Large-scale mutational analysis in the EXT1 and EXT2 genes for Japanese patients with multiple osteochondromas

Daichi Ishimaru1, Masanori Gotoh2, Shinichiro Takayama3, Rika Kosaki4, Yoshihiro Matsumoto5, Hisashi Narimatsu2, Takashi Sato2, Koji Kimata6, Haruhiko Akiyama1, Katsuji Shimizu7 and Kazu Matsumoto1*

Abstract
Background: Multiple osteochondroma (MO) is an autosomal dominant skeletal disorder characterized by the formation of multiple osteochondromas, and exostosin-1 (EXT1) and exostosin-2 (EXT2) are major causative genes in MO. In this study, we evaluated the genetic backgrounds and mutational patterns in Japanese families with MO.

Results: We evaluated 112 patients in 71 families with MO. Genomic DNA was isolated from peripheral blood leucocytes. The exons and exon/intron junctions of EXT1 and EXT2 were directly sequenced after PCR amplification. Fifty-two mutations in 47 families with MO in either EXT1 or EXT2, and 42.3% (22/52) of mutations were novel mutations. Twenty-nine families (40.8%) had mutations in EXT1, and 15 families (21.1%) had mutations in EXT2. Interestingly, three families (4.2%) had mutations in both EXT1 and EXT2. With regard to the types of mutations identified, 59.6% of mutations were inactivating mutations, and 38.5% of mutations were missense mutations.

Conclusions: We found that the prevalence of EXT1 mutations was greater than that of EXT2 mutations in Japanese MO families. Additionally, we identified 22 novel EXT1 and EXT2 mutations in this Japanese MO cohort. This study represents the variety of genotype in MO.

Keywords: Multiple hereditary exostoses, EXT1, EXT2, Mutational analysis

Background
Multiple osteochondromas (MO) is a relatively rare autosomal dominant skeletal disorder characterized by the formation of multiple osteochondromas and skeletal deformities, including limb length discrepancy, bowing deformities of the forearms, valgus deformity of the lower extremities, and scoliosis [1–3]. In Western countries, the prevalence of MO in the general population is one in every 50,000 individuals, and men tend to be affected more frequently than women [4, 5]. Osteochondroma is a benign bone tumor exhibiting cartilage-capped bone growth that typically originates from the metaphysis of long bones or surface of flat bones. Patients commonly feel pain or irritation of the tissues due to osteochondroma, and some patients may undergo multiple surgeries during their life in an attempt to relieve the symptoms of this disorder [6]. Malignant transformation of osteochondroma toward chondrosarcoma is a serious complication in MO and occurs in 0.38–7.0% of patients [6–9].

The exostosin-1 (EXT1) and exostosin-2 (EXT2) genes, which encode heparin sulfate glycosyltransferases, are major causative genes in MO [10, 11]. EXT1 is located on chromosome 8q23-q24 [12], and EXT2 is located on chromosome 11p11-p12 [13]; these genes are essential for heparan sulfate chain elongation. Approximately 90% of patients with MO harbor EXT1 or EXT2 germ-line mutations; however, the genetic background of patients with MO is heterogeneous. Several reports have described mutational variations and novel mutations in EXT1 and EXT2 genes in patients with MO in several different countries [14–17], and the distribution of
mutations in the *EXT1* and *EXT2* genes has been shown to vary. For example, in Spanish patients with MO, 74 % had mutations in *EXT1*, and 21 % had mutations in *EXT2* [14]. In contrast, in Polish patients with MO, 54.6 % had mutations in *EXT1* and 30.3 % had mutations in *EXT2* [15]. Most of these mutations are inactivating mutations, including nonsense, frameshift, and splice-site mutations [18]. However, only one study has described variations in genotypes for Japanese patients with MO [16].

Therefore, in this study, we sought to determine genetic backgrounds and mutational patterns in 71 Japanese families with MO; this report describes the genetic diagnostic results of the largest Japanese cohort of MO patients presented to date and identified several novel mutations in the *EXT1* and *EXT2* genes in MO.

