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Phenotypic and Molecular Spectrum of a Turkish Cohort with Hereditary Multiple Osteochondromas

Nilay Güneş[1], Dilek Uludağ Alkaya[1], Aslı Toylu[2], Püren Özüdoğru[3], Evrim Çifçi Sunamak[1], Ali Şeker[4], Bilal Demir[5], Sebuh Kuruğoğlu[6], Ercan Mıhçı[3], Beyhan Tüysüz[1]

[1]Department of Pediatric Genetics, İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, İstanbul, Turkey
[2]Department of Medical Genetics, Akdeniz University Faculty of Medicine, Antalya, Turkey
[3]Department of Pediatric Genetics, Akdeniz University Faculty of Medicine, Antalya, Turkey
[4]Department of Orthopedics and Traumatology, İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Istanbul, Turkey
[5]Department of Orthopedics and Traumatology, Metin Sabancı Baltalimanı Bone Diseases Training and Research Center, Health Sciences University, İstanbul, Turkey
[6]Department of Radiology, İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, İstanbul, Turkey

What is already known on this topic?

- *Hereditary multiple osteochondromas (HMO) is an autosomal dominant disorder characterized by the formation of multiple cartilage-capped bone growth, termed osteochondromas or exostoses. It is mainly caused by heterozygous pathogenic variants in EXT1 or EXT2.*

What does this study add on this topic?

- *This study reports the phenotype–genotype spectrum in a Turkish HMO cohort. There was no difference in the clinical severity in patients with pathogenic variants in EXT1 or EXT2. Four patients without any detectable EXT1 or EXT2 variants had mild phenotypes with class I disease.*

ABSTRACT

Objective: Hereditary multiple osteochondromas is an autosomal dominant disorder caused by heterozygous pathogenic variants in *EXT1* or *EXT2*. We aimed to evaluate the clinical and molecular findings of a Turkish cohort with hereditary multiple osteochondroma.

Materials and Methods: Thirty-two patients aged 1.3–49.6 years from 22 families were enrolled. Genetic analyses were made by *EXT1* and/or *EXT2* sequencing and chromosomal microarray analyses.

Results: We found 17 intragenic pathogenic variants in *EXT1* (13/17) and *EXT2* (4/17), 12 of which are novel. Four probands had *EXT1* deletions, including 2 patients with partial *EXT1* microdeletions involving exons 2–11 and 5–11, and 2 patients with whole-gene deletions. In 21 variants, the frequency of truncating and missense variants was 76.1% and 23.8%, respectively. Two families had no detectable variants in *EXT1* and *EXT2*. All patients had multiple osteochondromas at the long bones, mainly at the tibia, forearm, femur, and humerus. Bowing deformity of the forearms (9/32) and the lower extremities (2/32), and scoliosis (6/32) were observed. The clinical severity was not different between patients with *EXT1* or *EXT2* variants. One patient with an *EXT2* variant and another with an *EXT1* microdeletion had the most severe phenotype with class III disease. Four patients with no *EXT1* or *EXT2* variants had milder phenotypes. Intrafamilial variability in disease severity was not observed.

Conclusion: We report a hereditary multiple osteochondroma cohort with clinical and molecular data including 12 novel intragenic variants in *EXT1* or *EXT2*, and 4 microdeletions involving *EXT1*. Taken together, our data expand the existing knowledge of the phenotype–genotype spectrum in hereditary multiple osteochondroma.

