Genetics of Ollier disease and Maffucci syndrome

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Chapter 4

Maffucci Syndrome: A Genome-Wide Analysis Using High Resolution Single Nucleotide Polymorphism and Expression Arrays on Four Cases

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Abstract

Ollier disease and Maffucci syndrome are rare, nonhereditary skeletal disorders characterized by the presence of multiple enchondromas with (Maffucci) or without (Ollier) co-existing multiple hemangiomas of soft tissue. Enchondromas can progress toward central chondrosarcomas. *PTH1R* mutations are found in a small subset of Ollier patients. The genetic deficit in Maffucci syndrome is unknown. Here, we report the first genome-wide analysis using Affymetrix SNP 6.0 array on Maffucci enchondromas (n = 4) and chondrosarcomas (n = 2) from four cases. Results were compared to a previously studied cohort of Ollier patients (n = 37). We found no loss of heterozygosity (LOH) or common copy number alterations shared by all enchondromas, with the exception of some copy number variations. As expected, chondrosarcomas were found to have multiple genomic imbalances. This is similar to conventional solitary and Ollier-related enchondromas and chondrosarcomas and supports the multistep genetic progression model. Expression profiling using Illumina BeadArray-v3 chip revealed that cartilaginous tumors in Maffucci patients are more similar to such tumors in Ollier patients than to sporadic cartilage tumors. Point mutations in a single gene or other copy number neutral genomic changes might play a role in enchondromagenesis.

Introduction

Enchondromatosis patients have multiple skeletal enchondromas. Enchondromas are benign hyaline cartilage-forming tumors arising within the medulla of the bone (Lucas and Bridge, 2002; Bovee et al., 2010). Ollier disease is rare, nonhereditary, and characterized by the often unilateral occurrence of multiple enchondromas. Maffucci syndrome is extremely rare, also nonhereditary, and demonstrates multiple vascular tumors of soft tissue in addition to enchondromas (Maffucci, 1881; Mertens and Unni, 2002; Auyeung et al., 2003; Bovee et al., 2010; Pansuriya et al., 2010). According to Spranger et al. (1978), Ollier disease (type I) and Maffucci syndrome (type II) are the most common enchondromatosis subtypes. Diagnosis is based on a combination of clinical, radiological, and histological features (Lewis and Ketcham, 1973). Both the enchondromas and the vascular lesions may progress to malignancy, and the malignant transformation rate is the highest (25–100%) in Maffucci syndrome (Zwenneke et al., 2001; Silve and Juppner, 2006).

Because the genetic background of Maffucci syndrome is unknown and genetic studies are limited to a single case report (Matsumoto et al., 1986), we set out to perform whole-genome analysis on tumors from four Maffucci patients collected from the EuroBoNeT consortium (www.eurobonet.eu). We used a high-resolution Affymetrix SNP 6.0 array to detect copy number alterations (CNA) as well as loss of heterozygosity (LOH) and Illumina expression array to study six tumors of four patients.

Materials And Methods

Clinical Information

Patient 1

A 43-year-old man was originally diagnosed with Ollier disease, which was changed to Maffucci syndrome when later developing hemangiomas. An enchondroma in the femur had transformed to a chondrosarcoma grade II (L2195), which was removed by en bloc resection. Subsequently, he developed an enchondroma that was curetted from his finger. The patient is still alive without any sign of metastasis.

Patient 2

A 10-year-old girl was initially diagnosed with Ollier disease based on multiple enchondromas. An enchondroma from her left femur and 4 years later in the fourth and fifth digits of the left hand were curetted. At the age of 17 and 18, amputations of the fifth digit and of the left second toe, respectively, were required for histologically established grade I chondrosarcoma. Six months later, a biopsy from her left proximal tibia (L2097a) and her left fifth toe (L2097c) confirmed the presence of enchondromas. At the age of 19, she developed multifocal lesions involving her left ankle and a biopsy demonstrated cavernous spaces with intervening spindle cell proliferation mixed with some inflammatory cells. The diagnosis of hemangiomas associated with Maffucci syndrome was made. At 21 years, biopsies of the left fifth toe and fourth finger, respectively, revealed atypical enchondroma. One year later, her left hallux was curetted demonstrating an enchondroma.

Patient 3

A 29-year-old man was diagnosed with Maffucci syndrome. Radiologically, bilaterally distributed lesions involving the pelvis, femur, metacarpals, phalanges, carpal bones, metatarsals, and foot phalanges were found. A biopsy of the left distal femur demonstrated chondrosarcoma and an above the knee amputation was performed. A large expanding mass involving the metaphysis and epiphysis of the distal femur was seen; the diagnosis was grade II chondrosarcoma (L2102). In the same year, a biopsy of the left ileum showed an enchondroma (L2101). In the following years, biopsies confirmed enchondromas in multiple digits of the left hand as well as of the right distal femur. In addition, a spindle-cell hemangioendothelioma was excised from the hand, supporting the diagnosis of Maffucci syndrome (Figure 1). A grade II chondrosarcoma of the distal phalanx of the fifth digit of the right hand required amputation.

