Common Somatic Alterations Identified in Maffucci Syndrome by Molecular Karyotyping

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Key Words

Cancer · Chondrosarcoma · Copy number variation · Defect · Enchondromatosis · Enchondroma · Gene · Microarray · Mutation · Spindle cell hemangiomas

Abstract

Maffucci syndrome (MS) is a rare congenital disorder characterized by multiple central cartilaginous tumors (enchondromas) in association with cutaneous spindle cell hemangiomas. These patients have a high incidence of malignant transformation. No familial case is known and the etiopathogenic cause remains unknown. In enchondromatosis (Ollier disease, OD), which is comprised of enchondromas only, 4 mutations in the *PTH1* gene have been identified in 4 patients; 3 were somatic and 1 was germline. No *PTH1* mutations have been detected in MS, whereas somatic *IDH1* and, more rarely, *IDH2* mutations have been observed in 77% of patients with MS and 81% of patients with OD. These genetic alterations are shared with other tumors, including glioma, leukemia and carcinoma. To search for underlying somatic

genomic causes, we screened MS tissues using Affymetrix SNP-chips. We looked for CNVs, LOH and uniparental isodisomy (UPID) by performing pairwise analyses between allelic intensities in tumoral DNA versus the corresponding blood-extracted DNA. While common chromosomal anomalies were absent in constitutional DNA, several shared CNVs were identified in MS-associated tumors. The most frequently encountered somatic alterations were localized in 2p22.3, 2q24.3 and 14q11.2, implicating these chromosomal rearrangements in the formation of enchondromas and spindle cell hemangiomas in MS. In one chondrosarcoma specimen, large amplifications and/or deletions were observed in chromosomes 3, 6, 9, 10, 12, 13, and 19. Some of these genetic changes have been reported in other chondrosarcomas suggesting an etiopathogenic role. No LOH/UPID was observed in any Maffucci tissue. Our findings identify frequent somatic chromosomal rearrangements on 2p22.3, 2q24.3 and 14q11.2, which may unmask mutations leading to the lesions pathognomonic of MS.

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Odile Enjolras is deceased.

Table 1. Generalized enchondromatosis (Spranger classification)

Name of condition	Major radiographic features	Etiology	Chromosomal location	Gene	References
Ollier disease	multiple enchondromas of flat and tubular bones, vertebrae and calvaria evenly distributed	AD or sporadic	3p22p21.1	<i>PTHR1</i>	Hopyan et al., 2002; Couvineau et al., 2008
Maffucci syndrome	same as Ollier disease and multiple spindle cell hemangiomas	sporadic	–	somatic <i>IDH1</i> and <i>IDH2</i>	Couvineau et al., 2008; Amary et al., 2011; Pansuriya et al., 2011
Metachondromatosis	multiple enchondromas with prominent calcifications of short tubular bones	AD	12q24.1	<i>PTPN1</i>	Sobreira et al., 2010; Bowen et al., 2011
Spondyloenchondrodysplasia	irregularly distributed discrete enchondromas of long tubular bones, generalized severe platyspondyly, mild or no involvement of hands and feet	AR	19p13	<i>TRAP</i>	Briggs et al., 2011
Enchondromatosis with irregular vertebral lesions	multiple enchondromas of long tubular and flat bones, generalized irregular dysplasia of vertebral bodies, mild or no involvement of hands or feet	sporadic	–		Spranger et al., 1978
Generalized enchondromatosis	generalized evenly distributed enchondromas, severe involvement of hands and feet, mild platyspondyly, skull deformity	sporadic	12p11p23	dupl <i>PTHLH</i>	Spranger et al., 1978

AD = Autosomal dominant; AR = autosomal recessive.

Maffucci syndrome (MS) (OMIM 166000) is characterized by multiple enchondromas associated with superficial and deep vascular lesions, now called spindle cell hemangioma, a low-grade vascular tumor [Maffucci, 1881; Silva et al., 1986]. MS is rare with less than 200 cases reported; males and females are equally affected. The disorder appears in children aged between 4 and 5 years; however, 25% of cases are congenital. No familial inheritance has been shown [Boon and Vikkula, 2008].

