A Novel Nonsense Mutation of the *EXT1* Gene in an Argentinian Patient with Multiple Hereditary Exostoses

A Case Report

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Multiple hereditary exostoses (MHE), also known as multiple osteochondromatosis, is an autosomal-dominant O-linked glycosylation disorder recently classified as EXT1/EXT2-CDG in the congenital disorder of glycosylation (CDG) nomenclature[1]. MHE is characterized by the presence of multiple cartilage-capped tumors, called "osteochondromas," which usually develop in the juxta-epiphyseal regions of the long bones. The prevalence of MHE is estimated at 1:50,000 in the general population[2,3]. The Online Mendelian Inheritance in Man (OMIM) database classified it as either 133700 or 133701, according to whether the mutations occurred in the *EXT1* or the *EXT2* gene. These genes are located at 8q24 and 11p11-11p12, respectively, and they encode the co-polymerases responsible for heparan sulfate biosynthesis. *EXT1* and *EXT2* are tumor suppressor genes of the EXT gene family. The *EXT1* gene contains eleven exons with a coding region of 2238 base pairs (bp), and the *EXT2* gene contains sixteen exons with a coding region of 2154 bp[4-7]. These genes encode two glycosyltransferases involved in heparan sulfate biosynthesis, exostosin-1 (EXT1) (EC2.4.1.224) and exostosin-2 (EXT2) (EC2.4.1.225), whose impairment leads to the formation of exostoses[5,8-10]. Inactivating mutations (nonsense, frameshift, and splice site mutations) in *EXT1* and *EXT2* genes represent the majority of mutations that cause MHE. An overview of the reported variants is provided by the online Multiple Osteochondroma Mutation Database[11].

The most important complication of MHE is the malignant transformation of osteochondroma to chondrosarcoma, which is estimated to occur in 0.5% to 5% of patients[7]. Chondrosarcomas arise de novo (primary) or as a result of a preexisting cartilage lesion (secondary). The biological aggressiveness of chondrosarcomas can be predicted by means of a histological grading system (grade I to grade III), based on three parameters: cellularity, degree of nuclear atypia, and mitotic activity[12,13].

In our case report, we investigated the clinical, radiographic, and genetic aspects of a patient with MHE with a severe phenotype and malignant transformation to chondrosarcoma. The patient was informed that data concerning her case would be submitted for publication, and she provided consent.

Case Report

Our patient, a thirty-two-year-old woman with MHE, was first identified with this disorder when she was twenty-six years old in the Orthopedic and Imaging Diagnosis Department of Children's Hospital of Cordoba, Argentina. She had developed a malignant transformation of a sessile osteochondroma in the left iliopubic region. She was included in the Argentinian Multiple Osteochondromatosis Program, which consisted of studies that had been approved by the Ethics Committee of the Children's Hospital of Cordoba (CIEIS) Act No. 95/2007. The phenotypic data were based on clinical examinations and radiographic measurements and were analyzed according to variables representing lesion quality: count, morphology, and location. The quality of lesions was classified as: (a) type-I lesions (the size of the lesions correspond to ≤25% of native bone size); (b) type-II lesions (the size of the lesions correspond to 26% to 49% of native bone size); (c) type-III lesions (the size of the lesions correspond to 50% to 74% of native bone size); and (d) type-IV lesions (the size of the lesions correspond to ≥75% of native bone size)[14]. The severity of the disease was assessed with five factors: age of onset, number of exostoses, absence or presence of vertebral location, stature, and functional rating (good

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TABLE I Clinical and Radiographic Features*

Variable	Clinical and Radiographic Findings
Lesion quality	
No. of lesions	28 osteochondromas
Morphology	
Type of lesions	Type I (≤25% of native bone size), 100%
	Simple lesions, 96.5% (n = 27)
	Complex lesions, 3.5% (n = 1)
Lesion characteristics	
	Sessile osteochondromas, 86% (n = 24)
	Pedunculated osteochondromas, 14% (n = 4)
Location	
	In pelvis, 17% (n = 5)
	In other bones, 83% (n = 23)
Age at onset (more or less than 3 years old)	>3 years old (12 years old)
Severity of the disease†	
No. of exostoses (more or less than 10 osteochondromas)	>10 osteochondromas (n = 28)
Vertebral location of the exostoses (absence or presence)	Presence (9 mm × 5-mm osteochondroma on the fifth cervical vertebra, lateral)
Stature (more or less than 10th percentile)	<3rd percentile
Functional rating (good or fair)	Fair
Degree of severity	Group S (severe) (IVS) with secondary chondrosarcoma in pelvis