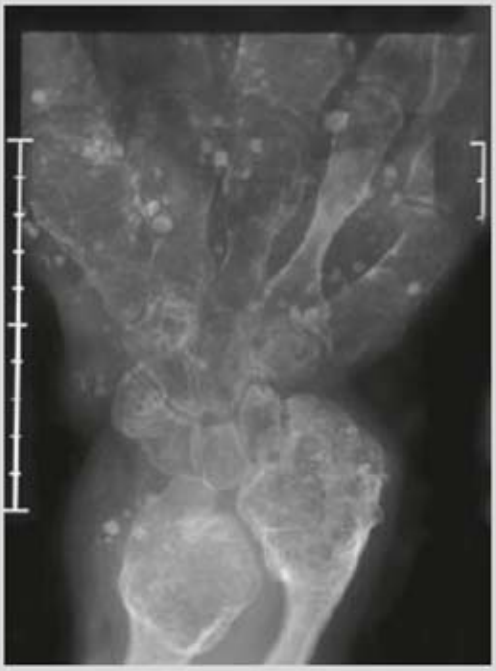
Patient 4

A 37-year-old woman was diagnosed with Maffucci syndrome based on the presence of histologically proven spindle-cell hemangiomas and multiple enchondromas. Histologically confirmed enchondromas were present in the phalangeal bones (L1684). In addition, the patient had superficial hemangiomas in the left thigh. At age 37, an enchondroma was curetted from her left third finger. The patient is alive and has not developed chondrosarcomas or lung metastases.

Sample Preparation

Fresh frozen tissues from six tumors and one normal muscle of the four patients were collected from the EuroBoNet network (Table 1). All samples were obtained according to the ethical guidelines of the host institution. Samples were coded, and all procedures were performed according to the ethical guidelines "Code for Proper Secondary Use of Human Tissue in The Netherlands" (Dutch Federation of medical Scientific Societies). All samples derived from primary tumors and all were graded (Evans et al., 1977).

Figure 1 Radiographic images from patient 3



Patient 3 with Maffucci syndrome. In addition to the multiple enchondromas, soft tissue calcifications are seen representing phleboliths in hemangiomas.

Single Nucleotide Polymorphism Array, Expression Array, and Data Analysis

DNA and RNA were isolated from tumors containing a minimum of 80% tumor cells. Affymetrix Genome-Wide Human SNP Array 6.0 was performed as described earlier (Pansuriya et al., 2011). The average call rate was 98.3%. Data analysis was performed using statistical language R version 2.8 and Nexus software version 4.1 (BioDiscovery, CA) on six tumors (Table 1) and 29 controls as described previously (Pansuriya et al., 2011). Sample preparation, hybridization to Illumina Human-6 v3.0 BeadChips, and data analysis were performed as previously described (Table 1) (Pansuriya et al., 2011). Data files are publicly available at NCBI's Gene Expression Omnibus under accession number GSE26675 (www.ncbi.nlm.nih.gov/geo/, accession number GSE26675).

Table 1 Clinicopathological Data of the Patients

Patient ID	Tumor	Diagnosis	Tumor location	Age	Gender	Application
Patient 1	L2195a	CSII	Femur	43	M	1,2,3
Patient 2	L2097a	EC	Tibia	18	F	1,2
Patient 2	L2097c	EC	Toe	18	F	1,2
Patient 3	L2102	CSII	Femur	29	M	1,2,3
Patient 3	L2101	EC	Ilium	29	M	1
Patient 4	L1684	EC	Phalanx	37	F	1,2,3

*Normal DNA available enabling paired analysis. Applications: samples used for (1) single nucleotide polymorphism array, (2) MLPA, (3) expression array. EC, enchondroma; CS, chondrosarcoma.

Validation with Multiplex Ligation-Dependent Probe Amplification and Fluorescence In Situ Hybridization

Because of the shared homologous sequences between the chromosomes 13 and 21 centromeric regions and the short arms of chromosomes 13 and 21, we performed MLPA to confirm the single nucleotide polymorphism (SNP) array results and performed FISH on metaphases of a normal individual to map the presently assigned 21p11.2 locus sequences by using BAC probes selected from the UCSC database. MLPA was performed for the *TPTE* locus (CTCACCTGTCATTGGGGCCGAGCTCAATGATGACTCCCGCCAGGTCAGTCGGATCAGGACTAAAGGACA) using 38 normal controls and five Maffucci tumor samples. There was not enough DNA from L2101 to allow further studies. Data analysis was done as described (Pansuriya et al., 2011) using SoftGenetics Gene Marker version 1.70. Apart from the region on chromosome arm 21p, candidate regions (*FAM86D* and *PRKG1*) that we found in Ollier tumors were also screened in Maffucci tumors (Pansuriya et al., 2011). FISH was performed on normal metaphase and interphase cells using blood derived from normal donors as reported (Pajor et al., 1998) to confirm the location of the *TPTE* and *BAGE* genes at chromosome arm 21p. We used BAC clone RP11-95E16 (Cy3 labeled) covering *TPTE* region, FITC-labeled L1.26 alphoid repeat probe specific for chromosome 13 and 21 centromeres, and a whole chromosome paint (wcp) probe specific for the long arm of chromosome 21 (Cy5 labeled).