MS lies in the spectrum of enchondromatoses, which were classified into 6 subtypes by Spranger, depending on the distribution of enchondromas, other signs and symptoms, and mode of inheritance: type I, Ollier disease; type II, Maffucci syndrome; type III, metachondromatosis; type IV, spondyloenchondrodysplasia; type V enchondromatosis with irregular vertebral lesions; and type VI, generalized enchondromatosis (table 1) [Spranger et al., 1978; Bhargava et al., 2005; Pansuriya et al., 2010; Briggs et al., 2011].

Enchondromas are hamartomatous proliferations of chondrocytes within long bones leading to an abnormal endochondral ossification of the diaphysis or the metaphysis. These tumors can appear anywhere, be unilateral or bilateral, but are always asymmetric. They are most often found on radiographic imaging performed for skeletal deformities, such as pathologic fracture, bony distortion, scoliosis, and short stature [Lewis and Ketcham, 1973; Kaplan et al., 1993; Albregts and Rapini, 1995]. On conventional radiographs, enchondromas are seen as multiple, oval-shaped osteolytic lesions with well-defined mar-

gins in the metaphysis and/or the diaphysis of long tubular and flat bones.

Ollier disease (OD) (Spranger type I) is mainly sporadic, although rare familial cases have been reported [Hopyan et al., 2002; Rozeman et al., 2004; Casal et al., 2010]. The most frequent location for enchondromas is the small bones of the hands and feet (fig. 1A, C). MS (Spranger type II) is a similar enchondromatosis, but it is associated with slow-flow vascular lesions, which are blue, noncompressible, subcutaneous or cutaneous nodules, typically occurring on the fingers or feet (fig. 1B). Phleboliths may also be present. The lesions consist of dilated capillary-venous vessels that are more cellular in appearance than venous malformations and contain a variable number of spindle cells. These lesions were once thought to be low-grade angiosarcomas, but are now considered to be benign spindle cell hemangiomas (fig. 1D) [Haga et al., 1998; Boon and Vikkula, 2008]. Lymphatic malformations can also be seen in patients with MS. The hemangioendotheliomas are sometimes associated with lymphatic malformations [Suringa and Ackerman, 1970; Haga et al., 1998; Colonna et al., 2002; Auyeung et al., 2003]. In contrast to patients with venous malformations, MS patients have normal D-dimer levels [Dompmartin et al., 2009]. This is probably due to the more cellular composition of spindle cell hemangiomas and differing etio-pathogenesis.

Patients with enchondromatosis, especially those with MS, have a significant risk (nearly 100%) of developing skeletal and nonskeletal malignant lesions after 40 years

Fig. 1. Clinical features of OD and MS. **A** Classic deformities of the left hand and the left foot of a patient with OD showing multiple enchondromas (arrows). **B** Enchondromas associated with spindle cell hemangiomas (arrows) in the right hand of a patient with MS. **C** Hematoxylin-eosin-stained section of enchondroma. **D** Spindle cell hemangioma demonstrating dilated vascular structures (asterisks). **Inset** Spindle cells.

of age [Auyeung et al., 2003]. Sarcomatous degeneration of enchondromas may occur; it is difficult to differentiate a benign enchondroma from a low-grade malignant chondrosarcoma. In MS patients, most chondrosarcomas are of low histological grade (grade 1 or 2) and are successfully managed by surgical resection [Schwartz et al., 1987]. Other mesodermal neoplasms include fibrosarcoma, glioma, astrocytoma, ovarian tumors, hemangiosarcoma, and leukemia [Albregts and Rapini, 1995]. In 30% of cases, non-mesodermal tumors, such as adenocarcinoma, also occur. Therefore, careful and prolonged clinical follow-up and imaging studies are necessary.