**Results**

**Identification of 22 novel genetic lesions**

In this study, all exons and intron/exon junctions in *EXT1* and *EXT2* were sequenced in 112 patients with MO from 71 families. Eighty (71.4 %) patients harbored 52 mutations in either *EXT1* or *EXT2*. All *EXT1* and *EXT2* mutations are shown in Table 1. Twenty-nine families (40.8 %) had mutations in *EXT1*, and 15 families (21.1 %) had mutations in *EXT2*. Interestingly, three families (4.2 %) had mutations in both *EXT1* and *EXT2*. Twenty-four families (33.8 %) did not have mutations in *EXT1* or *EXT2*. The distribution of mutations was as follows: 40.4 % (21/52) of patients had missense mutations, 30.8 % (16/52) of patients had frameshift mutations, 21.2 % (11/52) of patients had nonsense mutations, 5.8 % (3/52) of patients had splicing mutations, and 1.9 % (1/52) of patients had insertions. Of all 52 mutations, 22 mutations (42.3 %) were novel mutations that had not been registered in the Multiple Osteochondroma Mutation Database (MOdb) (http://medgen.ua.ac.be/LOVDv.2.0/home.php) [18]. Of these mutations, 17 mutations (77.3 %) were identified in the *EXT1* gene, while five mutations (22.7 %) were found in the *EXT2* gene. The distribution of novel mutations was as follows: 27.3 % (6/22) of patients had missense mutations, 31.8 % (7/22) of patients had frameshift mutations, 22.7 % (5/22) of patients had nonsense mutations, 9.1 % (2/22) of patients had splicing mutations, and 4.5 % (1/22) of patients had insertions.

**Characteristic genome mutations in five families with MO**

Interestingly, five families with MO showed unique genotypes (MO-11, -25, -44, -47, and -59) as illustrated in Fig. 1. In the family with MO-11, one MO patient harbored missense mutations in both exon 2 of *EXT1* and exon 2 of *EXT2*. Moreover, parents and children in the families with MO-25, -44, and -47 showed different genotypes. In the family with MO-59, a patient had a double missense mutation in *EXT1*.

**Discussion**

In this study, we evaluated the presence and features of *EXT1* and *EXT2* mutations in Japanese families with MO. Our data demonstrated that 29 families (40.8 %) had mutations in *EXT1*, and 15 families (21.1 %) had mutations in *EXT2*. Moreover, three families (4.2 %) had mutations in both *EXT1* and *EXT2*, and 24 families (33.8 %) did not have mutations in either *EXT1* or *EXT2*. Of the 52 mutations observed in this study, 34 mutations were identified in *EXT1*, and 18 mutations were identified in *EXT2*. Of the 52 mutations 22 novel mutations were identified. Thus, the data presented herein provides important insights into the genetic causes of MO in Japanese families.

