



Universiteit  
Leiden  
The Netherlands

## Genetics of Ollier disease and Maffucci syndrome

Pansuriya, T.C.

### Citation

Pansuriya, T. C. (2012, March 15). *Genetics of Ollier disease and Maffucci syndrome*. Retrieved from <https://hdl.handle.net/1887/18591>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/18591>

**Note:** To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/18591> holds various files of this Leiden University dissertation.

**Author:** Pansuriya, Twinkal C.

**Title:** Genetics of Ollier disease and Maffucci syndrome

**Issue Date:** 2012-03-15

# Chapter 1

General  
Introduction



Genetic disorders of the skeleton known as skeletal dysplasia shows diverse manifestations (1). Skeletal dysplasia is a group of skeletal disorders that result from disturbances in the complex processes of skeletal development and growth which constitute a diagnostic challenge due to the rarity of these diseases (2). Mutations are reported either in regulators of skeletal organogenesis, such as cellular signaling (growth factors and its receptors), or in matrix components that affect cartilage and bone tissues (3). There are two main classes of skeletal dysplasia: osteochondrodysplasia and dysostosis. Osteochondrodysplasia develops due to the abnormal growth and development of bone and/or cartilage. In contrast, dysostosis is a developmental anomaly resulting from localized problems in the migration of mesenchymal cells and their condensation (3). The Nosology and Classification of Genetic Skeletal Disorders provides an overview of different diagnostic entities based on clinical and radiographic features and molecular pathogenesis (2). In 2010, in total 456 conditions were described which were divided into 40 different groups. Presently, for 316 of these 456 conditions, the underlying genetic defects are known (2).

Within this classification (2), enchondromatosis syndrome is recognized as a form of osteochondrodysplasia. Patients with enchondromatosis syndrome have multiple enchondromas (benign cartilage forming tumors in the medulla of bone) in their skeleton. The focus of our study concerns the two main subtypes of enchondromatosis syndrome known as Ollier disease and Maffucci syndrome. In addition to multiple enchondromas present in Ollier disease, soft tissue hemangiomas are present in Maffucci syndrome.

#### **Ollier Disease**

Ollier disease (OMIM 166000) is a rare, non-familial skeletal disorder (4). Ollier disease was first described by Louis Ollier, a French surgeon in 1889. The disorder is characterized by the presence of at least three enchondromas with an asymmetric distribution and extreme clinical variability (size, number, location, age of onset and requirement of surgery) (4-6).

#### **Maffucci Syndrome**

Maffucci syndrome (OMIM 166000) is characterized by presence of multiple enchondromas, resulting in bone deformities, together with soft tissue haemangiomas especially spindle cell hemangiomas or rarely lymphangiomas (4;7;8).

#### **Chondrosarcomas**

Enchondromas in Ollier disease and Maffucci syndrome can undergo malignant transformation towards chondrosarcoma. Chondrosarcomas are defined in the 2002 WHO classification as a "Heterogeneous group of lesions with diverse morphological features and clinical behaviour" (9). Chondrosarcoma is the third most frequent malignant bone tumor, in which the tumor cells deposit a hyaline cartilaginous matrix (9). The incidence of chondrosarcoma is slightly increased in males compared to females (9). The age of onset varies from 30-60 years (9). The pelvis is the most commonly affected site followed by femur, humerus, and ribs. The small bones of hands and feet, spine and craniofacial bones are rarely affected. Symptoms involve pain and swelling. Myxoid changes, calcification or ossification may be present. Radiographically, the development of chondrosarcoma is manifested as a lytic lesion, cortical erosion or destruction, soft tissue extension and irregularity or indistinctness of the surface of tumor (10).

MRI is used to identify soft tissue extension and the extent of tumor while CT scan can be helpful to see calcified matrix. As chondrosarcomas are highly resistant to chemo- and radiotherapy, surgery is the only option to cure the patients so far (11).

### Chondrosarcoma subtypes

Chondrosarcomas can be divided mainly into 5 different subtypes including conventional (80-85%), dedifferentiated (6-10%), periosteal (2%), mesenchymal (2%) and clear cell chondrosarcomas (1%). Periosteal chondrosarcomas (juxtacortical chondrosarcomas) are malignant hyaline cartilage forming tumors located at the surface of the bone, arising from perichondrium and not connected to the original bone (9;12). Dedifferentiated chondrosarcoma contains two clearly distinct components: a well-differentiated cartilage tumor (either an enchondroma or a low grade chondrosarcoma), juxtaposed to a high-grade noncartilaginous sarcoma, with a sharp interface between the two components (13). Conventional chondrosarcomas are most frequent and arise *de novo* (primary) or from a benign precursor (secondary). Conventional chondrosarcomas are divided into two groups based on their anatomical location in the bone: i) secondary peripheral chondrosarcomas (10-15%) and ii) central chondrosarcomas (85-90%). Chondrosarcomas arising in patients with Ollier disease and Maffucci syndrome are classified as the conventional secondary central subtype.