Keywords: Osteochondroma, exostosis, *EXT1*, *EXT2*

INTRODUCTION

Hereditary multiple osteochondromas (HMO, OMIM 133700), also known as hereditary multiple exostoses, is characterized by the formation of several benign cartilage-capped bone tumors, typically located in the metaphyseal region of long bones.[1-4] Osteochondromas are rarely present at birth and grow in number and size during childhood until the closure of the growth plates and may cause various clinical manifestations including limb deformity, restricted joint motion, shortened stature, scoliosis, and compression of peripheral nerves.[1,4]

Corresponding author:
Beyhan Tüysüz
✉ beyhan@istanbul.edu.tr
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HMO is mainly caused by heterozygous pathogenic loss-of-function variants in the *EXT1* or *EXT2* genes.[4,5] *EXT1* and *EXT2* encode exostosin 1 (EXT1) and 2 (EXT2), respectively, which are 2 heparan sulfate glycosyltransferases involved in heparan sulfate synthesis and elongation, and thus, are implicated in chondrocyte proliferation and differentiation.[1,2] Variants in *EXT1* rather than *EXT2* have been reported more frequently in HMO patients, despite the variable prevalence among populations.[6-10] Most of these alterations are responsible for the premature termination and loss of function of EXT proteins. Although EXT1 and EXT2 are expressed in many tissues, the most common pathogenic effect of their alteration affects the growing bones.[11]

In patients with HMO, osteochondromas have been associated with a reduction in skeletal growth, bone deformities, functional limitations, premature osteoarthrosis, and compression of peripheral nerves. Malignant transformation of osteochondroma toward chondrosarcoma is the most serious secondary complication in HMO and occurs in 2%-5% of patients.[4] HMO is characterized by a wide intra- and interfamilial clinical variability, with great differences in the number and location of osteochondromas and varying degrees of deformities and functional impairments.[4,12] Furthermore, few genotype–phenotype correlation studies have been performed to date identifying a more severe HMO phenotype and a higher risk of malignant transformation in patients carrying *EXT1* variants.[4,8,12]

The aim of this study is to evaluate the phenotype severity of a Turkish HMO cohort and to investigate the phenotype–genotype relationship by comparing it with the molecular analysis results of the cohort.

MATERIALS AND METHODS

Thirty-two patients from 22 families followed up by our department with the clinical diagnosis of HMO were included in this study. The diagnosis of HMO was established when 2 or more osteochondromas were diagnosed upon physical and radiographic examinations. Each patient or the guardian of the patient gave their written consent according to the International Ethical Guidelines and Declaration of Helsinki for molecular analyses as well as for the publication of clinical findings, patient images, and molecular data. The study was approved by the institutional ethics committee of Cerrahpaşa Faculty of Medicine (Approval date/number: 23.02.2023/627600).

Genomic DNA was extracted from the blood samples of each proband using standard techniques. *EXT1* (NM_000127.3) and *EXT2* (NM_000401.3) primers for all exons and exon-intron flanking regions were designed. Sequencing analyses were performed by next-generation sequencing using the Ion S5 platform (Thermo Fisher Scientific). Sequence alignment, variant calling, and annotation were carried out using Ion Reporter software. Chromosomal microarray analysis (CMA) using the HumanCytoSNP-12 BeadChip array (Illumina Inc., San Diego, Calif, USA) was carried out according to the manufacturer's instructions. This array contains approximately 300 000 single nucleotide polymorphism (SNP) markers per sample with an average probe spacing of 72kb. The B-allele frequency and log R ratio data were analyzed with KaryoStudio software (Illumina Inc.). The genomic positions were determined using GRCh38/hg38, UCSC Genome Browser. Variant interpretations were made according to the American College of Medical Genetics and Genomics practice guidelines.[13] Bioinformatics tools (PolyPhen2, SIFT, Mutation Taster, DANN) and electronic data (dbSNP, ExAC, 1000G, ClinVar, Varsome, HGMD Professional version, DGV, DECIPHER) were used to identify the variant pathogenicity. All detected variants were searched in the Multiple Osteochondromas Mutation Database (MOdb) (https://databases.lovd.nl/shared/genes/EXT1, and https://databases.lovd.nl/shared/genes/EXT2) (accessed February 12, 2023).