*Clinical classification of osteochondromas based on variables published by Bovée[6]. †Evaluation of severity described in Francanet et al.[14].

or fair). The degree of severity was represented in two groups, M (moderate) or S (severe). Group S was classified into four clinical subgroups according to the increasing grade of severity (type IS, type IIS, type IIIS, and type IVS), especially with regard to the lower percentile[14-16]. In our patient, we defined the phenotype as severe (type IVS) based on the fair functional rating, the large number of exostoses (>25) and their vertebral locations, and very short stature (below the third percentile)[15]. In addition to the phenotype classification, clinical and radiographic variables were defined for the patient (Table I).

In our patient, multiple osteochondromas were detected by radiographs. A tibiofibular osteochondroma was observed on the proximal side of the right knee (Fig. 1-A). A large chondrosarcoma was detected on the left iliopubic bone (Fig. 1-B). In addition, there were left and right humeral osteochondromas, Madelung deformities in both radii (Fig. 1-C), and multiple tiny phalangeal osteochondromas. A pelvic magnetic

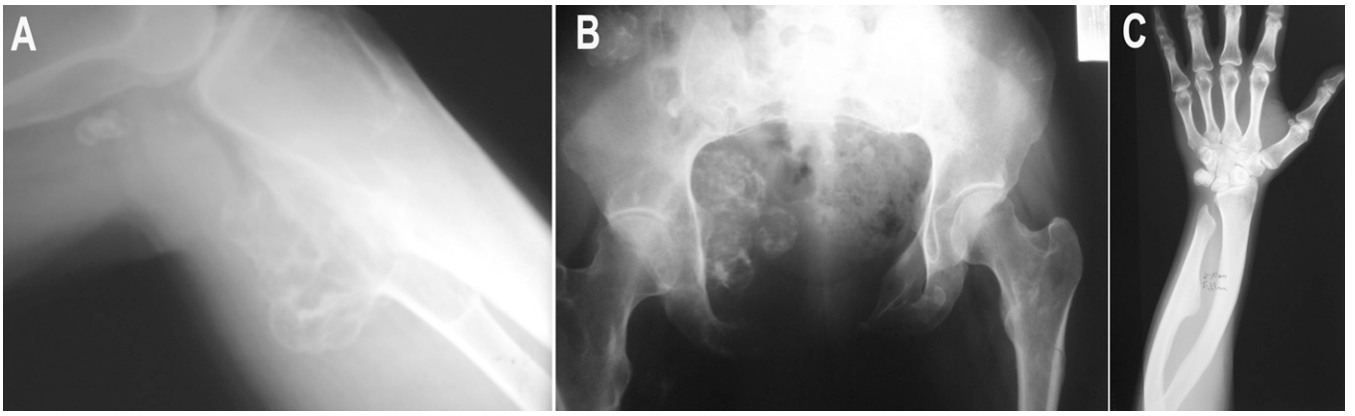

Fig. 1

Radiographs demonstrating multiple osteochondromas at the tibia and fibula: next to the knee (**Fig. 1-A**), at the pelvis and proximal part of the femur (**Fig. 1-B**), and at the radius and ulna (**Fig. 1-C**).

TABLE II Polymerase Chain Reaction Primer Sequences Used for *EXT1* and *EXT2* Genes*

