

GENOTYPE-PHENOTYPE STUDY IN 240 MO PATIENTS

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Introduction

Multiple osteochondromas (MO) is an autosomal dominant disease (with an incidence of at least one in 50000) characterized by the formation of multiple cartilage-capped, benign bone tumours (exostoses) typically located at the meta-epiphyseal area of the long bones. It is known to be an allelic heterogeneous disorder with a wide variation in the severity, also within a family. To evaluate if the severity and the risk of malignant transformation are related with any factors or genetic alterations we performed a genotype-phenotype correlation study using a DHPLC mutation screening protocol and a clinical classification based on deformity and functional limitations.

Group	Subgroup	Features
I	IA	No deformity - No functional limitations ≤ 5 sites with exostoses
	IB	
II	IIA	Deformity - No functional limitations ≤ 5 sites with deformity
	IIB	
III	IIIA	Deformity - Functional limitations Functional Limitation of 1 site
	IIIB	

Clinical classification

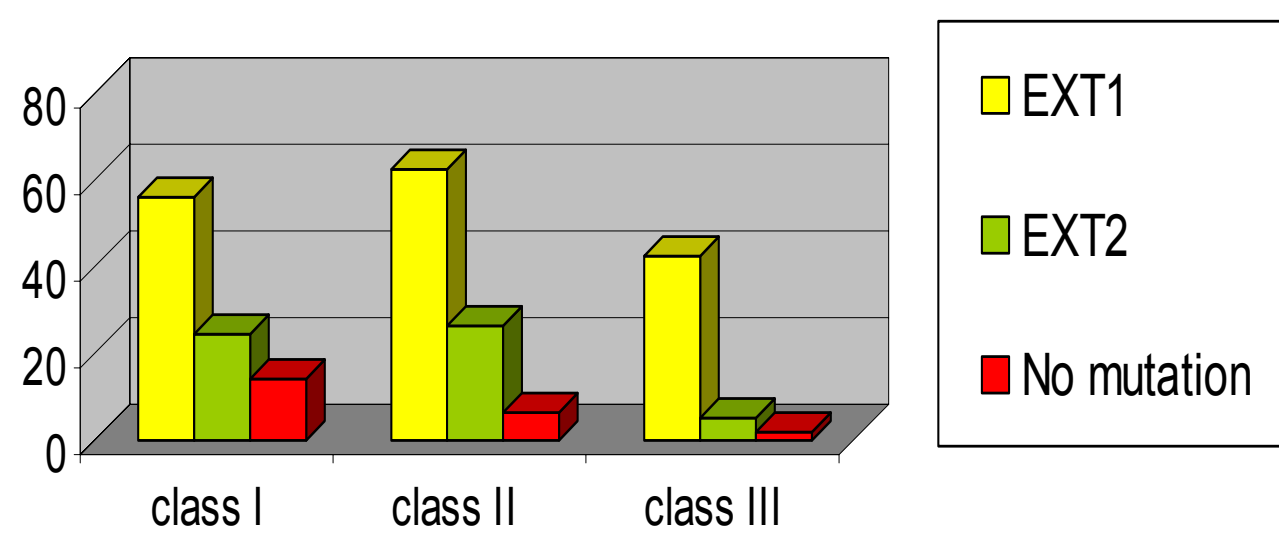
Methods

We investigated 240 patients (including 60 sporadic), 119 males and 121 females, with clinical and radiographic diagnosis of MO, for the presence of mutations in either EXT1 (8q24.1) or EXT2 (11p12-11) gene, using a DHPLC analysis method and subsequent direct sequencing of all samples with abnormal elution profile. In negative patients, all EXT1 and EXT2 exons and splice-site junction were directly sequenced. The severity of the disease has been characterized considering the presence of deformity and functional limitations as described in *Table 1*. Statistical analysis of data was performed using non-parametric tests.