Disease severity was divided into 3 classes according to the number of bone segments affected and the presence of skeletal deformities and/or functional limitations using the following criteria: class I: no deformities and no functional limitations (A: ≤5 sites with osteochondromas, B: >5 sites with osteochondromas), class II: deformities and no functional limitations (A: ≤5 sites with deformities, B: >5 sites with deformities), and class III: deformities and functional limitations (A: functional limitation of 1 site, B: functional limitation of >1 site).[14]

Statistical Analysis

Statistical analysis was performed with Statistical Package for Social Sciences statistical software (version 21.0 for Mac OS X; IBM corp., Armonk, NY, USA). The Kolmogorov–Smirnov test was used to determine the distribution of continuous variables. The Mann–Whitney *U* test was used to compare nonparametric data. Categorical data were compared using Chi-square or Fisher's exact tests. A *P*-value of less than .05 was considered statistically significant.

RESULTS

Clinical Characteristics

The median age of the study cohort including 17 males and 15 females was 11.1 years at the first examination. Nine patients were followed up with a median period of 3.5 years. The clinical features of the patients were summarized in Table 1. Seven (31.8%) of the 22 families in the study cohort were familial.

Patients' median standard deviation score (SDS) of height at their initial examination was −1.5 and the SDS values were in a broad range from −4.8 to 1.2. Six adult patients (P3, P4, P6, P15, P19, and P25) and 6 patients younger than 18 years of age (P13, P14, P18, P26, P29, and P31) had short stature. Ten (31.2%), 9 (28.1%), 6 (18.7%), 5 (15.6%), and 2 patients (6.2%) in the cohort had class IIB, IB, IA, IIA, and IIIA diseases, respectively. Protuberances at the ends of long bones were present in 7 patients. Osteochondromas were present at the long bones of the upper and/or lower limbs (32/32), scapulae (10/32), and phalangeal bones (9/32). The earliest age at which osteochondromas were noticed was around 1 year of age in P2 and P9. The median number of the affected body sites with osteochondromas in the total cohort was 6 (range: 2-13), while the median number of total osteochondromas was 11 (range: 2-32). Nine (28.1%) patients had bowing of the forearms (P3, P4, P6, P9, P14, P15, P21, P22, and P25). Six (18.7%) patients had Madelung's deformity (P3, P4, P6, P9, P14, P15). Two (6.2%) patients (P12, P15) had deformities of the lower extremities. Scoliosis was present in 6 (18.7%) patients (P8, P9, P10, P12, P13, and P30). Four (12.5%) patients (P9, P13, P20, and P31) complained of pain

