

Detection of exostosin glycosyltransferase gene mutations in patients with non-hereditary osteochondromas of the mandibular condyle

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Abstract. Exostosin glycosyltransferase (EXT) 1 and EXT2 have been identified as causative genes in osteochondroma; however, it is not known whether these genes are also involved in condylar osteochondromas. The aim of this study was to identify EXT1 and EXT2 mutations in patients with non-hereditary osteochondromas of the mandibular condyle. DNA was obtained from resected tissues (cartilage cap) of 12 patients with solitary condylar osteochondromas. The exons, 3',5'-untranslated regions and intron-exon boundaries of EXT1 and EXT2 were amplified by polymerase chain reaction and the products were sequenced directly. Through direct sequencing, four genetic variations of EXT1 in 4 cases and three variations of EXT2 in 5 cases were identified. The intronic alteration of the EXT2 gene, occurring in 2 cases, was novel, whereas the other alterations had been previously reported. Nonsense somatic mutations were detected in tumor DNA. Our study extended the mutational spectrum in EXT1 and EXT2 and may facilitate a better understanding of the pathophysiology of condylar osteochondromas.

Introduction

Osteochondroma is a cartilage-capped bony projection arising from the external surface of bone, containing a marrow cavity that is continuous with that of the underlying bone (1). Osteochondroma is the most common benign bone tumour,

mainly arising in the juxta-epiphyseal region of long bones (1). Approximately 15% of osteochondromas occur in the context of multiple osteochondromas (MO), previously referred to as hereditary multiple exostoses, which is an autosomal dominantly inherited disorder (2,3), while the majority of osteochondromas present as solitary (non-hereditary) lesions. Solitary and multiple osteochondromas are histologically indistinguishable (4). Osteochondromas have been associated with defects in the exostosin glycosyltransferase (EXT) 1 gene on chromosome 8q24.11-q24.13 (MIM 608177) and EXT2 on chromosome 11p12-p11 (MIM 608210) (5-7).

Osteochondroma may be an incidental finding, or diagnosed due to secondary events, such as esthetic or mechanical problems. Osteochondromas of the mandibular condyle have been associated with facial asymmetry, preauricular pain or edema, occlusal disorders, limitation of mouth opening, even condylar neck fracture. The most severe complication is malignant transformation into chondrosarcoma. According to Roychoudhury *et al* (8), by 2011 at least 108 cases had been reported in the English-language literature; those studies were mainly focused on clinical reports, such as clinical manifestations, radiological findings, treatment and prognosis, whereas the study on pathogenesis and molecular biology remains at the initial stage. However, it is not known whether the EXT genes are also involved in condylar osteochondromas. We performed a mutation analysis of the EXT1 and EXT2 genes in 12 cases of solitary osteochondroma of the condyle, to estimate the distribution of mutations that lead to the development of condylar osteochondromas.

Patients and methods

Patients. A total of 12 sporadic patients diagnosed with condylar osteochondroma were included in this study. Patients with a solitary osteochondroma were selected based on a review of their pathological, clinical and radiological data. After obtaining written informed consent, cartilage from the cap of the resected specimens was collected from each participant. The patients comprised 5 women and 7 men, with a mean age of 45.08 years (range, 23-76 years). The clinical characteristics of all the patients are summarised in Table I.

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Table I. Characteristics of 12 condylar osteochondroma patients.

Case	Gender	Age (years)	Side	Duration of symptoms	Symptoms
1	M	44	Right	>3 years	Facial asymmetry, LMO, occlusal disorder
2	F	76	Right	3 years	Facial asymmetry, LMO, occlusal disorder, pain
3	M	49	Left	1 months	Facial asymmetry, pain, clicking, occlusal disorder
4	M	65	Right	6 months	Numbness of right tongue base, pain, occlusal disorder
5	M	50	Right	8 months	Facial asymmetry, clicking, occlusal disorder
6	F	24	Left	4 months	Facial asymmetry, occlusal disorder
7	M	23	Left	2 years	Facial asymmetry, occlusal disorder
8	M	32	Right	>5 years	Facial asymmetry, LMO, pain
9	F	39	Left	7 years	Facial asymmetry, pain, occlusal disorder
10	F	61	Left	2 years	Facial asymmetry, clicking, LMO, occlusal disorder
11	M	39	Left	>2 years	LMO, pain
12	F	39	Right	2 months	Facial asymmetry

M, male; F, female; LMO, limitation of mouth opening.

Table II. Sequencing regions and primers of exostosin glycosyltransferase (EXT) 1 and EXT2 genes.