Results

Genetic Alterations in Maffucci enchondromas and Chondrosarcomas

Using an unpaired approach (29 control samples as a baseline) for enchondromas, we found in total nine regions with copy number gain and eight regions with copy number loss in a minimum of two patients (Table 2). Most of the identified regions were known to have common copy number variants in the DGV (Database of Genomic Variants) database. Selection of candidate regions for validation was done based on the copy number gain or loss in three or more enchondromas, with the same copy number event occurring in chondrosarcomas. One of the regions mapped to 21p11.2 containing the *TPTE* gene and a gene cluster (*BAGE2, BAGE3, BAGE4, BAGE5,* and *BAGE*) in three and four enchondromas, respectively.

There was no common genomic region with LOH (Figure 2) or CNA in all Maffucci enchondromas, consistent with previous findings in Ollier enchondromas (Pansuriya et al., 2011). Paired LOH and CNA analysis based on chondrosarcoma DNA and corresponding normal DNA was only possible for patient 1. This analysis revealed LOH with copy number loss at small regions of chromosome arms 1q, 7p, 7q, 8q, 12p, 12q, 13q, 14q, 21p, 21q, and copy neutral LOH at chromosome arm 9p. Copy number gains involving small regions were found at 7q and 15q. Unpaired analysis on the chondrosarcoma of patient 3 revealed LOH with copy number loss at small regions of chromosome arms 3p, 6q, 9p, 9q, 12q, 13p, 13q, and 14q. Copy number gains were observed for small areas of chromosome arms 16p and 16q.

Expression Array Findings

Expression array analysis demonstrated that the *TPTE* and *BAGE* genes were not highly expressed in the tumor samples. Because we had only one Maffucci enchondroma on the expression array, comparison between Ollier and Maffucci enchondromas was not possible. There were no significantly differentially expressed genes between grade II chondrosarcomas from Maffucci ($n = 2$) and Ollier patients ($n = 4$), possibly explained by small sample sizes; the top 50 genes with the lowest p-values are listed in Supporting Information Table 1. We performed cluster analysis on Ollier ($n = 16$), Maffucci ($n = 3$), and solitary ($n = 19$) tumors using the 100 most significantly differentially expressed genes, obtained by LIMMA analysis on Ollier versus solitary tumors (unpublished data). As expected, the Ollier and solitary tumors show good separation. The Maffucci tumors cluster together with Ollier tumors (Figure 3).

Verification of Gene CNA by MLPA and Location by FISH

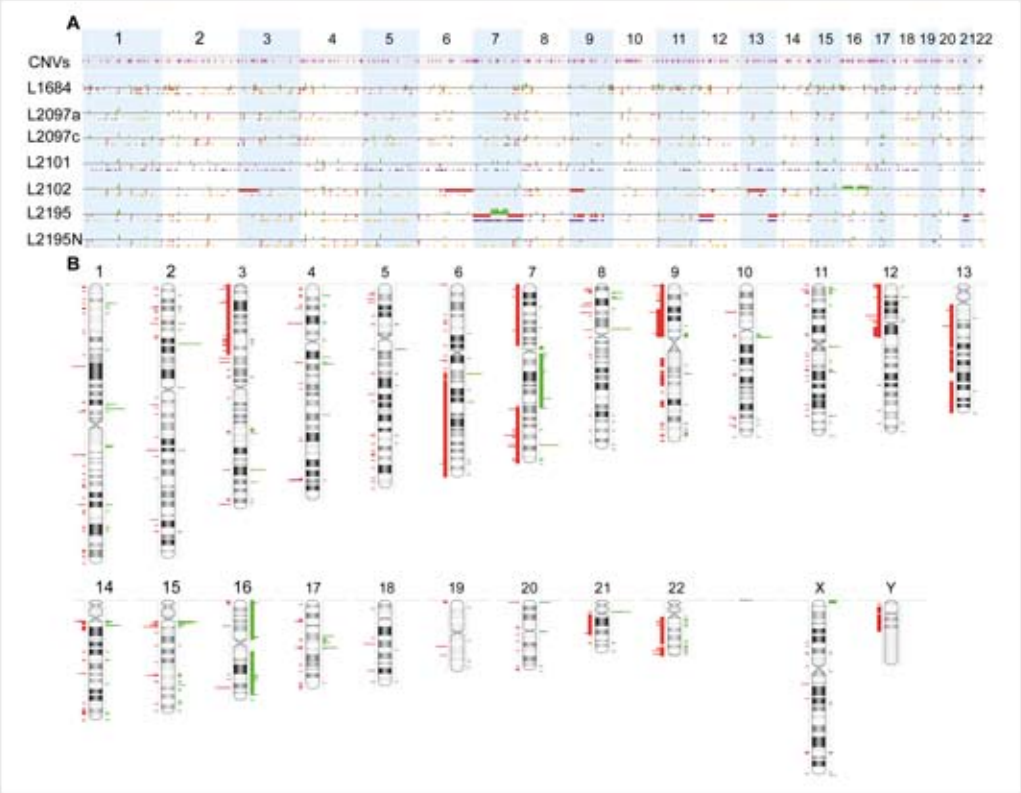
MLPA analysis did not confirm gain of *TPTE* at 21p11.2 containing *TPTE* genes in any of the Maffucci tumors at given threshold (0.8 loss, 1.2 gain). In addition, we screened Maffucci tumors for the candidate genes previously identified in Ollier disease (Pansuriya et al., 2011), and no copy number alteration (CNA) affecting *FAM86D* and *PRKG1* was seen. FISH results in line with the data from human genome sequencing project reporting polymorphic genome variant (DGV database: http://projects.tcag.ca/variation/) proved that indeed 21p11.2 region containing *TPTE* gene is polymorphic, as specific signals were observed both at chromosome 21 and 13 close to the centromeric signals (Supporting Information Figure 1).