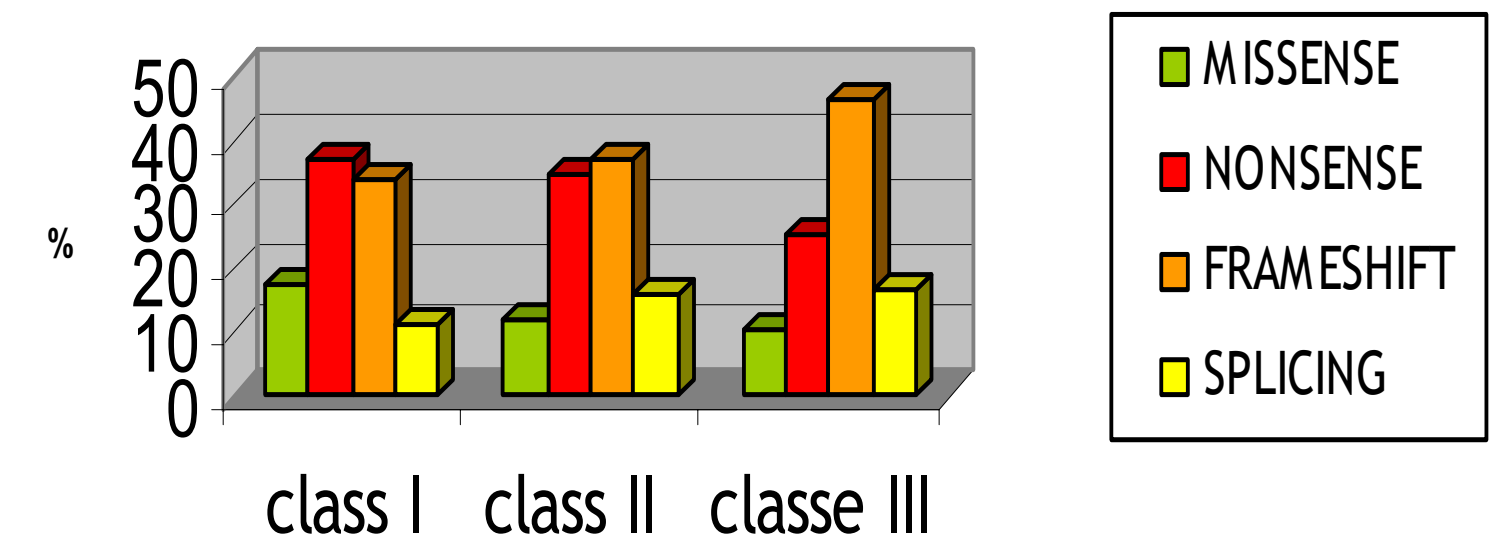
Different severity of disease in MO patients. Someone achieves normal height with little exostoses, whereas others are more affected

Results

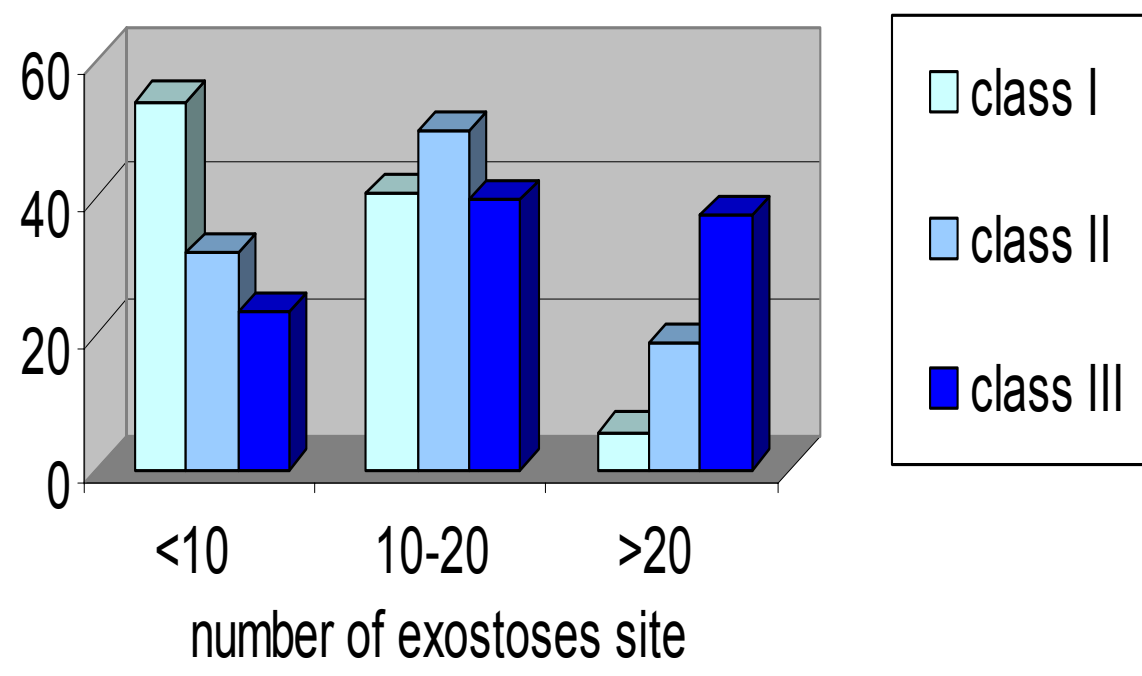
Mutational analysis showed 162 mutations in EXT1 (74%) and 56 in EXT2 (26%) with a total mutation frequency of 90%. The majority are responsible of EXT1/EXT2 protein truncation.



