Enchondromatosis (Ollier Disease, Maffucci Syndrome) Is Not Caused by the PTHR1 Mutation p.R150C

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HUMAN MUTATION 24:466–473 (2004)

RAPID COMMUNICATION

Enchondromatosis (Ollier Disease, Maffucci Syndrome) Is Not Caused by the PTHR1 Mutation p.R150C

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Communicated by Arnold Munnich

Enchondromatosis (Ollier disease, Maffucci syndrome) is a rare developmental disorder characterized by multiple enchondromas. Not much is known about its molecular genetic background. Recently, an activating mutation in the parathyroid hormone receptor type 1 (*PTHR1*) gene, c.448C>T (p.R150C), was reported in two of six patients with enchondromatosis. The mutation is thought to result in upregulation of the IHH/PTHrP pathway. This is in contrast to previous studies, showing downregulation of this pathway in other cartilaginous tumors. Therefore, we investigated PTHR1 in enchondromas and chondrosarcomas from 31 enchondromatosis patients from three different European countries, thereby excluding a population bias. PTHR1 protein expression was studied using immunohistochemistry, revealing normal expression. The presence of the described PTHR1 mutation was analyzed, using allele-specific oligonucleotide hybridization confirmed by sequence analysis, in tumors from 26 patients. In addition, 11 patients were screened for other mutations in the *PTHR1* gene by sequence analysis. Using both allele-specific oligonucleotide hybridization and sequencing, we could neither confirm the previously found mutation nor find any other mutations in the *PTHR1* gene. These results indicate that the *PTHR1* gene is not, in contrast to previous suggestions, the culprit for enchondromatosis. Hum Mutat 24:466–473, 2004. © 2004 Wiley-Liss, Inc.

KEY WORDS: Ollier disease; Maffucci syndrome; enchondromatosis; chondrosarcoma; PTHR1

DATABASES:
PTHR1 – OMIM: 168468, 166000 (enchondromatosis); GenBank: NM_000316.2, U22401, U22402, U22403, U22404, U22405, U22406, U22407, U22408, U22409

INTRODUCTION

Ollier disease (enchondromatosis, MIM# 166000) is a rare developmental disorder that seems to distribute in a nonhereditary manner. The syndrome is characterized by skeletal deformities and multiple enchondromas, often with unilateral predominance [Ollier, 1900]. Enchondromas are benign cartilaginous lesions, located in the metaphyseal medulla of bone. The solitary form of enchondroma is far more common than the rare occurrence within the context of enchondromatosis.

Maffucci syndrome is a rare variant of enchondromatosis characterized by both multiple enchondromas and benign hemangiomas [Maffucci, 1881]. Apart from these two enchondromatosis syndromes, recognized by the World Health Organization (WHO) [Mertens and Unni, 2002], there are other extremely rare distinct variants described, such as spondyloenchondromatosis (MIM# 271550) [Chagnon et al., 1985; Halal and Azouz, 1991; Uhlmann et al., 1998; Kozlowski and Masel, 2002].

Malignant transformation of enchondromas to chondrosarcomas occurs in <1% of solitary cases [Mulder et al., 1993], and 25 to 30% of enchondromatosis cases [Mulder et al., 1993; McDermott et al., 2001]. Chondrosarcomas can be either conventional central (83%) or secondary peripheral (17%), with central chondrosarcomas arising de novo, or as a result of malignant transformation of an enchondroma [Bertoni et al., 2002].

Received 4 February 2004; accepted revised manuscript 26 May 2004.

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Grant sponsor: ZonMW; Grant number: 908-02-018; Grant sponsor: Optimix Foundation for Fundamental Research.

DOI 10.1002/humu.20095
Published online in Wiley InterScience (www.interscience.wiley.com).

Secondary peripheral chondrosarcomas arise, by its definition, secondarily within the cartilaginous cap of its benign precursor, osteochondroma, which is solitary or occurs in the context of multiple osteochondromas (MO) syndrome [Bovée and Hogendoorn, 2002]. In contrast to enchondromatosis, MO demonstrates a clear autosomal dominant inheritance pattern, caused by *EXT1* or *EXT2* mutations. EXT mutations are postulated to disturb, via heparan sulfate proteoglycans, the Indian hedgehog (IHH)/parathyroid hormone–related peptide (PTHrP) negative feedback loop within the normal human growth plate [Bovée and Hogendoorn, 2002; Hogendoorn et al., 2003], and absence of these signaling molecules has been demonstrated in osteochondromas [Bovée et al., 2000b].