Karyotyping is usually normal in patients with MS and OD. Somatic LOH on 13q14 and 9p21, as well as deletion in 1p and inversion in chromosome 9, were found in rare patients with enchondromatosis [Ozisik et al., 1998; Bovée et al., 2000, 2001]. Overexpression of p53 has been noted in benign and metastatic cartilaginous tumors [Bovée et al., 1999, 2000, 2001]. Metachondromatosis (Spranger type III) is caused by germline mutations in *PTPN11* with a likely loss of the second allele due to a somatic second-hit [Sobreira et al., 2010; Bowen et al., 2011]. Missense mutations have been reported in the parathyroid hormone receptor 1 (*PTH1R*) gene in 5 patients with enchondromatosis (Spranger type I), but no change in 23 patients with MS [Hoppyan et al., 2002; Couvineau et al., 2008]. These findings suggest that, notwithstanding clinical overlap, MS and OD are not allelic disorders. Mutations

in *PTH1R* are infrequent even in OD; another study of 31 patients failed to identify any such mutation [Rozeman et al., 2004]. The complex phenotype of MS suggests that it is either a contiguous gene disorder caused by a deletion of multiple genes or that it is caused by a mutation in a gene with pleiotropic, growth-promoting effects. Whole genome screening could help identify loci and candidate genes involved in the development of enchondromas, vascular malformations and cancers. As SNP arrays offer high resolution and the possibility of distinguishing between 2 genotyped alleles, it is a powerful tool for the detection of allelic imbalances [Wang et al., 1998; Yamamoto et al., 2007; Amyere et al., 2013]. In this study, we identified several somatic CNVs in most of the tissue samples taken from patients with MS.

Materials and Methods

Recruitment of Patients

In collaboration with several clinicians, we collected blood and samples of resected tissues from patients with MS or OD. Informed consent was obtained from all patients in the study prior to participation, as approved by the ethical committee of the medical faculty at Université catholique de Louvain, Brussels, Belgium. The series comprised 9 blood and 9 frozen tumor samples from patients with MS as well as 1 blood and 2 frozen tissue samples from 2 patients affected by OD. Clinical information was collected by physicians who care for the patients (L.M.B., I.K., P.-L.D., O.E., and J.B.M.).

Table 2. Clinical data of patients with MS or OD

No.	Patient	Sex	Disease	Vascular anomalies in spindle cell hemangioma				Enchondroma				Cancer
				head/neck	UE	LE	trunk	head/neck	UE	LE	trunk	other anomaly
1	MAF-90	M	MS		+	+	+	+	+	+		chondrosarcoma
2	MAF-190	M	MS	+	+	+	+		+	+		
3	MAF-110	M	MS		+				+			
4	MAF-150	F	MS	+	+	+			+	+		
5	MAF-160	M	MS			+				+		AML and VWD
6	MAF-170	F	MS			+			+	+	+	
7	MAF-200	F	MS		+				+			
8	MAF-210	F	OD						+	+		
9	MAF-250	F	MS		+	+			+	+		
10	MAF-260	M	MS			+				+		
11	OLR-30	M	OD							+		

UE = Upper extremities; LE = lower extremities; AML = acute myeloid leukemia; VWD = von Willebrand disease.

DNA Isolation

Venous blood samples were drawn from participants and DNA was extracted from buffy coats using QIAamp DNA blood mini kit (Qiagen) or from whole blood using DNA purification kit (Gentra). DNA was extracted from pieces of frozen tissues using Puregene, DNA extraction kit (Gentra).

SNP Array Analysis

Eleven samples were investigated using SNP arrays (100K or SNP6.0), according to manufacturer's instructions (Affymetrix, Santa Clara, Calif., USA). Results were analyzed for copy number changes using Affymetrix genotyping console 4.1.4 (<http://www.affymetrix.com>) and CNAG 3.3.0.1 (<http://www.genome.umin.jp>). Genotype-calling algorithms MPAM Mapping or Birdseed v2 were used to generate the genotypes for 100K and SNP6.0 chips, respectively. When pairwise analysis (comparing tissue-extracted and blood-extracted DNA) from the same patient was employed, copy number status was inferred using algorithms based on the Hidden Markov Model [Nannya et al., 2005; Yamamoto et al., 2007]. Perfectly matched probes (for 100K and SNP6.0) and mismatched probes (for 100K only) were used to calculate the ratios between the 2 alleles.