Table 2 Regions with Copy Number Gains in Minimum Two Maffucci Patients with Enchondromas (Aggregate ¼ 50% EC)

Location	Start	End	Length	Event	Genes	Enchondromas	Patient ID	% of CNV Overlap
1q21.1	142,693,888	144,042,778	1,348,890	CN gain	12	L1684, L2097c	4,2	100
1q31.3	194,993,359	195,081,241	87,882	CN gain	2	L1684, L2097a	4,2	100
2p11.2–11.1	90,982,622	91,669,499	686,877	CN gain	3	L2097a,L2101	4,3	100
8p23.1	12,259,626	12,521,958	262,332	CN gain	3	L1684, L2097a, L2097c	4,2	100
8p11.23–11.22	39,347,411	39,506,777	159,366	CN gain	2	L2097a, L2097c, L2101	2,3	100
8p23.1	7,202,562	7,841,769	639,207	CN gain	26	L1684, L2097a, L2097c	4,2	100
11q11	55,133,136	55,209,319	76,183	CN gain	3	L1684, L2101	4,3	100
15q11.2	18,453,455	20,084,073	1,630,618	CN gain	11	L1684, L2101	4,3	100
21p11.2–11.1	9,758,730	10,195,652	436,922	CN gain	6	L1684, L2097a, L2097c	4,2	100
1q21.3	150,822,228	150,853,170	30,942	CN loss	2	L1684, L2101	4.3	100
2p24.1	20,614,169	20,751,564	137,395	CN loss	3	L1684, L2097a	4,2	29
12p11.21	31,005,629	31,040,097	34,468	CN loss	1	L1684, L2097a	4,2	100
15q23	65,858,075	65,937,824	79,749	CN loss	2	L1684, L2097a	4,2	0
17p11.2	18,299,660	18,352,373	52,713	CN loss	1	L1684, L2101	4,3	100
20p13	1,508,963	1,543,885	34,922	CN loss	1	L1684, L2101	4,3	100
22q13.31	44,356,090	44,430,291	74,201	CN loss	1	L1684, L2101	4,3	6
22q13.32	47,291,203	47,498,786	207,583	CN loss	1	L1684, L2097a	4,2	7

% of CNV overlap is according to DGV (Database of Genomic Variant) and calculated according to Nexus software. These regions are annotated using NCBI 36.1 (hg18).

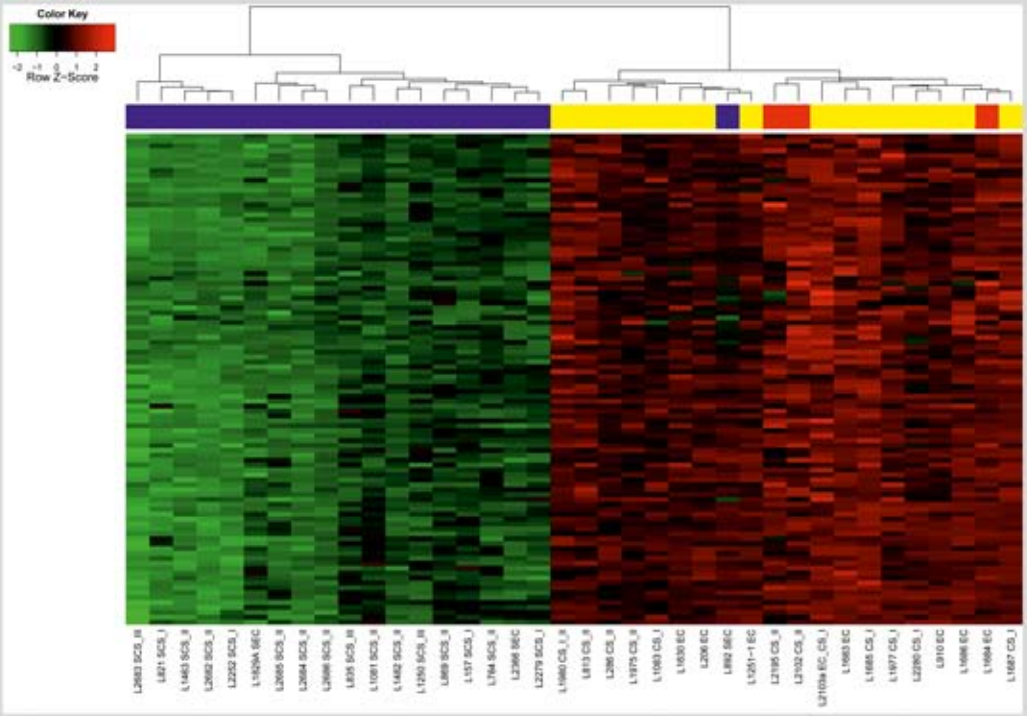
Figure 2 Genome-wide copy number alterations in the tumors and normal of Maffucci patients