Three genetic studies have been reported on enchondromatosis. Cytogenetic analysis showed a interstitial deletion at chromosomal region 1p in one case [Ozisik et al., 1998], loss of heterozygosity (LOH) at 13q14 and 9p21 and p53 overexpression in chondrosarcoma in another case [Bovée et al., 2000a], and recently, Hopyan et al. [2002] described a mutation in the parathyroid hormone receptor 1 (PTHR1, MIM# 168468), PTHR1 c.448C>T (p.R150C PTHR1), which was found in two of six patients with Ollier disease, one a germline and the other probably a somatic mutation. This mutation was absent in 50 solitary chondrosarcomas and 100 unaffected individuals [Hopyan et al., 2002]. The single nucleotide change in PTHR1 codes for an amino acid change, which results in a constitutively active receptor, with reduced translocation to the membrane (as shown by Western blot), reduced PTHrP binding (by cell transfection), and a decrease in chondrocyte differentiation (PTHR1 p.R150C mouse model). The authors argued that, by upregulation of IHH/PTHrP signaling, this leads to the formation of enchondromas [Hopyan et al., 2002].

It is difficult to perceive that upregulation of IHH/PTHrP signaling would lead to enchondroma formation, knowing that downregulation of IHH/PTHrP signaling, as a result of EXT mutation [McCormick et al., 1999; Bovée et al., 2000b; Bovée and Hogendoorn, 2002; Hogendoorn et al., 2003], plays a role in osteochondroma formation. We therefore investigated the role of PTHR1 in enchondromatosis, by studying the expression of the PTHR1 protein and screening for the specified p.R150C *PTHR1* mutation. In addition, in 11 patients, all exons of the *PTHR1* gene were screened for mutations.

MATERIALS AND METHODS

Patient Data

In total, 23 enchondromas and 18 chondrosarcomas from 31 patients with enchondromatosis were obtained. The samples were collected from the files of The Netherlands Committee for Bone Tumors (three patients; six samples), the Leiden University Medical Center (nine patients; 14 samples), Rizzoli Orthopedic Institute, Bologna, Italy (15 patients; 15 samples), and the Institute of Pathology, University of Bern, Switzerland (four patients; six samples).

Patient data were obtained by review of clinical charts and radiographs. Grading was performed according to Evans et al. [1977]. Patients were included if at least two different sites were affected by enchondromas and or chondrosarcomas [Mertens and Unni, 2002]. For patient descriptions, see Table 1. All procedures were performed according to the local ethical guidelines.

PTHR1 Immunohistochemistry

In 24 of the 31 cases, paraffin blocks were available for immunohistochemistry. From each patient, one tumor was used. Sections (4 μm) were stained with the polyclonal PTHR1 antibody from Babco (Eurogentec, San Diego, CA) in a 1 in 75 dilution, using a citrate antigen retrieval as previously described [Bovée et al., 1998]. As a positive control, skin was used, and vessel walls and osteoblasts served as an internal positive control. Two independent observers scored the sections.

DNA Isolation

Tumor DNA was isolated from paraffin-embedded (n = 24), as well as fresh frozen material (n = 12). From 13 patients normal DNA was also obtained (10 from paraffin, two from frozen tissue, and one from blood). The tumor percentage as determined by hematoxylin and eosin stained slides was at least 70%. DNA from paraffin-embedded material was isolated as described earlier [De Leeuw et al., 2000]. Some samples were microdissected to enrich for tumor percentage or to obtain normal DNA. Samples isolated from paraffin-embedded material with a low DNA concentration were concentrated using the DNA Clean and Concentrator kit (Zymo Research, Orange, CA). DNA from fresh frozen material was isolated using a wizard genomic DNA purification kit (Promega, Madison, WI), and DNA of blood was isolated using a salting out procedure according to Miller and Polesky [1988].

R150C PTHR1 PCR

Genomic DNA of the *PTHR1* gene (NM_000316.2) containing the position of the p.R150C PTHR1 mutation was amplified using the PCR primers 5′-TGACACACTCGCTGTAGTTGG-3′ (PTHR1-F) and 5′-TTGGAGCTAGGGGTTCAGTG-3′ (PTHR1-R), generating a 154-bp product. As a positive control, DNA isolated from a normal placenta was used, both to serve as a control for the PCR and as a wild-type control for the allele-specific oligonucleotide (ASO) hybridization.