Databases

UCSC Genome Browser (<http://genome.ucsc.edu>) and Database of Genomic Variants (<http://projects.tcag.ca/variation/>).

Results

Characteristics of the Patients

Nine patients with MS and 2 patients with OD were included in our analysis (table 2). The 9 MS patients had both spindle cell hemangiomas and multiple enchondromas. The spindle cell hemangiomas were located in

the feet (7/9), hands and fingers (6/9), head and neck (2/9), or trunk (2/9). Enchondromas were mainly located on the hands, feet and long bones. Patient MAF-190 had a chondrosarcoma located in the shoulder and patient MAF-170 developed acute myeloid leukemia. Family history for MS and OD was negative for all 11 patients.

Pairwise Copy Number Analysis

In order to detect possible candidate loci for MS and OD, we applied the GeneChip Human Mapping 100K or SNP6.0 on 10 blood-extracted and 11 tissue-extracted DNAs. Copy number analysis individually comparing each DNA sample to a set of 48 references did not reveal copy number changes in blood DNAs; however, several chromosomal imbalances were identified in tissular DNAs. Patient MAF-110 had a mosaic 1p deletion, found using pairwise copy number analysis (fig. 2). Three genomic variations (deletions or amplifications) were seen repeatedly: 2p22.3, 2q24.3 and 14q11.2 (fig. 3; table 3). These chromosomal alterations do not share a common breakpoint, and they have variable sizes (2.4–33.4 Mb) in 2p22.3, (1.1–6.5 Mb) in 2p24.3 and (1.5–25.6 Mb) in 14q11.2 (table 3). In one chondrosarcoma (MAF-190), we detected large chromosomal alterations by pairwise copy number analysis: deletions in 3q (26.1–29 Mb), 6q (13–27 Mb), 9p, 10q, 12p (13.33–12.1 Mb), and 13, and amplification of 12q12 (fig. 4) and deletion of 19p13.3q12 (data not shown).

Fig. 2. Chromosomal anomaly in enchondroma of one MS patient (MAF-110) using Affymetrix 100K SNP-chip pairwise copy number analysis. Somatic deletion of complete chromosome 1p by decreased individual SNP-probe signals (red dots) and intensity ratios of alleles in tissue versus blood represented by a blue curve in a logarithmic scale. This deletion is highlighted by the increase in discrepancy calls between alleles in tissue versus blood (small pink bars) confirmed by loss of one allele (green line) in tissue and the increase of the LOH curve in blue.

Fig. 3. Localization of common somatic amplifications/deletions in 10 Maffucci tissues genotyped using Affymetrix 100K SNP-chip and detected by pairwise copy number analysis. Deletions or amplifications in each tissue are shown by red bars. Arrows show common amplifications/deletions in Maffucci tissues.

Discussion

We applied SNP arrays for a genome-wide screen of LOH and DNA copy number changes in a collection of tissue samples from patients with MS or OD. We compared paired blood and tissue DNAs. We identified large and small somatic areas of allelic imbalance in the Maffucci samples, but none in the enchondromas of OD. This suggests a point mutation in *PTHRI* or another gene rather than a large deletion/amplification to explain the

pathophysiology of OD [Couvineau et al., 2008]. The loci identified in Maffucci samples did not contain any of the genes that have been linked to enchondromatous lesions: *PTHRI* (OD), *PTPN11* (metachondromatosis), *TRAP* (spondyloenchondrodysplasia), *PTHLH* (generalized enchondromatosis) (table 1).

