected genes found in methylation and expression analysis.

One of the most differentially methylated genes was *DLX5*. There was a trend for downregulation of *DLX5* but this was not significant in Ollier enchondromas versus controls (adj. p-value = 0.3, Supplementary Fig.2). The controls consisted of 2 growth plates and 4 articular/rib cartilage samples. The homeodomain transcription factor *DLX5* is a cell autonomous positive regulator of chondrocyte maturation during endochondral ossification, promoting the conversion of immature proliferating chondrocytes into hypertrophic chondrocytes [47, 48] *DLX5* also induces expression of Runx2 and osterix, promoting

osteogenic differentiation [49, 50]. Future studies should reveal whether down regulation 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 *IDH1* or, rarely, *IDH2* point mutations in tumor tissues of 81% of patients with Ollier disease and 77% of patients with Maffucci syndrome, and provide evidence for intraneoplastic and somatic mosaicism. Future studies using deep sequencing approaches should reveal whether the percentage of patients carrying somatic mosaic *IDH1* or *IDH2* mutations 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 should demonstrate 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, providing good *in vitro* models for functional studies to dissect the role of *IDH1* and *IDH2* in Ollier disease and Maffucci syndrome, but also in tumorigenesis in general.

Data Deposition

MIAME-compliant data of tiling arrays, expression arrays and methylation arrays have been deposited in the GEO database (www.ncbi.nlm.nih.gov/geo/, accession number GSE30844).

Materials and Methods

Patients and Clinical Specimens

Fresh frozen tumor tissues (n = 60) of 44 patients with multiple cartilage tumors (36 patients with Ollier disease and 8 patients with Maffucci syndrome) (Table 1, Supplementary Table 1) were collected from EuroBoNet consortium (http://www.eurobonet.eu) [8] and the Laboratory of Human Molecular Genetics at the de Duve Institute, UCL (Brussels, Belgium). In addition, paraffin embedded tumor tissues (n = 15) from 12 patients were obtained from the files of the Children's Hospital (Boston, USA). Samples were handled according to the ethical guidelines of the host institution. All samples were coded and the ethical guidelines “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 according to Evans et al [56]. Normal DNA derived from saliva, blood or muscle was available from 12 patients with Ollier disease. Patients' ages were documented at the time of operation. Demographic and survival data were obtained from the host institutions' patient records.

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), 37 peripheral cartilaginous tumors [solitary osteochondroma (n = 11), peripheral chondrosarcomas (n=20), multiple osteochondromas (n=6)], as well as 9 chondromyxoid fibromas, 7 chondroblastomas, and 2 osteochondroma-like lesions of metachondromatosis. Matching blood-derived DNA was also available from 24 cases as controls. Additionally, we included DNA from angiosarcomas (n = 14) since patients 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 patients.

DNA extraction and Mutation Analysis

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

PCR amplification was performed on *IDH1* exon 4 for all the samples. *IDH2* exon 4 was amplified in samples without *IDH1* mutation and *GNAS* exon 8 was studied in samples without *IDH1* and *IDH2* mutations. To correlate with possible *PTH1R* mutations we also amplified *PTH1R* exon 4 for G121E and A122T, exon 5 for R150C and exon 9 for R255H using DNA from 35 patients with Ollier disease and Maffucci syndrome. PCR was performed in 25μl reactions using 10ng DNA, 12.5 μl of iQ SYBR green Supermix (Bio-Rad, CA) and 10 pmol M13 tailed primers (Supplementary Table 5). The PCR was carried out in a CFX 96 ™ Real-Time PCR detection system (Bio-Rad, CA) at an initial denaturation step of 5 min 95°C followed by 40 cycles of 10 sec 95 °C, 10 sec 60 °C and 10 sec 72 °C. After a final elongation step of 10 min at 72 °C, a melt curve was obtained to check for the quality of the PCR products. PCR products were purified using the Qiagen MinElute ™ 96 UF PCR Purification kit (Qiagen) system and finally eluted in 25μl sterile water. PCR amplimers were sequenced by a commercial party using standard forward and reverse M13 primers (Macrogen Inc. Europe, Amsterdam). The sequence trace files were analyzed with Mutation Surveyor™ DNA variant software (version 3.97 SoftGenetics, PA).