Table 1. Summary of the Clinical Characteristics of the Total HMO Cohort

Family	Case	Sex	Age (*/**)	Height* (SDS)	Number of Affected Bone Segments	Number of Exostoses (a/b/c)	Bowing/ Dysmetria of the Forearms	Bowing/ Dysmetria of the Legs	Functional Disability	Pain	Scoliosis	Clinical Classification	Other Clinical Features
Family 1	P1	M	4 yr. 3 mo./N/A	109 (1.2)	5	3/8/–	–/–	–/–	–	–	–	IA	
Family 2	P2	F	1 yr. 4 mo./ 5 yr. 6 mo.	73 (–1.6)	6	3/4/3	–/–	–/–	–	–	–	IB	
	P3	F	29 yr. 6 mo./ 33 yr. 1 mo.	146 (–2.6)	8	4/4/–	+/+	–/–	–	–	–	IIB	
	P4	F	33 yr. 2 mo./ 36 yr. 9 mo.	146 (–2.6)	9	6/4/1	+/+	–/–	–	–	–	IIB	
Family 3	P5	M	4 yr. 6 mo./N/A	106 (0.2)	6	4/4/2	–/–	–/–	–	–	–	IB	
	P6	F	31 yr. 11 mo./N/A	150 (–2)	7	5/6/1	+/+	–/–	–	–	–	IIB	
Family 4	P7	F	9 yr. 4 mo./ 9 yr. 11 mo.	135 (0.1)	2	–/–	–/–	–/–	–	–	–	IA	Underwent costal resection
Family 5	P8	M	10 yr. 1 mo./N/A	140 (0.2)	7	5/8/–	–/–	–/–	–	–	+	IIB	Coxa valga deformity
Family 6	P9	F	1 yr. 4 mo./ 18 yr. 1 mo.	72 (–1.9)	12	16/14/2	+/+	–/–	+	+	+	IIIA	Down syndrome, operated due to VSD and scoliosis, hypothyroidism
Family 7	P10	M	5 yr. 8 mo./N/A	113 (0.06)	4	1/7/–	–/–	–/–	–	–	+	IIA	
Family 8	P11	M	11 yr. 8 mo./ 12 yr. 3 mo.	155 (1.1)	7	5/12/1	–/–	–/–	–	–	–	IB	
Family 9	P12	F	9 yr. 3 mo./N/A	133 (–0.08)	5	1/5/–	–/–	+/–	–	–	+	IIA	
Family 10	P13	F	13 yr. 7 mo./N/A	144 (–2.1)	13	8/10/1	–/–	–/–	–	+	+	IIB	
Family 11	P14	M	10 yr. 11 mo./N/A	128 (–2.1)	6	6/8/–	+/+	–/–	–	–	–	IIB	
Family 12	P15	F	34 yr. 5 mo./N/A	150 (–2)	7	6/7/–	+/+	–/–	–	–	–	IIB	Operated due to the bowing of the forearms
Family 13	P16	M	6 yr./N/A	115 (0.03)	7	6/11/–	–/–	–/–	–	–	–	IB	
	P17	M	35 yr./N/A	N/A	6	4/4/1	–/–	–/–	–	–	–	IB	
Family 14	P18	M	13 yr./N/A	136 (–2.4)	9	3/9/2	–/–	–/–	–	–	–	IB	
	P19	M	49 yr./N/A	153 (–3.2)	4	3/6/–	–/–	–/–	–	–	–	IA	
Family 15	P20	M	15 yr. 2 mo./N/A	171 (0.1)	6	8/3/–	–/–	–/–	–	+	–	IB	
	P21	M	6 yr. 3 mo./N/A	120 (0.7)	4	5/6/–	+/–	–/–	–	–	–	IIA	
	P22	F	38 yr./N/A	N/A	4	1/7/–	+/–	–/–	–	–	–	IIA	
Family 16	P23	M	1 yr 7 mo./ 2 yr. 1 mo	78 (–1.4)	6	5/2/1	–/–	–/–	–	–	–	IB	
	P24	M	32 yr. 11 mo./N/A	176 (–0.08)	5	1/7/1	–/–	–/–	–	–	–	IA	
Family 17	P25	M	35 yr./N/A	159 (–2.4)	6	4/14/–	+/–	–/–	–	–	–	IIB	Operated for exostoses of the lower extremities
	P26	F	13 yr. 4 mo./N/A	140 (–2.5)	7	4/13/1	–/–	–/–	–	–	–	IIB	Operated for phalangeal exostoses
	P27	M	11 yr. 4 mo./N/A	137 (–1)	7	6/12/–	–/–	–/–	–	–	–	IIB	

(*Continued*)

Table 1. Summary of the Clinical Characteristics of the Total HMO Cohort (*Continued*)

Family	Case	Sex	Age (*/**)	Height* (SDS)	Number of Affected Bone Segments	Number of Exostoses (a/b/c)	Bowing/ Dysmetria of the Forearms	Bowing/ Dysmetria of the Legs	Functional Disability	Pain	Scoliosis	Clinical Classification	Other Clinical Features
Family 18	P28	F	5 yr. 6 mo./N/A	110 (−0.1)	4	2/4/−	−/−	−/−	−	−	−	IA	
Family 19	P29	M	2 yr 7 mo./ 6 yr. 1 mo	103 (−2.4)	5	1/2/−	−/−	−/−	−	−	−	IA	Trichorhinophalangeal syndrome type II
Family 20	P30	F	1 yr 4 mo./ 3 yr. 4 mo	90 (−1.6)	4	1/3/−	−/−	−/−	−	−	+	IIA	Trichorhinophalangeal syndrome type II, intellectual disability
Family 21	P31	F	14 yr. 3 mo./N/A	129 (−4.8)	8	2/10/−	−/−	−/−	+	+	−	IIIA	Trichorhinophalangeal syndrome type II, intellectual disability
Family 22	P32	F	6 yr./N/A	115 (0.1)	12	9/14/3	−/−	−/−	−	−	−	IB	