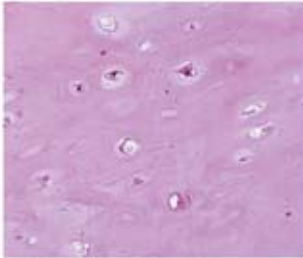
### Conventional Chondrosarcomas

i) Secondary peripheral chondrosarcoma develop within the cartilage cap of a pre-existing osteochondroma. Osteochondroma, a benign tumor, is a cartilage capped bony projection arising at the surface of bone (14). Multiple osteochondromas (MO, also known as hereditary multiple exostoses) is an autosomal dominant skeletal dysplasia caused by mutations in *EXT1* or *EXT2* (15-18) and characterized by the presence of multiple osteochondromas (19). Although the involvement of *EXT1* or *EXT2* inactivation in osteochondroma formation is beyond dispute, *EXT1* and *EXT2* are not involved in the progression towards secondary peripheral chondrosarcoma (20). The most frequent location of secondary peripheral chondrosarcoma is ilium, followed by scapula, tibia, femur, pubic bone and ribs.

ii) Central chondrosarcoma arises *de novo* in the medulla of the bone (primary) or from a preexisting benign enchondroma (secondary). Most of the central chondrosarcomas are believed to arise primary. Clinical signs suggestive of malignancy are presence of pain, cortical erosion and extension of the tumor into soft tissues (6;21). Central chondrosarcomas can be found in almost all parts of the skeleton which are formed by enchondral ossification. The most preferential sites include femur, followed by ribs and ilium. The distinction between enchondroma and low grade chondrosarcoma is difficult at radiological as well as at the histological level (22) and slightly subjected to interobserver variability (23;24). Criteria to distinguish include mucoid matrix degeneration of more than 20% and/or presence of host bone entrapment (24). In Ollier disease and Maffucci syndrome more cellularity and atypia is tolerated, making the histological distinction even more difficult.

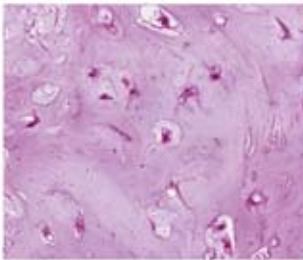
Histologically conventional chondrosarcomas can be divided into three grades (Grade I, II and III) (25) (Figure 1). Grading is so far the most important prognostic predictor for metastasis. The risk of developing metastasis increases with increase in tumor grade. Studies showed that metastases of grade I chondrosarcomas are rare or absent, while 10-33% of grade II and around 70% of grade III chondrosarcomas metastasize (25;26).

**Figure 1 Histology of enchondroma and different grades of chondrosarcomas**



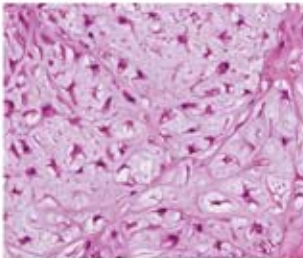
**Enchondroma**

encasement  
low cellularity  
no atypia



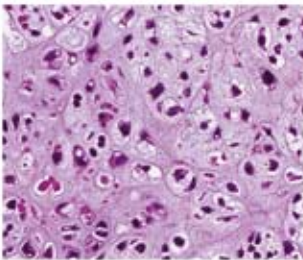
**Grade I chondrosarcoma**

bone entrapment of preexisting host bone  
presence of small, densely staining nuclei  
chondroid or myxoid matrix



**Grade II chondrosarcoma**

nuclei are of moderate size  
increased cellularity  
myxoid matrix  
presence of mitosis



**Grade III chondrosarcoma**

pleomorphic nuclei  
high cellularity  
myxoid matrix  
presence of mitosis  
spindle cell shaped cells at the edge of lobules

### **Approaches to understand complex diseases**

Complex diseases are caused by a combination of genetic and environmental factors. For most of these diseases it is difficult to identify the cause as they do not obey standard Mendelian patterns of inheritance. Genomics is the field of study helping to understand a role of the genome in the development of particular diseases. The Human Genome Project has changed the view of researchers to gain new insights in pathogenesis of diseases. The known role of copy-number alterations in sporadic genomic disorders, combined with emerging information about inherited copy-number variation, indicate the importance of assessing copy-number variants (CNVs), including common copy-number polymorphisms, involved in disease (27). In principle, complex disease can be explained by the presence of particular single nucleotide polymorphisms (SNPs) or CNVs or variation in noncoding sequences associated with diseases. As Ollier disease and Maffucci syndrome are non-inherited disorders, we first started off with a genome-wide analysis to search for candidate regions involved in these disorders. We used high resolution SNP array combined with expression arrays.

