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Original Article

Genetic analysis of seven pateints with Hereditary Multiple Osteochondromas (HMO)

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Abstract: Background: HMO (Hereditary Multiple Osteochondroma), an uncommon autosomal dominant disorder, is characterized by the development of multiple osteochondromas, which are nonmalignant cartilage-capped bone tumors growing outwards from long bone metaphyses. Methods: The present work retrospectively analyzed seven children with HMO who were enrolled for routine clinical diagnosis and treatment, including X-ray examination. Subsequent genetic detection was carried out using whole exome sequencing (WES). In addition, this work applied Sanger sequencing to be the validation approach. Moreover, this work also examined amino acid (AA) evolutionary conservatism under the influence of certain missense variants. Results: The clinical indications of all seven patients and their family members were thoroughly indexed. WES identified diagnostic variants in the *EXT1* or *EXT2* gene in these patients. In these variants, four were reported for the first time, namely *EXT1*: c.1285-2A>T, *EXT2*: c.1139delT, *EXT1*: c.203G>A, and *EXT1*: c.1645_1673del. Familial validation revealed that three of the variants were hereditary, while the other four were *de novo*, which was consistent with the phenotype in each case. Conclusion: Our results expanded HMO variation spectrum, and laid certain foundations for the precise counseling of those affected families.

Keywords: Hereditary multiple osteochondromas, *EXT1* gene, *EXT2* gene, Novel mutation, Whole-exome sequencing

Introduction

Hereditary multiple osteochondroma (HMO, MIM #133700/133701), called hereditary multiple exostoses (HME), as well multiple cartilaginous exostoses, or diaphyseal aclasis, is a rare monogenic skeletal disease with the feature of the development of multiple osteochondromas, which are nonmalignant cartilage-capped bone tumors growing outwards from long bone metaphyses [1]. HMO patients usually have persistent pain as a result of compression on the surrounding tissues, blood circulation disturbance, sometimes due to spinal compression. Nonetheless, malignant development of chondrosarcoma is its serious complication, which makes up around 3.9% of all HMO cases [2]. The exact incidence of HMO is not clear due to the presence of asymptomatic patients, yet it is estimated to be about 1/50,000 in western ethnicities [3-5]. Mordenti *et al.* classified HMOs in 3 categories based on whether deformity or functional limitation occurred [6].

EXT1 (chromosome 8q24.11; MIM no. *608-177) and *EXT2* (chromosome 11p11.2; MIM no. *608210) genes, encode the Golgi-associated glycosyltransferases, plus exostosin 1 and 2, which are involved with the heparan sulfate (HS) biosynthesis. Those two genes are the well-defined causative genes for HMO [1] in approximately 90% of patients, while another locus with potential pathogenicity has been postulated [7]. It is demonstrated that heparan sulfate proteoglycans (HSPGs), which are constituted by HS chains and core protein, are related to different physiological and developmental activities in the extracellular matrix

(ECM) or on cell surface [8], most importantly, the interaction of them with certain key signaling factors in osteogenic differentiation and bone homeostasis [9]. The fact that mutations in *EXT1* and *EXT2* genes can cause HMO was first identified by Ahn *et al.* in 1995 and Wuyts *et al.* in 1996, respectively [10, 11]. So far, over eight hundred pathogenic variants in these two genes which are found to be responsible for HMO have been reported (Human Gene Mutation Database, HGMD; http://www.hgmd.cf.ac.uk/). These variants include a variety of types, of which small insertions and missense mutations together account for more than half [2]. However, there are still ~10% of HMO patients without a clear molecular diagnosis, which makes their consultation and precise management challenging [12].

In the current study, seven families with HMO patients were enrolled. A comprehensive clinical evaluation including X-ray imaging and post-operative pathological examination was conducted; subsequently, this work was conducted with genetic analysis by whole exome sequencing (WES), followed by validation by Sanger sequencing to identify the causative variations.

Materials and methods

Subjects

The present work retrospectively enrolled seven pediatric cases presenting with HMO who were recruited in our centers between Jan 2014 and Dec 2021. A thorough clinical evaluation, X-ray imaging and post-operative routine pathological examination on each proband was carried out. Expanded examination on the family members with symptoms was conducted according to the needs of demanding families. Subsequently, the peripheral blood samples for genetic testing were taken in patients, corresponding parents and symptomatic family members.

The present work gained approval from the Ethics Committee of Shijiazhuang Obstetrics and Gynecology Hospital (No. 2021 0095). Each subject provided informed consent for participation. Each procedure was carried out following the Declaration of Helsinki 1964 together with the subsequent amendments or relevant ethical standards.

DNA extraction

QIAamp DNA Blood Mini-Kit (Qiagen Sciences, USA) was utilized for extracting genomic DNA (gDNA). Thereafter, 1% agarose gels were utilized to validate DNA quality by using Qbit DNA Assay Kit from Qubit 2.0 Flurometer (Life Technologies, CA, USA).

