



Hereditary Multiple Exostoses: Genotypic and Phenotypic Characteristics of a Turkish Family

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Abstract

Aim: Hereditary Multiple Exostoses has an autosomal dominance inheritance pattern and is classified as an O-xyloglucan glycosylation defect. Several Hereditary Multiple Exostoses patient series from Turkey have been published, but these did not include genetic analyses. This study is the largest genetic analysis of a Hereditary Multiple Exostoses patient series in Turkey reported to date and aims to determine the genotypic and phenotypic characteristics of Turkish Hereditary Multiple Exostoses family.

Material and Methods: Based on an index patient with at least two osteochondromas in the long bones found clinically and with a positive family history, a father and his five children underwent genetic analysis. Polymerase chain reaction amplification and direct sequencing of all coding exons of the *Exostosin glycosyltransferase* gene.

Results: Three of the children were male and two were female. Mutations were found in the father and four of the children, the exception being the third child (male). A heterozygotic c.1019G>A (p.Arg340His) mutation was found in the *Exostosin glycosyltransferase 1* gene of all of the patients. However, disease symptoms started earlier and were more severe in males than in females. Cranial bone involvement was detected in three patients.

Conclusion: Although the clinical findings of our patients were generally consistent with the literature, the frequency of cranial involvement was high in this series of Hereditary Multiple Exostoses patients, unlike those reported in previous studies and no Hereditary Multiple Exostoses patient with an *Exostosin glycosyltransferase 2* gene mutations has been reported in Turkey to date.

- What is Already Known? Several HME patient series from Turkey have been published, but these did not include genetic analyses.
- Some reports state that cranial bones are not involved, while others state that they are rarely involved.

What this Study Adds?

- This study is the first and widest genetically studied patient series reported from Turkey.
- Cranial involvement was high in this series of HME patients.
- There has been no *EXT2* gene mutation reported from Turkey so far.

Keywords: Hereditary Multiple Exostoses; *EXT1* gene; *EXT2* gene; Cranial involvement

Introduction

Hereditary Multiple Exostoses (HME) (OMIM 133700/133701) is a disease classified among the rarely found O-xyloglucan glycosylation defects and caused by mutation of the *Exostosin glycosyltransferase 1* (*EXT1*) or *Exostosin glycosyltransferase 2* (*EXT2*) gene [1,2]. The *EXT1* and *EXT2* genes encode the glucosyltransferase responsible for the synthesis of heparan sulfate and proteoglycans in the endoplasmic reticulum [3]. Mutation of the *EXT1* or *EXT2* gene results in insufficient synthesis of heparan sulfate, which facilitates chondrocyte proliferation, in turn giving rise to the development of multiple exostoses because of overdevelopment of neighboring bone parts [4]. Diagnosis of the HME requires at least two exostoses at the juxtaepiphyseal part of long bones, a positive family history, and mutation of at least one *EXT* gene [2].

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Table 1: Clinical, demographic findings and gene mutations of the patients.

	Father	1 st Child	2 nd Child	3 rd Child	4 th Child	5 th Child
Gender	Male	Male	Female	Male	Male	Female
Age (year)	40	17	15	12	10	6
Height (cm)	159	153	150	136	113	103
(Persantile)	(3P↓)	(3P↓)	(3-10 P)	(25P-50P)	(3P↓)	(25P-50P)
Beginning of exostoses (Month)	12	24	120	There isn't exostose	18	There isn't exostose
The number of exostoses	More than 20	17	10	There isn't exostose	5	There isn't exostose
Location of exostoses	Humerus Ulna Metacarp Femur Tibia Metatars	Humerus Metacarp Tibia Metatars Cranium	Humerus Tibia Cranium	There isn't exostose	Tibia, Clavicula, Cranium	There isn't exostose
Mutation	Heterozygot c.1019G>A (p.Arg340His)	Heterozygot c.1019G>A (p.Arg340His)	Heterozygot c.1019G>A (p.Arg340His)	There isn't mutation	Heterozygot c.1019G>A (p.Arg340His)	Heterozygot c.1019G>A (p.Arg340His)
Gene	EXT 1	EXT 1	EXT 1	There isn't mutation	EXT 1	EXT 1

EXT: Exostosin Glycosyltransferase; P: Persantile

The prevalence of HME is 1/50,000 of the general population. Deformities and functional losses are more severe in patients with the *EXT1* genotype and in males (male:female ratio, 1.5:1). The lower frequency in females might be due to a lighter phenotype and a probable omission. Moreover, 63% of patients have a family history [5].

Although mutations in the *EXT1* and *EXT2* genes have an autosomal dominance inheritance pattern, *de novo* mutations are not rare [5]. In HME patients, 70% to 95% of mutations are in the *EXT1* and *EXT2* genes; moreover, the mutation frequency of the former is almost twofold greater than that of the latter [4,6,7]. Exostoses can develop in all bones but are most frequently located in the knee, humerus, scapula, costa, elbow, hand, foot, and pelvis [8]. Regarding the involvement of craniofacial bones, reports are conflicting [3,5].

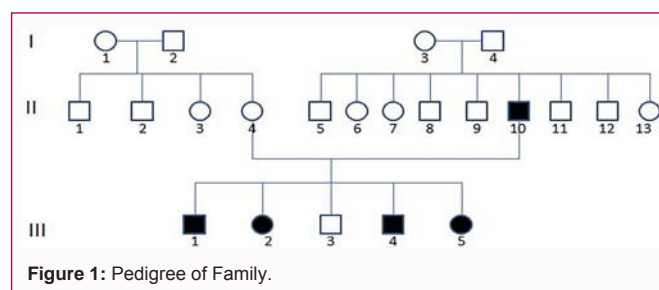
This article presents genotypic and phenotypic characteristics determined in Turkey's first and largest series of HME patients proven by genetic analysis, with some different clinical characteristics than those reported in the literature.