To validate the R132C and R132H *IDH1* mutations, we designed hydrolysis probes (Supplementary Table 6) assays using the Custom Taqman® Assay Design Tool (Applied Biosystems, Nieuwerkerk a/d Ijssel, NL). Assays were performed on 144 samples including tumors related to Ollier disease, Maffucci syndrome, solitary cartilaginous tumors, chondrosarcoma cell-lines, blood from Ollier patients as well as negative controls (healthy donor DNA) together with no template controls. qPCR was performed in 10 μL reactions as described earlier [57] in a CFX384™ Real-Time PCR Detection System (Bio-Rad, Veenendaal, NL) for 10 minutes at 95 °C and 40 cycles of 10 seconds at 92 °C and 30 seconds 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 allele R132C *IDH1* (c.394C>T) or R132H *IDH1* (c.395G>A) was set after subtracting the highest background signal from the negative controls.

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

Tiling Resolution Targeted Oligonucleotide Arrays

Custom designed Agilent tiling oligonucleotide array CGH (Agilent, Amstelveen, The Netherlands) containing 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 of patients with Ollier disease and Maffucci syndrome, with (n=14) and without (n=2) *IDH1* or *IDH2* mutations were selected. Labeling and hybridization of genomic DNA from freshly frozen tumor and data processing were performed as described earlier [58].

Immunohistochemistry

To determine the protein expression of the R132H IDH1 mutant allele, immunohistochemistry was performed as described earlier [8] using R132H IDH1 antibody (1:200 dilution 5% in non-fat milk, citrate antigen retrieval, blocking for 30′ with 5% non-fat milk) from Dianova (Hamburg, Germany). We used 403 tumors (Table 2) on 19 tissue microarrays (TMA), for which details were published previously [8, 59-61]. Additional cases from Ollier disease and Maffucci syndrome were collected through EMSOS, and clinical details for these patients are described separately [4]. 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 tumor and in surrounding normal tissues, we selected resection specimens from tumors expressing R132H IDH1 mutant protein (n = 7) and stained multiple tissue blocks from different areas. All except 9 tumors of patients with Ollier disease that were used for mutation analysis were also included in the TMA, and results were confirmed.

Statistical Analysis for Clinical Correlation

From 83 patients with solitary tumors, follow up data were available (range 2 to 335 months, mean 115.23). To investigate the relation of *IDH1* or *IDH2* mutations with patients' clinical features, multivariate survival analysis (Cox Regression) and cross-tabulations (Pearson Chi-Square) were performed using SPSS version 16.0 (Chicago, Illinois, USA). Statistical analysis was not performed for patients with Ollier disease because nearly all patients with available follow up data had *IDH1* or *IDH2* mutations. All the p-values reported are two-sided and p-values < 0.05 were considered to indicate statistical significance.