Whole exome sequencing (WES)

WES was carried out as previously described [13-15]. In brief, Sure Select Human Exon Sequence Capture Kit (Agilent, USA) was employed to enrich exonic sequences. Illumina DNA Standards and Primer PremixKit (Kapa Biosystems, USA) was adopted for quantifying sequencing libraries, followed by massive parallel-sequencing with Illumina Novaseq6000 platform. Afterwards, low-quality readings were sequenced and screened, whereas high-quality reads (Q30 percentage > 89%) were later compared with human genome reference sequence [hg19]. Those suspected pathogenic variants (https://software.broadinstitute.org/gatk/) were identified by GATK software. NCBI Reference Sequence using Chromas v2.33 was adopted for aligning sequences against NCBI to identify variations. For those variants identified, their pathogenicity was evaluated based on guideline released by the American Association of Medical Genetics and Genomics (ACMG) [16] with reference to several databases (1000g2015aug_eas, https://www.internationalgenome.org/; ExAC_EAS, http://exac.broadinstitute.org; gnomAD_exome_EAS, http://gnomad.broadinstitute.org/); HGMD: Human Gene Mutation Database (Professional Version 2019.4) with the Enliven® Variants Annotation Interpretation system (Berry Genomics, China).

Variant validation

By adopting ABI 3730 Automated Sequencer (Applied Biosystems, USA), Sanger sequencing was conducted for validating suspected diagnostic variants in line with specific instructions. MEGA7 (http://www.megasoftware.net) was utilized to examine amino acid (AA) evolutionary conservatism under the influence of certain missense variants by the use of default parameters.

Table 1. Clinical information of the seven patients with HMO

Patient No.	Gender*	Age at diagnosis	Brief clinical history of the proband in each family*	Family history and related issues of symptomatic members
1	M	6	Multiple, gradually enlarged phymas appeared initially on the extremities; Diagnosis was made, and subsequent resections of OC were performed for six times from 2014 to 2020.	None family history
2	M	8	Two previous HMO resections; Multiple OCs at the joints of the extremities; Chronic pain; Bilateral forearms varus deformity, both hips adduction flexion limited; 3 HMO resections in our center from 2019 to 2021.	None family history
3	M	4	Initial OCs in the right upper extremity; Progressive local pain; X-ray revealed HMOs in extremities; 1 resection at right distal ulna and bilateral distal femurs performed in Sept 2021.	Maternal HMO; 1 excision performed on the mother at 11 years old
4	F	3.5	Multiple OCs; 1st resection at right proximal tibia and distal fibula in May 2017; 2021, Left limping; X-ray revealed HMOs in lower extremities; Left common peroneal nerve injury by EMG; 2nd resection conducted in Jun 2021.	Familial HMO in father and paternal grandma; 1 excision performed on the father at 10 years old
5	M	6	Abnormal walking and right knee valgus for 2 years; X-ray revealed OCs in bilateral lower extremities; Resection conducted in Jul 2021.	Father and younger brother with HMOs; 1 excision performed on the brother in Oct 2021
6	M	7	Multiple OCs; Local pain; X-ray revealed OCs in lower extremities; 2 resections performed from 2018 to 2020.	None family history
7	M	6.5	Aged 30; Having undergone 3 OCs resections from 2000 to 2007.	None family history

*M, male; F, female; OC: osteochondroma.

Results

Clinical manifestations and family history

The key clinical information of each case was included in **Table 1**. The characteristic imaging manifestations were shown in **Figures 1** and **2**. Pedigree diagram for every family member indicating the relationship was demonstrated in **Figure 3**. According to our survey, three cases of the seven had family history of HMOs (Case 3, 4 and 5; see **Figure 3**), while the other four were sporadic. Like general HMO cases, in most of our patients the lesions were concentrated into long bone epiphyses, in particular lower extremities, which were also where the resections were carried out. Yet, there were two cases with additional rare onset sites, which were the right scapula in Case 2 and the left ring finger in Case 5 (**Figures 1** and **2**, red circles).

Genetic findings

WES identified the diagnostic variations in all seven cases. **Figure 3** shows the peak diagram (Sanger sequencing result) or Bam result (next-generation sequencing) for every variant analyzed in the present work. Altogether 6 cases carried heterozygous variants of *EXT1*, whereas another case had variation in *EXT2*. To be specific, Patient 1 had the splicing site variant, *EXT1*: c.1285-2A>T; Patient 2 carried a frameshift variant, *EXT2*: c.1139delT (p.I380Tfs*30); Patient 3 carried a missense variant, *EXT1*: c.1016G>A (p.G339D); Patient 4 carried a nonsense variant, *EXT1*: c.203G>A (p.W68*); Patient 5 carried a splicing site variant, *EXT1*: c.963-1G>T; Patient 6 carried a frameshift variant, *EXT1*: c.1469delT (p.L490Rfs*9); and Patient 7 carried a frameshift-causing coding sequence deletion, *EXT1*: c.1645_1673del (p.F550Rfs*6). The variant details were included in **Table 2**. Notably, four were reported for the first time, namely*EXT1*: c.1285-2A>T, *EXT2*: c.1139delT, *EXT1*: c.203G>A, and *EXT1*: c.1645_1673del.

According to verification results, co-separation of the variant and phenotype was found in all three familial HMO cases, while all the sporadic patients harbored *de novo* variants; and the specific carrying status was also shown in the pedigree diagrams in **Figure 3**. Additionally, **Figure 3G** indicated that the amino acid residue, EXT1: G339 affected by *EXT1*: c.1016G>A in Case 3, was maintained evolutionarily across species.