F, female; HMO, hereditary multiple osteochondromas; M, male; mo., months; N/A, not available; SDS, standard deviation score; VSD, ventricular septal defect; yr., years.
*At the first examination; **at the last examination; a, upper extremities; b, lower extremities; c, other body segments.

which is not associated with the number of osteochondromas. None of the patients were diagnosed with malignant tumors.

Three patients (P29, P30, and P31) had similar features including bulbous nose, long philtrum, thin upper lip, and cone-shaped epiphyses in addition to osteochondromas on radiographic examinations which were consistent with trichorhinophalangeal syndrome type II. Two of these patients (P30, P31) had intellectual disability. The clinical findings of these patients were published in our previous study.[15] A further patient with Down syndrome (P9) underwent a scoliosis repair and had a functional disability on both ankles. She also had hypothyroidism, congenital heart abnormality, and severe intellectual disability. None of the remaining patients had endocrinopathies or cardiac abnormalities.

Genetic Studies

The results of the genetic analyses of the patients were summarized in Table 2. Of 32 patients, 28 patients (87.5%) had the genetic diagnosis of HMO. Nineteen patients presented intragenic pathogenic *EXT1* variants, and 3 patients had pathogenic *EXT2* variants. Three patients from 1 family (P20, P21, and P22) had 8q24.11 microdeletions over 44 Kb involving the *EXT1* gene partially detected by CMA. P29, P30, and P31 had large microdeletions with variable breakpoints involving the TRPS1-EXT1 interval. No pathogenic variants were found in 4 patients (P18, P19, P23, and P24) by sequencing analyses. Karyotype analysis of the patient diagnosed with Down syndrome (P9) showed trisomy 21 caused by a 21;21 Robertsonian translocation.

In sequencing analyses, 17 variants including 13 *EXT1* variants and 4 *EXT2* variants were detected. One patient (P11) was heterozygous for both novel likely pathogenic *EXT2* variants. Segregation analysis in this family was not possible. While 8 out of 13 intragenic variants detected in *EXT1* were truncating variants (splicing, frameshift, and nonsense), all 4 *EXT2* variants were truncating variants. In *EXT1*, exon 1 was the most affected exon containing 2 missenses and 2 nonsense variants.

Genotype–Phenotype Correlation

The disease severity was not different between patients with *EXT1* and *EXT2* variants ($P > .05$). Four patients without any detectable pathogenic variants had a relatively mild phenotype with class IA (P19, P24) and class IB (P18, P23) disease. The patient (P31) with a partial microdeletion involving exons 2-11 of *EXT1* had class IIIA disease, whereas 2 patients with whole-gene deletions (P29, P30) were milder with class IA and IIA diseases, respectively. In familial cases in the total cohort, intrafamilial variability in clinical findings and disease severity was not observed.

DISCUSSION

In the current study, we studied *EXT1* and *EXT2* variant spectra in 32 HMO patients from 22 families. We found disease-causing intragenic variants in 16 families, including 13 families (81.2%) with *EXT1* variants, and 3 families (18.7%) with *EXT2* variants. Among the variants currently listed in the MOdb, variants in *EXT1* are more common than those in *EXT2*, with a rate of 63.4%, which is similar to our rate. Among the variants in the MOdb, missense variants are rare at around 20%, and most variants

