Mutation spectrum of EXT1 and EXT2 in the Saudi patients with hereditary multiple exostoses

Zayed Al-Zayed1,2, Roua A. Al-Rijjal3, Lamya Al-Ghofali2, Huda A. BinEssa3, Rajeev Pant1, Anwar Alrabiah1,2, Thamer Al-Hussainan1,2, Minjing Zou3, Brian F. Meyer3 and Yufei Shi3*

Abstract

Background: Hereditary Multiple Exostoses (HME), also known as Multiple Osteochondromas (MO) is a rare genetic disorder characterized by multiple benign cartilaginous bone tumors, which are caused by mutations in the genes for exostosin glycosyltransferase 1 (EXT1) and exostosin glycosyltransferase 2 (EXT2). The genetic defects have not been studied in the Saudi patients.

Aim of study: We investigated mutation spectrum of EXT1 and EXT2 in 22 patients from 17 unrelated families.

Methods: Genomic DNA was extracted from peripheral leucocytes. The coding regions and intron–exon boundaries of both EXT1 and EXT2 genes were screened for mutations by PCR-sequencing analysis. Gross deletions were analyzed by MLPA analysis.

Results: EXT1 mutations were detected in 6 families (35%) and 3 were novel mutations: c.739G > T (p. E247*), c.1319delG (p.R440Lfs*4), and c.1786delA (p.S596Afs*25). EXT2 mutations were detected in 7 families (41%) and 3 were novel mutations: c.541delG (p.D181Ifs*89), c.583delG (p.G195Vfs*75), and a gross deletion of approximately 10 kb including promoter and exon 1. Five patients from different families had no family history and carried de novo mutations (29%, 5/17). No EXT1 and EXT2 mutations were found in the remaining four families. In total, EXT1 and EXT2 mutations were found in 77% (13/17) of Saudi HME patients.

Conclusion: EXT1 and EXT2 mutations contribute significantly to the pathogenesis of HME in the Saudi population. In contrast to high mutation rate in EXT1 (65%) and low mutation rate in EXT2 (25%) in other populations, the frequency of EXT2 mutations are much higher (41%) and comparable to that of EXT1 among Saudi patients. De novo mutations are also common and the six novel EXT1/EXT2 mutations further expands the mutation spectrum of HME.

Keywords: EXT1, EXT2, Mutation, Exostoses, Osteochondromas

Introduction

Hereditary Multiple Exostoses (HME) or Multiple Osteochondromas (MO) is a rare autosomal-dominant pediatric disorder with an incidence of about 1 in 50,000 individuals and male-to-female ratio of about 1.5:1 [1, 2]. The disease is characterized by the development of two or more cartilage capped bony outgrowths within perichondrium in long bones and ribs, which can cause a variety of orthopedic deformities such as disproportionate short stature, shortened forearms, and unequal limb length. Although it is generally a benign skeletal tumor, 2.8% (0.5–5%) of patients undergo malignant transformation towards life-threatening chondrosarcomas or...
osteosarcomas due to their typical resistance to chemo- or radiation therapy [3, 4].

Germline heterozygous loss-of-function mutations in the EXT1 (exostosin-1, located on chromosome 8q23-q24) or EXT2 (exostosin-2, located on chromosome 11p11-p12) tumor suppressor genes are responsible for over 70–95% of HME cases [5, 6]. There are 566 EXT1 and 278 EXT2 mutations reported in the literature (HGMD database). The majority of these mutations (79% in EXT1 and 75% in EXT2) are frameshift, nonsense, and splice-site mutations, resulting in truncated proteins [5]. About 65% of the mutations occur in EXT1 and 25% in EXT2. In about 10–15% of HME cases, genomic alterations cannot be detected by the conventional method due to alterations such as intronic deletions, translocations or somatic mosaicism [7, 8]. The involvement of other genes or the putative EXT3 gene on chromosome 19 still needs investigation.

The genetic defects causing HME have not been systematically investigated in the Arab population. In the present study, we performed molecular analysis of 22 patients from 17 unrelated Saudi families with HME. EXT1 or EXT2 mutations were identified in 77% of patients (13/17) including six novel mutations.