Patients and Methods

In a family of 7 people with findings of the disease, DNA analysis was made for the father and five children in the form of direct sequencing of all the coding exons (exon 1-11) of *EXT1* gene with PCR amplification method. DNA isolation was performed by salt precipitation method from peripheral blood samples. PCR was performed according to standard protocols. PCR fragments that included aberrant fragments were reamplified and purified from agarose gels by use of the SephaGlas BandPrep Kit (Pharmacia Biotech). Written consent was obtained from the family.

Results

Genetic analysis was performed on all of the five children and the father of the family. Three of the children were males and two were females. With the exception of the third child (male), gene mutations were found in the father and four of the children. A heterozygot c.1019G>A (p.Arg340His) gene mutation in the *EXT1* gene was found in all of the patients. This gene mutation was previously described as pathogenic (NM_000127, p.R340H). When the pedigree is examined

**Figure 1:** Pedigree of Family.

it looks like a *de novo* mutation (Figure 1).

The symptoms of HME manifested earlier in male patients (2 years of age) than in one of the two female patients (10 years of age); the other female patient was 6 years of age and gene mutation-positive, but had not yet developed disease symptoms. The number of exostoses was markedly higher in male patients. In all of the patients, exostoses were concentrated around the larger joints. Although not frequently involved, exostoses were found in the cranium of three of five (60%) patients, of whom two were male and one female. The most severe exostoses were found in the father. The father and the oldest child had shortened upper extremities and a decreased range of motion of joints due to deformities, the father suffering more severely. None of the patients had any tissue decompression findings. Except for the disease-free male child, the father and all the male children had a short stature, while the female children had a normal stature.

The clinical and demographic findings and gene mutations of the patients are shown in Table 1. The clinical examples of exostoses are shown in Figure 2 and the radiographic images are displayed in Figure 3.

Discussion

Several HME patient series from Turkey have been published, but these did not include genetic analyses [9-11]. This study is the largest genetic analysis of an HME patient series in Turkey reported to date, the clinical features of which differ from those reported previously.

Although clinical and radiological findings of HME are usually first detected between the ages of 2 and 10 years, some are reported



Figure 2: Clinical examples of exostoses the cases.



Figure 3: Direct graphics of the cases.

at birth [12]. In the present study, findings started in all of the male patients before the age of 2 years, and in one of the female patients at 10 years of age. The other female patient was 6 years old and gene mutation-positive, but had not yet developed disease symptoms.

While HME can be asymptomatic [6,13], exostoses can cause bone deformities, joint restrictions, short stature, and early osteoarthritis development, and exert mechanical pressure on tissues [2,14]. The typical clinical manifestation of HME is single or multiple painful or painless swellings close to large joints [6]. In our patients, exostoses were concentrated around the larger joints. Although all of the patients harbored the same gene mutation, clinical symptoms were less severe in female patients, as reported previously [8]. Exostoses burden and deformities were more severe in male than in female patients.

Short stature is seen in 37% to 44% of HME patients. Short stature can be related to longitudinal non-growth of bones due to the development of exostoses in childhood or early puberty [15,16]. All of the male patients in this study had a short stature, while the female children had a normal stature.

HME patients can have less evident problems such as delayed wound healing, dental problems, and learning disability [8]. Our patients had no learning disability or evident dental problems.

The literature includes some conflicting statements about involvement of cranium and the frequency of *EXT 1-2* mutations [3-5,17,18].

Previous studies have yielded conflicting results regarding involvement of the cranium and facial bones. Some reports state that such bones are not involved, while others state that they are rarely involved. Publications stating that cranium and facial bones are rarely involved did not specify the frequency of involvement [3,5,18,19]. Clement and Porter published an anatomic location map of 4508 exostoses of 143 patients from 65 families (71 *EXT1* and 72 *EXT2* gene mutations) [18]. None of the patients had cranial involvement. Similarly, Ciavarella et al. [3] mapped the locations of exostoses involvement in 90 patients with *EXT1* and *EXT2* gene mutations; none had cranial involvement. On the other hand Ruiz and colleagues are reported 52 cases in literature which have facial or cranial osteochondroma [20]. In our case, there were four cranial exostoses, at least one in each of two male and one female patient (60%). This high cranial involvement rate differs our patients from previously reports.

EXT1 and *EXT2* mutations are present in 56% to 78% and 21% to 44%, respectively, of HME patients [3,4]. However, this ratio seems to vary according to geographical region and ethnicity. Chinese patients had a higher frequency of gene mutations in *EXT2* than in *EXT1* [7,17]. We found no previous study from Turkey involving a genetic analysis of HME patients. However, in their study of patients according to *EXT* gene mutation by country, Wuyts et al. [21] reported that three Turkish patients had a mutation in *EXT1*. To our knowledge, no mutation in the *EXT2* gene has been reported from Turkey to date.

Conclusion

Although the clinical findings of our patients were generally consistent with the literature, the frequency of cranial involvement was high in this series of HME patients, unlike those reported in previous studies. All of the patients had *EXT1* mutations, and no HME patient with an *EXT2* gene mutation has been reported in Turkey to date.

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