Primers	Reverse	Forward
EXT1 gene		
Exon 1.1	5′tgggaaacttgggtgattctt 3′	3′gggatcaattggaaaacgaggattc 5′
Exon 1.2	5′ggcttgcagtttagggcatcgag 3′	3′ttgttccacaagtggagactcttg 5′
Exon 1.3	5′ccagttgtcacctcagtatgtgc 3′	3′aacttcacacctggaccaag 5′
Exon 2	5′ tgcgtaaattcatgcacatgg 3′	3′ggtttaacattatcacctctcc 5′
Exon 3	5′tgcgtaaattcatgcacatgg 3′	3′ttcactttccaacaaaatctcc 5′
Exon 4	5′ctatatgctagaagccaaatgc 3′	3′gattcctgtacttggatatattg 5′
Exon 5	5′tccattactctctctgtcttg 3′	3′gtccatctaacaccctgcat 5′
Exon 6	5′cctgtcaggacataagaagc 3′	3′gcctcgttacacccttttca 5′
Exon 7	5′tctgccgttttgtcttgctg 3′	3′gctttgtgaccttgtgtgtat 5′
Exon 8	5′gtgaggatgggagaattgtc 3′	3′gttttaatcagcccgattcct 5′
Exon 9	5′gaattaatgtttcgccacagtc 3′	3′ctgatgtgtcaaatgtgtgttt 5′
Exon 10	5′catcatcattatcattaccattc 3′	3′ccaaggatgtaaagctctcc 5′
Exon 11	5′ttgctgcttgctcatttgcc 3′	3′cttagatgagcagaatgacaaa 5′
EXT2 gene		
Exon 4.1	5′tgagtgacagagtgaaaccc 3′	3′caaggtgtatatctatgctctg 5′
Exon 4.2	5′agccgacagtcccatccc 3′	3′cttctgctcttgagttggttt 5′
Exon 5	5′gttgggatttccaggagtttgc 3′	3′cctcctgaagatttagaagtg 5′
Exon 6	5′ccgagatgcgtgtataaggc 3′	3′gctgtctaccaagttttctaag 5′
Exon 7	5′tcaagaactgttcccaaataag 3′	3′ggtgtcttgtgttcgtgcaa 5′
Exon 8	5′agtattgcttggcgtcaacc 3′	3′gagagttagtacactggtcta 5′
Exon 9	5′aatggagctgtaagagaactc 3′	3′ggtttacttcctccactagat 5′
Exon 10	5′tggcttgaacagcagggag 3′	3′cctgataagggcagcataatt 5′
Exon 11	5′caccaagcctgccatgtttg 3′	3′catttctgagacagcatgcc 5′
Exon 12	5′acctttggatttgatgagagc 3′	3′gtgcgtaagagtgtgggtta 5′
Exon 13	5′tctccagaatcccattatgac 3′	3′cacgctcatagtagaaaatatg 5′
Exon 14	5′gtcacttgaccaaaagcattc 3′	3′ggactagataaactttaagctc 5′
Exon 15	5′cttgtgagttctgccgttgg 3′	3′gaatgctactcactcaatttgt 5′
Exon 16	5′acctgtcaacctttttaagaac 3′	3′gttgacctacttggatcttgg 5′

*This table was created based on the methods of Wuyts et al.[17]

resonance imaging (MRI) study demonstrated an iliac mass in the left iliopubic bone with a heterogeneous signal, with limb and pelvic compromise (Figs. 2-A and 2-B). The tissue had been resected with indistinct margins three times, but soon recurred. Clinically, the lesion behaved as a chondrosarcoma, and, in all three specimens obtained at the time of surgery, the

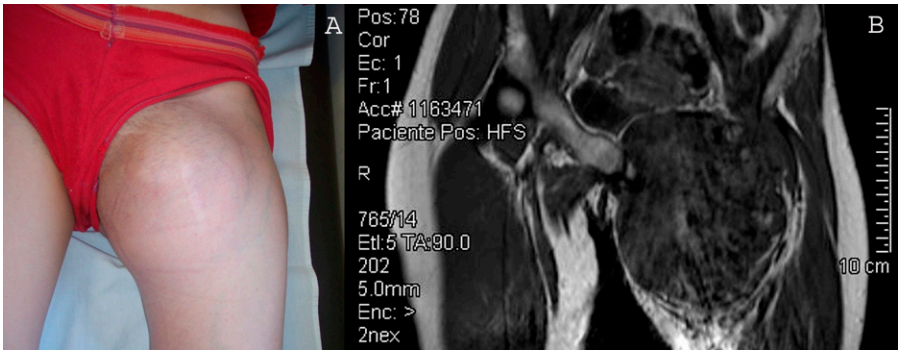

Fig. 2

Figs. 2-A and 2-B Our patient at age thirty-two. **Fig. 2-A** shows a chondrosarcoma in the pelvis and the proximal part of the femur. **Fig. 2-B** The coronal image of the pelvic MRI shows a large iliac mass on the left with a heterogeneous appearance, as well as shell and nodular calcifications growing into the inner pelvis, which produced lower-extremity compromise.

