Identification of Two Novel Frameshift Mutations in Exostosin 1 in Two Families with Multiple Osteochondromas

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Keywords

EXT1 · Mutation · Long bone deformation · Multiple osteochondromas

Abstract

Multiple osteochondromas (MO) is an autosomal dominant hereditary disorder, which typically manifests as skeletal dysplasia, mainly involving long bones and knees, ankles, elbows, wrists, shoulders, and pelvis. Previous studies have demonstrated that mutations in exostosin glycosyl transferase-1 (*EXT1*) and exostosin glycosyl transferase-2 (*EXT2*) were the main cause of MO. In this study, we enrolled 2 families with MO. Sanger sequencing revealed 2 novel frameshift mutations – c.1432_1433insCCCCCT; p.Lys479Profs*44 and c.1431_1431delC; p.S478PfsX10 – in the *EXT1* gene detected in 2 families, respectively. Both novel mutations, located in the conserved domain of *EXT1* and predicted to be disease causing by informatics programs, were absent in our 200 control cohorts and other public databases. Our study expanded the spectrum of *EXT1* mutations and contributed to genetic diagnosis and counseling of patients with MO.

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Introduction

Multiple osteochondromas (MO), also called hereditary multiple exostoses, is an inherited bone disorder, characterized by the presence of multiple benign cartilage-capped tumors, localized primarily in the long tubular bones, particularly in the humerus (10–50%), forearm (39–60%), knee (33%), and ankle (25%) [Wu et al., 2018; Chen et al., 2019]. The estimated prevalence of MO is 1 in 50,000; moreover, the approximate ratio of its morbidity between men and women is 3:2 [Hong et al., 2017; Xu et al., 2017; Yang et al., 2019]. The patients with MO often suffer from pain due to nerve and vascular compression, and 1–2% of patients may progress into osteosarcoma or chondrosarcoma, which is one type of malignant tumor and may threaten the health and lives of patients [Hong et al., 2017; Li et al., 2018].

Previous studies have revealed that MO is an autosomal dominant hereditary disorder with 2 main disease-causing genes identified, exostosin glycosyl transferase-1 (*EXT1*) and exostosin glycosyl transferase-2 (*EXT2*) [Santos et al., 2018; Chen et al., 2019; Oliver et al., 2019].

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Approximately 85–95% of the MO patients harbor variants of these 2 genes, but patients with *EXT1* pathogenic variants were found to have a greater number of exostoses, a greater incidence of limb malalignment with shorter limb segments and height, and more frequent pelvic and flat bone involvement than those with *EXT2* pathogenic variants [Pacifi, 2017; Piombo et al., 2018].

In China, the studies and investigations of MO are still lacking. In this study, 2 families with MO were enrolled and Sanger sequencing of *EXT1* and *EXT2* was performed to detect the genetic lesion of these 2 family members [Pacifi, 2018; Santos et al., 2018].

Materials and Methods

Subjects

Blood was collected from the probands and their family members; individuals were examined by X-rays.

DNA Extraction

Genomic DNA was prepared from peripheral blood of the patients and all other participants using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) as described [Fan et al., 2015].

Polymerase Chain Reaction

All the coding exons of the causative gene were amplified by PCR in 25 µL reaction mixture, which consisted of 2× Power Taq PCR MasterMix (Bioteke corporation, PR1702) and 50 ng of genomic DNA. The thermal cycling consisted of an initial denaturation at 95°C for 4 min, followed by 35 cycles of amplification consisting of denaturation at 95°C for 1 min, primer annealing at 55–61°C for 30 s, and primer extension at 72°C for 1 min. A final extension step was performed at 72°C for 7 min.

Mutation Sequencing

The entire coding regions, including the flanking intronic sequences of *EXT1* (NM_608177) and *EXT2* (NM_608210), were amplified with polymerase chain reaction (PCR; primer sequences can be provided upon request). Sequences of the PCR products were determined by the ABI 3100 Genetic Analyzer (ABI).