Subjects and methods

Patients

Seventeen Saudi families with HME were investigated (Fig. 1 and Table 1). The inclusion criteria were two or more exostoses diagnosed upon physical and radiographic examinations. Disease severity was divided into 3 classes based on the presence of skeletal deformities and 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] [9]. Blood samples were obtained from patients and available relatives for genomic DNA extraction after informed consent. The study was approved by the Ethics Committee of King Faisal Specialist Hospital and Research Centre (RAC # 2170 027). Written consent was obtained from the patients or guardian of the patients before enrollment.

Genomic DNA isolation

Genomic DNA from peripheral blood leukocytes was extracted as described previously [10].

DNA amplification and sequencing

DNA samples were analyzed for mutations in all the coding exons and intron–exon boundaries of EXT1 and EXT2 genes by polymerase chain reaction (PCR) and sequencing analysis. PCR primers and conditions were described previously and listed in Table 2 [11]. The resulting PCR products were directly sequenced with BigDye Terminator 3.1 Cycle Sequencing kit using an automated ABI PRISM 3700 sequencer (Applied Biosystems; Life Technologies, Foster City, CA).

Fig. 1 Radiology of patients with osteochondromas. Patient #1 has an osteochondroma at left hip joint; Patient #15 has an osteochondroma at right proximal humerus; Patient #18 has an osteochondroma at left distal radius; and Patient #21 has a right pelvic osteochondroma with malignant transformation. Osteochondroma is indicated by an arrow.
| Family | Pt # | DX Age (year) and Height (cm) | Onset Age (year) | Sex | Clinical features | EXT1 mutation NM_000127 | EXT2 mutation NM_207122 | Family history |
|--------|------|------------------------------|------------------|-----|-------------------|--------------------------|-------------------------|----------------|
| 1      | 1    | 32, 157                      | 18               | M   | Class IIIB severity and surgical excision of exostosis from left hip joint due to decreased range of motion and pain. Two exostoses were found | c.1469delT (p.L490fs*9), exon 6, reported [37] | ND | Younger sister is affected |
| 2      | 2    | 17, 156                      | 8                | M   | Class IIIB severity and surgical excision of exostosis from the head of fibula due to nerve compression. Four exostoses were found | #c.739G > T (p. E247*), exon 1, novel mutation (not reported in the literature) | ND | NO |
| 3      | 3    | 18, 149                      | 8                | M   | Class IIA severity and surgical excision of left distal tibia exostosis exostosis. Two exostoses were found | ND | ND | NO |
| 4      | 4    | 40, 165                      | N/A              | M   | Class IA severity (mild asymptomatic disease). Two exostoses were found | ND | c.626 + 2_626 + 5delTAGG, intron 3, reported [38] | |
| 5      | 5    | 14, 149                      | 10               | F   | Class IIIB severity and surgical excision of two exostoses on the right leg | ND | c.626 + 2_626 + 5delTAGG, intron 3, reported [38] | 2 siblings are affected |
| 6      | 6    | 18, 149                      | 11               | F   | Class IIIB severity and surgical excision of left proximal femur and tibia exostosis. Three exostoses were found | #c.1319delG (p.R440fs*4), exon 5, novel mutation | ND | NO |
| 7      | 7    | 55, 164                      | N/A              | N/A | Class IA severity (mild asymptomatic disease). Two exostoses were found | ND | c.541delG (p.D181fs*89), exon 3, novel mutation | |