A: Copy number alterations in enchondromas, chondrosarcomas, and one normal tissue of Maffucci patients. Gains are plotted in green above the baseline, and losses are plotted in red below the baseline. The X-axis corresponds to the genomic region from chromosomes 1 to 22, and the Y-axis represents the percentage of gains and losses of samples used in this study at the specific location in genome. The number and size of genomic alterations increases with increasing tumor grade. Also, the number and size of genomic alterations in enchondromas and in controls are comparable, which can be attributed to common copy number variation.

B: An overview of genomic aberrations observed in four enchondromas and two chondrosarcomas of Maffucci patients. The idiogram shows copy number gains in green on the right side of the chromosomes and copy number losses in red on the left side of the chromosomes in Maffucci enchondromas and chondrosarcomas.

Figure 3 Supervised clustering of enchondromas and chondrosarcomas occurring in context of solitary, Ollier and Maffucci syndrome



Heatmap depicting supervised clustering of Ollier (yellow, n = 16), Maffucci (red, n = 3), and solitary (blue, n = 19) tumor samples using the 100 most significantly differentially expressed genes (unpublished data), obtained by LIMMA analysis on Ollier versus solitary tumors (all genes have Benjamini and Hochberg FDR-adjusted P values < 0.00001). The heatmap was generated using the function heatmap 2 of R package *gplots* under standard settings. Negative z-scores are shown in green, while positive z-scores are shown in red. Maffucci tumors clustered together with Ollier tumors, indicating that the Maffucci tumors are more similar to tumors of Ollier disease than to solitary tumors. The solitary enchondroma L892 clusters together with the rest of the Ollier and Maffucci tumors, possibly because of misdiagnosis due to the undetected lesions. This cannot be proven, because the patient was lost to follow up.

Discussion

Enchondromatosis is a rare skeletal disorder for which seven subtypes have been described among which Ollier disease and Maffucci syndrome are the most common (Pansuriya et al., 2010). Both disorders can be differentiated from most of the other enchondromatosis subtypes by their sporadic nature and the predominantly unilateral occurrence of enchondromas. It is unclear whether these are different ends of a spectrum caused by mutations in a single gene or whether they represent different diseases. Some of the other enchondromatosis subtypes seem to be caused by genes involved in skeletal development and be inherited as Mendelian disorders, for example, metachondromatosis (*PTPN11*) (Sobreira et al., 2010; Bowen et al., 2011) and spondyloenchondrodysplasia (*ACP5*) (Briggs et al., 2011; Lausch et al., 2011) (Table 3). Two of our patients were originally diagnosed as having Ollier disease, developing hemangiomas only later in life, demonstrating that these two disorders might be closely related. Therefore, Ollier patients should be carefully checked for the presence of hemangiomas.

Table 3 Summary of Genetic Findings in Subtypes of Enchondromatosis

Candidate genes	Enchondromatosis type	Mutation type	References	Single nucleotide polymorphism array Maffucci (Present study)	Sequencing Maffucci (Present study)
PTH1R	Ollier disease	R150C, A1222T, R255H, G121E	Hopyan et al., 2002; Couvineau et al., 2008	-	Absent (unpublished data)
PRKG1	Ollier disease	copy number gain	Pansuriya et al., 2011	absent	-
FMA86D	Ollier disease	homozygous loss	Pansuriya et al., 2011	absent	-
PTPN11	Metachondromatosis	loss of function mutations	Sobreira et al., 2010; Bowen et al., 2011	absent	Absent (Bowen et al., 2011)
ACP5	Spondyloench-ondromatosis	homozygous loss	Lausch et al., 2011a; Briggs et al., 2011	absent	-
PTHLH	Symmetrical enchondromatosis	duplication	Collinson et al., 2010	absent	-

The pathogenesis underlying enchondroma development as well as the genetic cause of the different enchondromatosis subtypes are so far unknown, with the exception of *PTPN11* mutations causing autosomal dominant metachondromatosis in which enchondromas are combined with osteochondroma-like lesions (Sobreira et al., 2010; Bowen et al., 2011).