MO is an autosomal dominant disorder, and germline and heterozygous mutations conferring loss of function in the *EXT1* and *EXT2* genes are main causes of MO. Mutational variations in *EXT1* and *EXT2* are continuously being reported; as of January 2015, 432 mutations in *EXT1* and 223 mutations in *EXT2* were registered in the MOdb (http://medgen.ua.ac.be/LOVDv.2.0/home.php) [18]. Several studies have described the mutational variations in *EXT1* and *EXT2* in European countries and Asia. For example, in Spanish patients with MO, 74 % were found to have mutations in *EXT1*, and 21 % were found to have mutations in *EXT2* [14]. Additionally, in Polish patients with MO, 54.6 and 30.3 % were found to have mutations in *EXT1* and *EXT2*, respectively [15]. In an Italian cohort, 69 % of patients were found to have mutations in *EXT1*, and 27 % of patients were found to have mutations in *EXT2*. In a previous study of Japanese families with MO, 17 (40 %) of the 23 families had a mutation in *EXT1*, and six (14 %) of the 23 families had a mutation in *EXT2* [16]. In contrast, in Chinese families with MO, 13.9 and 33.3 % of the 36 families were found to have mutations in *EXT1* and *EXT2*, respectively [17]. In most studies, the prevalence of *EXT1* mutations has been reported to be higher than that of *EXT2* mutations. Similarly, in our current analysis, a greater proportion of *EXT1* mutations was observed (*EXT1*: 40.8 %, *EXT2*: 21.1 %). However, mutations in these genes were not identified in 24 families (33.8 %) with MO; this percentage was relatively high compared with that in European countries, where the proportion of patients without mutations in *EXT1* and *EXT2* has been shown to range from 4 to 24 % (Fig. 2a) [9, 14, 19, 20]. Further studies are needed to examine this finding such as MLPA assays because the families with MO harboring no mutations in this study might include deletion mutation. While the *EXT* family also includes three *EXT*-like genes (i.e., *EXTL1*, *EXTL2*, and *EXTL3*) [21–23], no reports have described the presence of gene mutations in these three
| Family number | Number of participants | Gene   | The number of the exon | Mutation | Amino acid change | Nucleotide change | Novel/Reported | Familial/Sporadic |
|---------------|------------------------|--------|------------------------|----------|------------------|-------------------|---------------|------------------|
| MO-1          | 4                      | EXT2   | Exon6                  | Missense | p.C339F          | c.1016G > T       | R             | F                |
| MO-2          | 4                      | EXT1   | Exon6                  | Frame shift | p.T488fs         | c.1462A > A       | N             | F                |
| MO-3          | 3                      | EXT1   | Exon6                  | Frame shift | p.T490fs         | c.1469T > A       | R             | F                |
| MO-4          | 2                      | EXT1   | Exon8                  | Frame shift | p.F550fs         | c.T1650A > A      | N             | F                |
| MO-5          | 1                      |        |                        |          |                  |                   |               |                  |
| MO-6          | 1                      | EXT1   | Exon5                  | Frame shift | p.R433fs         | c.A1297 > A       | R             | F                |
| MO-7          | 1                      | EXT2   | Exon5                  | Missense | p.R297H          | c.890G > A        | N             | F                |
| MO-8          | 2                      | EXT1   | Exon1                  | Nonsense | p.Q27X           | c.79C > T         | N             | F                |
| MO-9          | 2                      |        |                        |          |                  |                   |               |                  |
| MO-10         | 1                      |        |                        |          |                  |                   |               |                  |
| MO-11         | 1                      | EXT1   | Exon2                  | Missense | p.R341S          | c.1023G > C       | R             | F                |
| MO-12         | 2                      | EXT2   | Exon2                  | Missense | p.R128W          | c.382C > T        | R             | F                |
| MO-13         | 1                      | EXT2   | Exon8                  | Nonsense | p.W429X          | c.1286G > A       | R             | F                |
| MO-14         | 1                      | EXT1   | Exon6                  | Frame shift | p.L490fs       | c.1469T > A       | R             | F                |