Bioinformatics Analysis

The multiple *EXT1* protein sequences across mammals were aligned using the program MUSCLE (version 3.6, an online program at <http://www.ncbi.nlm.nih.gov>). The MutationTaster, SIFT, and PolyPhen-2 were used to predict the effect of the variant. The 1000 G, dbSNP132, ExAC, and GenomAD database were used to exclude the polymorphism sites.

Results

Family 1

Family 1 from Central-South China (Hunan Province) with 5 members across 3 generations participated in the

study (Fig. 1a). The proband (III:1), an 11-year-old boy, presented with osteochondromas in the femur, tibia, and fibula (Fig. 1b). Simultaneously, the proband suffered from long bone deformation, and an osteotomy was performed to correct the deformation (Fig. 1b). In addition, similar symptoms were also found in his mother (II:2) and grandmother (I:4), according to the description of his father.

Sanger sequencing identified a novel insertion, c.1432_1433insCCCCCT; p.K479PfsX44, in *EXT1* in the proband (Fig. 1c). This mutation was also found in the mother of proband (II:2), but not found in another family member (II:1). Unfortunately, since the proband's grandmother has passed away, we cannot be sure whether she carried the mutation. Co-separation analysis found that this mutation was present in affected individuals and absent in healthy members. This novel mutation, resulting in a premature stop codon at position 523 in exon 6 of the *EXT1* gene, was absent in our 200 healthy control cohorts as well as the 1000 Genomes project (1000 G, www.1000genomes.org), dbSNP132 (<http://www.ncbi.nlm.nih.gov/projects/SNP/>), ExAC (<http://exac.broadinstitute.org/>), and GenomAD database (<https://gnomad.broadinstitute.org/>). Further bioinformatics analysis revealed that this mutation is located in a conserved site of the third extracellular domain (Fig. 1d) and is predicted to be deleterious by 3 different algorithm-based bioinformatics programs. According to American College of Medical Genetics and Genomics (ACMG) guidelines, this mutation belongs to PVS1. We consider the mutation to be highly pathogenic. Genetic analysis indicated that this insertion, c.1432_1433insCCCCCT; p.Lys-479Profs*44, in *EXT1* was the genetic lesion of Family 1.

Family 2

In Family 2 (Fig. 2a), only the proband (II:2), a 17-year-old boy, presented with osteochondromas in femur, tibia, and fibula and suffered from long bone deformation (Fig. 2b). An osteotomy was performed to correct the deformation (Fig. 2b). Both his mother and father were healthy.

Mutation detection found a de novo mutation, c.1431_1431delC; p.S478PfsX10, in *EXT1* present in the patient (Fig. 2c). The novel deletion resulting in a premature stop codon at position 488 in exon 6 of the *EXT1* gene was also absent in our 200 healthy control cohorts as well as the 1000 Genomes project, dbSNP132, ExAC, and GenomAD database. Bioinformatics analysis revealed that this mutation is located in a conserved site of the third extracellular domain (Fig. 2d) and is predicted to be del-

Fig. 1. a Pedigree of the family affected with MO. Family members are identified by generations and number. Squares indicate male family members; circles, female members; a closed symbol, the affected member; open symbols, unaffected members; arrow, proband. **b** Radiographs of proband, osteochondromas in the femur, tibia. **c** Sequencing result of the *EXT1* mutation. A novel heterozygous frameshift mutation was detected in the exon 6 of the *EXT1* gene and defined as c.1432_1433insCCCCCCT; p.K479PfsX44; **d** Comparison of the functional domains of *EXT1* proteins encoded by mutated and normal *EXT1* genes.

eterious by bioinformatics programs. According to ACMG guidelines, this mutation belongs to PVS1. We consider the mutation to be highly pathogenic. Genetic analysis indicated that this deletion, c.1431_1431delC; p.S478PfsX10, in *EXT1* was the genetic lesion of the patient.