IDH mutations, *IDH1* (R132C) and *IDH2* (R172S) were found in 40 and in 50% of tested Maffucci tissues, respectively (table 3). The presence of these mutations did not correlate with clinical features [Avakian et al., 2010; Shal-

Table 3. Somatic genetic changes in Maffucci and Ollier patients

Patient	Tissue type	<i>IDH1</i> mutation	<i>IDH2</i> mutation	Chromosomal alterations		
				2p22.3 (bp)	2q24.3 (bp)	14q11.2 (bp)
MAF-90	SCH	NT	NT	normal	amp (167.828.122–168.934.370)	amp (21.831.221–24.468.411)
MAF-190	chondrosarcoma	no	NT	normal	normal	normal
MAF-110	ENC	R132C	no	normal	amp (161.032.150–167.488.990)	amp (19.171.760–25.131.920)
MAF-150	SCH	no	R172S	amp 31.000.000–34.750.000)	amp (165.250.000–166.750.000)	amp (21.500.000–23.750.000)
MAF-160	SCH	NT	NT	del (32.100.010–36.600.000)	amp (166.372.448–168.344.925)	amp (20.637.329–22.183.224)
MAF-170	SCH	NT	NT	del (29.998.250–32.426.700)	normal	amp (20.141.600–23.570.000)
MAF-200	SCH	R132C	no	amp (36.121.760–41.446.880)	amp (164.072.560–169.397.680)	amp (0–25.619.440)
MAF-210	ENC	no	no	normal	normal	normal
MAF-250	ENC	no	R172S	normal	amp (161.488.204–166.396.329)	normal
MAF-260	ENC	NT	NT	amp (29.857.045–37.908.824)	normal	normal
OLR-30	ENC	R132C	NT	normal	normal	normal

amp = Amplification; del = deletion; ENC = enchondroma; NT = not tested; SCH = spindle cell hemangioma.

aby et al., 2011]. Heterozygous hotspot mutations in *IDH1/2* are frequently observed in several types of cartilaginous tumors, as well as in secondary glioblastomas, acute myeloid leukemia, and intrahepatic cholangiocarcinomas. *IDH1/2* mutations cause epigenetic modifications, which can result in altered, pathophysiological expression of genes [Pansuriya et al., 2010; Cohen et al., 2013; Lee et al., 2013; Schaap et al., 2013]. However, novel potential driver mutations in the Capicua (*CIC*) have been identified as the key event to oligodendroglioma tumorigenesis in a background of *IDH1/2* mutations [Yip et al., 2012].

Deletion/amplification of chromosomal DNA is thought to be one of the mechanisms that activate cancer-related genes in tumors. Three regions, 2p22.3, 2q24.3 and 14q11.2, were frequently altered in Maffucci tissues. They have been reported as CNVs and in the Mitelman Database of Chromosomal Aberrations and Gene Fusions in Cancer. The 2p22.3 region contains human microRNA (has-mir-558) predicted to target 182 genes, including *PTPN11*, that cause metachondromatosis (http://www.targetscan.org/vert_50/). This region also contains the *RASGRP3* gene, reported to promote activation of the ETS oncogene family member (*ELK1*) in a prostate cancer cell line [Schwartz et al., 1987]. 2p22.3 is reported to be associated with acute myeloblastic leukemia, adenocarcinoma and leiomyoma (Cancer Genome Anatomy Project (CGAP) <http://cgap.nci.nih.gov/Chromosomes/>

Mitel_Search?structural=on&numerical=on&breakpoint=&neopl=1101&tissue=&type=&page=1#MARK).

The 2q24.3 region is associated with acute lymphoblastic leukemia and follicular lymphoma. Chromosomal imbalances in this region are detected in highly malignant human colon cancer cell lines [Ranger and Szymczak, 2009]. The third locus, 14q11.2, is associated with lymphoma, meningioma and adenocarcinoma. The locus contains the defender against cell death (*DAD1*) gene that encodes a regulator of programmed cell death and the T-cell antigen receptor (*TCRA*) gene, strongly associated with CNVs in lymphocytes and neutrophils, and involved in T-lymphocyte response with MHC. *TCR* genes are assembled through V(D)J recombination, a site-specific recombination process directed by the lymphoid-specific recombinase and ubiquitously expressed DNA repair proteins [Yulug et al., 1995; Krangel, 2009; Dash et al., 2011]. We also observed this alteration in ependymoma tumors and Burkitt lymphoma. Thus, the presence of this somatic CNV may reflect clonal expansion of cells rather than being the cause of the disorder. The other recurrent somatic chromosomal alterations identified in our study could unmask driver mutations for enchondromas. This hypothesis could be addressed with a target deep sequencing of these regions.