| MO-15         | 1                      |        |                        |          |                  |                   |               |                  |
| MO-16         | 1                      | EXT2   | Exon3                  | Nonsense | p.R182X          | c.544A > T        | R             | F                |
| MO-17         | 2                      |        |                        |          |                  |                   |               |                  |
| MO-18         | 1                      |        |                        |          |                  |                   |               |                  |
| MO-19         | 1                      |        |                        |          |                  |                   |               |                  |
| MO-20         | 1                      | EXT2   | Exon5                  | Insertion | p.V282ins       | c.846A Ins        | N             | S                |
| MO-21         | 2                      | EXT1   | Exon2                  | Missense | p.R340H          | c.1019G > A       | R             | F                |
| MO-22         | 1                      |        |                        |          |                  |                   |               |                  |
| MO-23         | 2                      |        |                        |          |                  |                   |               |                  |
| MO-24         | 1                      | EXT2   | Exon5                  | Missense | p.R299H          | c.896G > A        | R             | F                |
| MO-25         | 1                      | EXT1   | Exon5                  | Frame shift | p.P466fs       | c.1395del del.   | R             | F                |
| MO-26         | 2                      |        |                        |          |                  |                   |               |                  |
| MO-27         | 1                      | EXT2   | Exon2                  | Frame shift | p.F309fs       | c.888A > A        | N             | F                |
| MO-28         | 1                      | EXT1   | Exon2                  | Missense | p.R340L          | c.1019G > T       | R             | F                |
| MO-29         | 2                      | EXT1   | Exon3                  | Missense | p.C355Y          | c.1086G > A       | N             | F                |
| MO-30         | 1                      |        |                        |          |                  |                   |               |                  |
| MO-31         | 2                      | EXT1   | Exon1                  | Nonsense | p.W304X          | c.912G > A        | R             | F                |
| MO-32         | 3                      | EXT1   | Exon9                  | Nonsense | p.W612X          | c.1797G > A       | R             | F                |
| MO-33         | 2                      | EXT1   | Exon1                  | Frame shift | p.L26fs         | c.78T > A       | N             | F                |
| MO-34         | 2                      |        |                        |          |                  |                   |               |                  |
| MO-35         | 1                      | EXT2   | Intron7                | Splicing mutation |   | c.(1173 + 1)G > A | R             | F                |
| MO-36         | 1                      | EXT2   | Exon3                  | Nonsense | p.R182X          | c.544C > T        | R             | F                |
| MO-37         | 2                      | EXT1   | Exon1                  | Nonsense | p.Q165X          | c.493C > T        | R             | F                |
| MO-38         | 1                      |        |                        |          |                  |                   |               |                  |
| MO-39         | 1                      |        |                        |          |                  |                   |               |                  |
| MO-40         | 1                      | EXT1   | Exon2                  | Missense | p.R340H          | c.1019G > A       | R             | F                |
| MO-41         | 2                      |        |                        |          |                  |                   |               |                  |
EXT-like genes in families with MO. Therefore, further studies are needed to determine whether these three genes may be causative genes in families with MO who do not harbor mutations in \textit{EXT1} and \textit{EXT2} genes.

