Received: 20 December 2020 | Revised: 4 August 2021 | Accepted: 10 August 2021

DOI: 10.1002/jcla.23968

CASE REPORT

WILEY

Identification of a novel *EXT2* frameshift mutation in a family with hereditary multiple exostoses by whole-exome sequencing

Mei Yang[1,2] | Hanbing Xie[1,2] | Bocheng Xu[1,2] | Qinqin Xiang[1,2] | He Wang[1,2] | Ting Hu[1,2] | Shanling Liu[1,2]

[1]Department of Obstetrics & Gynecology, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, China

[2]Key Laboratory of Birth Defects and Related Diseases of Women and Children, Ministry of Education, Sichuan University, Chengdu, Sichuan, China

Correspondence
Ting Hu and Shanling Liu, No. 20, Section 3, Renminnan Road, Chengdu, Sichuan, 610041, China.
Emails: huting4123@163.com (T. H.); sunny630@126.com (S. L.)

Funding information
This work was supported by the National Key Research and Development Program of China (2018YFC10022003) and Technology Research & Development Program of Science and Technology Department of Sichuan Province (2017SZ0125, 2018SZ0127)

Abstract

Background: Hereditary multiple exostoses (HME), also referred to as multiple osteochondromas, is an autosomal dominant skeletal disease characterized by the development of multiple overgrown benign bony tumors capped by cartilage and is associated with bone deformity, joint limitation, and short stature. Mutations in exostosin glycosyltransferase (*EXT*)1 and *EXT2* genes, which are located on chromosomes 8q24.1 and 11p13, contribute to the pathogenesis of HME.

Methods: In the present study, a genetic analysis of a four-generation Chinese family with HME was conducted using whole-exome sequencing (WES), followed by validation using Sanger sequencing.

Results: A novel heterozygous frameshift mutation in exon 5 of *EXT2* (c.944dupT, p.Leu316fs) was identified in all affected individuals but was not detected in any unaffected individuals. This mutation results in a frameshift that introduces a premature termination codon at position 318 (p.Leu316fs) with the ability to produce a truncated EXT2 protein that lacks the last 433 amino acids at its C-terminal to indicate a defective exostosin domain and the absence of the glycosyltransferase family 64 domain, or to lead to the degradation of mRNAs by nonsense-mediated mRNA decay, which is critical for the function of *EXT2*.

Conclusion: Our results indicate that WES is effective in extending the *EXT* mutational spectra and is advantageous for genetic counseling and the subsequent prenatal diagnosis.

KEYWORDS
bone tumor, *EXT2*, frameshift mutation, hereditary multiple exostoses, whole-exome sequencing

Ting Hu and Shanling Liu contributed equally.

1 | INTRODUCTION

Hereditary multiple exostoses (HME), also referred to as multiple osteochondromas, is an autosomal dominant skeletal disease. The disease is characterized by the development of multiple overgrown benign bony tumors capped by cartilage, generally occurring at the metaphyses of the long bones.[1] Exostoses may be asymptomatic or result in pain, bone deformity, joint limitation, and short stature.[2,3] The incidence of exostoses is estimated to be 1/50,000 in the western population,[4] which occurs more frequently in males than in females (~1.5:1).[5] The most serious complication of HME is malignant transformation into chondrosarcoma or osteosarcoma, occurring in 1%–2% of the patients.[6,7]

Although the molecular mechanisms associated with HME are still not fully understood, previous studies have reported that mutations in the exostosin glycosyltransferase 1 (*EXT1*) (OMIM: 608177) and *EXT2* (OMIM: 608210) genes, which are located on chromosome 8q24.1 and 11p13, respectively, contribute to the pathogenesis of HME.[8,9] To date, more than 862 mutations in *EXT1* (568) and *EXT2* (294) have been identified, most of which are nonsense, frameshift, or splice site mutations that result in the synthesis of truncated *EXT* protein with dysfunctional activity.

With the rapid development of next-generation sequencing technologies, whole-exome sequencing (WES) has already been applied in the investigation of rare genetic diseases to reveal novel pathogenic single-nucleotide variants (SNVs).[10,11] In this study, a genetic analysis of a four-generation Chinese family with HME was conducted using WES, followed by validation using Sanger sequencing. A novel heterozygous frameshift mutation (c.944dupT) was identified in exon 5 of *EXT2*, which may lead to structural changes in the EXT2 protein (p. Leu316fs).