Table 2. The Results of Genetic Analyses of the HMO Cohort

Family	Proband Code	Family History	Detected Heterozygous Variants	Variant Type	Variant Pathogenicity/ Novelty	Variant Localization
Family 1	P1	Maternal inheritance	EXT1:c.1219C>G, p.Gln407Glu	Missense	VOUS/novel	Exon 4/11
Family 2	P2, P3, P4	Familial	EXT1:c.2115delG, p.Met705IlefsTer13	Frameshift	Pathogenic/novel	Exon 11/11
Family 3	P5, P6	Familial	EXT1:c.1978delC, p.Leu660TrpfsTer5	Frameshift	Pathogenic/novel	Exon 10/11
Family 4	P7	*De novo*	EXT1:c.1019G>A, p.Arg340His	Missense	Pathogenic/known	Exon 2/11
Family 5	P8	Maternal inheritance	EXT1:c.493C>T, p.Gln165Ter	Nonsense	Likely pathogenic/known	Exon 1/11
Family 6	P9	*De novo*	EXT2:c.764dup, p.Tyr255Ter	Nonsense	Likely pathogenic/novel	Exon 4/14
Family 7	P10	*De novo*	EXT1:c.552G>A, p.Trp184Ter	Nonsense	Likely pathogenic/known	Exon 1/11
Family 8	P11	*De novo*	EXT2:c.1273-1G>C; EXT2:c.1179-1G>C	Splicing	Likely pathogenic/novel; Likely pathogenic/novel	Intron 7/13; Intron 6/13
Family 9	P12	*De novo*	EXT2:c.515_518del, p.Asp172ValfsTer130	Frameshift	Likely pathogenic/novel	Exon 2/14
Family 10	P13	*De novo*	EXT1:c.1632+2T>G	Splicing	Likely pathogenic/novel	Intron 7/10
Family 11	P14	Paternal inheritance	EXT1:c.1820del, p.Gly607AspfsTer14	Frameshift	Likely pathogenic/novel	Exon 9/11
Family 12	P15	*De novo*	EXT1:c.803G>T, p.Gly268Val	Missense	Pathogenic/novel	Exon 1/11
Family 13	P16, P17	Familial	EXT1:c.166C>T, p.Pro56Ser	Missense	VOUS/novel	Exon 1/11
Family 14	P18, P19	Familial	No pathogenic variants in *EXT1* and *EXT2*	—	—	—
Family 15	P20, P21, P22	Familial	arr[GRCh38]8q24.11 (117778822-117822898)x1	Large deletion (over 44 Kb)	Pathogenic	Exons 5-11 of *EXT1*
Family 16	P23, P24	Familial	No pathogenic variants in *EXT1* and *EXT2*	—	—	—
Family 17	P25, P26, P27	Familial	EXT1:c.2084del, p.Pro695LeufsTer11	Frameshift	Likely pathogenic/known	Exon 11/11
Family 18	P28	*De novo*	EXT1:c.1021A>G, p.Arg341Gly	Missense	Pathogenic/known	Exon 2/11
Family 19	P29	*De novo*	arr[GRCh38]8q23.3q24.12 (113028744-118670887)x1	Large deletion (over 5600 Kb)	Pathogenic	Whole gene (*EXT1*)
Family 20	P30	*De novo*	arr[GRCh38]8q23.3q24.12 (112473987-125160640)x1	Large deletion (over 12 600 Kb)	Pathogenic	Whole gene (*EXT1*)
Family 21	P31	*De novo*	arr[GRCh38]8q23.1q24.11 (107382797-117861338)x1	Large deletion (over 10 400 Kb)	Pathogenic	Exons 2-11 of *EXT1*
Family 22	P32	*De novo*	EXT1:c.1383T>A, p.Tyr461Ter	Nonsense	Likely pathogenic/novel	Exon 5/11

HMO, hereditary multiple osteochondromas; Kb, kilobase; N/A, not available.