| 8      | 8    | 29, 156                      | 10               | F   | Class IIA severity with mental retardation, epilepsy and developmental disorder. Two exostoses were found | ND | c.541delG (D181fs*89), exon 3, novel mutation | 5 siblings are affected |
| 9      | 9    | 9, 133                       | 7                | M   | Class IA severity (mild asymptomatic disease). Two exostoses were found | ND | c.541delG (D181fs*89), exon 3, novel mutation | |
| 10     | 10   | 24, 180                      | 18               | M   | Class IA severity (mild asymptomatic disease). Two exostoses were found at distal right femur | ND | c.544C > T, p.R182*, exon 3, reported [39] | All of his 5 brother and 4 of 7 sisters are affected |
| 11     | 11   | 23, 160                      | 10               | F   | Class IA severity (mild asymptomatic disease). Four exostoses were found | ND | 10 kb homozygous deletion including promoter and exon 1, novel mutation | NO |
| Family | Pt # | DX Age and Height (year) | Onset Age (year) | Sex | Clinical features | EXT1 mutation NM_000127 | EXT2 mutation NM_207122 | Family history |
|--------|------|------------------------|-----------------|-----|-------------------|------------------------|------------------------|-----------------|
| 9      | 12   | 62, 156 N/A            | N/A             | F   | Class IA severity (mild asymptomatic disease). Two exostoses were found | ND                     | homozgyous c.540G > A (p.W180*), exon 3, reported [11] | Her brother is affected with no symptoms |
| 13     | 36   | 36, 160 15            | F               | Class IIB severity and surgical excision of exostoses and deformity correction. Three exostoses were found | ND                     | c.540G > A (p.W180*), exon 3, reported [11] | |
| 10     | 14   | 49, 165 N/A           | M               | Class IA severity (mild asymptomatic disease). Two exostoses were found | c.1021A > G, (p.R341G), exon 2, reported [5] | ND                     | Yes, all of his 4 brothers and one sister are affected with mild form of the disease |
| 15     | 23   | 23, 170 18            | M               | Class IIB severity and surgical excision of exostoses from both tibia, femur, and radius. Currently complaining of left hip pain. Three exostoses were found | c.1021A > G (p.R341G), exon 2, reported [5] | ND                     | |
| 11     | 16   | 22, 171 18            | F               | Class IIA severity and surgical excision of exostoses from head of the fibula. Three exostoses were found | ND                     | ND                     | NO |
| 12     | 17   | 29, 162 10            | M               | Class IIB severity and surgical excision of exostoses from right proximal tibia, right distal tibia and 4th rib excision. Three exostoses were found | ND                     | ND                     | NO |
| 13     | 18   | 15, 159 10            | F               | Class IIA severity. Two exostoses were found in the upper extremities | ND                     | ND                     | NO |
| 14     | Father | 42 M normal         | nd              | ND  | ND                 | ND                     | ND                     | |
| 19     | Mother | 41 M normal          | nd              | ND  | ND                 | ND                     | ND                     | |
| 15     | 20   | 29, 169 8            | M               | Class IIB severity and surgical excision of exostoses from left and right knees. Five exostoses were found | ND                     | ND                     | NO |
| 15     | 20   | 29, 169 8            | M               | Class IIB severity and surgical excision of exostoses from left and right knees. Five exostoses were found | ND                     | ND                     | NO |
Table 1 (continued)