2 | MATERIALS AND METHODS

2.1 | Patients and clinical data

A four-generation Chinese family with 10 affected members, including a deceased individual, was investigated in this study. The proband was a 28-year-old woman who sought genetic counseling before pregnancy, whose chief complaint was a left forearm deformity, which led to limited physical activity, including pronation and supination, as well as bony prominences around the right knee without movement limitation. Her height was 1.65 m, weight was 48 kg, and body mass index was 17.6 kg/m^2. The pedigree of this family was drawn, and the complaint was found to be related to a typical autosomal dominant disorder (Figure 1). The diagnostic criteria for HME were at least two exostoses detected by radiography or palpated in the metaphysis of long bones.[5] After the related clinical examinations, including X-ray imaging (Figure 2) and physical examinations, were carried out in three affected individuals (II-2, III-5, III-6), the diagnosis of HME was confirmed. Blood samples (4 ml) were collected from the affected members (II-2, II-6, II-7, II-9, III-5, III-6) and unaffected members (II-3, II-4, II-10). Clinical data of the patients with HME in the family are shown in Table 1.

This study was approved by the Medical Ethics Committee of West China Second University Hospital, Sichuan University, and written informed consent was obtained from all participants.

2.2 | Whole-exome sequencing

Genomic DNA of the family members was extracted from the blood according to the manufacturer's instructions (QIAamp DNA Blood Mini

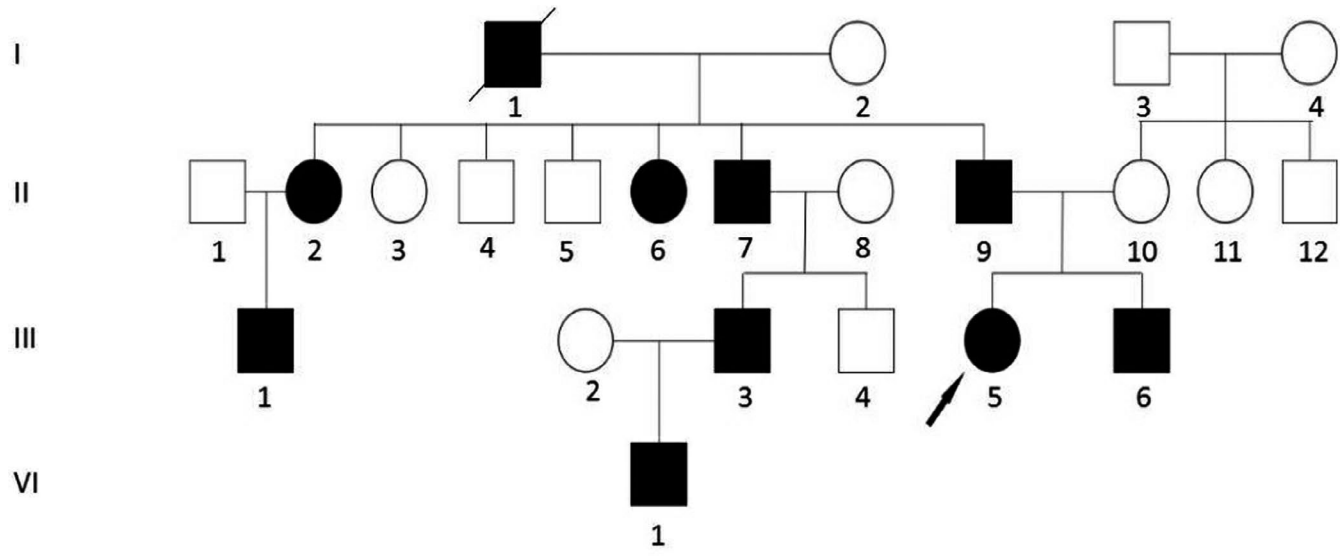

FIGURE 1 Pedigree of a four-generation non-consanguineous family with hereditary multiple exostoses (HME). Generations are shown as I–IV. The black arrow notes the proband (III-5). Empty symbols indicate unaffected individuals and filled symbols indicate affected individuals with HME. The oblique line indicates a deceased individual. Squares indicate male, and circles indicate female

