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20 novel point mutations and one large deletion in *EXT1* and *EXT2* genes: Report of diagnostic screening in a large Italian cohort of patients affected by hereditary multiple exostosis

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ABSTRACT

Background: Hereditary multiple exostosis represents the most frequent bone tumor disease in humans. It consists of cartilage deformities affecting the juxta-ephyseal region of long bones. Usually benign, exostosis could degenerate in malignant chondrosarcoma form in less than 5% of the cases. Being caused by mutations in the predicted tumor suppressor genes, *EXT1* (chr 8q23-q24) and *EXT2* (chr 11p11-p12) genes, HMEs are usually inherited with an autosomal dominant pattern, although "*de novo*" cases are not infrequent.
Aim: Here we present our genetic diagnostic report on the largest Southern Italy cohort of HME patients consisting of 90 subjects recruited over the last 5 years.
Results: Molecular screening performed by direct sequencing of both *EXT1* and *EXT2* genes, by MLPA and Array CGH analyses led to the identification of 66 mutations (56 different occurrences) and one large *EXT2* deletion out of 90 patients (74.4%). The total of 21 mutations (20 different occurrences, 33.3%) and the *EXT2* gene deletion were novel. In agreement with literature data, *EXT1* gene mutations were scattered along all the protein sequence, while *EXT2* lesions fell in the first part of the protein. Conservation, damaging prediction and 3-D modeling, in-silico, analyses, performed on three novel missense variants, confirmed that at least in two cases the novel aminoacidic changes could alter the structure stability causing a strong protein misfolding.
Conclusions: Here we present 20 novel *EXT1*/*EXT2* mutations and one large *EXT2* deletion identified in the largest Southern Italy cohort of patients affected by hereditary multiple exostosis.

Abbreviations: HME, hereditary multiple exostosis; EXT1, exostosin 1; EXT2, exostosin 2; EXT3, exostosin 3; MLPA, multiple ligation probes dependent amplification; MO, multiple osteochondrosarcomas; PCR, polymerase chain reaction; Array GH, array genomic hybridization; SNP Array, single nucleotide polymorphism array.

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1. Introduction

Hereditary forms of multiple exostosis (HME) are the most common benign bone tumors in humans and they represent up to the 15% of all the cases of multiple osteochondrosarcomas (MO) (Hennekam, 1991). Onset of the disease is variable ranging from 2–3 years up to 13–15 years with an estimated prevalence of 1/50,000 in European countries (Schmale et al., 1994), although different prevalence has been identified in a specific sub-population from Guam (Chamorro, 1:1000) (Krooth et al., 1961) and from a Canadian Indian community (Pauingassi, 1:77) (Black et al., 1993).

Clinical description of HME consists of the formation of several cartilage-capped bone tumors, usually benign and localized in the juxta-ephyseal region of long bones, although a wide body dissemination in severe cases is not uncommon. Due to the growth in number and size, exostoses can cause skeletal deformities with severe functional rebounds, such as blood or nerve compression and they can, even rarely (up to the 5% of cases), degenerate in malignant forms such as peripheral chondrosarcoma (Wicklund et al., 1995).

HMEs are usually inherited with an autosomal dominant pattern (but *de-novo* cases are not rare) and they are caused by mutations in two predicted tumor suppressor genes, namely *EXT1* (OMIM 133700, chr 8q23-q24) (Ahn et al., 1995) and *EXT2* (OMIM 133701, chr 11p11-p12) (Stickens et al., 1996; Wuyts et al., 1996). A third locus (*EXT3*) has been identified on the short arm of the chromosome 19 (Le Merrer et al., 1994), although so far, fine mapping linkage studies on patients have resulted negative at the screening of the first two genes, and failed in identifying the third one. *EXT1* gene mutations take into account about the 56–78% (Jennes et al., 2009) of all the HME cases, while *EXT2* for the 21–44% (Jennes et al., 2009). Mutation distribution across the genes is variable with a wide dissemination along the 11 exons for the *EXT1* but with a particular concentration in the first part of the *EXT2* gene. Both genes codify glycotransferase enzymes of the endoplasmic reticulum assigned to the synthesis of heparansulfate chain and proteoglycans (Busse et al., 2007).