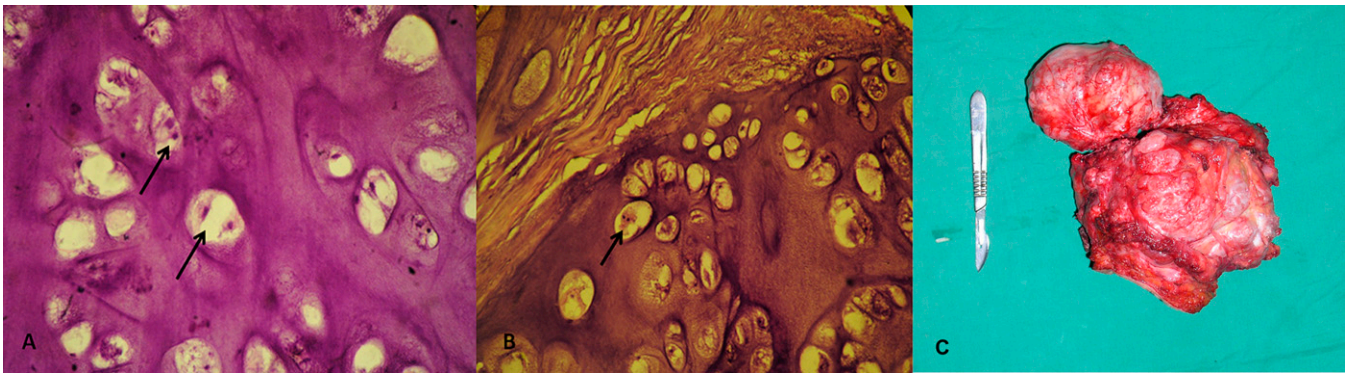

Fig. 3

Figs. 3-A and 3-B Histologic findings (**Fig. 3-A**, hematoxylin and eosin stain, 100× magnification; **Fig. 3-B**, hematoxylin and eosin stain, 40× magnification) of the neoplasm excised from the chondrosarcoma showed a mesenchymal neoplasm with cartilaginous matrix production. The arrows point to nuclear enlargement and occasional binucleation of the atypical chondrocytes. **Fig. 3-C** A photograph of the resected tissue from the first incomplete excision.

histological findings demonstrated a low-grade neoplasia (grade-I chondrosarcoma).

Gross and microscopic evaluation of the pelvic tumor tissue showed a mesenchymal neoplasm with cartilaginous matrix production (Figs. 3-A and 3-B). Infiltration of cartilaginous islands in underlying bone tissue, with a profile of an expansive invasion, was also observed. Tissue that was excised during the first operation is shown in Figure 3-C. The family history revealed that the patient's mother (fifty-three years old) and deceased grandfather both had a similar clinical presentation of MHE but without malignant transformation to chondrosarcoma (Fig. 4-A). The mother had the same clinical and imaging studies as the daughter, and the previously described variables also were analyzed.

We observed the same fair functional rating in the mother as in our patient, but, based on the absence of vertebral locations and her taller stature (below the 10th percentile), the degree of severity was classified as group IIS.

Genetic studies were performed for both our patient and her mother. The DNA taken from the patient's blood and the tumor tissue was studied through direct mutational analysis with use of primer pairs to amplify the coding regions and the flanking intronic sequences of the *EXT1* and *EXT2* genes, modified from the methods of Wuyts et al.[17] (Table II). Polymerase chain reaction products were sequenced on an ABI Prism DNA Analyzer 3700 or 3730 (Applied Biosystems), with use of BigDye Terminator chemistry (Applied Biosystems)[17]. Loss of heterozygosity was assessed in the tumor DNA[18].

A novel heterozygous mutation in the genomic DNA of the patient was identified: a substitution in exon 1 of the *EXT1* gene (c.848T>A) changed a leucine codon into a stop codon (p.L283X) in the EXT1 protein (Fig. 4-B). The patient was also heterozygous for the single nucleotide polymorphism rs11546829 in exon 3 of the *EXT1* gene (Table III). Loss of heterozygosity was not observed in the analysis of DNA in the tumor tissue. The same heterozygous nonsense mutation in exon 1 (c.848T>A; p.L283X) was detected in the patient's mother.