DNA Methylation Profiling

Total 12 samples which includes 8 enchondromas with *IDH1* mutation (4 Ollier enchondromas, 2 Maffucci enchondromas and 2 solitary enchondromas) and 4 enchondromas (1 Ollier enchondroma, 3 solitary enchondromas) without *IDH1* or *IDH2* mutations were used. Of these 4 enchondromas without *IDH1* mutation, one had R132G *IDH1* mutated cells present in the subpopulation, below the threshold level of Sanger sequencing. Bisulfite treatment was performed using EZ DNA Methylation™ Kit (Zymo Research, Orange, CA). Bisulfite converted DNA was then hybridized to Illumina HumanMethylation27 BeadChip (Illumina Inc., San Diego, CA) by following manufacturer's instructions. Infinium Unsupervised clustering analysis was performed using the Ward's clustering algorithm based on Euclidian distance. The 2000 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, 4 grade II) of Ollier disease and Maffucci syndrome as well as 1 solitary enchondroma and 4 solitary chondrosarcomas grade II and 6 controls (2 growth plates, 4 normal cartilage) were used. We determined differential expression between tumors with *IDH1* or *IDH2* mutations (n = 18) versus tumors without detectable *IDH1*or *IDH2* mutation using Sanger sequencing (n=3). Two of these demonstrated subthreshold peaks for R132G and R132C *IDH1* mutation suggesting a mutation in a minor subpopulation of tumor cells. Experimental procedures using the 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 (adj*P*) < 0.05 and a log fold change (logFC) > 0.1 were considered to be significantly differentially expressed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to all of the patients and their families for their participation. The study is financially supported by The Netherlands Organization for Scientific Research (917-76-315 to JVMGB and TCP), the Liddy Shriver Sarcoma Initiative (to JVMGB and JO), the Interuniversity Attraction Poles initiated by the Belgian Federal Science Policy, network 6/05; the National Institute of Health, Program Project P01 AR048564; and the F.R.S.-FNRS (Fonds de la Recherche Scientifique)(all to MV), the Manton Center for Orphan Disease Research at Children's Hospital Boston (to KK) and is performed within EuroBoNet, a European Commission granted Network of Excellence for studying the pathology and genetics of bone tumors (018814). 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, D. van der Geest and T. Krenács for constructing TMAs, 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 (Rizzoli Institute, Italy) for kindly providing peripheral dedifferentiated chondrosarcomas and vascular tumors, T. Kalinski (Otto-von-Guericke-University, Magdeburg, Germany) for C3842, M. Namba (Okayama University Medical School, Shikata, Japan) for OUMS27, Dr. T. Ariizumi (Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan) for NDCS1, and A. Llombart Bosch for CH2879 cell lines. Drs. J. Mulliken, J. Upton, and S. Fishman of the Vascular Anomalies Center at Children's Hospital Boston kindly provided spindle cell hemangiomas. S. Verdegaal, A.H.M. Taminiau and M.A.J. van de Sande are acknowledged for contributing patient data. We are thankful to S. Boeuf (Heidelberg University, Germany) R. Forsyth (Ghent University, Belgium) P. Mainil-Varlet (Bern University, Switzerland) W. Wuyts (University of Antwerp, Belgium) for providing a single Ollier or Maffucci case, or control samples. The continuous support of the Netherlands Committee on Bone Tumors is highly acknowledged.