#### **Genome-wide approach**

##### **Single Nucleotide Polymorphism (SNP) Arrays**

SNP arrays offer an opportunity to identify copy number changes together with loss of heterozygosity (LOH) events occurring in the tumor, throughout the genome. SNPs are variable positions in the genome with two different allelic types when the frequency of the minor allele exceeds 1% in at least one population (28;29). SNP arrays are an ideal platform to map somatic and germline genetic alterations (30-33). Only SNPs which are heterozygous in germline are informative as an indication of possible LOH. Paired normal DNA is not always available and therefore, an advantage of SNP arrays are that it provide marker densities that enable the identification of LOH regions, without using germline paired DNA. SNP frequency has an uneven distribution throughout the whole genome. Including copy number variation (CNV) probes to a SNP array platform like Affymetrix SNP 6.0 array can compensate for the unevenness of the SNPs. SNPs are much less frequent inside coding areas including exomic regions, therefore the detection rate of genetic changes that encompass only one or a few exons especially for small genes is rather limited. Also, balanced translocations and point mutations can not be detected by SNP arrays.

##### **Gene Expression Array**

Microarrays are used as a routine tool for molecular profiling, identification of new targets and biomarker discoveries in biomedical research (34). Microarray technology has allowed the abundance of thousands of different mRNAs to be measured simultaneously in a given sample using a single hybridization reaction (35;36). Therefore, analysis of individual genes has provided an opportunity to analyze large sets of genes and relationships in their expression (35). Interpretation of the results to gain insight into biological mechanisms is still challenging. There are some critical issues such as correct selection of samples, proper experimental design, sample collection, preparation of targeted RNA, integrity and purity of RNA related to microarrays in order to guarantee the quality and reproducibility of the obtained data (36).

Genomic changes (amplification or deletions) often comprise 10s 100s of genes, therefore integration of copy number changes and expression arrays might be more informative.

Expression changes (up or down regulation) of genes residing in copy number alteration (CNA) regions of the genome might identify genes important in the pathogenesis of tumors.

### **Methylation array**

DNA methylation plays a critical role in regulating gene expression and cellular functions during normal development as well as in carcinogenesis (37). Methylation is largely known as epigenetic modification of DNA (38). Methylation of DNA occurs exclusively in 5-cytosine and in mammals, the majority of cytosine methylation occurs in CpG sites. Non-CpG methylation is rare (39). CpG islands are present in ~70% of human promoters (40). Epigenetic changes (which alter the gene expression) have been recognized as one of the most important molecular signatures of the tumors in recent years. These alterations comprise of hypermethylation of tumor suppressor genes or hypomethylation of oncogenes (38). The exact mechanism of aberrant methylation is still unknown. Methylation profiling helps to understand the nature of gene regulation in cells, and also the epigenetic mechanisms of interactions between cells and environment (37). There are three methods available for DNA methylation profiling which includes i) discrimination of bisulfite induced C to T transition, ii) cleavage of genomic DNA by methylation sensitive restriction enzymes and iii) immunoprecipitation with methyl-binding proteins or antibodies against methylated cytosine (37).

Some of these approaches permit the investigation of the limited number of methylated regions at a time. Whereas, microarray and sequencing based DNA methylation profiling technologies have been developed in order to assess methylation status for a large number of genes or even the entire genome.

### **Hypothesis driven approach**

Based on the literature related to chondrocyte differentiation and enchondral bone formation, one could postulate a number of candidate genes for Ollier disease and/or Maffucci syndrome.

#### **1. NDST1**

*EXT1* or *EXT2* are known to be involved in osteochondroma (14-16). While it is evident that inactivation of *EXT1* or *EXT2* is the driving force for the development of benign peripheral cartilaginous tumors, they are not involved in central chondrosarcoma and expression of these genes was comparable to the growth plate (41). The EXT proteins are glycosyltransferases responsible for the elongation of heparan sulfate (HS) chains (42;43). HS is a large complex carbohydrate that binds various growth factors and enzymes and its assembly involves three steps i) chain initiation ii) chain elongation iii) chain modification.

i) chain initiation occurs when four sugars are attached to specific serine residue of the core proteins. ii) elongation steps involve N-acetyl glucosamine and glucuronic acids which are alternatively added by copolymerases encoded by *EXT1* or *EXT2*. iii) chain modification involves Ndeacetylation/ N-sulfation (*NDST1*), C5-epimerization, 2-O sulfation of uronic acids and 3-O and 6-O sulfation of glucosamine residues. Presto et al. proposed a GAGosome model in which cells over-expressing *NDST1* and *EXT2*, *NDST1* competes with *EXT1* to bind to *EXT2* and will form heteroduplex (44). Binding of more *NDST1* to *EXT2* might alter formation and localization of HS. The role of *EXT1*, *NDST1* and HS is unknown in enchondromas and chondrosarcomas related to Ollier disease and Maffucci syndrome.