(around 60%) are truncating variants. Similarly, the frequency of missense variants was (5/21) 23.8% in our study. Truncating variants in *EXT1* and *EXT2* were the frameshift (5/21), nonsense (4/21), splicing (3/21), and gross deletion variants (4/21). In the MOdb, frameshift alterations have been listed with an approximate rate of 30%, similar to our results. In the literature, genomic alterations cannot be detected in about 10%-15% of HMO patients by conventional methods due to alterations such as intronic deletions, translocations, or somatic mosaicism.[4,6,16] Correspondingly, 2 families (9.0%) in our study did not have intragenic variants in either *EXT1* or *EXT2*.

In HMO, it has been observed that osteochondromas are diagnosed before 3 years of age in 50% of patients and before the end of the first decade in more than 80% of cases.[1,4] Our patients were not suitable for age-based comparison as they showed heterogeneity in age. HMO may involve any bone which grows from endochondral ossification, and the most common region for osteochondromas is the lateral side of the growth plate of a long bone.[1] A mean of 6 osteochondromas per patient has been reported in previous studies, but the number of osteochondromas or involved bones, and the degree of the deformity vary.[3,4] The bones commonly affected by the disease are the long bones including the femur, radius and ulna, and tibia.[4] Correspondingly, the median number of osteochondromas per patient in our cohort was 11, despite the relatively younger age of our cohort. In our patients, the tibia, forearm, femur, and humerus were the commonly affected bones. No osteochondromas were detected in bones developed by intramembranous ossification. Angular deformities of the forearms (about 39%) and/or lower limbs (about 10%) and inequality in limb length (about 10%) are common orthopedic complications in HMO.[4] In our study, bowing deformity and dysmetria of the forearms were present in 6 (18.7%) patients. Two patients (6.2%) had deformities of the lower extremities.

In several studies on the genotype–phenotype correlation in HMO, pathogenic *EXT1* variants are associated with a severe

phenotype, including greater numbers of osteochondromas, skeletal deformities, and short stature and may have a higher risk for chondrosarcoma.[3,4,12] Furthermore, it has been reported that the presence of *EXT2* variants, the absence of *EXT1* and *EXT2* variants, and the female sex were associated with a mild phenotype.[12] In our study, we did not observe a difference in the clinical severity in our cases with *EXT1* or *EXT2* variants. Moreover, contrary to the study of Pedrini et al.[12] the most severe patient in our study was a female with class III disease carrying a likely pathogenic variant in *EXT2* (P9). The second severe patient with class III disease was also a female with partial *EXT1* microdeletions involving exons 2-11 (P31). On the other hand, 4 patients without any detectable pathogenic variants in *EXT1* and *EXT2* had a relatively mild phenotype with class I disease, in accordance with the previous studies.[8,12]

In the *EXT1* gene, 4 out of 13 intragenic variants were detected in exon 1, including 2 nonsense and 2 missense variants. Three further missense variants in *EXT1* were in exons 2 (c.1019G>A, and c.1021A>G) and 4 (c.1219C>G). The variants in exon 2 were pathogenic and their region has been known as a cluster region for missense variants.[5] On the other hand, the missense variant in exon 4 was a variant of unknown clinical significance. In the study of Santos et al.[9] all missense variants in *EXT1* were found in exons 1 and 2. They postulated that this is an important region since it contains the exons (exons 1 to 3) that encode the exostosin domain of the EXT1 protein. All detected *EXT2* variants in our study were novel and they were located in regions that are involved in encoding amino acids of the exostosin domain. It has been previously reported in several studies that *EXT2* pathogenic variants are mainly located in the first 8 exons.[4-6,9,10]

CONCLUSION

In the current study, a total of 87.5% of the HMO cohort presented *EXT1* or *EXT2* variants. All patients had multiple osteochondromas at the long bones. The most common complication of osteochondromas was bowing deformity and dysmetria of the long bones. The clinical severity was not different between the patients with *EXT1* or *EXT2* variants. Although HMO is a disease with characteristic clinical and radiological features and molecular genetic testing is not usually required to make the diagnosis, molecular confirmation can be a valid diagnostic tool for a definitive diagnosis.