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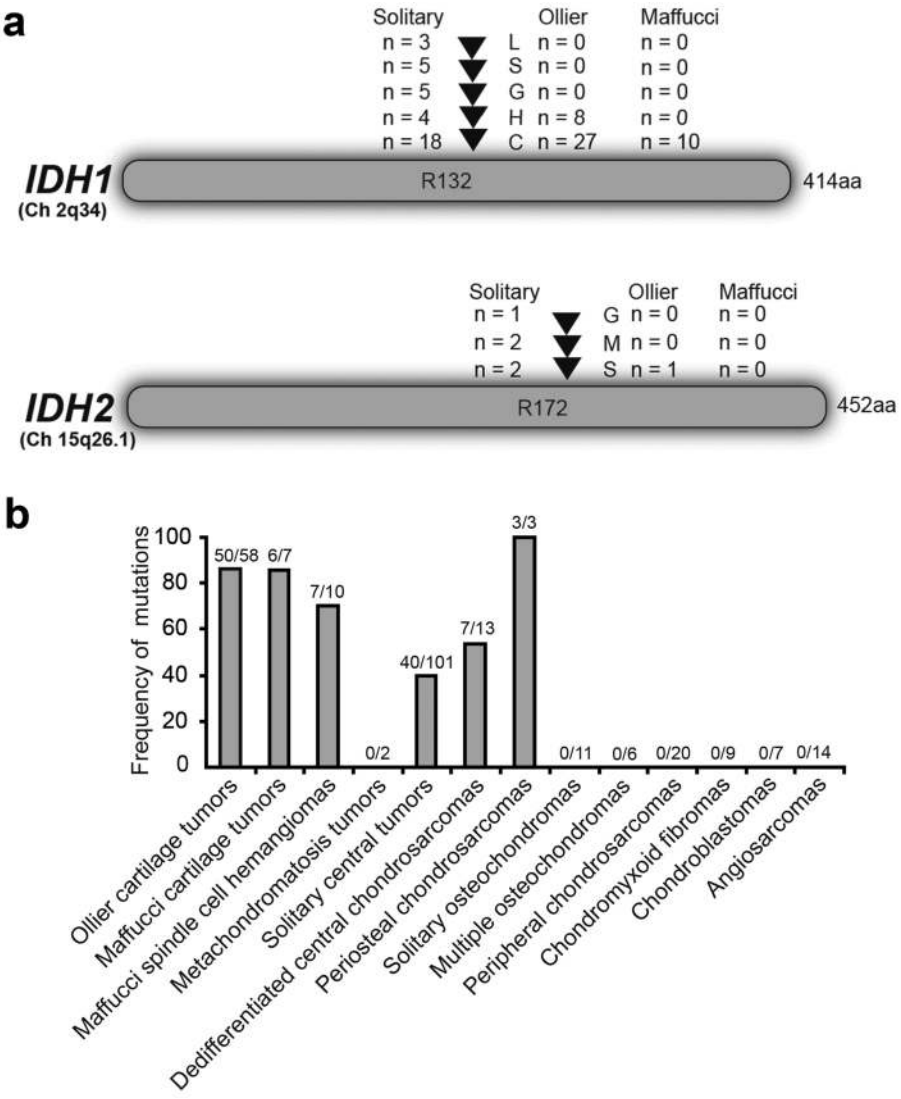

Figure 1. Frequency of *IDH1* and *IDH2* mutations

a) Distribution of the different R132 *IDH1* and R172 *IDH2* mutations among the patients with Ollier disease, Maffucci syndrome and solitary tumors. b) Frequency of somatic heterozygous *IDH* (*IDH1* and *IDH2*) mutations in tumors of patients with Ollier disease and Maffucci syndrome, in comparison with different subtypes of solitary cartilaginous tumors and angiosarcomas.