Discussion

In 1814, Alexis Boyer first depicted HMO [17]. HMO has different clinical presentations, such as limited range of motion (ROM), chronic pain syndromes, short stature, limb malformation, scoliosis or neurovascular change [18]. HMOs

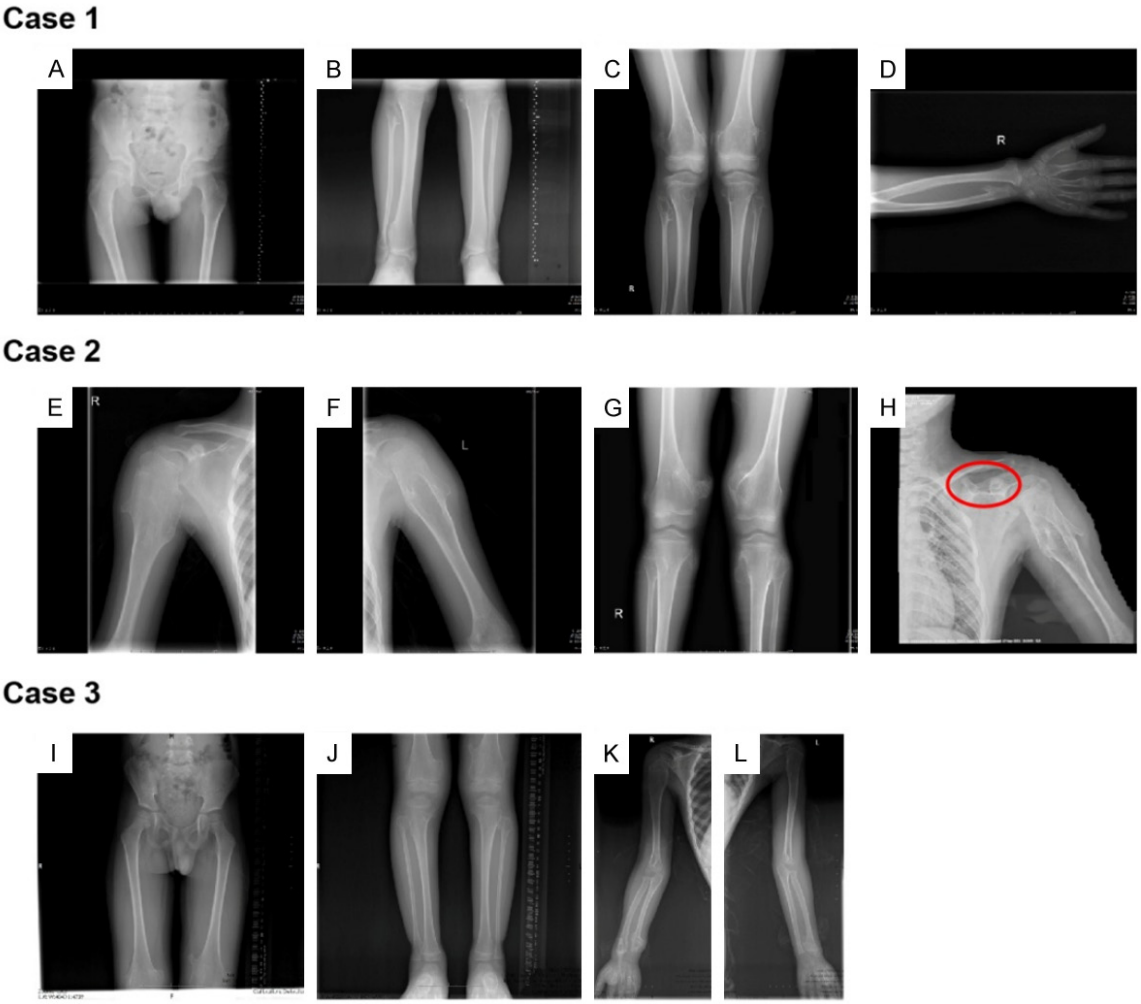

Figure 1. Clinical manifestations of the HMO patients (Part I). Case 1: the X-ray images of the patient's hip (A), tibiofibulas (B), knees (C) and right forearm (D) showing multiple exostoses. Case 2: the X-ray images of the patient's both humerus (E, F), knees (G), and left scapula (H) with multiple exostoses. (The red circle indicates rare lesion) Case 3: the X-ray images of the patient's hip (I), tibiofibulas and knees (J), both arms (K, L) showing multiple exostoses at the metaphysis of long bones.

can also occur in bones during endochondral ossification, usually the long bones [2]. Comparatively, HMO at birth is uncommon, but it can occur in children and adolescents, and it stops growing after the maturation of epiphyseal plates.

In general, *de novo* pathogenic variants account for the cause of around 10% of HMO cases affected [12, 21, 22]. Yet in our study, *de novo* mutations occurred in three of the seven cases, and this higher rate may be related to the proportion bias caused by our small sample size. Apart from long bone epiphyses, particularly within lower extremities, less common on-set sites for HMO include the hands, ribs, scapula, vertebrae and pelvis [23]. The clinical indications of our patients were generally consistent with usual HMOs, but some relatively rare lesions also appeared, such as scapula involvement of Case 2, and Case 5 had a chondroma on his left ring finger. Current pathological results and follow-up showed that our patients did not have the rare malignant transformation, but this risk should not be ignored during the lifetime of all patients.