In MO, the most common mutation types in the \textit{EXT1} and \textit{EXT2} genes are inactivating mutations, such as frameshift, nonsense, and splice-site mutations [24]. Similarly, in data reported in MOdb from 2009, approximately 80 % of mutations in \textit{EXT1} and 77 % of mutations in \textit{EXT2} were found to be inactivating mutations (\textit{EXT1}: frameshift 44 %, nonsense 24 %, splice-site 11 %; \textit{EXT2}: frameshift 42 %, nonsense 22 %, splice-site 13 %) [18]. In Spanish patients with MO, the prevalence of inactivation mutations was reported to be 79.5 % [14]. In our study, 52 mutations were found in \textit{EXT1} and \textit{EXT2}, and 59.6 % (31/52) of these mutations were inactivating mutations (with 30.8, 23.1, and 5.8 % of mutations being frameshift, nonsense, and splice-site mutations, respectively). The proportion of missense mutation was approximately 38.5 %, which was relatively higher in this study than in previous reports (Fig. 2b) [14, 18]. The differences in gene mutations between patients with MO in Japan and other

| Table 1 \textit{Ext1} and \textit{Ext2} mutations in Japanese MO families (Continued) |
| MO-43 | 2 | \textit{EXT1} | Exon2 | Missense | p.R341S | c.1023G > C | R | F |
| MO-44 | 2 | \textit{EXT2} | Exon3 | Missense | p.A202V | c.605C > T | R | F |
| MO-45 | 4 | \textit{EXT1} | Exon1 | Nonsense | p.G24X | c.70G > T | N | F |
| MO-46 | 1 | \textit{EXT1} | Exon1 | Frame shift | p.R314fs | c.941G (+2nt) | N | F |
| MO-47 | 1 | \textit{EXT2} | Exon2 | Missense | p.E74X | c.220G > T | N | F |
| MO-48 | 1 | \textit{EXT1} | Exon1 | Missense | p.E139X | c.415G > T | N | F |
| MO-49 | 4 | - | - | - | - | - | - | F |
| MO-50 | 2 | \textit{EXT2} | Exon7 | Nonsense | p.Y374X | c.1122C > A | N | F |
| MO-51 | 1 | \textit{EXT2} | Exon2 | Frame shift | p.S121fs | c.361TΔ2nt | R | F |
| MO-52 | 2 | \textit{EXT2} | Exon4 | Missense | p.D227N | c.679G > A | R | F |
| MO-53 | 1 | \textit{EXT1} | Exon3 | Frame shift | p.M359fs | Δ8nt | N | F |
| MO-54 | 1 | - | - | - | - | - | - | F |
| MO-55 | 1 | - | - | - | - | - | - | F |
| MO-56 | 1 | \textit{EXT1} | Exon10 | Nonsense | p.Q685X | c.2053C > T | R | F |
| MO-57 | 2 | \textit{EXT1} | Exon1 | Frame shift | p.K321fs | c.960GΔ1nt | N | F |
| MO-58 | 1 | \textit{EXT2} | Exon6 | Missense | p.F341T | c.1021C > A | N | F |
| MO-59 | 1 | \textit{EXT1} | Exon1 | Missense | p.J221V | c.661A > G | N | F |
| MO-60 | 2 | \textit{EXT1} | Exon2 | Missense | p.R340H | c.1019G > A | R | F |
| MO-61 | 2 | \textit{EXT1} | Exon1 | Frame shift | p.K218fs | Δ14nt | R | F |
| MO-62 | 1 | - | - | - | - | - | - | F |
| MO-63 | 1 | \textit{EXT1} | Exon1 | Frame shift | p.K218fs | Δ18nt | R | F |
| MO-64 | 1 | - | - | - | - | - | - | F |
| MO-65 | 1 | \textit{EXT1} | Exon10 | Splicing mutation | intron/exon10 | G > A | N | F |
| MO-66 | 2 | \textit{EXT1} | Exon1 | Nonsense | p.E139X | c.415G > T | N | F |
| MO-67 | 1 | \textit{EXT2} | Exon4 | Missense | p.D227N | c.679G > A | R | F |
| MO-68 | 1 | \textit{EXT1} | IVS9 | Splicing mutation | intron/exon5 | G > T | N | F |
| MO-70 | 1 | \textit{EXT1} | IVS5 | Splicing mutation | intron/exon10 | G > A | N | F |
| MO-71 | 1 | \textit{EXT2} | Exon1 | Missense | p.E139X | c.415G > T | N | F |
| MO-72 | 1 | \textit{EXT2} | Exon4 | Splicing mutation | intron/exon5 | G > T | N | F |
| MO-73 | 1 | \textit{EXT2} | Exon1 | Nonsense | p.E139X | c.415G > T | N | F |
| MO-74 | 3 | - | - | - | - | - | - | F |
| MO-75 | 2 | \textit{EXT2} | Exon4 | Missense | p.D227N | c.679G > A | R | F |
| MO-76 | 1 | - | - | - | - | - | - | F |
| MO-77 | 1 | \textit{EXT1} | IVS5 | Splicing mutation | exon5/intron | G > T | N | F |

* indicates no mutations detected
countries may be related to the differences in the prevalence rates of MO or the severity of skeletal abnormalities, including scoliosis, in the various countries. Further studies are required to determine the phenotype-genotype relationships in Japanese patients with MO.

In the present study, approximately 7.0% (5/71) of families with MO showed characteristic genotypes, e.g., one patient bearing two mutations and a parent and child bearing different mutations as shown in Fig. 1. Especially, in MO-25, MO-44 and MO-47, there was

---

**Fig. 1** Characteristic mutations and hereditary types in Japanese families with MO. Black mark represents patient with MO. White mark represents healthy person. NA: DNA not available. Written informed consents to publish were obtained from each participants described in this figure before study participation.

---

**Fig. 2** Comparison of mutation frequencies. **a** The proportions of EXT1 and EXT2 mutations. **b** The proportion of missense mutations.
and Ext2 were evaluated by Ext2 (2016) 17:52  and et al. BMC Genetics and Ext2 and Ext2 genes were not amplified by Ext2 Ext2 mutation Ext2 genes in 71 Japanese families and Ext2 and Ext2 procedures, and all participants obtained written informed development, and Kyusyu University. Ethics Committee of University, National Center for Child Health and Development, and Kyusyu University. Ethics Committee of Gifu University (Approval No. 22-221) approved all procedures, and all participants obtained written informed consent before any research procedures. In case of the participant under the age of 16 year old, written informed consents (child assent and parental consent) were obtained. In addition, written informed consents to publish were obtained from all patients before study participation, and in the case of the participant under the age of 16 year old, written informed consents to publish (child assent and parental consent) were obtained.