| Family | Pt # | DX Age (year) and Height (cm) | Onset Age (year) | Sex | Clinical features                           | EXT1 mutation NM_000127 | EXT2 mutation NM_207122 | Family history |
|--------|------|------------------------------|-----------------|-----|---------------------------------------------|--------------------------|--------------------------|-----------------|
| 16     | 21   | 42, 170                      | 20              | M   | Class IIIB severity with malignant transformation to osteochondrosarcoma at right pelvic. Three exostoses were found | ND                       | c.583delG (p.G195Vfs*75), exon 3, novel mutation | Yes, several nephews of his are affected but none of them required clinical intervention |
| 17     | 22   | 7, 120                       | 7               | M   | Class IIA severity with symptomatic bone deformities. Two exostoses were found | ^heterozygous deletion of exon 2–11, reported [41] | ND                       | NO              |

ND: not detected. #de novo mutations. Disease severity is divided into 3 classes 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) [9]
### Table 2  
**EXT1 and EXT2 primer sequences and PCR conditions**

| Exons | **EXT1-Forward** | **EXT1-Reverse** | Annealing (°C) | **EXT2-Forward** | **EXT2-Reverse** | Annealing (°C) |
|-------|------------------|------------------|----------------|------------------|------------------|----------------|
| Exon 1a | 5’ggaagccgctacgagaa-ggt-3’ | 5’cgtccgcaaggtggaatc-gaa-3’ | 58 | 5’-cagtccgctcctctctctttc-3’ | 5’-agtgccctggccaacat-gac-3’ | 62 |
| Exon 1b | 5’ttcgctctcttgatcaaatc-3’ | 5’cgtgctctgggagacatc-cctta-3’ | 56 | 5’-tgtgctggttctctctctct-3’ | 5’-gtgtggttgtttcctctctct-3’ | 54 |
| Exon 1c | 5’gagttgcttctctccataatc-3’ | 5’acacccctctttctacatc-3’ | 58 | 5’-gtgtaagggagacact-tactg-3’ | 5’-gtgtaagggagacact-tactg-3’ | 58 |
| Exon 2 | 5’-cagctgatctagctg-3’ | 5’-cagctgatctagctg-3’ | 56 | 5’-ctgtaagggagacact-tactg-3’ | 5’-ctgtaagggagacact-tactg-3’ | 58 |
| Exon 3 | 5’-ctctgtcctgtgctgctg-3’ | 5’-ctctgtcctgtgctgctg-3’ | 58 | 5’-ctgtaagggagacact-tactg-3’ | 5’-ctgtaagggagacact-tactg-3’ | 58 |
| Exon 4 | 5’-ctctgtcctgtgctgctg-3’ | 5’-ctctgtcctgtgctgctg-3’ | 58 | 5’-ctgtaagggagacact-tactg-3’ | 5’-ctgtaagggagacact-tactg-3’ | 58 |
| Exon 5 | 5’-ctctgtcctgtgctgctg-3’ | 5’-ctctgtcctgtgctgctg-3’ | 58 | 5’-ctgtaagggagacact-tactg-3’ | 5’-ctgtaagggagacact-tactg-3’ | 58 |
| Exon 6 | 5’-ctctgtcctgtgctgctg-3’ | 5’-ctctgtcctgtgctgctg-3’ | 58 | 5’-ctgtaagggagacact-tactg-3’ | 5’-ctgtaagggagacact-tactg-3’ | 58 |
| Exon 7 | 5’-ctctgtcctgtgctgctg-3’ | 5’-ctctgtcctgtgctgctg-3’ | 58 | 5’-ctgtaagggagacact-tactg-3’ | 5’-ctgtaagggagacact-tactg-3’ | 58 |
| Exon 8 | 5’-ctctgtcctgtgctgctg-3’ | 5’-ctctgtcctgtgctgctg-3’ | 58 | 5’-ctgtaagggagacact-tactg-3’ | 5’-ctgtaagggagacact-tactg-3’ | 58 |
| Exon 9 | 5’-ctctgtcctgtgctgctg-3’ | 5’-ctctgtcctgtgctgctg-3’ | 58 | 5’-ctgtaagggagacact-tactg-3’ | 5’-ctgtaagggagacact-tactg-3’ | 58 |
| Exon 10 | 5’-ctctgtcctgtgctgctg-3’ | 5’-ctctgtcctgtgctgctg-3’ | 58 | 5’-ctgtaagggagacact-tactg-3’ | 5’-ctgtaagggagacact-tactg-3’ | 58 |
| Exon 11 | 5’-ctctgtcctgtgctgctg-3’ | 5’-ctctgtcctgtgctgctg-3’ | 58 | 5’-ctgtaagggagacact-tactg-3’ | 5’-ctgtaagggagacact-tactg-3’ | 58 |
| Exon 12 | 5’-ctctgtcctgtgctgctg-3’ | 5’-ctctgtcctgtgctgctg-3’ | 58 | 5’-ctgtaagggagacact-tactg-3’ | 5’-ctgtaagggagacact-tactg-3’ | 58 |
| Exon 13 | 5’-ctctgtcctgtgctgctg-3’ | 5’-ctctgtcctgtgctgctg-3’ | 58 | 5’-ctgtaagggagacact-tactg-3’ | 5’-ctgtaagggagacact-tactg-3’ | 58 |
| Exon 14 | 5’-ctctgtcctgtgctgctg-3’ | 5’-ctctgtcctgtgctgctg-3’ | 58 | 5’-ctgtaagggagacact-tactg-3’ | 5’-ctgtaagggagacact-tactg-3’ | 58 |

**PCR conditions:** 50 ng of DNA were denatured at 95 °C for 5 min on initial cycle followed by 35 cycles of denaturation, annealing, and extension at 1 min on each step.

### Analysis of copy number variation

Copy number variation in genomic DNA was analyzed by MLPA (Multiplex Ligation-dependent Probe Amplification) analysis as described previously [12].