Construction of the Mutant Sequence

A plasmid containing the PTHR1 fragment with the c to t substitution (c.448C>T) was constructed using mutation specific PCR [Ho et al., 1989]. Detailed description of the construction can be obtained on request.

ASO Hybridization

For detection of the p.R150C PTHR1 mutation, PCR fragments of tumor and control DNA were electrophoresed on a 2% agarose gel and blotted to nylon membranes (Hybond™-N+; Amersham Biosciences, Piscataway, NJ) as described earlier [Devilee et al., 1991]. A separate blot was made with a dilution series of a mix of wild-type and constructed mutant PCR products in different concentrations. The blots were hybridized with α-^{32}P oligonucleotides specific for wild-type (5′-ACGCTGTGACCGCAATGGCA-3′) or mutant (5′-ACGCTGTGACTGCAATGGCA-3′). Oligonucleotides were labeled in 6 μl containing 20 pmol oligonucleotide, 1μl [α-^{32}P] ATP (10 μCi), 1× kinase buffer (70 mM Tris, pH 7.6; 10 mM $MgCl_2$), 9 units T4 PNK kinase (USB; US Biochemicals, Cleveland, OH) for 1 hr at 37°C and 5 min at 65°C. The blot was hybridized overnight with a hybridization mix containing 0.5 M $NaHPO_4/NaH_2PO_4$ (pH 7.0), 7% SDS at 65°C (wild-type) and 68°C (mutant). After washing the filters

TABLE 1. Patient Descriptions and PTHR1 Protein Expression Data

Pat.	M/F	Syndrome	Family history	Sample	Age at operation (yrs)	Diagnosis	Location	Abnormal spine	Other affected sides	PTHR1 protein expression
1	F	Ollier	No	1	25	EC	Hand	No	Hand L+R, humerus R, ribs	
2[a]	F	Ollier	No	2.1	30	CS-I	Distal femur R	No	Prox. tibia R, prox. femur R, prox. humerus R	
3[a]	M	Ollier	No	3.1	24	EC	Hand R	No	Hand L	
4[a]	F	Maffucci	Yes[b]	4.1	41	CS-dediff.	Prox. humerus L	No	Hand R+L	
5[a]	F	Ollier	No	5.1	38	EC	Prox. humerus L	No	Hand R+L, pubis, prox. Femur, prox. humerus L	
6	M	Ollier	No	6	5	EC	Prox. humerus L	No	femur L	+
7[a]	M	Maffucci	Yes[c]	7.1	36	CS-III	Pelvis	No	Hand R+L, prox. femur R	
8	F	Maffucci	No	8	38	CS-II	Pelvis	No	Humerus R+L, femur R+L	+
9	F	Ollier	No	9	15	EC	Hand	No	Hand L+R, pelvis	–
10	M	Ollier	No	10	30	EC	Hand R	No	Femur L, tibia L, hand L	
11[a]	M	Ollier	No	11.1	23	CS-dediff.	Spinal column	Tumor		–
12	F	Ollier	No	12.1	6	EC	Femur L	No	Os ilium L, 3 locations in tibia L	–
				12.2	6	EC	Tibia L			
13	M	Ollier	No	13.1	12	EC	Hand phalanx L	No	Hand phalanges dig 1-4 L	++
				13.2	12	EC	Hand R			
14	M	Ollier	No	14	8	EC	Distal fibula R	No		–
15[a]	F	Ollier	No	15.1	27	EC	Pelvis L	No	Femur L	
				15.2	27	CS-II	Prox. fibula R			
				15.3	28	EC	Hand phalanx R			
16[a]	F	Ollier	No	16.1	62	CS-I	Distal femur L	No	Tibia L, pelvis L	
				16.2	62	CS-I	Distal femur L			
17[a]	M	Ollier	No	17.1	42	CS-I	Hand metacarpal L	No	Foot L, hand L+R, femur L+R, humerus L, rib L+R, acetabelum R, ulna L	++
				17.2	47	CS-I	Foot metatarsal L			
18	F	Ollier	No	18	15	CS-I	Humerus R	No	Femur R, scapula R,	
19[a]	M	Ollier	No	19.1	40	CS-I	Prox. tibia R	No	Femur R, fibula L, scapula L+R	
				19.2	40	CS-II	Scapula R			
20[a]	F	Ollier	No	20.1	23	CS-II	Distal femur	No	Femur L+R, tibia L+R, ankle L, lung metastases	++
21[a]	M	Ollier	No	21.1	26	CS-I	Left tibia L	No	Skull, rib, tibia L+R, fibula L+R, femur L, hand L, foot L	++
				21.2	26	EC	Femur			
22	F	Ollier	No	22.1	25	EC	Hand phalanges R	No	Humerus L+R, rib R, os pubis R, hand L+R, femur R, tibia R	++
				22.2	16	EC	Hand			
23	M	Ollier	No	23.1	68	CS-II	Humerus	No		++
				23.2		CS-III	Humerus			
24	M	Ollier	No	24	18	CS-I	Femur R	No	Tibia R	
25[a]	M	Ollier	No	25.1	15	EC	Hand phalanx L		Hand L+R	++
26	M	Ollier	No	26	14	EC	Distal femur L	Yes[d]	Femur L	++
27	F	Ollier	No	27	26	EC	Hand R	No	Humerus R, ribs	
28	M	Ollier	No	28	31	EC	Prox. humerus R	No	Prox. tibia R, prox. femur R	
29	F	Ollier	No	29	38	EC	Prox. tibia L	No	Hand L+R	
30	F	Ollier	No	30	41	EC	Prox. femur R	No	Hand L+R, humerus L, os pubis	
31	M	Ollier	No	31	26	EC	Hand R	No	Hand L	