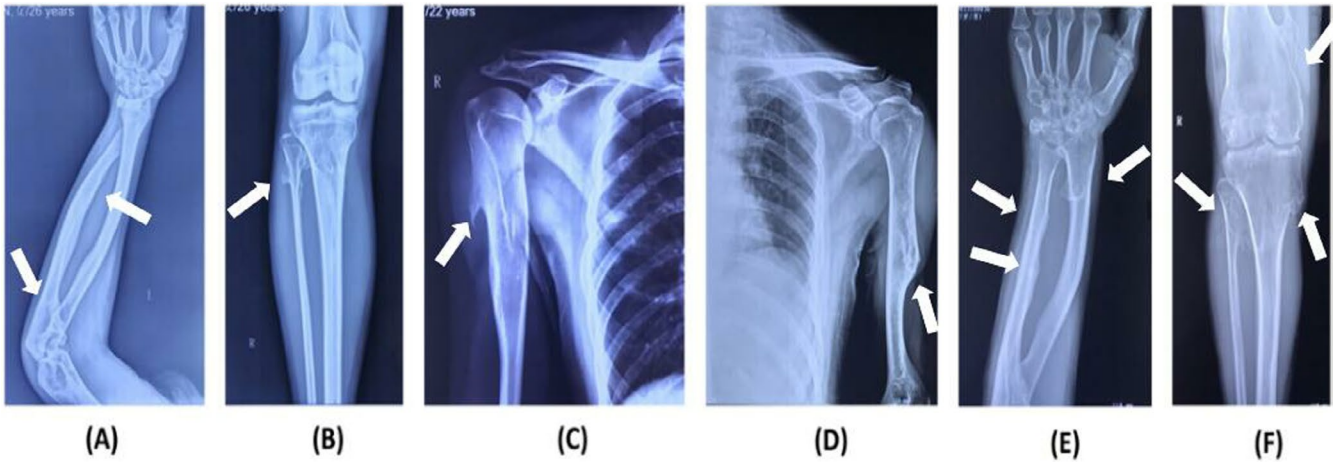

FIGURE 2 Radiograph images of patients, III-5 (the proband) (A, B), III-6 (C), and II-9 (D–F). Exostoses were indicated by white arrows. (A) A relatively curved left ulna and radius, with exostoses on the ulna; (B) exostoses on the right proximal fibulae; (C) exostosis on the right proximal humerus; (D) exostosis on the left humerus; (E) exostosis on the left proximal humerus radius; (F) exostoses on the left distal femur, proximal tibiae, and fibulae

TABLE 1 Clinical data on patients with HME in the family

Patient	Gender	Age, years	Age of onset, years	Height, m	Involved sites
I-1	Male	decreased	unknown	unknown	forearm, humerus, femur
II-2	Female	51	<10	1.58	humerus, ulna, femur
II-6	Female	46	<10	1.54	humerus, ulna, femur
II-7	Male	45	<10	1.67	humerus, femur, tibiae, fibulae
II-9	Male	48	<10	1.65	humerus, ulna, radius, femur, tibiae, fibulae
III-1	Male	21	2	1.72	humerus, femur
III-3	Male	29	2	1.71	humerus, femur, ulna
III-5	Female	26	3	1.65	ulna, fibulae
III-6	Male	22	2	1.74	humerus, femur
IV-1	Male	0.5	At birth	0.62	rib

Kit). Genomic DNA of the family members in trio (proband and both parents [III-5, II-9, II-10]) was subjected to WES. The Nano WES Human Exome V1 (Berry Genomics) was used to capture the sequences. The Illumina NovaSeq6000 platform with 150-bp paired-end reads was used to sequence the enriched library. Next, the Burrows–Wheeler Aligner software tool was used to align the sequencing reads with hg18/GRCh38. Subsequently, local alignment and recalibration of the base quality of the Burrows–Wheeler aligned reads were performed using the GATK Indel Realigner and the GATK Base Recalibrator, respectively (broadinstitute.org/). Then, the GATK Unified Genotyper (broadinstitute.org/) was used to identify SNVs and small insertions or deletions (InDels). Finally, variants were annotated and interpreted using ANNOVAR and the Enliven Variants Annotation Interpretation System, which was authorized by Berry Genomics. The quality control of WES has been illustrated in supplementary table (Table S1).

During the interpretation and analysis of the data, we selected the variations if their minor allele frequencies were <0.05 in gnomAD (http://gnomad.broadinstitute.org/), the 1000 Genomes Project (1000G) (http://browser.1000genomes.org), and the Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org/). SNVs with minor allele frequency (MAF) ≥1% for a recessive model or ≥0.1% for a dominant model were excluded. The detected SNVs were systemically evaluated for pathogenicity based on scientific and medical literature and disease databases obtained from the following resources: PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar), OMIM (http://www.omim.org), Human Gene Mutation Database (http://www.hgmd.org), and Human Genome Variation Society (http://www.hgvs.org/dblist/dblist.html). SNVs were classified into five categories: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign, according to the guidelines of ACMG based on population data, computational and predictive data, functional data, segregation data, de novo data, and allelic data.[12] The detailed process for identifying candidate variants is shown in Figure 3.[13-16]

2.3 | Sanger sequencing

To validate the identified variants by WES, Sanger sequencing was performed to confirm candidate variants in each independent gDNA