Patients and clinical studies
From April 2010 to September 2014, patients with MO were recruited for genetic testing of the Ext1 and Ext2 genes. A total of 116 patients (51 women and 65 men) from 74 families with MO were recruited. Clinical diagnosis was performed based on accurate family histories and physical examinations of the patients, including palpation tests for osteochondromas or joint deformities. Ethics Committee of Gifu University approved all procedures, and all participants obtained written informed consents before all procedures.

Mutation analysis
Genomic DNA was isolated from peripheral blood leukocytes of all patients with MO using a Wizard Genomic DNA purification kit (Promega, Madison, WI, USA). All exons and exon/intron junctions in the Ext1 and Ext2 genes (GenBank accession numbers NM_000127.2 and NM_207122.1) were amplified by PCR. After confirming amplification of the DNA fragments by agarose gel electrophoresis and purifying the amplified DNA fragments using a Wizard SV gel and PCR Clean-up System (Promega), the amplified DNA fragments were directly sequenced using a BigDye Terminator v1.1Cycle Sequencing Kit (ABI). Sequence analyses were then performed with an ABI PRISM 3100 Genetic Analyzer (ABI). Mutations in Ext1 and Ext2 were evaluated by comparing DNA sequences of normal Ext1 and Ext2 genes with the obtained sequences using Sequencher software (Hitachi Software Engineering Co., Ltd., Tokyo, Japan). Primer sequences are shown in Additional file 1: Table S1. The detected mutations in Ext1 and Ext2 were examined to determine whether they had been reported previously by consulting the MOdb (http://med-gen.ua.ac.be/LOVDv.2.0/home.php) [18].

In four patients of three families (MO-62, 63, 64), DNA sequence analysis could not be performed because Ext1 and Ext2 genes were not amplified by polymerase chain reaction (PCR). Finally, 112 patients of 71 MO families (48 women and 64 men), DNA sequence analysis was performed. All procedures were approved by Ethics Committee of Gifu University, and all participants obtained written informed consents before all procedures.

Conclusions
In this study, we evaluated and characterized mutations in the Ext1 and Ext2 genes in 71 Japanese families with MO. A total of 52 mutations in Ext1 and Ext2 were identified, with 22 of these mutations being reported here for the first time. Additionally, we identified several characteristics of gene mutations in Ext1 and Ext2. Approximately 60 % of Japanese families with MO had inactivating mutations in Ext1 and Ext2. Interestingly, these results differed somewhat from those from other countries and represented the variety of genotype in MO. Further studies are needed to determine the reasons for these differences. This study provides important insights into our understanding of the genetic features of MO in Japanese individuals.

Methods
Study design and ethical approval
In this study, we performed a multicenter study at Gifu University, National Center for Child Health and Development, and Kyusyu University. Ethics Committee of Gifu University (Approval No. 22-221) approved all procedures, and all participants obtained written informed consent before any research procedures. In case of the participant under the age of 16 year old, written informed consents (child assent and parental consent) were obtained. In addition, written informed consents to publish were obtained from all patients before study participation, and in the case of the participant under the age of 16 year old, written informed consents to publish (child assent and parental consent) were obtained.

Patients and clinical studies
From April 2010 to September 2014, patients with MO were recruited for genetic testing of the Ext1 and Ext2 genes. A total of 116 patients (51 women and 65 men) from 74 families with MO were recruited. Clinical diagnosis was performed based on accurate family histories and physical examinations of the patients, including palpation tests for osteochondromas or joint deformities. Ethics Committee of Gifu University approved all procedures, and all participants obtained written informed consents before all procedures.

Mutation analysis
Genomic DNA was isolated from peripheral blood leukocytes of all patients with MO using a Wizard Genomic DNA purification kit (Promega, Madison, WI, USA). All exons and exon/intron junctions in the Ext1 and Ext2 genes (GenBank accession numbers NM_000127.2 and NM_207122.1) were amplified by PCR. After confirming amplification of the DNA fragments by agarose gel electrophoresis and purifying the amplified DNA fragments using a Wizard SV gel and PCR Clean-up System (Promega), the amplified DNA fragments were directly sequenced using a BigDye Terminator v1.1Cycle Sequencing Kit (ABI). Sequence analyses were then performed with an ABI PRISM 3100 Genetic Analyzer (ABI). Mutations in Ext1 and Ext2 were evaluated by comparing DNA sequences of normal Ext1 and Ext2 genes with the obtained sequences using Sequencher software (Hitachi Software Engineering Co., Ltd., Tokyo, Japan). Primer sequences are shown in Additional file 1: Table S1. The detected mutations in Ext1 and Ext2 were examined to determine whether they had been reported previously by consulting the MOdb (http://med-gen.ua.ac.be/LOVDv.2.0/home.php) [18].