## 2. *PTH1R*

Enchondroma might arise as a result of abnormal regulation of pathways involved in chondrocyte proliferation and differentiation. One of the most important signaling pathways is the Indian Hedgehog/Parathyroid Hormone Like Hormone IHH/PTH1R negative feedback loop (45). Prehypertrophic chondrocytes secrete IHH which will bind to its receptor Patched (PTCH) which will result in increased secretion of PTH1R. PTH1R will bind to its receptor PTH1R which will inhibit further differentiation of chondrocytes by up-regulating BCL2, resulting in less IHH producing cells (46). PTH1R and IHH pathways are tightly coupled and therefore reduced PTH1R signaling could lead to impaired chondrocyte proliferation and differentiation.

Previously, a R150C *PTH1R* (3p22 (47)-p21.1) point mutation was reported in two out of six patients with constitutively active IHH signaling (48) but an elaborative study on 28 Ollier patients failed to detect any mutations in *PTH1R* by our group (49). G121E *PTH1R*, A122T *PTH1R* and R255H *PTH1R* mutations were subsequently found in 3 out of 14 Ollier patients (50). Two heterozygous mutations, G121E *PTH1R* and A122T *PTH1R* were present only in enchondroma from an Ollier patient while R255H *PTH1R* was present in tumor as well as in leukocyte DNA. All these mutations were claimed to alter the ligand affinity of the receptor as well as its expression at the cell surface and ultimately impaired its function (50). In total four different heterozygous mutations were reported. Thus, heterozygous *PTH1R* mutations may contribute or act as a modifier in small subset of Ollier patients (48-50).

## 3. *IDH1* and *IDH2*

Gliomas are the most frequent non-cartilaginous tumors associated with Ollier disease (51;52). Also, six patients with Maffucci syndrome having a glioma have been reported in the literature (52-57). Glioma is the most common type of primary brain tumor (58). Heterozygous mutations at the R132 codon of isocitrate dehydrogenase 1 (*IDH1*) and R172 codon of isocitrate dehydrogenase 2 (*IDH2*), mutations were described for up to 70% of gliomas (59). Mutations in *IDH1* and *IDH2* are mutually exclusive with rare exceptions, which suggest mutation in either of these two isoforms is sufficient to confer growth advantage and/or cell survival (60). Mutations in *IDH1* or *IDH2* were also reported for solitary central and periosteal chondrosarcomas as well as for few patients with enchondromatosis syndrome (61).

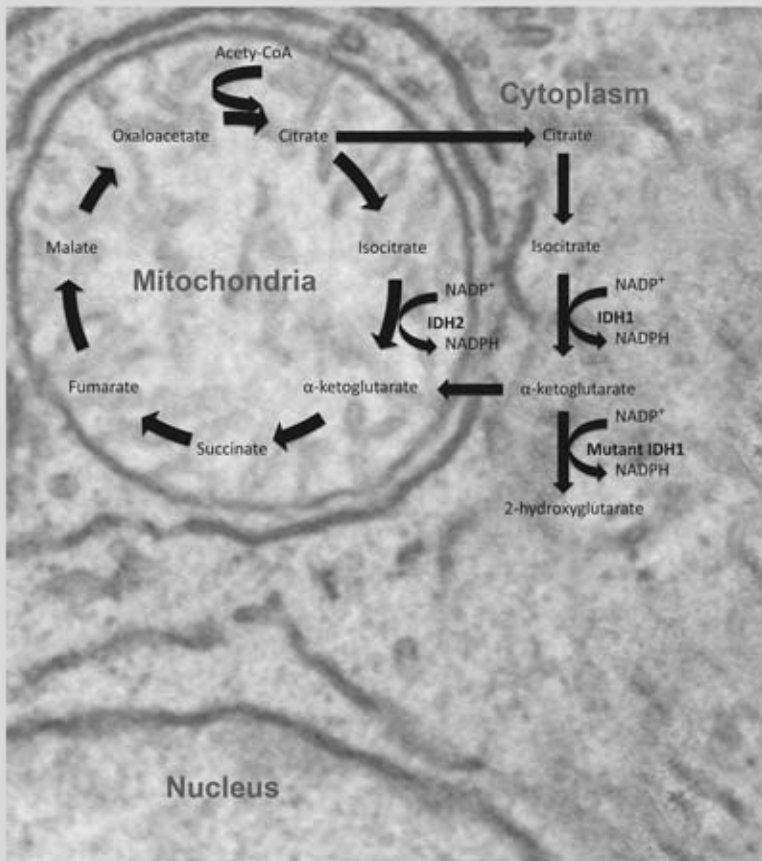
IDH catalyzes the oxidative decarboxylation of isocitrate to  $\alpha$ -ketoglutarate ( $\alpha$ -KG) and reduce NAD(P<sup>+</sup>) to NAD(P)H (Figure 2). *IDH1* is located in the cytoplasm and in the peroxisome while *IDH2* is located in the mitochondria. They are involved in lipid metabolism and in the Krebs cycle (Figure 2). Mutant *IDH1* or *IDH2* leads to gain of function by producing 2-hydroxyglutarate (2HG), a structural analogue of  $\alpha$ -KG (62) and ultimately lead to reduction in  $\alpha$ -KG production (Figure 2). Based on the increased incidence of gliomas and *IDH* mutations in solitary central cartilaginous tumors, we hypothesized that *IDH1* and/or *IDH2* mutations may occur in patients with Ollier disease and/or Maffucci syndrome.