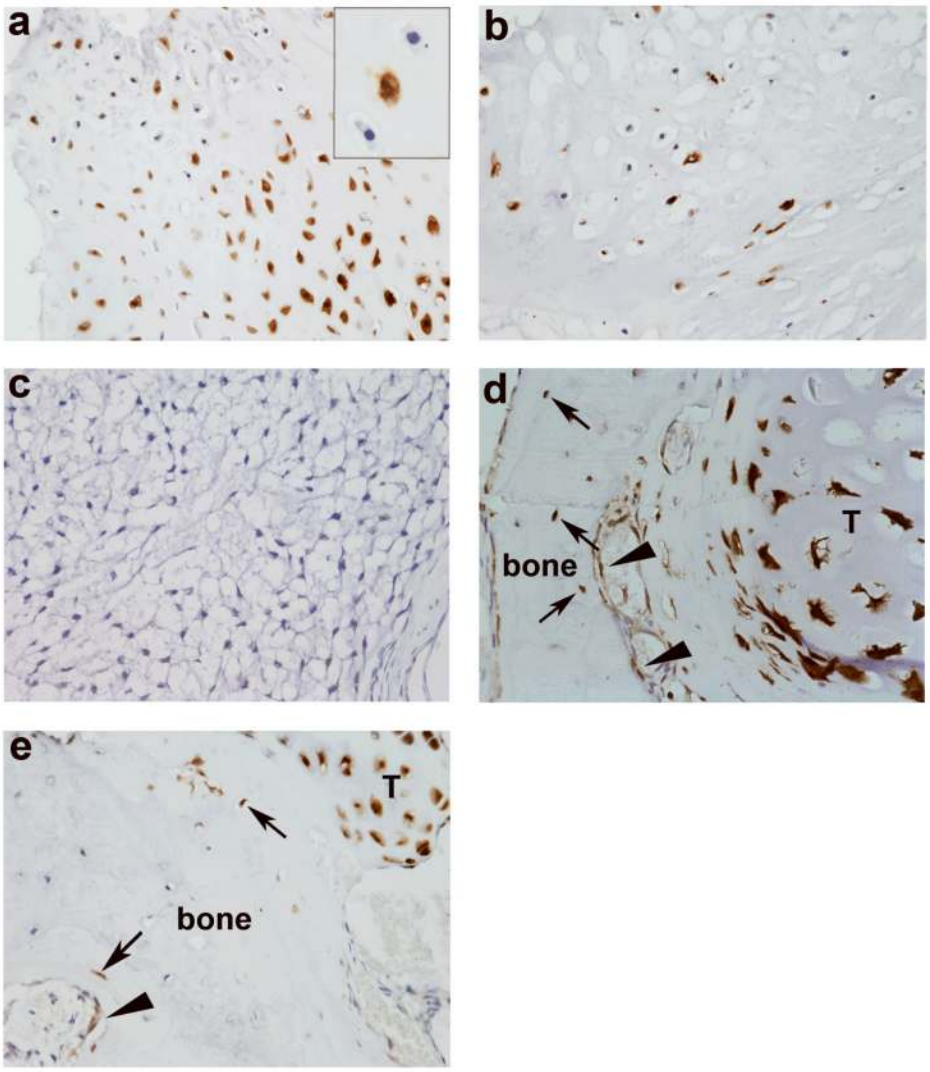

Figure 2. Immunohistochemistry for R132H IDH1 mutant protein

a,b) Enchondroma (L1490) of patient with Ollier disease demonstrating strong cytoplasmic and nuclear staining of R132H IDH1 mutant protein. Note the mixture of wild-type and mutated cells indicating intraneoplastic mosaicism. Overall the percentage of positive tumor cells ranged from 50% to 95%. Insets show vitality of the negative cells at higher magnification. c) Grade II chondrosarcoma negative for R132H IDH1 mutant protein. d and e) Enchondromas from patients with Ollier disease demonstrating occasional positive cells in the surrounding normal bone. Some positive osteocytes (arrows) and osteoblasts (arrowheads) are seen. T: tumor tissue. (Magnification 400×)