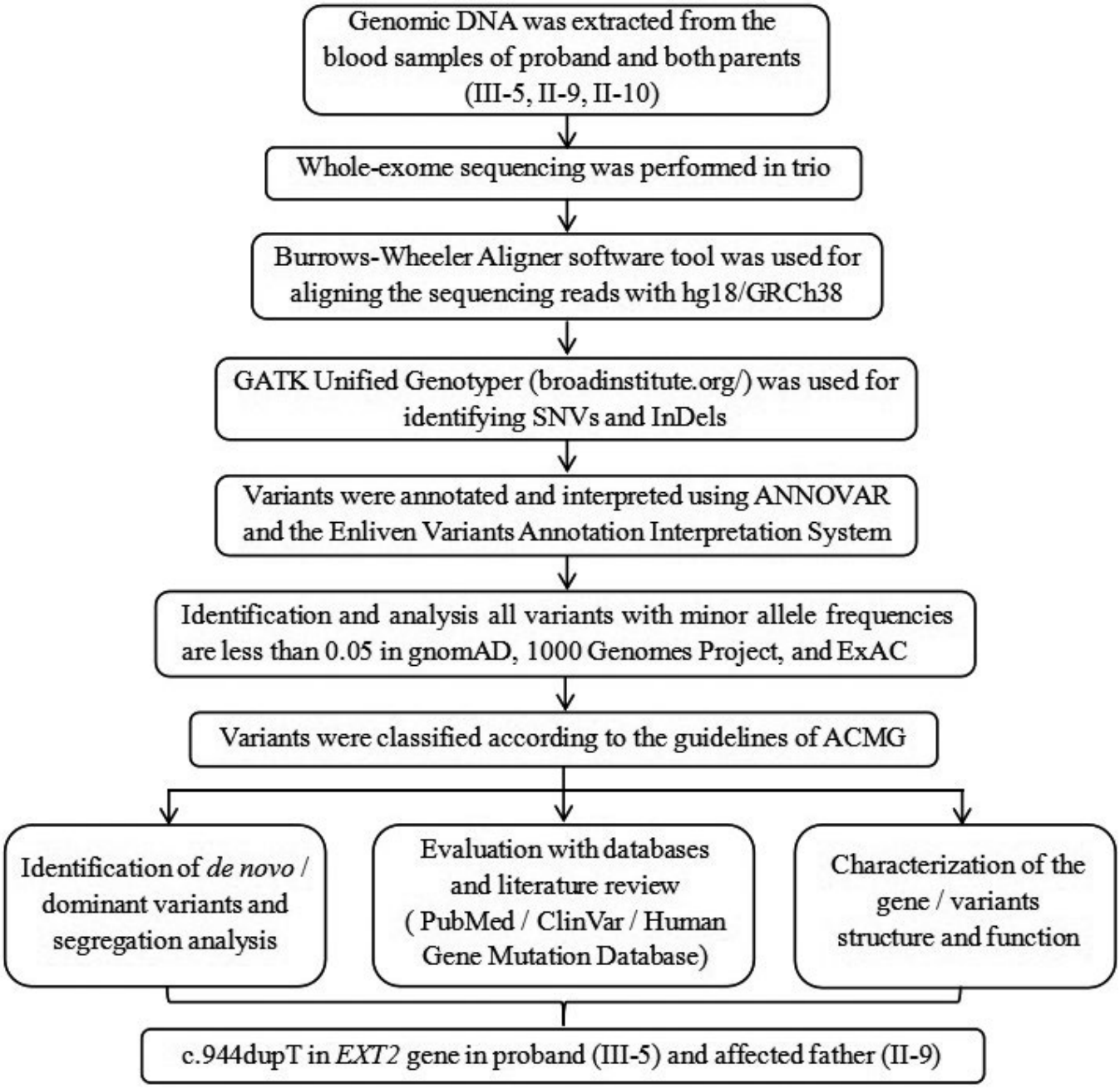

FIGURE 3 The detailed process for identifying candidate variants

sample (II-9, II-10, III-5). The segregation analysis of candidate variants was traced through other affected individuals (II-2, II-6, II-7, III-6) and unaffected individuals (II-3, II-4), also by using Sanger sequencing.

The primers were designed for standard polymerase chain reaction assays using Primer 3 software (version 0.4.0; http://bioinfo.ut.ee/primer3-0.4.0/). For the *EXT2* mutation (GenBank NM_000401.3, c.944dupT, p. Leu316fs), primers were designed as follows: forward 5′-TGG AGG TGA AGA CTG GTA A-3′, reverse 5′-TTG CGA GAG GTA ATG AAT GA-3′. PCR was performed in a total volume of 25 μl containing 5 μl of DNA, 2.5 μl of 10× buffer ($MgCl_2$ plus), 0.5 μl of dNTPs, 1.5 μl of each primer, 0.5 μl of rTaq, and 13.5 μl of H_2O. The PCR program was performed on a Veriti® 96-Well Thermal Cycler (Thermo Fisher) as follows: 95°C for 10 min, followed by 35 cycles of 94°C for 30 s, 62°C for 30 s, 72°C for 45 s, and 72°C for 5 min. PCR products were sequenced using an ABI 3500 Genetic Analyzer (Thermo Fisher Scientific) for c.944dupT.

2.4 | In Silico analysis

Possible changes in the amino acids, which may result from the mutation c.944dupT identified in patients with HME, were predicted using PROVEAN (Protein Variation Effect Analyzer, version 1.1.3).