Gene	Exon	Sense (5'-3')	Antisense (3'-5')	Fragment size (bp)
EXT1	1	CGAGCGCAGGAGTAAACAC	ATTGATCCCAAGGAACGAA	824
	1	GAAAGGCATCCAGAGAAGG	GACTCAGGACAAAGAGGCAC	614
	1	AAAACGGCTTCAAAGTCTACG	TTGCTCAGTTCAGGCTCA	768
	2	GGGGTGGGGAACAAGAA	GGAAGTGGAGACAATGAAG	906
	3	GAAATGGGGTTTTAGCA	GTTATTGAAAGGGGTGG	626
	4	GAAGTGCTTGGGAGATAA	CGAAGGATGCCATTGAG	834
	5	AGTCTATTTTGGAAATGAGC	GAGATATTGGGATTGTGA	804
	6	GCTCTTCCTTTCACCTTT	TCTCTGTAACCCATCCCT	794
	7	CGGACACAGTTGGTTTT	CCACTTTGTAGATGAGGAA	624
	8	GATTTATCTTTGTACCCTCTTTGAC	ATGCAGAACACGCACCC	675
	9	AGATGTGTTTGTGTCTCACG	CCAAGTAAAATGTTACTCTAC	744
10	GGGAGTAATAATAGAACCTG	CAAATGGACTAAGACAAACT	711	
11	TCAGTTGCTAAGTCGTG	AACAAAGAAGTCTGGTTT	817	
EXT2	1	CGCCTGCCTGGGAAAAC	GGCTAGGAGAACAGGTGGGTA	548
	2	CTGCTGGGTCGGGACAA	GTTCCCACCGAATGTAACAAA	794
	3	TGGTCACAGTTACTTGGG	GGCAGACTACTCTTCACG	997
	4	TAACCAGGCTTCTCTAATG	CGCTACCTTCTCTCAGTAA	751
	5	GGGAAGTAAGGAAAGGGTAT	CTAAGGGCAATGTGAAGC	815
	6	AACTGTTCCTCAAATAAGATG	GGGGGTAAAAGCAAGATA	763
	7	AAGGTAGGCTGAGGTAAG	GTAAGGAAGGGACACG	776
	8	GTAGGGAGTGGGAGGTAAA	AATGGGGTGTGAGAAGGT	818
	9	ACATGGCTATTCTCATCAT	TGCCTCCTTACTTATCTCT	668
	10	GGGTTTGGGGAGAGAAT	GAGCAGAGATAAGAAAGGAGA	826
	11	TTGAAGCCAATTTGTTC	CTTTGTTTGTGAGTGTGCG	877
	12	TAATACAAATCAGGGCAGTT	GGCTCACAATACAATCCA	883
	13	TAATGCCTCCTTTTACC	GCCTTTATTCTGATACTGA	533
	14	GAAAGAGGAGAAAGAGCG	CCCTGAAAAATAATCCAGTA	827
	15	CATCTCCTGTTCACGTTCT	GCTGGTGTCTTTCCTGT	949
15	TGGAGAAGAGAAGCGTGT	GCAAAGCAGTTGTATAGCAG	736	

Table III. Characters of EXT genetic variations in condylar osteochondroma.

Gene	Case	Genetic variation	Identity in dbSNP	Region	Allele freq
EXT1	1	chr8_118830894_G/A	rs4876757	Intron	0.2688
	2	chr8_118830820_A/G	rs10955837	Intron	0.4446
	1	chr8_118834952_G/A	rs4355803	Intron	0.2848
	8	chr8_118819578_G/A	rs7837891	Exon 9	0.3681
EXT2	3	chr11_44117372_C/G	rs12800404	5'UTR	0.0403
	7	chr11_44117899_G/T	Novel	Intron	-
	12	chr11_44117899_G/T	Novel	Intron	-
	1	chr11_44129290_C/A	rs4755228	Exon 3	0.0893
	2	chr11_44129290_C/A	rs4755228	Exon 3	0.0893

EXT, exostosin glycosyltransferase; dbSNP, single-nucleotide polymorphism database; UTR, untranslated region.

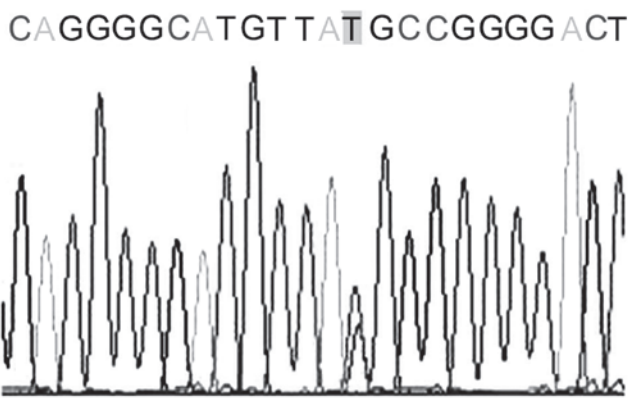


Figure 1. Novel polymorphic loci of exostosin glycosyltransferase 2 in samples 7 and 12.