[a]Of these patients normal DNA was available.
[b]One of the parents affected by Ollier disease.
[c]One of the parents affected by Maffucci syndrome.
[d]Radiographical evidence of spinal deformation, no platyspondyly.
pat, patient; M, male; F, female; EC, enchondromas; CS, chondrosarcoma; dediff, dedifferentiated; prox, proximal; R, right; L, left; I, II, III are grade I, grade II, grade III; –, no PTHR1 protein staining; +, weak PTHR1 protein staining; ++, positive PTHR1 protein staining.

with 1 × SSC/0.5% SDS, they were exposed to a Phosphor Imager screen (Amersham Biosciences, Piscataway, NJ).

The blot containing the samples was first hybridized with the mutant probe, then stripped at 68°C with 0.1 × SSC/0.5% SDS and subsequently hybridized with wild-type probe. Complete stripping of the blot was checked by phosphor imaging.

Signal intensities were scored as peak heights as detected by ImageQuant (Molecular Dynamics, Sunnyvale, CA).

Sequencing for the p.R150C PTHR1 Mutation

For 19 cases, the absence of the mutation, as detected by ASO hybridization, was confirmed by sequence analysis of the PCR fragment, using the forward and/or reverse PCR primer. Among the samples chosen were those tumor samples with a relative signal comparable and lower to the wild-type sample. The PCR products of these samples were purified using QIAGEN QIAquick PCR Purification Kit (QIAGEN, Germantown, MD) prior to sequencing.

Sequencing was performed using the ABI PRISM® Big Dye Terminators v. 2.0 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Samples were run on an ABI 3700 Genetic Analyzer (Applied Biosystems).

Sequencing of the Entire PTHR1 Gene

Fresh frozen tissue to obtain high molecular weight DNA was available for 11 patients affected by Ollier disease; five of which were not analyzed by the ASO hybridization. Genomic DNA was subjected to direct sequencing for the PTHR1 gene (NM_000316.2). Patient data are resumed in Table 1 (Patients 17, 19, 21, 22, 24, 25, and 27–31). All coding exons, including exon–intron boundaries, were amplified according to primers described by Schipani et al. [1995] and with additional primers described in Table 2, based on the sequences U22401–U22409.

TABLE 2. Sequence of the Additional Designed Nucleotide Primers of Each Exon of *PTHR1*