In this study, six patients carried *EXT1* variations with various types, including one nosense, one missense, two splicing-site, one frameshift and one microdeletion leading to pre-termination of protein translation. The other patient,

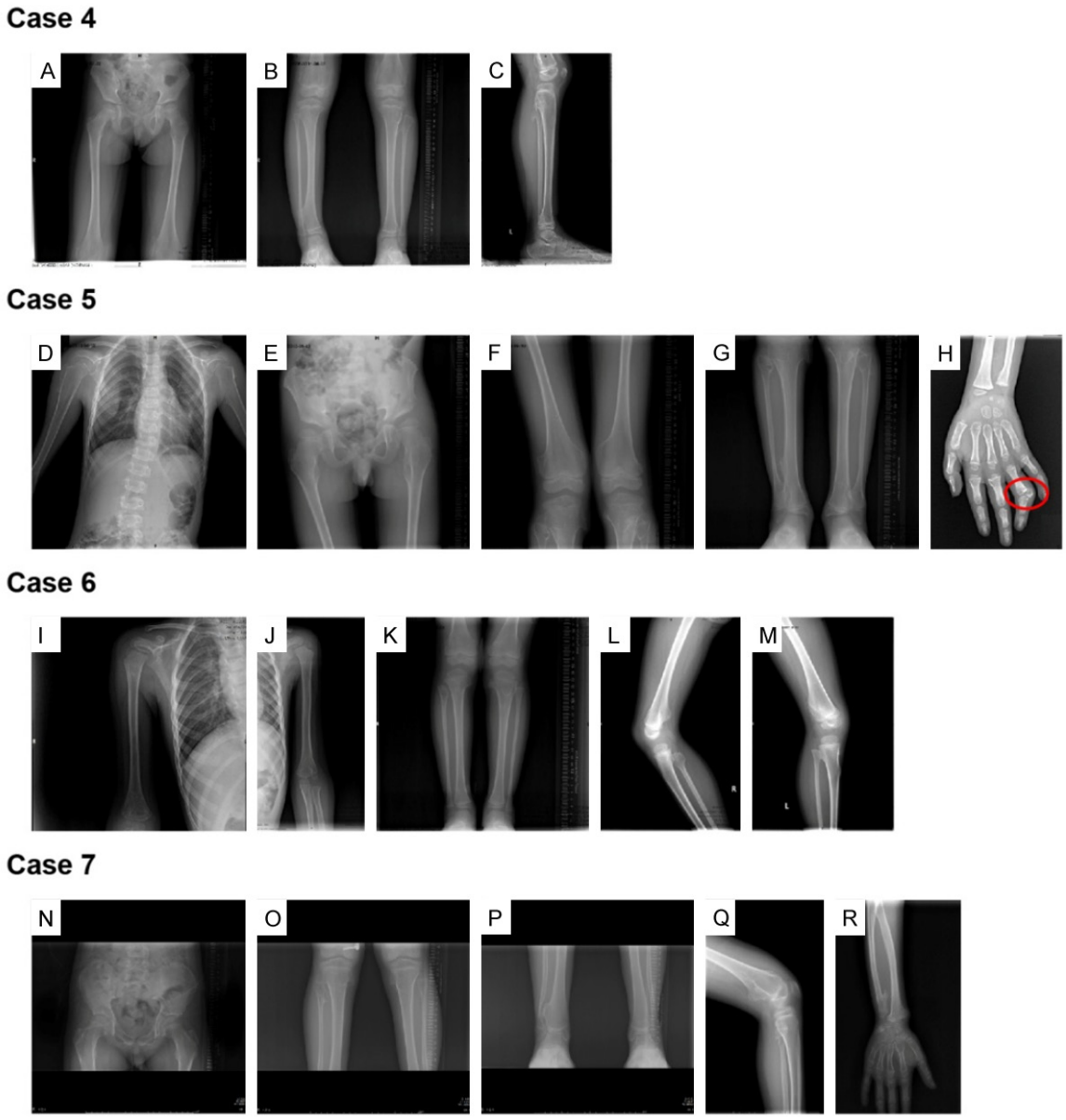

Figure 2. Clinical manifestations of the HMO patients (Part II). Case 4: the X-ray images of the patient's hip (A), tibiofibulas and knees (B, front; C, side) with exostoses at the metaphysis regions. Case 5: the X-ray images of the patient's stem (D, scoliosis), hip (E), knees (F), tibiofibulas (G) and left palm (H) showing multiple exostoses. (The red circle indicates rare lesion) Case 6: the X-ray images of the patient's upper arms (I, J), tibiofibulas and knees (K) and knees (L, M) showing exostoses at the metaphysis regions. Case 7: the X-ray images of the patient's hip (N), tibiofibulas (O), knees (P, backwards), left knee (Q, side) and left forearm (R) with multiple exostoses.