We excluded the presence of *PTPN11* mutations in our four patients (Bowen et al., 2011). Duplication involving *PTHLH* was recently reported for a patient with symmetrical enchondromatosis (Collinson et al., 2010). We did not see any copy number gain at the *PTHLH* locus at 12p11 in enchondromas from Maffucci patients. Four point mutations in *PTH1R* (R150C, A1222T, R255H, and G121E) leading to impaired function have been reported in 8% of patients with Ollier disease (Hopyan et al., 2002; Rozeman et al., 2004; Couvineau et al., 2008). PTH1R is involved in the IHH-PTHLH negative feedback loop regulating endochondral bone formation. In total, 26 patients (17 tumors and 9 blood samples) with Maffucci syndrome have been screened for *PTH1R* mutations, but none was detected (Rozeman et al., 2004; Couvineau et al., 2008). We screened our four patients for the four known *PTH1R* mutations and did not find any of these point mutations (data not shown). Nor were there any CNA in other genes involved in the IHH-PTHLH pathway in the tumors from our Maffucci patients.

Overall, the enchondromas did not show any large genomic alterations. The 21p11.2 region is known to contain copy number variants, and copy number changes are therefore not specific for the disease. Using FISH, we showed that the current annotation of the pericentromeric regions of acrocentric chromosomes might not be correct and should be handled with care. Two chondrosarcomas grade II showed more genomic aberrations. CNA at *FAM86D* and *PRKG1*, which we previously identified in a subset of Ollier enchondromas (Pansuriya et al., 2011), were absent in Maffucci enchondromas.

In conclusion, we report the first genome-wide study of enchondromas and chondrosarcomas from four individuals with Maffucci syndrome. Similar to Ollier-associated enchondromas, Maffucci associated enchondromas do not show LOH or common CNA. For the enchondromagenesis, small mutations and/or copy number neutral genomic alterations might be causative, which can be further investigated using a next-generation sequencing approach. An increased number of genetic alterations are found in Maffucci chondrosarcomas, supporting the multistep genetic progression model for chondrosarcomagenesis.

Acknowledgments

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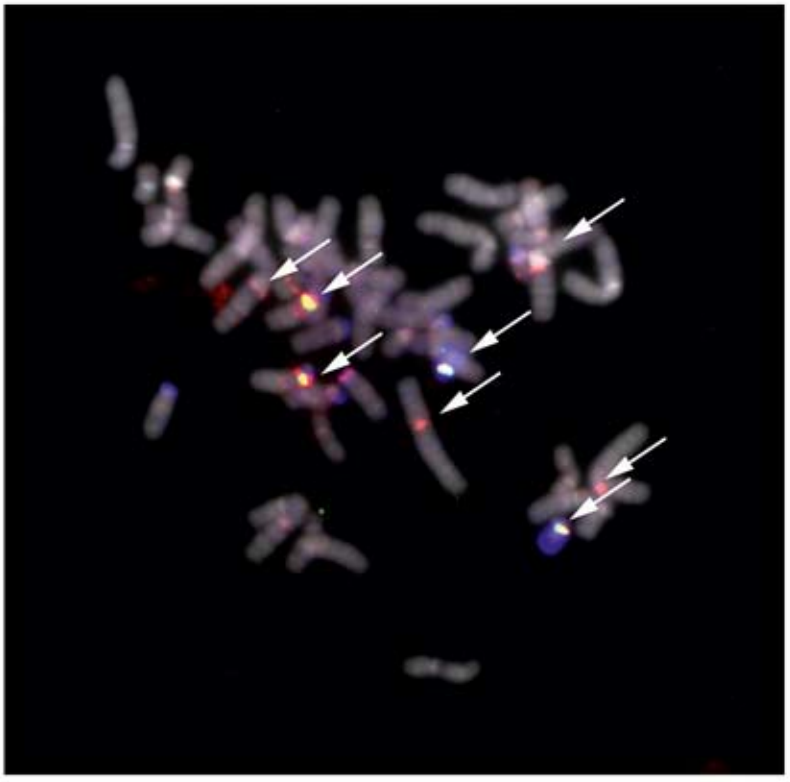
References