Ethics Committee Approval: This study was approved by Ethics Committee of İstanbul University-Cerrahpaşa (Approval No: 627600, Date: 23.02.2023).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

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REFERENCES

1. Beltrami G, Ristori G, Scoccianti G, Tamburini A, Capanna R. Hereditary Multiple exostoses: a review of clinical appearance and metabolic pattern. *Clin Cases Miner Bone Metab.* 2016;13(2):110-118. [CrossRef]
2. Bukowska-Olech E, Trzebiatowska W, Czech W, et al. Hereditary multiple exostoses-A review of the molecular background, diagnostics, and potential therapeutic strategies. *Front Genet.* 2021;12:759129. [CrossRef]
3. D'Arienzo A, Andreani L, Sacchetti F, Colangeli S, Capanna R. Hereditary multiple exostoses: current insights. *Orthop Res Rev.* 2019;11:199-211. [CrossRef]
4. Wuyts W, Schmale GA, Chansky HA, Raskind WH. Hereditary multiple osteochondromas. *GeneReviews* [internet]. In: Adam MP, Everman DB, Mirzaa GM, et al., eds. Seattle, WA: University of Washington 1993.
5. Vink GR, White SJ, Gabelic S, Hogendoorn PC, Breuning MH, Bakker E. Mutation screening of EXT1 and EXT2 by direct sequence analysis and MLPA in patients with multiple osteochondromas: splice site mutations and exonic deletions account for more than half of the mutations. *Eur J Hum Genet.* 2005;13(4):470-474. [CrossRef]
6. Al-Zayed Z, Al-Rijjal RA, Al-Ghofaili L, et al. Mutation spectrum of EXT1 and EXT2 in the Saudi patients with hereditary multiple exostoses. *Orphanet J Rare Dis.* 2021;16(1):100. [CrossRef]
7. Ishimaru D, Gotoh M, Takayama S, et al. Large-scale mutational analysis in the EXT1 and EXT2 genes for Japanese patients with multiple osteochondromas. *BMC Genet.* 2016;17:52. [CrossRef]
8. Kim S, Lee CH, Choi SY, Kim MK, Jung ST. A genotype-phenotype study of multiple hereditary exostoses in forty-three patients. *J Clin Med.* 2022;11(13) [CrossRef]
9. Santos SCL, Rizzo IMPO, Takata RI, Speck-Martins CE, Brum JM, Sollaci C. Analysis of mutations in EXT1 and EXT2 in Brazilian patients with multiple osteochondromas. *Mol Genet Genomic Med.* 2018;6(3):382-392. [CrossRef]
10. Sarrión P, Sangorrin A, Urreizti R, et al. Mutations in the EXT1 and EXT2 genes in Spanish patients with multiple osteochondromas. *Sci Rep.* 2013;3:1346. [CrossRef]
11. Fusco C, Nardella G, Fischetto R, et al. 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(13):2133-2142. [CrossRef]
12. 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 Am.* 2011;93(24):2294-2302. [CrossRef]
13. Richards S, Aziz N, Bale S, et al. 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. [CrossRef]
14. Mordenti M, Ferrari E, Pedrini E, et al. Validation of a new multiple osteochondromas classification through Switching Neural Networks. *Am J Med Genet A.* 2013;161A(3):556-560. [CrossRef]
15. Güneş N, Usluer E, Yüksel Ülker A, et al. The clinical and molecular spectrum of trichorhinophalangeal syndrome types I and II in a Turkish cohort involving 22 patients. *Turk Arch Pediatr.* 2023;58(1):98-104. [CrossRef]
16. Szuhai K, Jennes I, de Jong D, et al. Tiling resolution array-CGH shows that somatic mosaic deletion of the EXT gene is causative in EXT gene mutation negative multiple osteochondromas patients. *Hum Mutat.* 2011;32(2):E2036-E2049. [CrossRef]