3 | RESULTS

3.1 | Novel EXT2 mutation in a Chinese family with HME

To reveal the genetic determinants of HME in this family, WES was performed in triplicate. A heterozygous frameshift mutation in exon 5 of *EXT2* was detected (NM_000401.3, c.944dupT) in the proband, which was inherited from the patient's affected father; no mutation was detected in her unaffected mother. No secondary findings associated with the genes recommended by ACMG were detected in our study.[17]

The presence of the mutation was further validated by Sanger sequencing and was confirmed in all affected members (II-2, II-6, II-7, II-9, III-5, III-6) but was not detected in any unaffected members (II-3, II-4, II-10) (Figure 4).

3.2 | In silico analysis

To understand the potential impact of the c.944dupT mutation on *EXT2* function, an in silico analysis was performed. The mutation (c.944dupT) resulted in a frameshift that led to a change in leucine to threonine at amino acid position 316 and a premature stop codon at amino acid position 318 (p. Leu316fs). This mutation produces a truncated EXT2 protein that lacks the last 433 amino acids at its C-terminus.

The EXT2 protein (NP_000392.3), composed of 751 amino acids, contains two vital domains [the protein families (Pfam) database (http://pfam.xfam.org)]: an exostosin domain (134–413 amino acids) and a glycosyltransferase family 64 domain (490–723 amino acids). Amino acid 316 is located within the exostosin domain and prior to the glycosyltransferase domain.

The mutated protein contained an aberrant exostosin domain and lacked the glycosyltransferase family 64 domain (Figure 5). PROVEAN (Protein Variation Effect Analyzer, version 1.1.3) was used to predict the pathogenicity of the mutation (p. Leu316fs) in *EXT2*, which revealed the mutation to be pathogenic (PROVEAN: deleterious, with a score of −1267.928).

4 | DISCUSSION

HME is an autosomal dominant disorder with variable clinical characteristics, including symmetrical bony protrusions in long bones, disproportionate limbs, and short stature,[18] even with a wild-type clinical heterogeneity in the same family.[1,4] Although the exact etiology of HME is still under investigation, approximately 70%–95% of patients with HME have mutations in the *EXT1* or *EXT2* gene.[19,20] Mutations in *EXT1* account for 56%–78% of the patients with HME, whereas mutations in *EXT2* are detected in 21%–44% of the patients.[5] However, *EXT2* mutations are more common than *EXT1* mutations in the Chinese population.[21] Several studies have indicated that patients with *EXT1* mutations have a more severe phenotype than those with *EXT2* mutations.[22-24] The most commonly affected bones with exostoses were the long bones of the upper and lower limbs, while the second most commonly affected bones were those in the chest.[24]

Whether the severity of HME is associated with sex is controversial. In our study, it seemed that a larger number of lesions and more severe phenotypes were observed in men than in women, which is consistent with previous literature.[25,26] However, Porter et al. and Fusco et al. demonstrated that the severity of HME did not differ significantly with sex.[23,24] Thus, a larger cohort study should be conducted in the future.

The *EXT* gene encodes a type II transmembrane protein that forms a heterooligomeric complex that catalyzes the polymerization of heparan sulfate (HS), a key component in the adjustment of chondrocyte proliferation and endochondral ossification.[27] The complex encoded by the *EXT* gene is an essential factor in a signal transduction cascade that regulates chondrocyte differentiation, ossification, and apoptosis.[28,29] Thus, loss-of-function mutations in *EXT1* and *EXT2* cause HS deficiency to trigger the initial development of exostoses.[30,31] The majority of mutations in *EXT1* and *EXT2* are frameshift (42%–44%), nonsense (22%–24%), and splice site mutations (11%–13%), while in the *EXT2* gene, a majority of the mutations are frameshift (42%) and nonsense mutations (22%).[6]

In this study, a four-generation family with HME was investigated, and trio-WES was performed in the proband and her parents. All nine affected individuals (except one deceased individual) were confirmed to carry a novel heterozygous frameshift mutation (c.944dupT) in *EXT2* genes (p. Leu316fs). This mutation was, however, not detected in any of the unaffected members by Sanger sequencing. In addition, according to the distribution of the individuals with HME in this family pedigree (Figure 1), the mutation indicates an autosomal dominant pattern of inheritance from the proband's deceased grandfather to her father.