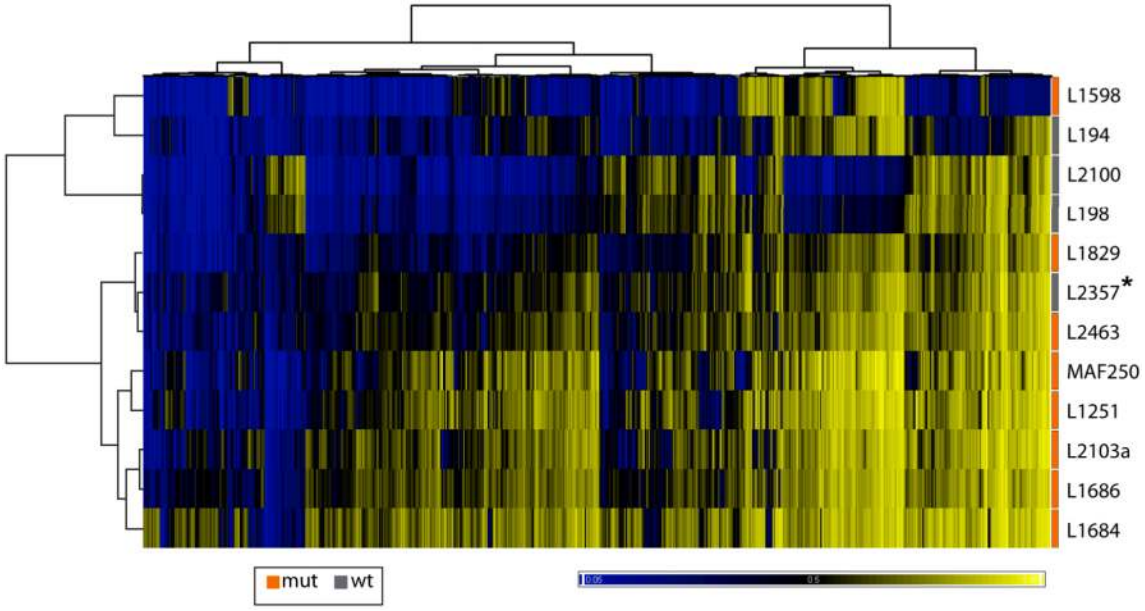

Figure 3. CpG island Methylator Phenotype in enchondromas with *IDH1* mutations

Heatmap depicting unsupervised clustering analysis based on the 2000 most variable CpG sites of enchondromas with *IDH1* mutations (orange, n = 8) and without *IDH1* mutation (gray, n=4). The level of DNA methylation (beta value) for each probe (columns) in each sample (rows) is represented by color scale as shown in the picture ranging from 0 (0% methylation, blue) to 1 (100% methylation, yellow). Asterisk indicates sample L2357 in which the R132G *IDH1* mutant allele was detected in a subpopulation of cells. However, the mutation escaped detection at Sanger sequencing, and therefore the sample is labeled "wild-type".

Nat Genet. Author manuscript; available in PMC 2012 August 27.

Table 1

Results of *IDH1* and *IDH2* mutation analysis

	Total	Gender (M:F) (median age, years)	*IDH1* mutation (%)	R132C *IDH1* (CGT>TGT)	R132H *IDH1* (CGT>CAT)	*IDH2* mutation (%)	Total *IDH1*+*IDH2* mutation
Ollier Disease							
Number of patients	43	21:21 *(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 patients	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	
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 ** (35%)	2 (29%)	2 (29%)	0	7 (35%)
Central chondrosarcoma grade II	57		18 **(32%)	9 (50%)	1 (6%)	3 (5%)	21 (37%)
Central chondrosarcoma grade III	15		7 ** (47%)	5 (71%)	0	0	7 (47%)
Dedifferentiated chondrosarcoma	13		6 ** (46%)	3 (50%)	1 (17%)	1 (8%)	7 (54%)
Periosteal chondrosarcoma	3		3 (100%)	3	0	0	3 (100%)

* unknown gender for one patient

** also other types of mutations than R132C or R132H

Table 2
Immunohistochemistry for R132H mutant protein expression

	Total nr of tumors	R132H positive
Ollier Disease		
Enchondroma	46	14/43 *(32%)
Chondrosarcoma grade I	22	3/17 * (18%)
Chondrosarcoma grade II	10	0/8 *
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 * (10%)
Central chondrosarcoma grade II	36	1/32 * (3%)
Central chondrosarcoma grade III	14	0/11 *
Central dedifferentiated chondrosarcoma	26	1/24 * (4%)
Periosteal chondrosarcoma	6	1/6 (17%)
Solitary osteochondroma	20	0/17 *
Multiple osteochondroma	7	0/7
Peripheral chondrosarcoma	45	0/35 *
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

* not all tumors included were evaluable due to tissue loss on tissue microarray

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Table 3

***IDH1* or *IDH2* mutations in solitary central chondrosarcoma cell lines and primary culture**

Cell line	Tumor type	Tumor Grade	Passage	*IDH1*	*IDH2*	Reference
SW1353	Solitary central	CSII	p12	Wt	R172S	ATCC
JJ012	Solitary central	CSII	p15	R132G	Wt	[51]
CH2879	Solitary central	CSIII	p16	G105G	Wt	[52]
OUMS27	Solitary central	CSIII	p18	Wt	Wt	[53]
L835	Solitary central	CSIII	p38	R132C	Wt	Home made
C3842	Ollier disease	CSII	p32	Wt	Wt	[54]
L2975	Dedifferentiated CS		p31	Wt	R172W*	Home made
NDCS1	Dedifferentiated CS		p12	Wt	Wt	[55]

* L2975 showed R172W *IDH2* homozygous mutation.

CS : chondrosarcoma