PCR product	Size (bp)	Primer sequence
S	226	5′-AGCTCTGCACCCCCTACC-3′
		5′-GCGTGCCTTAGACCTACTCC-3′
E1	232	5′-AAAGTCCTGCCTGTGGTCTG-3′
		5′-AGCCTTCACCTGGCTCTGTA-3′
E2	167	5′-AGGGAAGCCCAGGAAAGATA-3′
		5′-TCACATCAGAGGGACAGTGC-3′
E3	216	5′-TCCCCTACCCTGTCTGTCTC-3′
		5′-GAGGTCTCGAGGCACTGAAC-3′
G	237	5′-TTGGAGCTAGGGGTTCAGTG-3′
		5′-GTGTGGGTGGGAGTGAATTT-3′
M1	153	5′-GCCTCTTGCTCTTACCCTGA-3′
		5′-GATGAGCACAGCTACGGTGA-3′
M2	175	5′-TGTTCCTGTCCTTCATGCTG-3′
		5′-GGCAGAGGGGTACTCACGTA-3′
M3	176	5′-CCCTGCCCTCTGACTAACAC-3′
		5′-TGTGAAGCCCCACAGGTACT-3′
M4	173	5′-GCTGTGTGGGTCAGTGTCAG-3′
		5′-GGCTGGACTGAGAACTCCTG-3′
EL2	153	5′-ACTTCCAAAGAGGCCTGTGA-3′
		5′-TTGAGGCATTAGCTCCCATC-3′
M5	194	5′-CACTCCCCACAGCTCAACTT-3′
		5′-ATGGGCCACTGTCTTTCACT-3′
M6-7	243	5′-CATTGTCTTCATGGCCACAC-3′
		5′-GCCCTATGCCAACACTGTCT-3′
T1	162	5′-TTGGGAGACACACCTGACTG-3′
		5′-TCACACTTGTGTGGGACACC-3′
T2	150	5′-CTGCCCTGCTACAGGAAGAG-3′
		5′-TTCCCTGTTTTTCCTCTTGG-3′

RESULTS

Patient Data

In total, 41 tumors from 31 patients with enchondromatosis were collected. Clinical details of all patients are shown in Table 1.

PTHR1 Immunohistochemistry

Staining results are shown in Table 1. Due to loss of attachment of the tissue section from the slide, as a result of the antigen retrieval procedure, 10 of the 24 samples could not be evaluated. Eight out of 14 samples were scored as positive for the presence of PTHR1 protein, five demonstrated only weak positivity, while one tumor was completely negative with a positive internal control. The staining was mainly found in the nucleus, but cytoplasmic staining was also found (Fig. 1). The results were similar to those obtained for 20 sporadic chondrosarcoma cases [Bovée et al., 2000b].

ASO Hybridization: p.R150C PTHR1 Mutation

Of the 36 samples from 26 patients (Patients 1–26), 33 samples resulted in a PCR product of the expected size, though the amount of final PCR product varied considerably. The three samples failing were DNA samples isolated from paraffin-embedded tissues, one failing normal and tumor sample, the third only failing the tumor sample.

Blots containing PCR products from the PTHR1 fragment were hybridized for the mutant sequence, resulting in strong hybridization signals with intensities that decreased linear according to a dilution series of PCR products containing the constructed mutant as shown in Figure 2a. However, we did see cross-hybridization of the mutant probe with wild-type PCR products that could not be overcome by more stringent hybridization (Fig. 2a). The wild-type probe showed strong signals with the wild-type control (placental DNA from a healthy donor) and samples, but no cross-hybridization with the constructed mutant (Fig. 2b).

In the dilution experiment, the mutant sequence could be detected in a background of wild-type sequence containing 90% wild-type PCR product and 10% constructed mutant PCR product. Using this experiment, a cutoff value for the probes was determined (0.20). The signal threshold of the mutant oligonucleotide was defined as the strongest signal obtained by phosphor imager analysis, for the wild-type control fragment with the mutant oligonucleotide divided by the signal of the wild-type oligonucleotide for that sample. All patient samples showed a signal for the mutant oligonucleotide/wild-type oligonucleotide ratio below this threshold, ranging from 0.03 to 0.19. In contrast, the signal of constructed mutant, even in a 1 in 10 dilution, was above this threshold (value 0.26).

Sequencing for the p.R150C PTHR1 Mutation

A total of 19 samples were selected for sequencing to confirm the results found in the ASO hybridization. All