## 4. *GNAS*

Like gliomas, juvenile ovarian granulosa cell tumors show an increased incidence in patients with Ollier disease and Maffucci syndrome (52;53;63;64).

Mutations in *GNAS* at R201 were reported in 30% of juvenile ovarian granulosa cell tumors (65). Apart from this, somatic mosaic *GNAS* mutations are also found in McCune-Albright syndrome, which is a non hereditary disorder characterized by polyostotic fibrous dysplasia combined with endocrinopathies (66). Based on the association of juvenile ovarian granulosa cell tumors with Ollier disease and Maffucci syndrome, we hypothesized that *GNAS* mutations might be present in these patients.

**Figure 2 Role of IDH1 and IDH2**



Enzymes of TCA cycle in the mitochondria and mutated IDH in the cytosol are represented. Mutant IDH1 produces 2HG and reduces the amount of  $\alpha$ -KG.

### **Aim of the investigation and outline of the thesis**

The main purpose of the studies described in this thesis is to find the genetic deficit in Ollier disease and Maffucci syndrome and understand their functional consequences. As Ollier disease and Maffucci syndrome are very rare, non-inherited syndromes with a unilateral predominance of the multiple enchondromas, we hypothesized the presence of somatic mosaicism with an early post zygotic mutation resulting in asymmetric involvement of skeletal structures, similar to McCune Albright syndrome caused by somatic mosaic *GNAS* mutations, (67).

We first searched the literature and present a detailed overview of all different subtypes of enchondromatosis syndrome in **Chapter 2**.

In **Chapter 3** and **4**, the Affymetrix Genome-Wide Human SNP Array 6.0 platform was used to identify candidate gene/genes for Ollier disease (**Chapter 3**) and Maffucci syndrome (**Chapter 4**). The arrays contain 1.8 million reporters, including more than 906,600 SNPs and 946,000 probes for the detection of CNV. We compared genotypes between tumor and paired blood or saliva DNA. We have integrated copy number variation results with Illumina genome-wide expression v3 array and selected few candidate genes for enchondroma development.

In **Chapter 5** and **6**, a hypothesis driven approach was used to study the five genes as outlined above. As described earlier in detail, *NDST1* was selected as a candidate gene and other components of heparan sulfate pathway were analyzed in **Chapter 5**. Since a small subset of patients with Ollier disease showed mutations in *PTH1R*, we also performed mutation analysis as described in **Chapter 6**. Based on the increased incidence of gliomas and juvenile granulosa cell tumors in Ollier disease and Maffucci syndrome, and mutation studies on non-syndromal chondrosarcomas, we evaluated the occurrence of *IDH1*, *IDH2* and *GNAS* mutations. In addition we performed epigenetic studies to investigate the mechanism of enchondroma development using Illumina HumanMethylation arrays in Ollier disease and Maffucci syndrome as described in **Chapter 6**. Finally, results are summarized and discussed in **Chapter 7**.