The novel mutation (c.944dupT) was identified to be located in the exostosin domain of the *EXT2* gene, resulting in a frameshift and the introduction of a premature termination codon (PTC) at position 318 (p. Leu316fs). We proposed the possible mechanisms inducing HME in the family to be as follows: (1) the mutation (c.944dupT) produces a truncated EXT2 protein that lacks the last 433 amino acids at its C-terminus, indicating a defective exostosin domain and the absence of the glycosyltransferase family 64 domain. The glycosyltransferase family 64 domain plays a crucial role in HS biosynthesis; therefore, its absence leads to the loss of the function of the EXT2 protein. PROVEAN (deleterious, with a score of −1267.928) predicted that this mutation was a disease-causing mutation. (2) The PTC at position 318 (p. Leu316fs) may lead to the degradation of mRNAs by nonsense-mediated mRNA decay (NMD). It is well known that NMD is a quality control pathway that degrades mRNAs containing PTCs to exert protective effects by preventing the production of a toxic

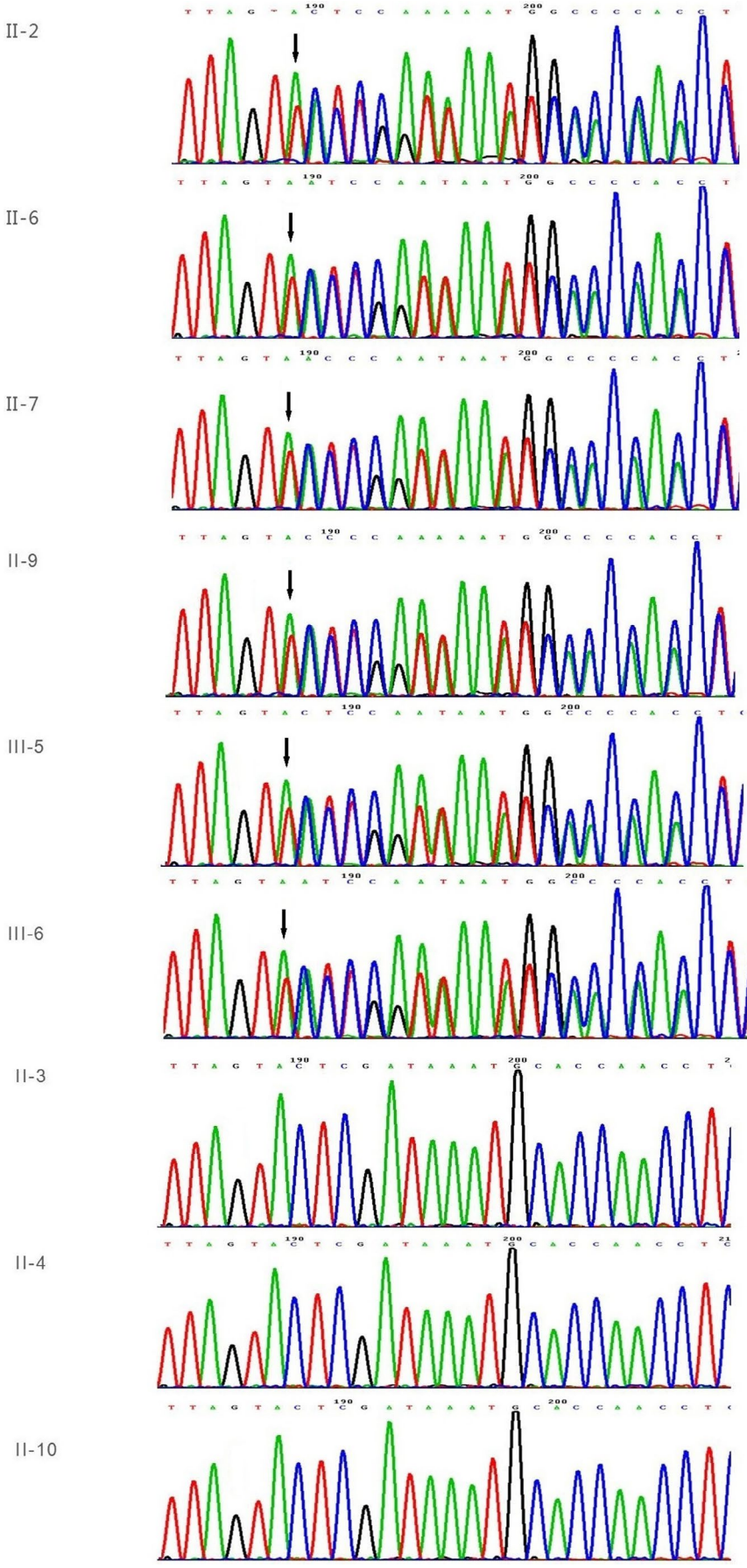

FIGURE 4 Whole-exome sequencing identified a novel *EXT2* frameshift mutation (c.944dupT, p. Leu316fs) in a family with hereditary multiple exostoses (HME). The mutation was detected in all affected individuals (II-2, II-6, II-7, II-9, III-5, III-6) but not in any unaffected members (II-3, II-4, II-10) by Sanger sequencing. The black arrows indicate the point of mutation (dupT)