Case 2, carried a frameshift variant in *EXT2*. According to the ACMG guideline, they were all interpreted as "pathogenic" or "likely pathogenic" rating level (**Table 2**). Additionally, the evolutionary conservatism of EXT1: p.G339 residue affected by the c.1016G>A (p.G339D) variant strongly supports its pathogenicity. To some extent, this reflects the omnipotence of WES detection technology in identifying causative variations, as in our previous study [24]. Four of the seven variants identified in our study were novelly reported, which expands the mutation spectrum of HMO. In terms of the other three known pathogenic variations, they have been reported with association to the HMO phenotype at least one time [12, 25-29]. For each

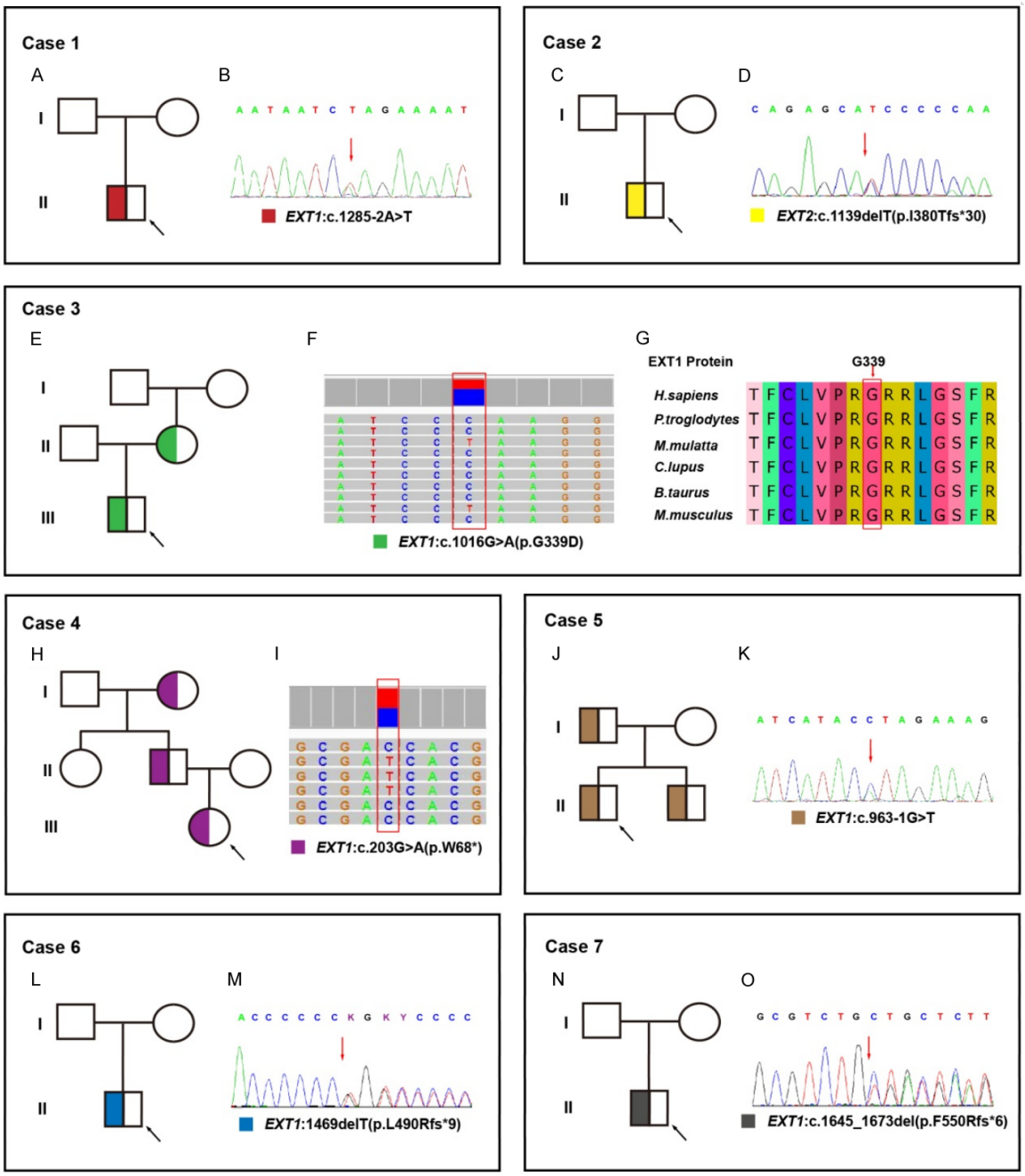

Figure 3. The pedigree diagrams, genetic variants, and the corresponding carrying status in each family. Case 1: the pedigree diagram (A), and the Sanger peak image of *EXT1*:c.1285-2A>T variant (B). Case 2: the pedigree diagram (C), and the Sanger peak image of *EXT2*: c.1139delT variant (D). Case 3: the pedigree diagram (E) and bam image of *EXT1*: c.1016G>A variant (F); (G) the evolutionary conservatism of the EXT1: G339 residue among species. Case 4: the pedigree diagram (H), and bam image of *EXT1*: c.203G>A variant (I). Case 5: the pedigree diagram (J), and Sanger peak image of *EXT1*: c.963-1G>T variant (K). Case 6: the pedigree diagram (L), and Sanger peak image of *EXT1*: c.1469delT variant (M). Case 7: the pedigree diagram (N), and Sanger peak image of *EXT1*: c.1645_1673del variant (O). Black arrows indicate probands, and red arrows or boxes indicate mutation sites.

familial HMO case (Case 3, 4, and 5), the couples still have a risk up to 50% of being affected in future pregnancies, so genetic counseling and interventional fertility options, like prenatal or pre-implantation diagnosis, should be considered [30]. Actually, based on our follow-up,