### Results

**EXT1** and **EXT2** mutations were identified in 13 out of 17 (77%) unrelated patients and 18 of total 22 patients (82%) (Table 1). Among them, 7 were **EXT1** mutations including 1 recurrent mutation in one related family member (35%, 6/17 unrelated patients or 32%, 7/22 total patients); 11 were **EXT2** mutations including 4 recurrent mutations from 4 family members (41%, 7/17 unrelated patients, or 50%, 11/22 total patients) (Table 1). Among 13 different mutations, 7 were previously reported mutations (Table 1, Fig. 2) and 6 were novel mutations (Fig. 3). Three novel mutations occurred in the **EXT1:** c.739G>T (p.E247*), c.1319delG (p.R440Lfs*4), and c.1786delA (p.S596afs*25) and in the **EXT2:** c.541delG (p.D181fs*89), c.583delG (p.G195Vfs*75) and a gross homozygous deletion of approximately 10 kb including promoter and exon 1 (Table 1, Fig. 3). In the patient with the homozygous deletion, we were able to amplify exon 2 to 14 successfully, but could not amplify exon 1 and its untranslated region of about 10 kb, indicating a 10 kb deletion of exon 1 and the promoter region. Five patients from unrelated families were found to have mutations without any family history of the disease and these mutations were thus de novo mutations (29%, 5/17). Interestingly, 4 of them were also novel mutations (Table 1). MLPA analysis was performed to detect large deletions in the patients who had no mutation detected by PCR-sequencing analysis. One large heterozygous deletion involving exons 2–11 was detected (Table 1). Among 13 different mutations, 6 were single nucleotide deletions, 3 were nonsense mutations, 1 missense mutation, 1 splice donor site mutation, and 2 large deletions. Therefore, all the mutations except for one missense mutation (92%, 12/13) are predicted to result in frameshift and truncated proteins devoid of enzymatic activity.
Compared to the patients with EXT2 mutations, most patients with EXT1 mutations had more severe phenotype and required surgery. Germline homozygous EXT2 mutations were identified in two patients (patient #11 and 12 in Table 1) who presented only mild asymptomatic disease and no clinical intervention was required. Furthermore, significant heterogeneity in clinical presentations were demonstrated among family members carrying the same mutations. For example as shown in Table 1, patient#12 carried a homozygous EXT2 c.540G>A mutation with only mild asymptomatic disease whereas her daughter (patient#13) had a heterozygous EXT2 c.540G>A mutation and required multiple operations to remove exostosis and correct bone deformity.

**Discussion**

In the present study, we have studied EXT1 and EXT2 mutation spectrum in 22 patients from 17 unrelated Saudi families. Disease-causing mutations are identified in 77% of patients (13/17) including 6 novel mutations. The frequency of EXT1 mutation is lower than EXT2: 35% (6/17) for EXT1 and 41% (7/17) for EXT2. Twenty-nine percent of patients (5/17) have de novo mutations, which account for 39% (5/13) of mutations identified.

**Fig. 2** Sequence analysis of EXT1 and EXT2 in the patients with hereditary multiple exostoses. Representative electropherograms of previously reported EXT1 and EXT2 mutations are shown. Heterozygous mutations are present in the patients and affected family members except for the affected mother (patient#12) in Family 9 who carries a homozygous mutation whereas her daughter (patient#13) has a heterozygous mutation. The mutation is indicated by an arrow.

**Previously reported EXT1 and EXT2 mutations**
exostosin interaction domain in the center and a catalytic domain at the C-terminal end. EXT1 and EXT2 form a hetero-oligomeric complex in vivo that leads to accumulation of both proteins in the Golgi apparatus. The Golgi-localized EXT1/EXT2 complex possesses substantially higher glycosyltransferase activity than EXT1 or EXT2 alone, suggesting that the hetero-oligomeric complex is the biological form of the enzyme for heparan sulfate biosynthesis and explains mutations in either EXT1 or EXT2 gene would result in the loss of enzymatic activity and disease development [19–21].

HME is a rare childhood-onset skeletal disease caused by germline mutations in the tumor suppressor gene EXT1 or EXT2. Most HME patients carry a germline heterozygous loss-of-function mutation in the EXT1 or EXT2 and display a 50% reduction of systemic heparin sulfate [22]. It is generally believed that exostosis formation and associated defects, such as growth retardation

![Detection of novel EXT1 and EXT2 mutations](image)
and skeletal deformities, require loss-of-heterozygosity or a second hit in the affected cells [23, 24]. Mice with single heterozygous deletion of Ext1± or Ext2± are normal. Compound heterozygous Ext1+/−; Ext2+/− deletion mice and conditional Ext1 knockout mice display multiple osteochondromas and closely resemble human HME [25–27]. However, a second hit in the EXT1 or EXT2 gene are not common in most cases (more than 60%), suggesting that mechanisms other than EXT genetic alterations may play a role in the disease development [28, 29]. In our patients, homozygous germline EXT2 mutations were detected in two patients (patient #11 and 12 (Table 1, Fig. 2 and 3b). To our knowledge, homozygous germline EXT1/EXT2 mutations have not been reported in the literature. Interestingly, the presence of homozygous germline EXT2 mutations does not associated with severity of the disease since both patients have mild asymptomatic disease. Furthermore, no significant difference in clinical presentations or disease progression is found between patients with mutation and those without mutation. In fact, significant heterogeneity in disease development and progression are observed among patients with or without mutations. This is even demonstrated among family members carrying the same mutations, indicating epigenetic and/or environmental factors may contribute to the disease development and progression.