## References

1. Superti-Furga A, Bonafe L, Rimoin DL. Molecular-pathogenetic classification of genetic disorders of the skeleton. *Am J Med Genet* 2001;106(4):282-93.
2. Warman ML, Cormier-Daire V, Hall C, Krakow D, Lachman R, Lemerrer M, et al. Nosology and classification of genetic skeletal disorders: 2010 revision. *Am J Med Genet A* 2011;155A(5):943-68.
3. Cotran RS, Robbins SL. *Pathologic Basis of Disease*. 7 ed. Philadelphia: WB Saunders Company; 2010. Chapter 26:1210.
4. Spranger J, Kemperdieck H, Bakowski H, Opitz JM. Two peculiar types of enchondromatosis. *Pediatr Radiol* 1978;7(4):215-9.
5. World Health Organization Classification of Tumors. *Pathology and Genetics of Tumors of Soft Tissue and Bone*. Lyon: IARC Press; 2002.
6. Silve C, Juppner H. Ollier disease. *Orphanet J Rare Dis* 2006;1:37.
7. Malfucci A. Di un caso encondroma ed angioma multiplo. *Movimento medico-chirurgico, Napoli* 1881;3:399-412; 565-575.
8. Mertens F, Unni KK. Congenital and inherited syndromes associated with bone and soft tissue tumors: Enchondromatosis. In: Fletcher CDM, Unni KK, Mertens F, editors. *World Health Organisation classification of tumors. Pathology and genetics of tumors of soft tissue and bone*. Lyon: IARC Press; 2002. p. 356-7.
9. Bertoni F, Bacchini P, Hogendoorn PCW. Chondrosarcoma. In: Fletcher CDM, Unni KK, Mertens F, editors. *World Health Organisation classification of tumors. Pathology and genetics of tumors of soft tissue and bone*. Lyon: IARC Press; 2002. p. 247-51.
10. Unni KK. Cartilaginous lesions of bone. *J Orthop Sci* 2001;6(5):457-72.
11. Gelderblom H, Hogendoorn PCW, Dijkstra SD, van Rijswijk CS, Krol AD, Taminiau AH, et al. The clinical approach towards chondrosarcoma. *Oncologist* 2008;13(3):320-9.
12. Norman A, Sissons HA. Radiographic hallmarks of peripheral chondrosarcoma. *Radiology* 1984;151(3):589-96.
13. Milchgrub S, Hogendoorn PCW. Dedifferentiated chondrosarcoma. In: Fletcher C.D.M., Unni KK, Mertens F, editors. *World health organization classification of tumors. Pathology and genetics. Tumors of soft tissue and bone*. 2002. p. 252-4.
14. Khurana J, Abdul-Karim F, Bovée JVMG. Osteochondroma. In: Fletcher CDM, Unni KK, Mertens F, editors. *World Health Organization classification of tumors. Pathology and genetics of tumors of soft tissue and bone*. Lyon (France): IARC Press; 2002. p. 234-6.
15. Ahn J, Ludecke H-J, Lindow S, Horton WA, Lee B, Wagner MJ, et al. Cloning of the putative tumor suppressor gene for hereditary multiple exostoses (EXT1). *Nature Genet* 1995;11:137-43.
16. Stickens D, Clines G, Burbee D, Ramos P, Thomas S, Hogue D, et al. The EXT2 multiple exostoses gene defines a family of putative tumor suppressor genes. *Nature Genet* 1996;14:25-32.
17. Bovee JVMG, Cleton-Jansen AM, Taminiau AHM, Hogendoorn PCW. Emerging pathways in the development of chondrosarcoma of bone and implications for targeted treatment. *Lancet Oncology* 2005;6(8):599-607.
18. Bovee JVMG, Hogendoorn PCW, Wunder JS, Alman BA. Cartilage tumors and bone development: molecular pathology and possible therapeutic targets. *Nat Rev Cancer* 2010;10(7):481-8.
19. Wicklund LC, Pauli RM, Johnston D, Hecht JT. Natural history study of hereditary multiple exostoses. *Am J Med Genet* 1995;55:43-6.
20. de Andrea CE, Reijnders CM, Kroon HM, de JD, Hogendoorn PC, Szuhai K, et al. Secondary peripheral chondrosarcoma evolving from osteochondroma as a result of outgrowth of cells with functional EXT. *Oncogene* doi: 10.1038/onc.2011.311
21. Pfeleiderer AG, Thomson P, Milroy CM. View from beneath: pathology in focus. ENT presentation of Ollier's disease. *J Laryngol Otol* 1991;105(2):148-50.
22. Mirra JM, Gold R, Downs J, Eckardt JJ. A new histologic approach to the differentiation of enchondroma and chondrosarcoma of the bones. A clinicopathologic analysis of 51 cases. *Clin Orthop* 1985;214-37.
23. Reliability of Histopathologic and Radiologic Grading of Cartilaginous Neoplasms in Long Bones. *J Bone Joint Surg Am* 2007;89-A(10):2113-23.