Table 2. Information of the identified variations in the seven patients

Patient No.	Gene (Transcript version)	Genomic alteration	Peptide alteration	Frequency in three databases*	Prediction by REVEL*	HGMD*/ClinVar* rating	Pathogenicity rating* (Evidences)
1	*EXT1* (NM_000127)	c.1285-2A>T	exon skipping?	0; 0; 0	-	Not indexed	Pathogenic (PVS+PS2+PM2+PM5)
2	*EXT2* (NM_207122)	c.1139delT	p.I380Tfs*30	0; 0; 0	-	Not indexed	Pathogenic (PVS1+PS2+PM2)
3	*EXT1* (NM_000127)	c.1016G>A	p.G339D	0; 0; 0	P	DM/P	Pathogenic (PS4+PM1+PM2+PM5+PP3)
4	*EXT1* (NM_000127)	c.203G>A	p.W68*	0; 0; 0	-	Not indexed/P	Likely pathogenic (PVS1+PM2)
5	*EXT1* (NM_000127)	c.963-1G>T	exon skipping?	0; 0; 0	-	DM/Not indexed	Pathogenic (PVS1+PS4+PM2)
6	*EXT1* (NM_000127)	c.1469delT	p.L490Rfs*9	0; 0; 0	-	DM/P	Pathogenic (PVS1+PS2+PM2)
7	*EXT1* (NM_000127)	c.1645_1673delCGTTTTCTGCCCTACGACAACATCATCAC	p.F550Rfs*6	0; 0; 0	-	Not indexed	Pathogenic (PVS1+PS2+PM2)

*Three databases: 1000g2015aug_eas, https://www.internationalgenome.org/; ExAC_EAS, http://exac.broadinstitute.org; gnomAD_exome_EAS, http://gnomad.broadinstitute.org/; REVEL: A comprehensive tool for predicting pathogenicity of missense variation [PMID: 27666373]; HGMD: Human Gene Mutation Database (Professional Version 2019.4, http://www.hgmd.cf.ac.uk/ac/index.php); ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/; DM: Disease causing mutation; P: Pathogenic; VUS: Variant of uncertain significance; B: benign; Pathogenicity rating: Based on the common guideline issued by the American Association of Medical Genetics and Genomics (ACMG) (Ref. 15; Richards *et al.*, 2015).

two families (Case 4 and 5) have already decided on a reproductive intervention in their next pregnancies, i.e., pre-implantation diagnosis (PGD), and had already entered the cycle. For the other four sporadic cases, there may be parental gonad mosaicism, and the probability of subsequent pregnancy involvement may be about 1%, so it should not be taken lightly [29].

HS chains, which are composed of proteoglycans, have diverse physiological and developmental activities [31]. The HS backbone is formed under the action of 2 glycosyltransferases coded via genes *EXT1* and *EXT2* [32]. HS chains can be attached to the "core protein" to form one type of proteoglycans called HSPG. HSPGs can be exposed onto cell membrane surface, the pericellular and the ECM, which play key roles in the signal transduction of many molecules [33]. Each signaling protein contains a certain HS-binding domain for regulating skeletal growth and development [9, 34]. Those critical signaling proteins include bone morphogenetic proteins (BMPs), fibroblast growth factor (FGF), Hedgehog and Wnt signaling proteins [9, 31]. When the above pathways are severely interfered with, it will induce osteochondroma development [18].

Furthermore, one "two-hit" model adopted for developing exostosis has long been set up and elucidated, and the germline mutation and somatic mutation together cause EXT1/EXT2 functional losses or the later carcinogenesis [8, 35, 36]. The potential second hits are loss-of-heterozygosity (LOH), mutations or aneuploidy of additional genes [36-38]. As for second-hit somatic mutations, together with the subsequent second gene copy's random inactivation, the synthesis of functional HS is terminated in cells. Consequently, EXT1/EXT2 gene mutations induce seriously impaired biosynthesis and elongation of HS chains, as presented by reduced HS level within ECM and surrounding cells [39]. HS impairs chondrocyte growth and differentiation factors, including IHH or those related to osteogenesis like FGF, BMPs, and Wnt signaling proteins [9, 40-42]. Therefore, abnormal bone growth may take place since HS chains completely lose or are shortened by chondrocyte growth and differentiation.

This work is mainly limited by the small sample size as well as lack of in-depth functional experiments. The genotype-phenotype association cannot be fully established and analyzed. Further research based on the "second hits" theory may hold promise for the treatment of HMO.

In summary, this study recruited seven HMO cases, conducted a thorough clinical evaluation, and performed a definitive genetic diagnosis on them. Our results expanded HMO variation spectrum and laid a certain foundation for counseling and reproduction for affected families. Further studies are still required to better understand the nosogenesis and to facilitate to develop new therapeutic drugs on HMO.

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Disclosure of conflict of interest

None.

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References

[1] Wuyts W, Schmale GA, Chansky HA and Raskind WH. Hereditary Multiple Osteochondromas. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2022. 2000 Aug 3 [Updated 2020 Aug 6].

[2] Bukowska-Olech E, Trzebiatowska W, Czech W, Drzymala O, Frak P, Klarowski F, Klusek P, Szwajkowska A and Jamsheer A. Hereditary multiple exostoses-A review of the molecular background, diagnostics, and potential therapeutic strategies. Front Genet 2021; 12: 759129.