1. Auyeung J, Mohanty K, Tayton K. 2003. Maffucci lymphangioma syndrome: An unusual variant of Ollier's. J Pediatr Orthop B 12:147–150.
2. Bovee JVMG, Hogendoorn PCW, Wunder JS, Alman BA. 2010. Cartilage tumours and bone development: Molecular pathology and possible therapeutic targets. Nat Rev Cancer 10:481–488.
3. Bowen ME, Boyden ED, Holm IA, Campos-Xavier B, Bonafe L, Ikegawa S, Cormier- Daire V, Bove´e JVMG, Pansuriya TC, De Sousa SB, Savarirayan R, Vikkula M, Garavelli L, Kozakewich HP, Kasser JR, Seidman JD, Kurek KC, Warman ML. 2011. Loss-of-function mutations in PTPN11 causes metachondromatosis, but not Ollier disease or Maffucci syndrome.
4. Briggs TA, Rice GI, Daly S, Urquhart J, Gornall H, Bader-Meunier B, Baskar K, Baskar S, Baudouin V, Beresford MW, Black GC, Dearman RJ, de ZF, Foster ES, Frances C, Hayman AR, Hilton E, Job-Deslandre C, Kulkarni ML, Le MM, Linglart A, Lovell SC, Maurer K, Musset L, Navarro V, Picard C, Puel A, Rieux-Laucat F, Roifman CM, Scholl-Burgi S, Smith N, Szynkiewicz M, Wiedeman A, Wouters C, Zeef LA, Casanova JL, Elkon KB, Janckila A, Lebon P, Crow YJ. 2011. Tartrate-resistant acid phosphatase deficiency causes a bone dysplasia with autoimmunity and a type I interferon expression signature. Nat Genet 43:127–131.
5. Collinson M, Leonard SJ, Charlton J, Crolla JA, Silve C, Hall CM, Oglivie C, James MA, Smithson SF. 2010. Symmetrical enchondromatosis is associated with duplication of 12p11.23 to 12p11.22 including PTHLH. Am J Med Genet A 152:3124–3128.
6. Couvineau A, Wouters V, Bertrand G, Rouyer C, Gerard B, Boon LM, Grandchamp B, Vikkula M, Silve C. 2008. PTHR1 mutations associated with Ollier disease result in receptor loss of function. Hum Mol Genet 17:2766–2775.
7. Evans HL, Ayala AG, Romsdahl MM. 1977. Prognostic factors in chondrosarcoma of bone. A clinicopathologic analysis with emphasis on histologic grading. Cancer 40:818–831.
8. Hopyan S, Gokgoz N, Poon R, Gensure RC, Yu C, Cole WG, Bell RS, Juppner H, Andrulis IL, Wunder JS, Alman BA. 2002. A mutant PTH/PTHrP type I receptor in enchondromatosis. Nat Genet 30:306–310.
9. Lausch E, Janecke A, Bros M, Trojandt S, Alanay Y, De LC, Hubner CA, Meinecke P, Nishimura G, Matsuo M, Hirano Y, Tenoutasse S, Kiss A, Rosa RF, Unger SL, Renella R, Bonafe L, Spranger J, Unger S, Zabel B, Superti-Furga A. 2011. Genetic deficiency of tartrate-resistant acid phosphatase associated with skeletal dysplasia, cerebral calcifications and autoimmunity. Nat Genet 43:132–137.
10. Lewis RJ, Ketcham AS. 1973. Maffucci's syndrome: Functional and neoplastic significance. Case report and review of the literature. J Bone Joint Surg Am 55:1465–1479.
11. Lucas DR, Bridge JA. 2002. Chondromas: Enchondroma, periosteal chondroma, and enchondromatosis. In: Fletcher CDM, Unni KK, Mertens F, editors. World Health Organization classification of tumours. Pathology and genetics of tumours of soft tissue and bone. Lyon: IARC Press. pp. 237–240.
12. Maffucci A. 1881. Di un caso encondroma ed angioma multiplo. Movimento Medico-chirurgico, Napoli 3:399–412; 565–575.
13. Matsumoto N, Fukushima T, Tomonaga M, Imamura M. 1986. [Maffucci's syndrome with intracranial manifestation and chromosome abnormalities–a case report]. No Shinkei Geka 14:403–410.
14. Mertens F, Unni KK. 2002. Enchondromatosis: Ollier disease and Maffucci syndrome. In: Fletcher CDM, Unni KK, Mertens F, editors. World Health Organization Classification of Tumours. Pathology and genetics of tumours of soft tissue and bone. Lyon: IARC Press. pp. 356–357.
15. Pajor L, Szuhai K, Mehes G, Kosztolanyi G, Jakso P, Lendvai G, Szanyi I, Kajtar P. 1998. Combined metaphase, interphase cytogenetic, and flow cytometric analysis of DNA content of pediatric acute lymphoblastic leukemia. Cytometry 34:87–94.
16. Pansuriya T, Kroon HM, Bovee JVMG. 2010. Enchondromatosis: Insights on the different subtypes. Int J Clin Exp Pathol 3:557–569.
17. Pansuriya TC, Oosting J, Krenacs T, Taminiau AH, Verdegaal SH, Sangiorgi L, Sciot R, Hogendoorn PC, Szuhai K, Bovee JV. 2011. Genome-wide analysis of Ollier disease: Is it all in the genes? Orphanet J Rare Dis 6:2.