24. Eefting D, Schrage YM, Geirnaerd MJ, Le CS, Taminiau AH, Bovee JV, et al. Assessment of interobserver variability and histologic parameters to improve reliability in classification and grading of central cartilaginous tumors. *Am J Surg Pathol* 2009;33(1):50-7.
25. Evans HL, Ayala AG, Romsdahl MM. Prognostic factors in chondrosarcoma of bone. A clinicopathologic analysis with emphasis on histologic grading. *Cancer* 1977;40:818-31.
26. Björnsson J, McLeod RA, Unni KK, Ilstrup DM, Pritchard DJ. Primary chondrosarcoma of long bones and limb girdles. *Cancer* 1998;83:2105-19.
27. McCarroll SA, Altshuler DM. Copy-number variation and association studies of human disease. *Nat Genet* 2007;39(7 Suppl):S37-S42.
28. Dutt A, Beroukhi R. Single nucleotide polymorphism array analysis of cancer. *Curr Opin Oncol* 2007;19(1):43-9.
29. Botstein D, Risch N. Discovering genotypes underlying human phenotypes: past successes for mendelian disease, future approaches for complex disease. *Nat Genet* 2003;33 Suppl:228-37.
30. Tuefferd M, de BA, Van dW, I, Talloen W, Verbeke T, Carvalho B, et al. Genome-wide copy number alterations detection in fresh frozen and matched FFPE samples using SNP 6.0 arrays. *Genes Chromosomes Cancer* 2008;47(11):957-64.
31. Zhao X, Weir BA, LaFramboise T, Lin M, Beroukhi R, Garraway L, et al. Homozygous deletions and chromosome amplifications in human lung carcinomas revealed by single nucleotide polymorphism array analysis. *Cancer Res* 2005;65(13):5561-70.
32. Beleza-Meireles A, Kockum I, Yuan QP, Picelli S, Wetterberg L, Gustavson KH, et al. Complex aetiology of an apparently Mendelian form of mental retardation. *BMC Med Genet* 2008;9:6.
33. Suzuki M, Kato M, Yuyan C, Takita J, Sanada M, Nannya Y, et al. Whole-genome profiling of chromosomal aberrations in hepatoblastoma using high-density single-nucleotide polymorphism genotyping microarrays. *Cancer Sci* 2008;99(3):564-70.
34. Kauffmann A, Huber W. Microarray data quality control improves the detection of differentially expressed genes. *Genomics* 2010;95(3):138-42.
35. Kuhn K, Baker SC, Chudin E, Lieu MH, Oeser S, Bennett H, et al. A novel, high-performance random array platform for quantitative gene expression profiling. *Genome Res* 2004;14(11):2347-56.
36. Brentani RR, Carraro DM, Verjovski-Almeida S, Reis EM, Neves EJ, de Souza SJ, et al. Gene expression arrays in cancer research: methods and applications. *Crit Rev Oncol Hematol* 2005;54(2):95-105.
37. Bibikova M, Fan JB. Genome-wide DNA methylation profiling. *Wiley Interdiscip Rev Syst Biol Med* 2010;2(2):210-23.
38. Cheung HH, Lee TL, Rennert OM, Chan WY. DNA methylation of cancer genome. *Birth Defects Res C Embryo Today* 2009;87(4):335-50.
39. Ramsahoye BH, Biniszkievicz D, Lyko F, Clark V, Bird AP, Jaenisch R. Non-CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a. *Proc Natl Acad Sci U S A* 2000;97(10):5237-42.
40. Saxonov S, Berg P, Brutlag DL. A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters. *Proc Natl Acad Sci U S A* 2006;103(5):1412-7.
41. Schrage YM, Hameetman L, Suzhai K, Cleton-Jansen AM, Taminiau AHM, Hogendoorn PCW, et al. Aberrant heparan sulfate proteoglycan localization, despite normal exostosin, in central chondrosarcoma. *Am J Pathol* 2009;174(3):979-88.
42. Lind T, Tufaro F, McCormick C, Lindahl U, Lidholt K. The putative tumor suppressors EXT1 and EXT2 are glycosyltransferases required for the biosynthesis of heparan sulfate. *J Biol Chem* 1998;273(41):26265-8.
43. McCormick C, Duncan G, Goutsos KT, Tufaro F. The putative tumor suppressors EXT1 and EXT2 form a stable complex that accumulates in the golgi apparatus and catalyzes the synthesis of heparan sulfate. *Proc Natl Acad Sci USA* 2000;97(2):668-73.
44. Presto J, Thuvesson M, Carlsson P, Busse M, Wilen M, Eriksson I, et al. Heparan sulfate biosynthesis enzymes EXT1 and EXT2 affect NDST1 expression and heparan sulfate sulfation. *Proc Natl Acad Sci U S A* 2008;105(12):1045-55.
45. Van der Eerden BCJ, Karperien M, Gevers EF, Lowik CWGM, Wit JM. Expression of Indian Hedgehog, PTHrP and their receptors in the postnatal growth plate of the rat: evidence for a locally acting growth restraining feedback loop after birth. *J Bone Miner Res* 2000;15(6):1045-55.
46. Bovee JVMG, Van den Broek LJCM, Cleton-Jansen AM, Hogendoorn PCW. Up-regulation of PTHrP and Bcl-2 expression characterizes the progression of osteochondroma towards peripheral chondrosarcoma and is a late event in central chondrosarcoma. *Lab Invest* 2000;80:1925-33.