Here we report our diagnostic experience on a large cohort of 90 HME affected patients recruited in the past 5 years at our genetic counseling center. We report on novel *EXT1*/*EXT2* mutations and on

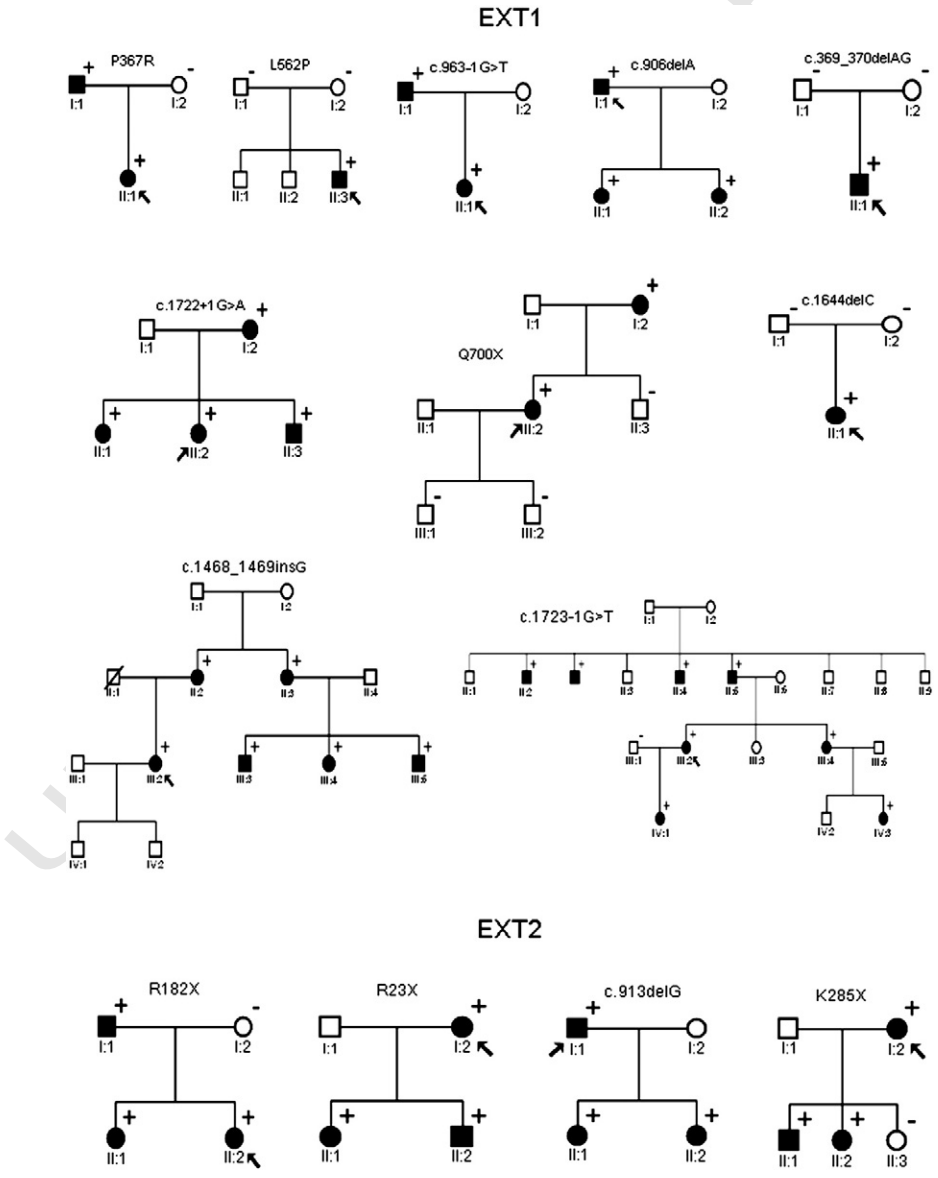

Fig. 1. Pedigree of some of the Families under study. The arrow indicates the proband; where available the mutation carrier status is indicated with "+" or "−".

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