Mutation analysis of EXT genes. DNA was extracted from the cartilage cap of resected specimens using the DNeasy Tissue kit (cat. no. 69581; Qiagen). DNA was polymerase chain reaction (PCR)-amplified with 13 pairs of PCR primers for 11 exons, 3',5'-untranslated regions (UTR) and intron-exon boundaries of the EXT1 gene, and 16 pairs for 15 exons, 3',5'-UTRs and intron-exon boundaries of the EXT2 gene (Table II). PCR cycling was performed on the 2720 Thermal Cycler (Applied Biosystems) using the Taq PCR Mastermix kit (Takara Biotechnology). All PCR programs included an initial denaturation of 5 min at 94°C, followed by 32 cycles of 15 sec at 94°C, 35 sec at 55-60°C, and 60 sec at 72°C; and a final extension step of 5 min at 72°C. The products were purified with QIAquick PCR Purification kit (cat. no. 28106; Qiagen) and the purified PCR products were sequenced using the forward and reverse primers. Automated sequencing was performed on an ABI PRISM® 3730XL Genetic Analyzer (Applied Biosystems).

Results

Genetic variations of EXT1. Through direct sequencing of all exons, intron-exon boundaries and 3',5'-UTRs, four genetic variations of EXT1 were identified, of which one was a synonymous coding variation (chr8_118819578_G/A in case 8),

and three were in intronic regions (chr8_118830894_G/A and chr8_118834952_G/A in case 1, and chr8_118830820_A/G in case 2), which have been previously reported (Table III).

Genetic variations of EXT2. Five variations of EXT2 were detected, two of which were synonymous coding variations (chr11_44129290_C/A in cases 1 and 2), two were in intronic regions (chr11_44117899_G/T in cases 7 and 12) and one was in the 5'-UTR (chr11_44117372_C/G in case 3). The genetic variations (chr11_44117899_G/T in cases 7 and 12) in EXT2 were novel (Fig. 1, Table III). All the mutations were confirmed by repeat PCR and sequencing.

Discussion

Osteochondroma was previously considered as a perversion in the direction of normal bone growth, resulting from aberrant epiphyseal development (9). However, later studies demonstrated that loss or mutation of EXT1 or EXT2 are crucial in the pathogenesis of solitary as well as hereditary osteochondromas (10,11). Germline mutations of either the EXT1 or EXT2 gene may be identified in >85% of the analyzed MO cases and MO families (12-16). Approximately 77-80% of intragenic EXT1 and EXT2 mutations are inactivating mutations (44-66% associated with EXT1 and 27% with EXT2) (16,17). These alterations result in truncated (non-functional) EXT proteins (18,19). The protein products of EXT1 and EXT2 are type II transmembrane glycoproteins and comprise a Golgi-localized hetero-oligomeric complex involved in heparan sulphate proteoglycan (HSPG) biosynthesis. Hameetman *et al* (20) found that EXT1 or EXT2 mRNA expression in osteochondromas was decreased; however, in non-hereditary tumors, only decreased EXT1 mRNA expression was detected. Decreased EXT1 or EXT2 mRNA expression in osteochondromas was associated with intracellular accumulation of HSPGs in the Golgi apparatus. It has been shown that a lack of HSPGs on the cell surface affects growth signaling pathways in the growth plate [e.g., Indian hedgehog (IHH) signaling] and, possibly, those in osteochondromas (21-23). In the growth plate, IHH requires interaction with HSPGs to diffuse through the extracellular matrix to its receptor (21).

Somatic mutations in the EXT genes are extremely rare in non-hereditary osteochondromas and have been described in only 3 cases (24–26). However, the observation that loss of heterozygosity (LOH) and clonal rearrangement at 8q24 (EXT1 locus) are as frequent in non-hereditary osteochondromas as are EXT1 gene mutations in patients with hereditary osteochondromas, suggests that EXT1 may be involved in the development of non-hereditary osteochondromas (10,11,27). By contrast, LOH at the EXT2 locus has been reported in only 1 case of non-hereditary osteochondroma (28).

Structural changes in the EXT1 locus have been reported in 10/30 non-hereditary and in 1/13 hereditary osteochondromas (10,11). LOH detected by microsatellite analysis using DNA isolated from the cartilaginous cap was found almost exclusively at the EXT1 locus (27). Fluorescence *in situ* hybridization revealed loss of the 8q24.1 locus in 27/34 (79%) osteochondromas (29). Finally, in 7/8 solitary osteochondromas, homozygous deletion of EXT1 was detected (30). Of note, EXT2 was found to be affected only in MO and not in solitary lesions. In our study, we analyzed 12 patients with solitary osteochondroma for the presence of mutations in the EXT1 and EXT2 genes. A novel nonsense mutation (chr11_44117899_G/T in the intronic region in cases 7 and 12) was identified. The other 7 variations were already in the databases. Apart from known polymorphisms, no sense somatic or germline mutations were detected in tumor DNA. The results were consistent with the rarity of EXT gene mutations in non-hereditary osteochondromas. As the LOH and clonal rearrangement in EXT genes are common, we should analyse the incidence of deletions and rearrangement in condylar osteochondromas.

In conclusion, we screened EXT1 and EXT2 mutations in 12 patients with non-hereditary osteochondromas of the mandibular condyle. To the best of our knowledge, this is the first study to identify mutations of EXT1 and EXT2 in condylar osteochondromas. Our study extended the mutational spectrum in EXT1 and EXT2 and may facilitate a better understanding of the pathophysiology of condylar osteochondromas.

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