47. Adams V, Hany MA, Schmid M, Hassam S, Briner J, Niggli FK. Detection of t(11;22)(q24;q12) translocation breakpoint in paraffin-embedded tissue of the Ewing's sarcoma family by nested reverse transcription-polymerase chain reaction. *Diagn Mol Pathol* 1996;5(2):107-13.
48. Hopyan S, Gokgoz N, Poon R, Gensure RC, Yu C, Cole WG, et al. A mutant PTH/PTHrP type I receptor in enchondromatosis. *Nat Genet* 2002;30(3):306-10.
49. Rozeman LB, Sangiorgi L, Bruijn IH, Mainil-Varlet P, Bertoni F, Cleton-Jansen AM, et al. Enchondromatosis (Ollier disease, Maffucci syndrome) is not caused by the PTHR1 mutation p.R150C. *Hum Mutat* 2004;24(6):466-73.
50. Couvineau A, Wouters V, Bertrand G, Rouyer C, Gerard B, Boon LM, et al. PTHR1 mutations associated with Ollier disease result in receptor loss of function. *Hum Mol Genet* 2008;17(18):2766-75.
51. Ranger A, Szymczak A. Do intracranial neoplasms differ in Ollier disease and maffucci syndrome? An in-depth analysis of the literature. *Neurosurgery* 2009;65(6):1106-13.
52. Pansuriya TC, Kroon HM, Bovee JVMG. Enchondromatosis: insights on the different subtypes. *Int J Clin Exp Pathol* 2010;3(6):557-69.
53. Schwartz HS, Zimmerman NB, Simon MA, Wroble RR, Millar EA, Bonfiglio M. The malignant potential of enchondromatosis. *J Bone Joint Surg Am* 1987;69(2):269-74.
54. Jirarattanaphochai K, Jitpimolmard S, Jirarattanaphochai K. Maffucci's syndrome with frontal lobe astrocytoma. *J Med Assoc Thai* 1990;73(5):288-93.
55. Goto H, Ito Y, Hirayama A, Sakamoto T, Kowada M. [Maffucci's syndrome associated with primary brain tumor: report of a case]. *No Shinkei Geka* 1987;15(9):971-5.
56. Cremer H, Gullotta F, Wolf L. The Maffucci-Kast Syndrome. Dyschondroplasia with hemangiomas and frontal lobe astrocytoma. *J Cancer Res Clin Oncol* 1981;101(2):231-7.
57. Lewis RJ, Ketcham AS. Maffucci's syndrome: functional and neoplastic significance. Case report and review of the literature. *J Bone Joint Surg Am* 1973;55(7):1465-79.
58. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO classification of tumors of the central nervous system. *Acta Neuropathol* 2007;114(2):97-109.
59. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, et al. IDH1 and IDH2 mutations in gliomas. *N Engl J Med* 2009;360(8):765-73.
60. Dang L, Jin S, Su SM. IDH mutations in glioma and acute myeloid leukemia. *Trends Mol Med* 2010;16(9):387-97.
61. Amary MF, Bacsi K, Maggiani F, Damato S, Halai D, Berisha F, et al. IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumors. *J Pathol* 2011;224(3):334-43.
62. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* 2009;462(7274):739-44.
63. Rietveld L, Nieboer TE, Kluijvers KB, Schreuder HW, Bullen J, Massuger LF. First case of juvenile granulosa cell tumor in an adult with Ollier disease. *Int J Gynecol Pathol* 2009;28(5):464-7.
64. Leyva-Carmona M, Vazquez-Lopez MA, Lendinez-Molinos F. Ovarian juvenile granulosa cell tumors in infants. *J Pediatr Hematol Oncol* 2009;31(4):304-6.
65. Kalfa N, Ecochard A, Patte C, Duvallard P, Audran F, Pienkowski C, et al. Activating mutations of the stimulatory g protein in juvenile ovarian granulosa cell tumors: a new prognostic factor? *J Clin Endocrinol Metab* 2006;91(5):1842-7.
66. Lietman SA, Ding C, Levine MA. 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* 2005;87(11):2489-94.
67. Cohen MM, Jr., Siegal GP. McCune-Albright syndrome. In: Fletcher C.D.M., Unni KK, Mertens F, editors. *World Health Organization Classification of Tumors. Pathology & Genetics. Tumors of Soft Tissue and Bone*. Lyon: IARC Press; 2002. p. 357-9.