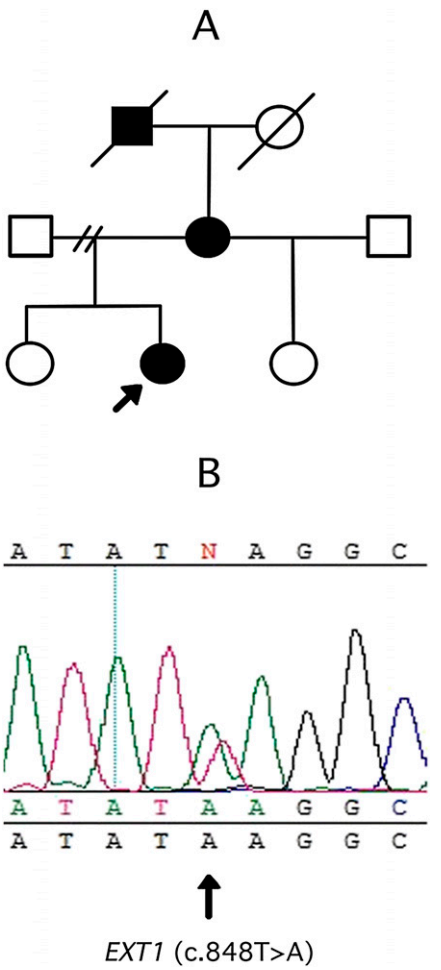

Fig. 4

Figs. 4-A and 4-B Pedigree of the family studied and mutation detection in the *EXT1* gene. **Fig. 4-A** The patient is marked with an arrow, and other affected members are indicated by black squares or circles. **Fig. 4-B** Exon 1 partial sequence of the *EXT1* gene. The arrow indicates the precise mutation in the patient (c.848T>A).

TABLE III *EXT1* Gene Changes Detected in Our Patient by Polymerase Chain Reaction and Direct Sequencing*

Gene	Exon/Intron	DNA Change	Protein Change	Ref SNP_ID†	Patient's Genotype	MAF†
EXT1	Exon 1	c.848T>A	p.L283X	—	T/A	—
EXT1	Exon 3	c.1035C>T	p.C355C	rs11546829	C/T	0.233
EXT1	Exon 9	c.1761G>A	p.E587E	rs7837891	A/A	0.367

*No changes were found in *EXT2*. †Reference single nucleotide polymorphism. ‡Minor allele frequencies in a CEU population (dbSNP).

Discussion

According to previous reports, the identification of novel and private mutations in a single family or patient support the strong allelic heterogeneity of *EXT1* or *EXT2* genes in patients with MHE[19]. To our knowledge, our case represents the first clinical and genetic report in the hereditary multiple osteochondromatosis program in Argentina and other Latin American countries, in collaboration with an interdisciplinary Spanish research group. The patient and her mother presented a heterozygous transversion in exon 1 (c.848T>A), resulting in a nonsense mutation (p.L283X) with loss of function in the amino-terminal region of the protein, which led to a prematurely truncated *EXT1*. The mutated protein only conserves 30.4% of the amino acids compared with the wild-type protein. Most patients with MHE present a wide spectrum of mutations in the *EXT1* gene, most of which are null[11,15,17,19].

We observed differences in the grade of severity between the patient and her mother, similar to the intrafamilial variability reported previously in other families with MHE[2]. This finding supports the variation in the expressivity of MHE that is in favor of genetic anticipation in this disease[15].

It has been shown that hereditary osteochondromas and secondary chondrosarcomas are associated with a second mutational hit in the *EXT1* gene[20,21]. In our patient, we did not observe loss of heterozygosity as indicative of a somatic deletion in the DNA of the tumor. This situation rules out a complete deletion of the second *EXT1* allele. A screening with a combined multiplex ligation-dependent probe amplification protocol could be performed to assess the presence of genetic rearrangements affecting other parts of the *EXT1* gene[22].

Although a basic molecular defect is known in most patients with MHE (a heterozygous mutation in either *EXT1* or *EXT2*), the pathogenic mechanism is still unclear. Despite the fact that heparan sulfate proteoglycans have many roles in cell physiology and in different tissues, the loss of their specific function in chondrocytes and their deregulation in bone growth plate seem to be the principal cause of this pathology. ■

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