It has been reported that EXT1 mutation is more common (about 65%) than EXT2 (about 30%) and its protein is less tolerant to the damaging mutations [5, 30]. This may explain EXT1 mutations usually result in more severe disease phenotype. Indeed, most of our patients with EXT1 mutations have more severe phenotype and require surgery. In contrast to the higher EXT1 mutation rate reported in the literature, the frequency of EXT1 mutation appears to be lower than EXT2 in our current study. It remains to be determined whether this is due to small sample size or population-specific.

The most common type of mutations in the EXT1 and EXT2 genes are inactivating mutations, such as frameshift, nonsense, and splice-site mutations [6, 31, 32]. Based on the HGMD® Professional 2020.1 (Accessed on August 10, 2020), approximately 79% EXT1 mutations and 75% EXT2 mutations are inactivating mutations: frameshift 47% (268/566), nonsense 22% (123/566), splice-site 10% (58/565) in the EXT1; frameshift 43% (119/278), nonsense 22% (60/278), splice-site 10% (29/278) in the EXT2. The remaining EXT1 mutations are missense (12%, 68/566), gross deletions (7%, 40/566), and complex rearrangements (1%, 7/566) whereas remaining EXT2 mutations are missense (14%, 40/278) and gross deletions (9%, 26/278). In our current study of Saudi patients, the overall frequency of inactivating EXT1 and EXT2 mutations is 92% (12/13); frameshift 46% (6/13), nonsense 23% (3/13), splice-site 8% (1/13), gross deletion (15%, 2/13), which is higher than the overall rate documented in the HGMD (78%, 657/843). This is probably due to small sample size in our study. All of these mutations (92%, 11/12) are predicted to result in truncated proteins devoid of enzymatic activity. Four patients were not found to have EXT1/EXT2 mutations (Patient# 3, 16, 17, 18). Although HME may be confused with enchondroma which is a benign cartilage tumor, enchondroma often affects the cartilage that lines the inside of long bones in the hands and feet. The clinical and radiographic features of our patients (multiple bony outgrowths on the external surface in the metaphysis of long bones) do not support the diagnosis of enchondromas. The involvement of additional genes other than EXT1/EXT2 or other mechanisms may contribute to the disease development [7, 8].

De novo EXT1 and EXT2 mutations have been reported to account for approximately 10% of patients [5, 33]. However, higher frequency are reported in other populations: Polish (21%) [34], English (33%) [35], and Chinese (30%) [36]. The high de novo mutation rate in the Saudi patients (29%) indicates that family history should not be relied upon heavily in the diagnosis of the disease.

**Conclusions**

We have investigated genetic defects of EXT1 and EXT2 in the Saudi HME patients. EXT1 and EXT2 mutations are detected in 77% of patients. De novo EXT1 and EXT2 mutations are common. The current study further expands the mutation spectrum of HME.

**Abbreviations**

HME: Hereditary Multiple Exostoses; MO: Multiple Osteochondromas; EXT1: Exostosin glycosyltransferase 1; EXT2: Exostosin glycosyltransferase 2; PCR: Polymerase chain reaction; MLPA: Multiplex ligation-dependent probe amplification.

**Acknowledgements**

Not applicable.

**Authors’ contributions**

Study design: ZA, LA, and YS. Patient data collection: ZA, LA, RP, and TA; Laboratory investigation: RAA, HAB, and MZ. Data analysis: ZA, LA, MZ, BFM, and YS; Drafted manuscript: ZA and YS. Revised manuscript: LA, RP, TA, MZ, and BFM. All authors read and approved the final manuscript.

**Funding**

This study is supported by a KACST Biotech grant 13-MED1765-20.

**Availability of data and materials**

Data supporting the findings of the study are included in the manuscript.
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