In four patients of three families (MO-62, 63, 64), DNA sequence analysis could not be performed because Ext1 and Ext2 genes were not amplified by polymerase chain reaction (PCR). Finally, 112 patients of 71 MO families (48 women and 64 men), DNA sequence analysis was performed. All procedures were approved by Ethics Committee of Gifu University, and all participants obtained written informed consents before all procedures.
Availability of data and materials
The Datasets used in this paper can be found at http://medgen.ua.ac.be/LOVDv2.0/home.php [18]. All supporting data are included in the manuscript as well as additional files in the supplementary section.

Additional file

Additional file 1: Table S1. Primer sequences for PCR amplification of the exons and exon/intron junctions of EXT1 and EXT2. (DOCX 21 kb)

Competing interests
There are no conflicts of interest to declare, and all the authors certify that they have no commercial associations that might pose a conflict of interest in connection with the study.

Authors’ contributions
DL acquired data, performed the data analysis, and drafted the manuscript; ST, RK, and YM acquired the data from the patients; MG, HN, and TS performed the data analysis; KM, KK, HA, and KS helped interpret the data and drafted the manuscript; KM was involved in the conception and design of the study, analysis and interpretation of the data, and drafting the manuscript. All authors read and approved the final manuscript.

Acknowledgments
We thank Yu Yamaguchi and Fumitoshi Irue for valuable discussions and useful comments. This study was supported by a Grant-in-Aid for Scientific Research of Japan (H22 nanchippan-209 to K.S.).

Author details
1Department of Orthopaedic Surgery, Gifu University, Graduate School of Medicine, 1-1, Yanagido, Gifu 501-1194, Japan. 2Research Center for Medical Glycoscience (RCMG), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan. 3Department of Orthopedic Surgery, National Research Institute for Child Health and Development, Tokyo, Japan. 4Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan. 5Advanced Medical Research Center, Aichi Medical University, Nagakute, Aichi, Japan. 6Spine Center, Gifu Municipal Hospital, Gifu, Japan.