The most severe clinical presentations are significantly associated with EXT1 mutations ($p=0,021$), with a trend towards frameshift mutations ($p=0,077$). Patients without EXT mutations seems to be related to mild phenotypes, especially class I.

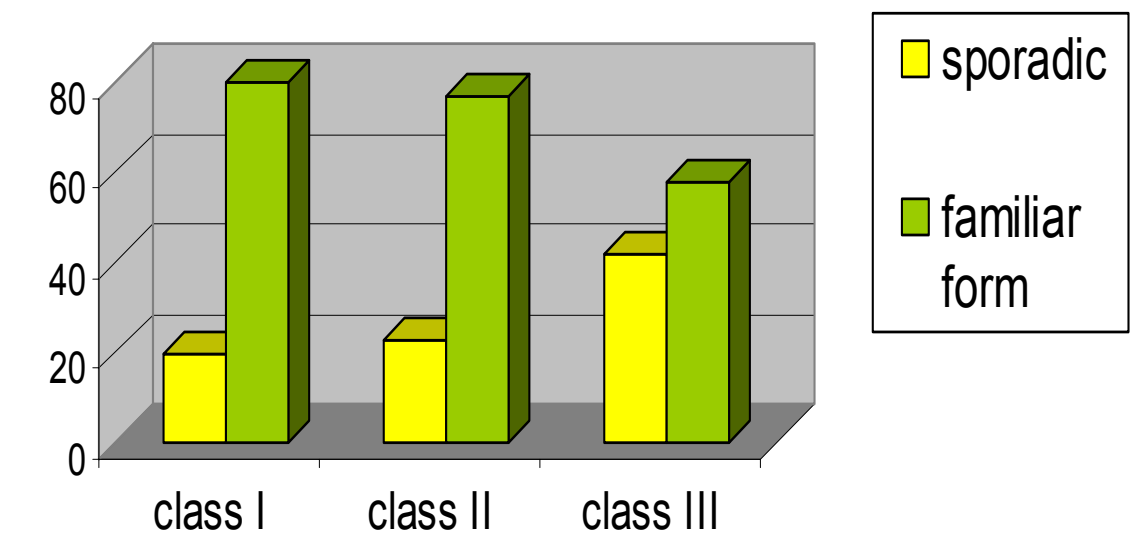


Considering the height of patients, it has an inverse relationship with the clinical severity: the mean height of individuals of group III is below the 10° percentile; the same behaviour is observed in 25% of group I patients ($p=0,005$). Smaller patients are related with EXT1 mutations.