One Maffucci patient had a mosaic, complete 1p deletion (fig. 2). Rare chromosome 1 alterations, 1p11p21 in-

Fig. 4. Chromosomal anomalies in chondrosarcoma (MAF-190) using Affymetrix 100K SNP-chip pairwise copy number analysis. From the top, individual SNP probe signals (red dots); intensity ratio of alleles in tissue versus blood (blue curve in logarithmic scale); cytoband of each chromosome (black/white rectangles). Heterozygous SNPs are represented by green bars, and discrep-

ancy calls between tissue and its corresponding blood by pink bars. Pairwise copy numbers between the same allele in tissue and in blood are represented by green and red lines, respectively, and the LOH curve is shown in blue. Deletions are delineated by horizontal black lines and amplifications by double horizontal lines.

version (MS) and 1p11p31.2 deletion (OD), have been reported. 1p36 and 1p13.2p22.1 deletions have been detected in chondrosarcomas [Matsumoto et al., 1986; Ozisik et al., 1998; Rozeman et al., 2004]. Thus, chromosome 1p may contain genetic material that is involved in the pathogenesis of the lesions in MS.

Mutation on arginine 132 of *IDH1* has been found in chondrosarcoma [Amary et al., 2011]. However, the majority of the alterations identified on the single chondro-

sarcoma analyzed were large somatic chromosomal alterations: 3q26q29, 6q13q27, 9p, 10q, 12p and 13. Similar genetic anomalies have been reported in other chondrosarcomas, suggesting to be pathogenic [Rozeman et al., 2006] (table 1). The 10q deletion region contains the *RET* oncogene, which has been implicated in the pathogenesis of multiple endocrine neoplasia and papillary thyroid carcinomas, and LOH on 10q has been found in early-stage chondrosarcomas [Nannya et al., 2005]. Monosomy

of chromosome 13 involves *COL4A2*, *RAB20* and *ING* genes. *COL4A2* is a structural component of basement membrane involved in (cartilaginous) differentiation [Romeo et al., 2007]. Missense mutations in this gene are associated with ocular defects and vascular stability, whereas *RAB20* and *ING* genes are tumor suppressors [Amillet et al., 2006; Feng et al., 2006; Favor et al., 2007]. We also identified amplifications 12q12 and 19p13.3q12 gain as reported in a chondrosarcoma of OD [Casorzo et al., 2003; Rozeman et al., 2006], and it is also frequently seen in the Mitelman cancer database.

No common abnormality was detected among the enchondromas and spindle cell hemangiomas. Tissue heterogeneity greatly impedes the detection of somatic alterations limited to a subset of cells. In this situation, the sensitivity of the currently available algorithms for LOH detection by SNP arrays is restricted [Amyere et al., 2013].

The array data was also analyzed for possible UPID, a mechanism well known to be involved in some cancers, such as neuroblastoma [George et al., 2007]. We have shown that it is the most frequent mechanism leading to complete localized glomerulin dysfunction in glomerulonephrosis malformations [Amyere et al., 2013]. Nevertheless, no somatic copy number neutral LOH region >85 kb was detected in the Maffucci samples.

In conclusion, we identified frequent somatic alterations in 2p22.3, 2q24.3 and 14q11.2 that may play a role in causing enchondroma and spindle cell hemangioma. These regions probably contain genes involved in pathways that are dysregulated in MS. In addition, large chromosomal alterations were detected in a chondrosarcoma from a Maffucci patient; some of these changes are recurrent in other published studies. We could not detect any LOH or UPID region larger than 85 kb, excluding this as a likely pathogenic mechanism. As we did not identify shared gains or losses of a single DNA segment in all of the individuals, the underlying genetic basis of MS may be a single gene defect with pleiotropic effects.

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