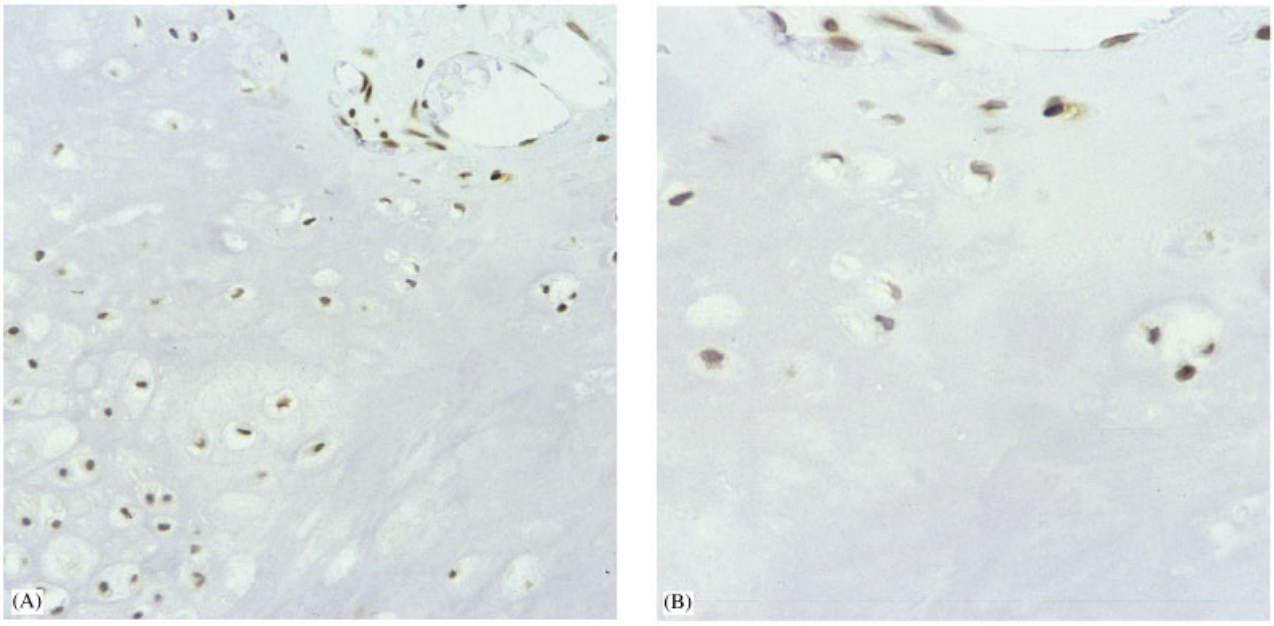

FIGURE 1. **Immunohistochemical staining for the PTHR1 protein. Sample 22.1, an enchondroma of the phalanx, showing positive PTHR1 expression in both nucleus and cytoplasm of tumor cells. The endothelial cells, serving as an internal control, also show PTHR1 expression. A: 50 ×. B: 100 ×. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]**

19 samples showed the wild-type sequence (reviewed but not shown).

Sequencing of the Entire PTHR1 Gene

From the 11 patients for whom the whole PTHR1 gene was sequenced, all PCR reactions resulted in sequencing products. No mutations and/or polymorphisms were detected in the coding exons of the gene.

DISCUSSION

The PTHR1 protein is important in chondrogenesis and skeletogenesis and is involved in the IHH/PTHrP feedback loop present in the growth plate [Lanske et al., 1996; Van der Eerden et al., 2000; de Crombrugghe et al., 2001; Hogendoorn et al., 2003]. IHH binds to its receptor Patched (Ptc) after diffusion, presumably under mediation of heparan sulfate proteoglycans (HSPG) of which the biosynthesis is mediated by EXT. The binding results in PTHrP expression, which then binds to PTHR1 in the late proliferating zone [Erlebacher et al., 1995], resulting in upregulation of Bcl-2 [Amling et al., 1997; Van der Eerden et al., 2000]. This signaling regulates the pace of chondrocyte differentiation by delaying the progression of chondrocytes towards the hypertrophic zone, allowing longitudinal bone growth [Van der Eerden et al., 2000].

Deregulation of this feedback loop can result in many different syndromes. For instance, in patients with Blomstrand chondrodysplasia (MIM# 215045), inactivating mutations have been identified in *PTHR1* [Gardella and Juppner, 2001], resulting in accelerated chondrocyte differentiation and premature ossification [Leroy et al., 1996]. In contrast, constitutive active *PTHR1* mutations have been identified in patients with Jansen metaphyseal chondrodysplasia (MIM# 156400) [Gardella and Juppner, 2001]. These patients have a delay in chondrocyte differentiation, in vascular invasion, and a reduction or absence of mineralization of bone elements that are formed through the endochondral process [Schipani et al., 1997]. This syndrome shares some radiographical and histological features with enchondromatosis, like the presence of radiolucent areas containing noncalcified cartilage [Jaffe, 1972].