[3] Bovée JV. Multiple osteochondromas. Orphanet J Rare Dis 2008; 3: 3.

[4] Ryckx A, Somers JF and Allaert L. Hereditary multiple exostosis. Acta Orthop Belg 2013; 79: 597-607.

[5] Pacifici M. Hereditary multiple exostoses: new insights into pathogenesis, clinical complications, and potential treatments. Curr Osteoporos Rep 2017; 15: 142-152.
[6] Mordenti M, Ferrari E, Pedrini E, Fabbri N, Campanacci L, Muselli M and Sangiorgi L. Validation of a new multiple osteochondromas classification through Switching neural networks. Am J Med Genet A 2013; 161A: 556-560.
[7] Le Merrer M, Legeai-Mallet L, Jeannin PM, Horsthemke B, Schinzel A, Plauchu H, Toutain A, Achard F, Munnich A and Maroteaux P. A gene for hereditary multiple exostoses maps to chromosome 19p. Hum Mol Genet 1994; 3: 717-722.
[8] Li J, Wang Z, Han Y, Jin C, Cheng D, Zhou YA and Zhen J. Genetic and functional analyses detect an EXT1 splicing pathogenic variant in a Chinese hereditary multiple exostosis (HME) family. Mol Genet Genomic Med 2022; 10: e1878.
[9] Billings PC and Pacifici M. Interactions of signaling proteins, growth factors and other proteins with heparan sulfate: mechanisms and mysteries. Connect Tissue Res 2015; 56: 272-280.
[10] Ahn J, Lüdecke HJ, Lindow S, Horton WA, Lee B, Wagner MJ, Horsthemke B and Wells DE. Cloning of the putative tumour suppressor gene for hereditary multiple exostoses (EXT1). Nat Genet 1995; 11: 137-143.
[11] Wuyts W, Van Hul W, Wauters J, Nemtsova M, Reyniers E, Van Hul EV, De Boulle K, de Vries BB, Hendrickx J, Herrygers I, Bossuyt P, Balemans W, Fransen E, Vits L, Coucke P, Nowak NJ, Shows TB, Mallet L, van den Ouweland AM, McGaughran J, Halley DJ and Willems PJ. Positional cloning of a gene involved in hereditary multiple exostoses. Hum Mol Genet 1996; 5: 1547-1557.
[12] Fusco C, Nardella G, Fischetto R, Copetti M, Petracca A, Annunziata F, Augello B, D'Asdia MC, Petrucci S, Mattina T, Rella A, Cassina M, Bengala M, Biagini T, Causio FA, Caldarini C, Brancati F, De Luca A, Guarnieri V, Micale L, D'Agruma L and Castori M. Mutational spectrum and clinical signatures in 114 families with hereditary multiple osteochondromas: insights into molecular properties of selected exostosin variants. Hum Mol Genet 2019; 28: 2133-2142.
[13] Zhang J, Hu H, Mu W, Yu M, Chen W, Mi D, Yang K and Guo Q. Case report: exome sequencing identified a novel compound heterozygous variation in PLOD2 causing bruck syndrome type 2. Front Genet 2021; 12: 619948.
[14] Huang YX, Gao CY, Zheng CY, Chen X, Yan YS, Sun YQ, Dong XY, Yang K and Zhang DL. Investigation of a novel LRP6 variant causing autosomal-dominant tooth agenesis. Front Genet 2021; 12: 688241.
[15] Yang F, Yang RJ, Li Q, Zhang J, Meng YX, Liu XJ and Yao YF. Whole-exome sequencing facilitates the differential diagnosis of Ehlers-Danlos syndrome (EDS). Mol Genet Genomic Med 2022; 10: e1885.
[16] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K and Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17: 405-24.
[17] Hennekam RC. Hereditary multiple exostoses. J Med Genet 1991; 28: 262-266.
[18] D'Arienzo A, Andreani L, Sacchetti F, Colangeli S and Capanna R. Hereditary multiple exostoses: current insights. Orthop Res Rev 2019; 11: 199-211.
[19] Vanhoenacker FM, Van Hul W, Wuyts W, Willems PJ and De Schepper AM. Hereditary multiple exostoses: from genetics to clinical syndrome and complications. Eur J Radiol 2001; 40: 208-217.
[20] Pedrini E, Jennes I, Tremosini M, Milanesi A, Mordenti M, Parra A, Sgariglia F, Zuntini M, Campanacci L, Fabbri N, Pignotti E, Wuyts W and Sangiorgi L. Genotype-phenotype correlation study in 529 patients with multiple hereditary exostoses: identification of "protective" and "risk" factors. J Bone Joint Surg Am 2011; 93: 2294-2302.
[21] Gentile M, Agolini E, Cocciadiferro D, Ficarella R, Ponzi E, Bellacchio E, Antonucci MF and Novelli A. Novel exostosin-2 missense variants in a family with autosomal recessive exostosin-2-related syndrome: further evidences on the phenotype. Clin Genet 2019; 95: 165-171.
[22] Heinritz W, Huffmeier U, Strenge S, Miterski B, Zweier C, Leinung S, Bohring A, Mitulla B, Peters U and Froster UG. New mutations of EXT1 and EXT2 genes in German patients with multiple osteochondromas. Ann Hum Genet 2009; 73: 283-291.
[23] Wells DE, Hill A, Lin X, Ahn J, Brown N and Wagner MJ. Identification of novel mutations in the human EXT1 tumor suppressor gene. Hum Genet 1997; 99: 612-615.
[24] Yang K, Shen M, Yan Y, Tan Y, Zhang J, Wu J, Yang G, Li S, Wang J, Ren Z, Dong Z, Wang S, Zhang M and Tian Y. Genetic analysis in fetal skeletal dysplasias by trio whole-exome sequencing. Biomed Res Int 2019; 2019: 2492590.