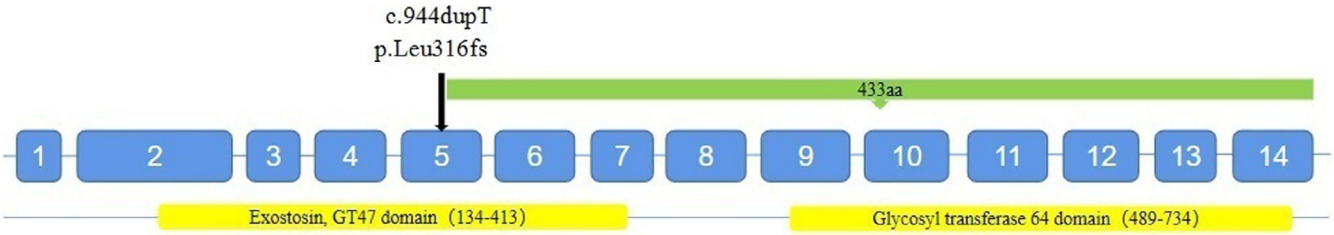

FIGURE 5 The frameshift mutation (c.944dupT) in the *EXT2* gene results in the premature termination of transcription (p. Leu316fs) and produces a truncated EXT2 protein lacking the last 433 amino acids at its C-terminus

truncated protein to ameliorate disease.[32,33] Conversely, in some conditions, NMD tends to aggravate the clinical phenotype in genetic diseases and increase the deleterious effects of the truncating variants.[34,35] NMD may have a negative effect on the HME patients of the family. Thus, further functional tests, such as the RNA synthesis rate, are needed to determine the exact mechanism of the mutation.

In conclusion, a novel heterozygous frameshift mutation in *EXT2* (c.944dupT, p. Leu316fs) was identified in a four-generation family with HME, which was revealed to produce a dysfunctional EXT2 protein following in silico analysis. The present study indicates that WES is effective in the diagnosis of monogenic disorders, such as HME, and extends the *EXT* mutational spectra. Furthermore, our results are advantageous for genetic counseling and the subsequent prenatal diagnoses.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

MY, TH and SLL designed the study. HBX, BCX, and QQX performed the experiments. MY, HW, and TH conducted data analysis. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The present study was approved by the Medical Ethics Committee of West China Second University Hospital of Sichuan University. Written informed consent to participate was obtained from the family members of the patient.

PATIENT CONSENT FOR PUBLICATION

Written informed consent for publication was obtained from the family members of the patient.

ORCID

Ting Hu https://orcid.org/0000-0002-4642-3848
Shanling Liu https://orcid.org/0000-0001-8788-7991

REFERENCES

1. Wicklund CL, Pauli RM, Johnston D, et al. Natural history study of hereditary multiple exostoses. *Am J Med Genet*. 1995;55(1):43-46.
2. Jones KB, Datar M, Ravichandran S, et al. Toward an understanding of the short bone phenotype associated with multiple osteochondromas. *J Orthop Res*. 2013;31(4):651-657.
3. Wu Y, Xing X, Xu S, et al. Novel and recurrent mutations in the EXT1 and EXT2 genes in Chinese kindreds with multiple osteochondromas. *J Orthop Res*. 2013;31(9):1492-1499.
4. Schmale GA, Conrad EU 3rd, Raskind WH. The natural history of hereditary multiple exostoses. *J Bone Joint Surg Am*. 1994;76(7):986-992.
5. Legeai-Mallet L, Munnich A, Maroteaux P, et al. Incomplete penetrance and expressivity skewing in hereditary multiple exostoses. *Clin Genet*. 1997;52(1):12-16.
6. Jennes I, Pedrini E, Zuntini M, et al. Multiple osteochondromas: mutation update and description of the multiple osteochondromas mutation database (MOdb). *Hum Mutat*. 2009;30(12):1620-1627.
7. Tian C, Yan R, Wen S, et al. A splice mutation and mRNA decay of EXT2 provoke hereditary multiple exostoses. *PLoS One*. 2014;9(4):e94848.
8. Wu YQ, Heutink P, de Vries BB, et al. Assignment of a second locus for multiple exostoses to the pericentromeric region of chromosome 11. *Hum Mol Genet*. 1994;3(1):167-171.
9. Wuyts W, Van Hul W, Wauters J, et al. Positional cloning of a gene involved in hereditary multiple exostoses. *Hum Mol Genet*. 1996;5(10):1547-1557.
10. Yang Y, Muzny DM, Reid JG, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *N Engl J Med*. 2013;369(16):1502-1511.
11. Wright CF, FitzPatrick DR, Firth HV. Paediatric genomics: diagnosing rare disease in children. *Nat Rev Genet*. 2018;19(5):253-268.
12. Richards S, Aziz N, Bale S, et al. 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(5):405-424.
13. Han P, Wei G, Cai K, et al. Identification and functional characterization of mutations in LPL gene causing severe hypertriglyceridaemia and acute pancreatitis. *J Cell Mol Med*. 2020;24(2):1286-1299.
14. Zhang R, Chen S, Han P, et al. Whole exome sequencing identified a homozygous novel variant in CEP290 gene causes Meckel syndrome. *J Cell Mol Med*. 2020;24(2):1906-1916.
15. Dai Y, Liang S, Dong X, et al. Whole exome sequencing identified a novel DAG1 mutation in a patient with rare, mild and late age of onset muscular dystrophy-dystroglycanopathy. *J Cell Mol Med*. 2019;23(2):811-818.
16. Zheng Y, Xu J, Liang S, et al. Whole exome sequencing identified a novel heterozygous mutation in HMBS gene in a Chinese patient with acuteintermittent porphyria with rare type of mild anemia. *Front Genet*. 2018;9:129.

17. Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med*. 2017;19(2):249-255.
18. Bovée JV. Multiple osteochondromas. *Orphanet J Rare Dis*. 2008;3:3.
19. Ahn J, Ludecke HJ, Lindow S, et al. Cloning of the putative tumour suppressor gene for hereditary multiple exostoses (EXT1). *Nat Genet*. 1995;11(2):137e43.
20. Stickens D, Clines G, Burbee D, et al. The EXT2 multiple exostoses gene defifines a family of putative tumour suppressor genes. *Nat Genet*. 1996;14(1):25e32.
21. Xu L, Xia J, Jiang H, et al. Mutation analysis of hereditary multiple exostoses in the Chinese. *Hum Genet*. 1999;105(1–2):45-50.
22. Francannet C, Cohen-Tanugi A, Le Merrer M, et al. Genotype-phenotype correlation in hereditary multiple exostoses. *J Med Genet*. 2001;38(7):430-434.
23. Porter DE, Lonie L, Fraser M, et al. Severity of disease and risk of malignant change in hereditary multiple exostoses: a genotype-phenotype study. *J Bone Joint Surg Br*. 2004;86(7):1041-1046.
24. Fusco C, Nardella G, Fischetto R, et al. Mutational spectrum and clinical signatures in 114 families with hereditary multiple properties of selected exostosin variants. *Hum Mol Genet*. 2019;28:2133-2142.
25. Pedrini E, Jennes I, Tremosini M, et al. Genotype-phenotype correlation study in 529 patients with multiple hereditary exostoses: identification of "protective" and "risk" factors. *J Bone Joint Surg Br*. 2011;24:2294-2302.
26. Alvarez C, Tredwell S, De Vera M, et al. The genotype-phenotype correlation of hereditary multiple exostoses. *Clin Genet*. 2006;70(2):122-130.
27. Takei Y, Ozawa Y, Sato M, et al. Three Drosophila EXT genes shape morphogen gradients through synthesis of heparan sulfate proteoglycans. *Development*. 2004;131(1):73-82.
28. Duncan G, McCormick C, Tufaro F. The link between heparan sulfate and hereditary bone disease: fifinding a function for the EXT family of putative tumor suppressor proteins. *J Clin Invest*. 2001;108(4):511-516.
29. Busse M, Feta A, Presto J, et al. Contribution of EXT1, EXT2, and EXTL3 to heparan sulfate chain elongation. *J Biol Chem*. 2007;282(45):32802-32810.
30. Esko JD, Selleck SB. Order out of chaos: assembly of ligand binding sites in heparan sulfate. *Annu Rev Biochem*. 2002;71:435-471.
31. Zak BM, Crawford BE, Esko JD. Hereditary multiple exostoses and heparan sulfate polymerization. *Biochim Biophys Acta*. 2002;1573(3):346-355.
32. Lykke-Andersen S, Jensen TH. Nonsense-mediated mRNA decay: an intricate machinery that shapes transcriptomes. *Nat Rev Mol Cell Biol*. 2015;16(11):665-677.
33. Popp MW, Maquat LE. Leveraging rules of nonsense-mediated mRNA decay for genome engineering and personalized medicine. *Cell*. 2016;165(6):1319-1322.
34. Lindeboom RGH, Vermeulen M, Lehner B, et al. The impact of nonsense-mediated mRNA decay on genetic disease, gene editing and cancer immunotherapy. *Nat Genet*. 2019;51(11):1645-1651.
35. Supek F, Lehner B, Lindeboom RGH. To NMD or Not To NMD: Nonsense-Mediated mRNA Decay in Cancer and Other Genetic Diseases. *Trends Genet*. 2021;37(7):657-668.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Yang M, Xie H, Xu B, et al. Identification of a novel *EXT2* frameshift mutation in a family with hereditary multiple exostoses by whole-exome sequencing. *J Clin Lab Anal*. 2021;35:e23968. https://doi.org/10.1002/jcla.23968