18. Rozeman LB, Sangiorgi L, Bruijn IH,Mainil-Varlet P, Bertoni F, Cleton- Jansen AM, Hogendoorn PCW, Bove´e JVMG. 2004. Enchondromatosis (Ollier disease,Maffucci syndrome) is not caused by the PTHR1 mutation p.R150C. Hum Mutat 24:466–473.
19. Silve C, Juppner H. 2006. Ollier disease. Orphanet J Rare Dis 1:37.
20. Sobreira NL, Cirulli ET, Avramopoulos D, Wohler E, Oswald GL, Stevens EL, Ge D, Shianna KV, Smith JP, Maia JM, Gumbs CE, Pevsner J, Thomas G, Valle D, Hoover-Fong JE, Goldstein DB. 2010. Whole-genome sequencing of a single proband together with linkage analysis identifies a Mendelian disease gene. PLoS Genet 6:e1000991.
21. Spranger J, Kemperdieck H, Bakowski H, Opitz JM. 1978. Two peculiar types of enchondromatosis. Pediatr Radiol 7:215–219.
22. Zwenneke FH, Ginai AZ, Wolter OJ. 2001. Best cases from the AFIP. Maffucci syndrome: Radiologic and pathologic findings. Armed Forces Institutes of Pathology. RadioGraphics 21:1311– 1316.

Figure S1 FISH of chromosomes 13 and 21



FISH results indicated chromosomes 13 and 21 in metaphase. Probe RP11-95E16 (Cy3 labeled, red) and centromeric probe binds to the chromosome 13 and 21 (FITC, green) and wcp 21 (blue). A colocalization of all 4 red and green signals was seen indicating the presence of RP11-95E16 signals both at chromosome 21 and 13; in addition several secondary signals of the RP11-95E16 clone were observed at other chromosomes.

Table S1 Top fifty genes with the lowest p-value between Ollier and Maffucci chondrosarcoma grade II

Gene Symbol	Probe ID	logFC	p-value	adj. p-value
C11orf87	7510619	-1.04410334	3.89E-06	0.111094124
NOMO2	2650040	0.765193013	1.25E-05	0.15298495
ZP4	7400392	-0.49531778	1.61E-05	0.15298495
ARL4A	7560615	-0.92348809	2.50E-05	0.157254509
PTGFR	2480139	-2.1611789	2.96E-05	0.157254509
PTGFR	770551	-2.00072338	3.93E-05	0.157254509
CNTNAP2	1400520	-2.48996817	4.16E-05	0.157254509
TNFSF10	870202	-0.83818461	4.41E-05	0.157254509
CSPG5	160397	-0.52438993	6.53E-05	0.20696256
TCF7L1	5050678	-0.31782816	8.05E-05	0.229817111
SELP	4810468	-0.70240586	9.35E-05	0.242399961
RORB	2370561	-0.68969674	0.0001544	0.367116161
CASP4	1450136	-0.91852242	0.0001706	0.374490797
SLC26A7	6760221	-0.48107142	0.000241	0.469874082
GORAB	830441	-0.93710499	0.000247	0.469874082
FLJ14213	4610672	-0.62513607	0.0002934	0.515246514
ADAMTS18	3290133	-0.29706855	0.000307	0.515246514
C6orf141	3450632	-0.26495298	0.0003508	0.556052553
THNSL1	5260520	-0.42022175	0.000379	0.56094917
PRG2	1580195	-0.54328552	0.0003932	0.56094917
SLCO1C1	2510554	-0.43268572	0.0004278	0.581158031
PLOD2	460338	1.312897386	0.0004555	0.582134774
MAP3K1	4230373	-0.65515382	0.0004703	0.582134774
TP63	6060131	-0.90703834	0.0005088	0.582134774
LOC25845	2760068	-0.72352259	0.0005494	0.582134774

Table S1 (Continue)

Gene Symbol	Probe ID	logFC	p-value	adj. p-value
EYA1	5720040	-1.61785141	0.0005548	0.582134774
CES1	2680056	-0.99532331	0.000556	0.582134774
KCTD14	2940632	0.567228862	0.0005999	0.582134774
CNR1	4010152	-0.28482701	0.0006165	0.582134774
SALL4	5050270	-0.45354179	0.0006362	0.582134774
ARL4A	1410113	-2.26725707	0.0006541	0.582134774
CCL20	4220246	-2.39865682	0.0006695	0.582134774
FAM148B	5860093	-1.72158352	0.0006733	0.582134774
EYA1	4850377	-0.20387975	0.0007209	0.587165546
PTPN13	3060609	-0.7534637	0.000749	0.587165546
TCP11L1	1070753	0.401831792	0.0007517	0.587165546
LOC649553	4040563	0.190343381	0.0007615	0.587165546
GGT5	670538	-1.20784023	0.0008607	0.639070504
HOXA3	2900048	-0.18387521	0.0008736	0.639070504
EMID1	6100487	-1.45511301	0.0009094	0.648682531
MYL10	4730427	-0.18551934	0.0010966	0.729648862
NOSTRIN	7210113	-1.245946	0.0011105	0.729648862
HES5	6590300	-0.4334798	0.0011293	0.729648862
MEOX1	1230594	-0.79694545	0.0011413	0.729648862
GRB10	5960725	0.385577604	0.0011508	0.729648862
KCNB1	6900196	-0.3336151	0.0012007	0.74472513
MAGEC1	7650731	-0.28558901	0.0013058	0.78699823
SLC22A3	6200333	-0.23877922	0.001324	0.78699823
SNTB2	840053	-0.33481726	0.0014166	0.797789001
ABCG2	7160220	-0.28215736	0.0014351	0.797789001