[25] Li Y, Wang D, Wang W, Wang J, Li H, Wang J, Wang X and Fu Q. Identification of four novel EXT1 and EXT2 mutations in five Chinese pedigrees with hereditary multiple exostoses. Genet Test Mol Biomarkers 2009; 13: 825-830.
[26] Ciavarella M, Coco M, Baorda F, Stanziale P, Chetta M, Bisceglia L, Palumbo P, Bengala M, Raiteri P, Silengo M, Caldarini C, Facchini R, Lala R, Cavaliere ML, De Brasi D, Pasini B, Zelante L, Guarnieri V and D'Agruma L. 20 novel point mutations and one large deletion in EXT1 and EXT2 genes: report of diagnostic screening in a large Italian cohort of patients affected by hereditary multiple exostosis. Gene 2013; 515: 339-348.
[27] Li Y, Wang J, Tang J, Wang Z, Han B, Li N, Yu T, Chen Y and Fu Q. Heterogeneous spectrum of EXT gene mutations in Chinese patients with hereditary multiple osteochondromas. Medicine (Baltimore) 2018; 97: e12855.
[28] Li Y, Wang J, Wang Z, Tang J and Yu T. A genotype-phenotype study of hereditary multiple exostoses in forty-six Chinese patients. BMC Med Genet 2017; 18: 126.
[29] Sarrión P, Sangorrin A, Urreizti R, Delgado A, Artuch R, Martorell L, Armstrong J, Anton J, Torner F, Vilaseca MA, Nevado J, Lapunzina P, Asteggiano CG, Balcells S and Grinberg D. Mutations in the EXT1 and EXT2 genes in Spanish patients with multiple osteochondromas. Sci Rep 2013; 3: 1346.
[30] Xian C, Zhu M, Nong T, Li Y, Xie X, Li X, Li J, Li J, Wu J, Shi W, Wei P, Xu H and Tang YP. A novel mutation in ext2 caused hereditary multiple exostoses through reducing the synthesis of heparan sulfate. Genet Mol Biol 2021; 44: e20200334.
[31] Sarrazin S, Lamanna WC and Esko JD. Heparan sulfate proteoglycans. Cold Spring Harb Perspect Biol 2011; 3: a004952.
[32] Busse-Wicher M, Wicher KB and Kusche-Gullberg M. The exostosin family: proteins with many functions. Matrix Biol 2014; 35: 25-33.
[33] Lopes CC, Dietrich CP and Nader HB. Specific structural features of syndecans and heparan sulfate chains are needed for cell signaling. Braz J Med Biol Res 2006; 39: 157-167.
[34] Xu D and Esko JD. Demystifying heparan sulfate-protein interactions. Annu Rev Biochem 2014; 83: 129-157.
[35] Hall CR, Cole WG, Haynes R and Hecht JT. Reevaluation of a genetic model for the development of exostosis in hereditary multiple exostosis. Am J Med Genet 2002; 112: 1-5.
[36] Hecht JT, Hayes E, Haynes R, Cole WG, Long RJ, Farach-Carson MC and Carson DD. Differentiation-induced loss of heparan sulfate in human exostosis derived chondrocytes. Differentiation 2005; 73: 212-221.
[37] Huegel J, Sgariglia F, Enomoto-Iwamoto M, Koyama E, Dormans JP and Pacifici M. Heparan sulfate in skeletal development, growth, and pathology: the case of hereditary multiple exostoses. Dev Dyn 2013; 242: 1021-1032.
[38] Bovée JV, Cleton-Jansen AM, Wuyts W, Caethoven G, Taminiau AH, Bakker E, Van Hul W, Cornelisse CJ and Hogendoorn PC. EXT-mutation analysis and loss of heterozygosity in sporadic and hereditary osteochondromas and secondary chondrosarcomas. Am J Hum Genet 1999; 65: 689-698.
[39] Alvarez C, Tredwell S, De Vera M and Hayden M. The genotype-phenotype correlation of hereditary multiple exostoses. Clin Genet 2006; 70: 122-130.
[40] Yoon BS, Pogue R, Ovchinnikov DA, Yoshii I, Mishina Y, Behringer RR and Lyons KM. BMPs regulate multiple aspects of growth-plate chondrogenesis through opposing actions on FGF pathways. Development 2006; 133: 4667-4678.
[41] Jones KB, Pacifici M and Hilton MJ. Multiple hereditary exostoses (MHE): elucidating the pathogenesis of a rare skeletal disorder through interdisciplinary research. Connect Tissue Res 2014; 55: 80-88.
[42] Mundy C, Bello A, Sgariglia F, Koyama E and Pacifici M. HhAntag, a Hedgehog signaling antagonist, suppresses chondrogenesis and modulates canonical and non-canonical BMP signaling. J Cell Physiol 2016; 231: 1033-1044.