In patients with MO mutations in the *EXT* genes are found, postulated to lead to a downregulation of the IHH/PTHrP pathway [McCormick et al., 1999; Bovée and Hogendoorn, 2002]. Indeed, in osteochondromas, that are histologically comparable to the human growth plate, absence of IHH/PTHrP signaling was demonstrated [Bovée et al., 2000b]. Upon malignant transformation of osteochondromas, upregulation of PTHrP and Bcl-2 was detected, and this was also found during progression of low- toward high-grade conventional central chondrosarcomas.

Surprisingly, the p.R150C mutation is described to lead to an upregulation of IHH [Hopyan et al., 2002]. It is difficult to understand that both upregulation (in enchondromatosis) and downregulation (in MO) of the same IHH/PTHrP pathway would cause benign cartilaginous tumors. We therefore wanted to further investigate PTHR1 in a large series of enchondromatosis patients. We looked at the PTHR1 protein expression in 14 patients with enchondromatosis, revealing normal expression in 13 cases. Three of five weakly-staining samples originated from young patients (ages 6, 15, and

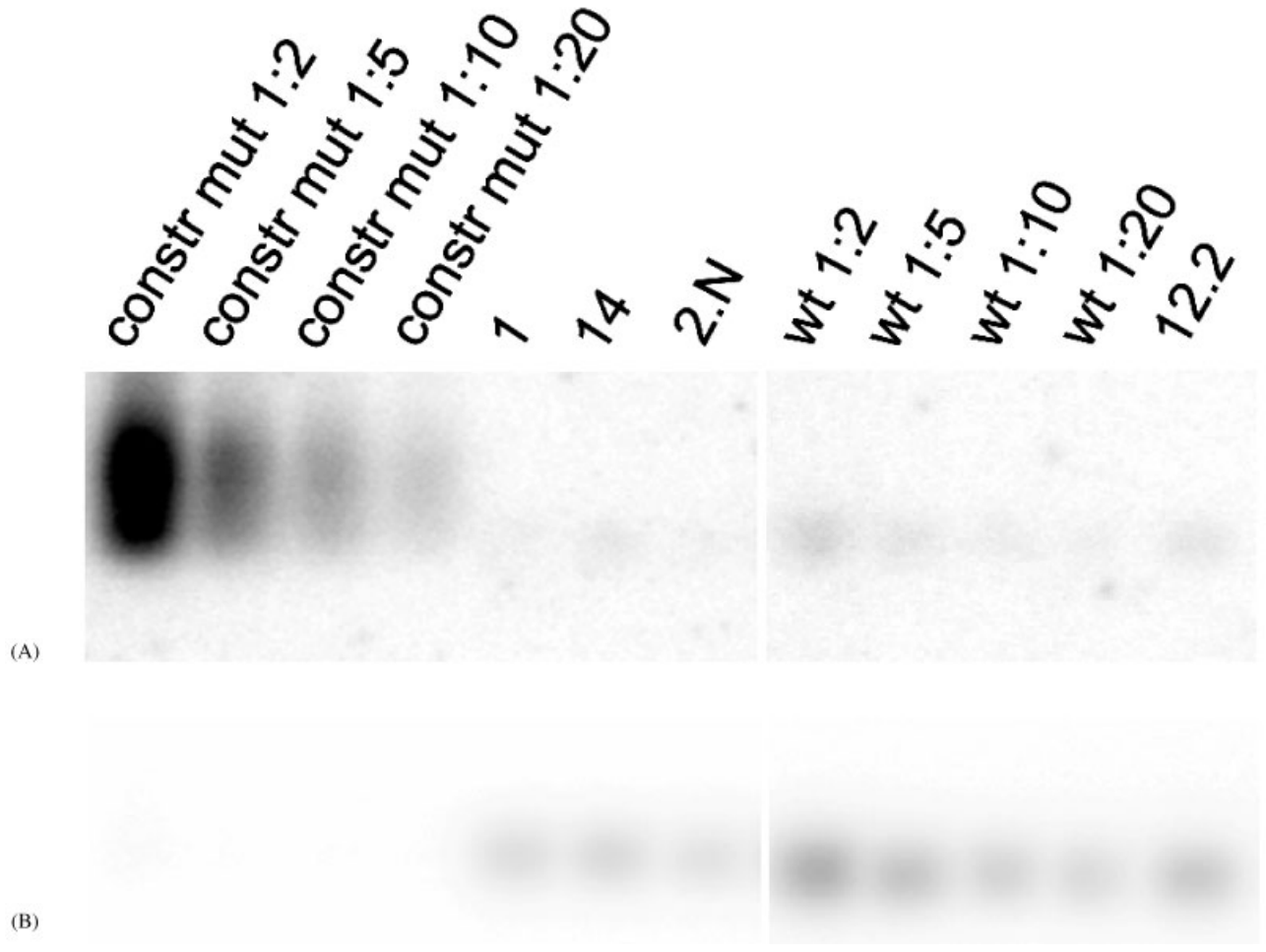

FIGURE 2. **ASO hybridization of the samples with labeled γ-^{32}P mutant and wild-type oligonucleotides. A: Blot of ASO hybridization with mutant oligonucleotide. B: Blot of ASO hybridization with wild-type oligonucleotide, showing the same samples as seen in A. constr mut, constructed mutant; wt, wild type.**

23 years). This suggests that there may be an age-related expression of PTHR1.

The mutation described by Hopyan et al. [2002] in two of six (33%) enchondromatosis patients was not found in the 31 enchondromatosis patients that were the subject of this article. Sequencing of all exons, including exon–intron boundaries, of the PTHR1 gene in 11 of these patients also did not reveal any other mutations. The possible presence of an intronic splice mutation, located outside the sequenced products, is not likely since this would most probably lead to inactivation of the PTHR1 protein, as seen in Blomstrand chondrodysplasia [Zhang et al., 1998; Jobert et al., 1998], and not to a receptor with increased signaling, as was described by Hopyan et al. [2002]. This indicates that the *PTHR1* gene is not involved in Ollier disease and Maffucci syndrome in our large multinational series. A possible explanation for this discrepancy could be that the p.R150C mutation described by Hopyan et al. [2002] is specific for the Canadian population, i.e., a founder mutation.