Received: 19 July 2015 Accepted: 1 March 2016
Published online: 09 March 2016

References
1. Akita S, Murase T, Yonenobu K, Shimada K, Masada K, Yoshikawa H. Long-term results of surgery for forearm deformities in patients with multiple cartilaginous exostoses. J Bone Joint Surg Am. 2007;89(9):1993–9.
2. Matsumoto T, Matsumoto K, Harimaya K, Okaia S, Doi T, Iwamoto Y. Scoliosis in patients with multiple hereditary exostoses. Eur Spine J. 2015;24:1568–73.
3. Matsumoto K, Irie T, Mackem S, Yamaguchi Y. A mouse model of chondrocyte-specific somatic mutation reveals a role for Ext1 loss of heterozygosity in multiple hereditary exostoses. Proc Natl Acad Sci U S A. 2010;107(24):10952–7.
4. Schmide GA, Conrad 3rd EU, Raskind WH. The natural history of hereditary multiple exostoses. J Bone Joint Surg Am. 1994;76(7):986–92.
5. Bovee JV. Multiple osteochondromas. Orphanet J Rare Dis. 2008;3:3.
6. Czajka CM, DiCaprio MR. What is the Proportion of Patients With Multiple Hereditary Exostoses Who Undergo Malignant Degeneration? Clin Orthop Relat Res. 2015;473:2355–61.
7. Goud AL, de Lange J, Scholtes VA, Bulstra SK, Ham SJ. Pain, physical and social functioning, and quality of life in individuals with multiple hereditary exostoses in The Netherlands: a national cohort study. J Bone Joint Surg Am. 2012;94(11):1013–20.
8. Legeal-Mallet L, Munnoch A, Maroteaux P, Le Merre M. Incomplete penetrance and expressivity skewing in hereditary multiple exostoses. Clin Genet. 1997;52(1):12–6.
9. Pedrini E, Jennes I, Tremosini M, Milanesi A, Mordenti M, Parra A, 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(4):2294–302.
10. Ahi J, Ludecke HJ, Lindow S, Horton WA, Lee B, Wagner MJ, Horsthemke B, Wells DE. Cloning of the putative tumour suppressor gene for hereditary multiple exostoses (EXT1). Nat Genet. 1995;11(2):137–43.
11. Stickens D, Clines G, Burbee D, Ramos P, Thomas S, Hogue D, Hecht JT, Lovett M, Evans GA. The EXT2 multiple exostoses gene defines a family of putative tumour suppressor genes. Nat Genet. 1996;14(1):25–32.
12. Ludecke HJ, Ahi J, Lin X, Hill A, Wagner MJ, Schomburg L, Horsthemke B, Wells DE. Genomic organization and promoter structure of the human EXT1 gene. Genomics. 1997;40(2):351–9.
13. Clines GA, Ashley JA, Shah S, Lovett M. The structure of the human multiple exostoses 2 gene and characterization of homologs in mouse and Caenorhabditis elegans. Genome Res. 1997;7(4):359–67.
14. Sarrion P, Sangorin A, Urezzet R, Delgado A, Artuch R, Martorell L, Armstrong J, Anton J, Torner F, Vilaseca MA, et al. Mutations in the EXT1 and EXT2 genes in Spanish patients with multiple osteochondromas. Sci Rep. 2013;3:1346.
15. Jamsheer A, Socha M, Sowinska-Seidler A, Telega K, Trzebiak T, Latos-Bielskowska A. Mutation screening of EXT1 and EXT2 genes in Polish patients with hereditary multiple exostoses. J Appl Genet. 2014;55(2):183–8.
16. Seki H, Kubota T, Ikegawa S, Haga N, Fujioka F, Ohteki S, Waku K, Yoshikawa H, Takaoka K, Fukushima Y. Mutation frequencies of EXT1 and EXT2 in 43 Japanese families with hereditary multiple exostoses. Am J Med Genet. 2001;99(1):59–62.
17. Xu L, Xia J, Jiang H, Zhou J, Li H, Wang D, Pan Q, Long Z, Fan C, Deng HK. Mutation analysis of hereditary multiple exostoses in the Chinese. Hum Genet. 1999;105(1-2):45–50.
18. Signori E, Massi E, Matera MG, Poscente M, Gravina C, Falcone G, Rusa MA, Rinaldi M, Wuys W, Setipa D, et al. A combined analytical approach reveals novel EXT1/2 gene mutations in a large cohort of Italian multiple osteochondromas patients. Genes Chromosomes Cancer. 2007;46(5):470–7.
19. Jennes I, Entus MM, Van Hul E, Parra A, Sangiorgi L, Wuys W. Mutation screening of EXT1 and EXT2 by denaturing high-performance liquid chromatography, direct sequencing analysis, fluorescence in situ hybridization, and a new multiplex ligation-dependent probe amplification probe set in patients with multiple osteochondromas. J Mol Diagn. 2008;10(1):85–92.
20. Van Hul W, Wuys W, Hendrickx J, Speelman F, Wauters J, De Boule K, Van Roy N, Bossuyt P, Willems PJ. Identification of a third EXT-like gene (EXTLI) belonging to the EXT gene family. Genomics. 1998;47(2):230–7.
21. Wuys W, Van Hul W, Hendrickx J, Speelman F, Wauters J, De Boule K, Van Roy N, Van Agtmajer T, Bossuyt P, Willems PJ. Identification and characterization of a novel member of the EXT gene family, EXTL2. Eur J Hum Genet. 1997;5(6):382–9.
22. Wise CA, Clines GA, Massa H, Trask BJ, Lovett M. Identification and localization of the gene for EXTL1, a third member of the multiple exostoses gene family. Genome Res. 1997;7(1):10–6.
23. Wuys W, Van Hul W. Molecular basis of multiple exostoses: mutations in the EXT1 and EXT2 genes. Hum Mutat. 2000;15(5):220–7.