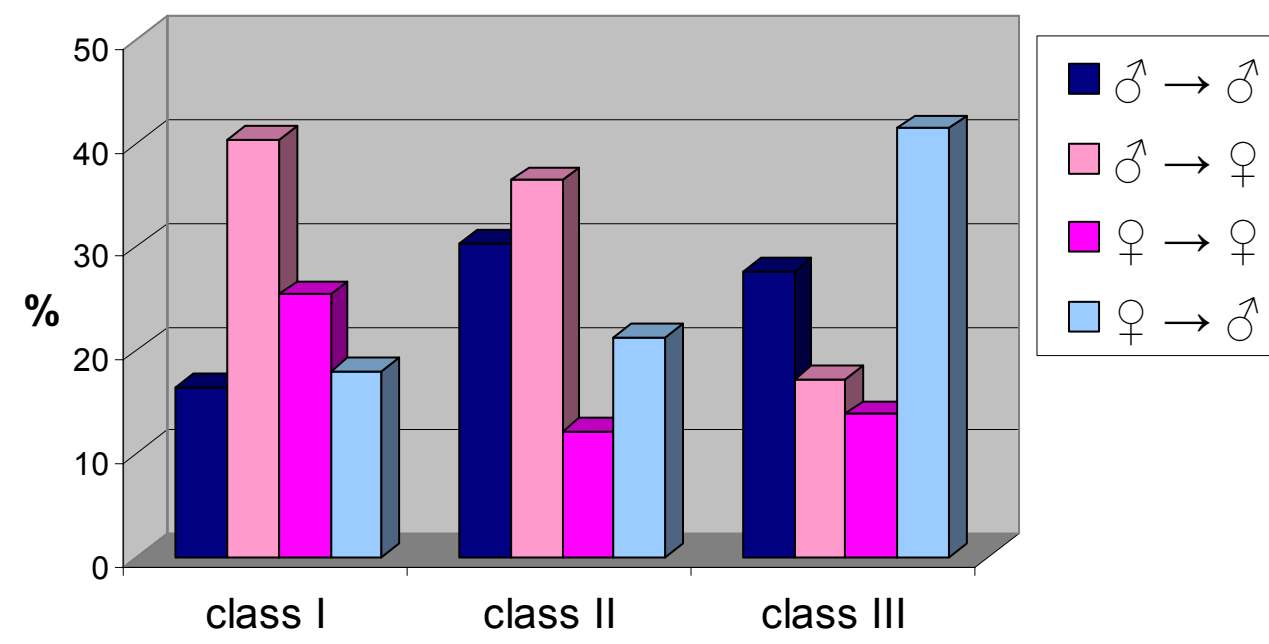
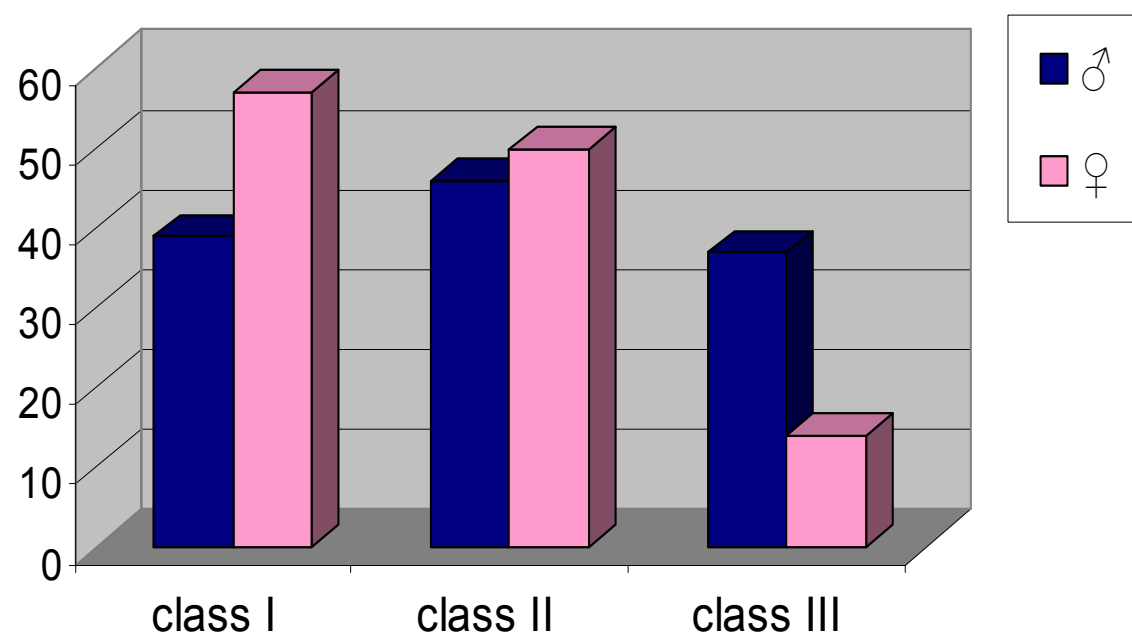


There is an ordinal correlation between the number of skeletal sites with exostoses and the severity of MO ($p=0,000$, Test Tau-b of Kendall). Most of the patients with a mild disease have a number of exostoses sites below 10, whereas patients with more than 20 exostoses sites (strictly related with EXT1 mutations) had significantly more severe disease.

The most of sporadic cases is statistically related to more severe phenotype ($p=0,010$)



The most severe clinical presentations are related with men ($p=0,001$) rather than women which seem to be associated with mild forms. If we consider separately sporadic form or a proband for each family, the statistical significance remains only for the association of males with class III. This implies that way of transmission could be relevant for the "protection" of women.



Analyzing intrafamilial variability (associated both with EXT1 and EXT2 mutations), ingravescence of disease is more frequently observed in the mother-son transmission (which increases the risk for men to belong to class III). In our study the association between the gender of both proband and parent affected is important and statistically significant ($p=0,029$) not only for 'men risk' but also for 'women protection' which is more consistent if related with maternal-transmission.

Malignant transformation of osteochondroma occurred in 16 patients (more than 5%) which are all with a MO positive family history but it is not related to the severity of disease, EXT1 or EXT2 gene, mutation type or number of exostoses.

Conclusions

Due to the great number of patients analyzed, it has been possible to obtain interesting information about MO which may provide an useful tool both in predicting patient outcome and in defining the right follow-up program (increasing clinical examination for patients with higher risk to belong to class III). We have found a strong relationship between more severe forms of MO and EXT1 mutations showing also the important role of the association of proband and parents gender which could increase or reduce the risk of developing a mild or severe form. In contrast with previous observations there is no evidence of an association between malignant degeneration and EXT1 mutations or more severe forms; the absence of any prognostic marker in malignant transformation requires a regular screening for every patient with MO.