Technically, one could argue that the level of contamination with normal tissue in our tumor samples is just too high for detection of somatic mutations by sequence analysis (in our samples the tumor percentage was at least 70%). However, the detection level for the p.R150C mutation using ASO hybridization is high enough to detect even those cases in which the mutated sequence is present in only 10% of the total sample as shown by the dilution experiment. Thus, with the mutation described by Hopyan et al. [2002] as being heterozygous, we should have been able to detect the mutation if it was present.

Another explanation for the discrepancy between the results of Hopyan et al. [2002] and our results may be found in the exact definition of the clinical syndrome, the classification of which is confusing. Enchondromatosis can be divided using several subclassifications [Halal and Azouz, 1991; Uhlmann et al., 1998; Kozlowski and Masel, 2002]. The two most important ones are Ollier disease and Maffucci syndrome, both accepted by the WHO [Mertens and Unni, 2002].

Extremely rare is spondyloenchondromatosis, which was described to be autosomal recessive. Its radiographic features include irregularly distributed, mostly discrete enchondromas of long tubular bones and generalized severe platyspondyly with mild or no involvement of hands and feet [Schorr et al., 1976; Halal and Azouz, 1991]. Generalized enchondromatosis, with patients having platyspondyly and metaphyseal manifestations of enchondromatosis with severe involvement of the hands and feet, has also been described [Spranger et al., 1978; Halal and Azouz, 1991].

If the patient carrying the germline p.R150C PTHR1 mutation [Hopyan et al., 2002], belongs to one of these rare subclasses of enchondromatosis instead of having Ollier disease, this mutation may be specific for this rare variant of enchondromatosis. Hopyan et al. [2002] describe that one of the two patients having the p.R150C PTHR1 mutation inherited the mutation from his father, who had short statue, similar to a patient described by Halal and Azouz [1991] who was diagnosed with "generalized enchondromatosis," and following an autosomal recessive inheritance pattern. Our population consisted strictly of patients with Ollier disease or Maffucci syndrome, lacking platyspondyly after reviewing their clinical charts and radiographs.

In conclusion, in our large, well-characterized, multinational group of enchondromatosis patients, we cannot confirm the involvement of mutations in the PTHR1 gene, indicating that PTHR1 is not causative for enchondromatosis in contrast to previous reports.

ACKNOWLEDGMENTS

We thank Roland Fischer for collecting part of the patient data, and Ayse Yavas, Shohreh Keshtkar, and Veronica Maini for expert technical assistance.

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