Discussion and Conclusion

The human *EXT1* gene encoding exostosin glycosyl transferase-1 protein is located at chromosome 8q24.11, and it consists of 11 exons, spanning approximately 31.7 kb [Liang et al., 2020]. As a tumor-suppressor gene, loss of function or dysregulation of this may lead to MO and

chondrosarcoma. In this study, we identified 2 novel frameshift mutations – c.1432_1433insCCCCCCT; p. K479PfsX44 and c.1431_1431delC; p.S478PfsX10 – in *EXT1* in 2 families with MO. Our study was consistent with previous studies that pathogenic mutations in *EXT1* may lead to MO. In addition, an osteotomy was performed to correct the long bone deformation.

EXT1 contains 2 crucial domains: the exostosin domain (aa110–396) and the glycosyltransferase domain (aa480–729) [Bai et al., 2020]. Previous studies have proven that *EXT1* is a tumor-suppressor gene and the C-terminus glycosyltransferase domain of *EXT1* may play a critical role in heparan sulfate biosynthesis, which is the necessary signaling of fibroblast growth factors, vascular endothelial growth factor, and transforming growth

Fig. 2. **a** Pedigree of the family affected with MO. Family members are identified by generations and number. Squares indicate male family members; circles, female members; a closed symbol, the affected member; open symbols, unaffected members; arrow, proband. **b** Radiographs of proband, osteochondromas in the femur, tibia. **c** Sequencing result of the *EXT1* mutation. A novel heterozygous deletion mutation was detected in the exon 6 of the *EXT1* gene and defined as c.1431_1431delC; p.S478PfsX10; **d** Comparison of the functional domains of *EXT1* proteins encoded by mutated and normal *EXT1* genes.

factor- β , as well as the gradient formation of morphogens such as hedgehog or bone morphogenetic proteins [Sethi et al., 2019; Yang et al., 2019]. In our study, both novel mutations result in a premature stop codon at C-terminus glycosyltransferase domain, which may induce nonsense-mediated mRNA decay and lead to haploinsufficiency of the glycosyl transferase. The reduced level of glycosyl transferase may affect biosynthesis of heparan sulfate, which may disrupt the downstream signaling pathways such as bone morphogenetic proteins and result in the development of osteochondroma.

In 1995, a 1-bp deletion in the *EXT1* gene was identified and co-separated in 2 of 23 unrelated families with MO [Liu et al., 2019; Oliver et al., 2019]. Since then, more

than 500 mutations have been identified in patients with MO. In addition, some reports also revealed that mutations in *EXT1* can cause chondrosarcoma and trichorhinophalangeal syndrome [Chen et al., 2019]. Most of these 500 mutations are nonsense, frameshift, or splice-site mutations, which result in truncation, premature termination, premature degradation, and nearly complete loss of function of the *EXT1* protein. In our study, both novel mutations may induce mRNA degradation and lead to haploinsufficiency of the glycosyl transferase and result in MO.

At present, the treatment of MO may rely on scrape and bone graft. If the MO patients present with limb deformities, orthopedic correction will be an effective meth-

od [Liang et al., 2020]. In our study, both patients had long bone deformation and osteotomy was performed to correct the malformation.

In summary, we identified 2 novel mutations – c.1432_1433insCCCCCT; p.K479PfsX44 and c.1431_1431delC; p.S478PfsX10 – in *EXT1* in 2 families with MO. Our study expanded the spectrum of *EXT1* mutations and contributed to genetic diagnosis and counseling of patients with MO.

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Statement of Ethics

The study was approved by the Xiangya Hospital of the Central South University ethics committee and was performed according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

C.-Y.W. performed the Sanger sequencing and wrote the manuscript; F.Y. enrolled the patients; J.-Y.J. and L.-L.F. performed the DNA extraction; J.-Y.T. and R.X. designed and supported the project.