***Multiple osteochondromas–with contribution of 19 Bulgarian families***

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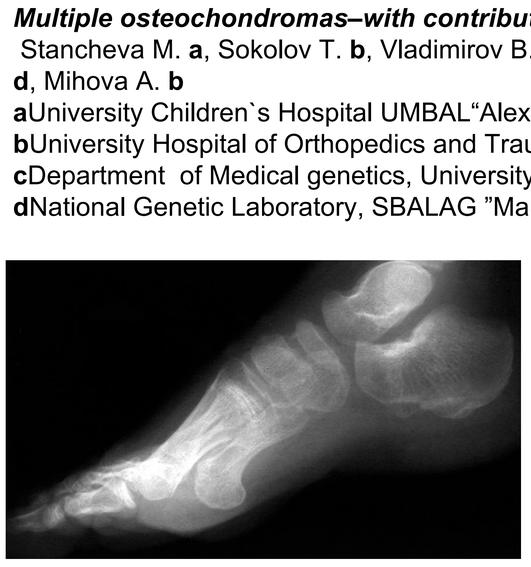
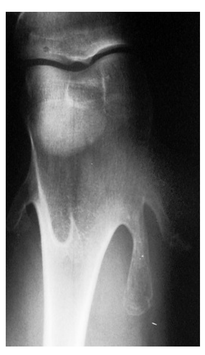
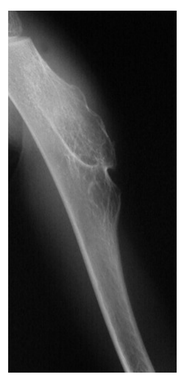
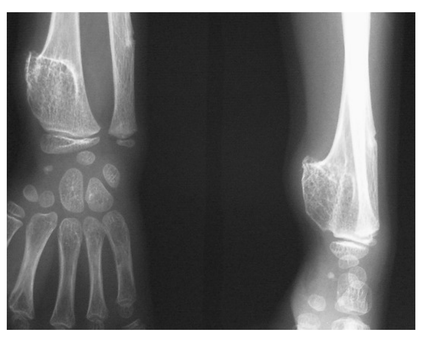
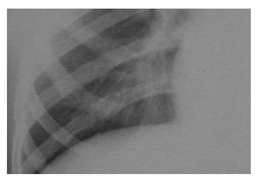
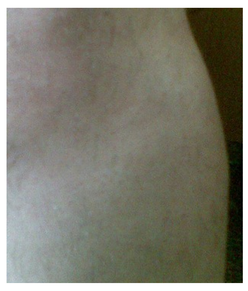
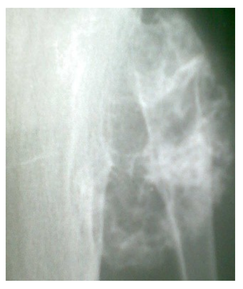
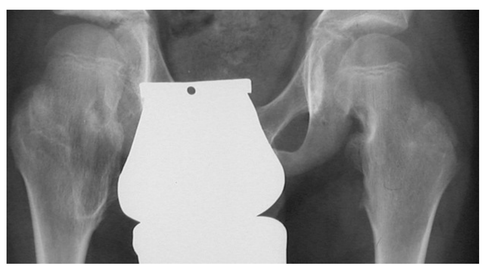
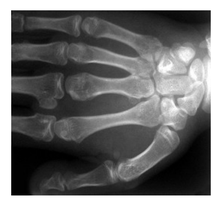
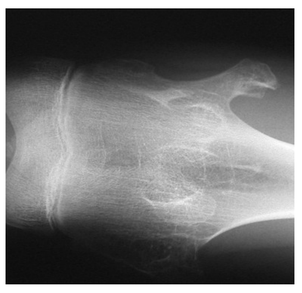

Localizations of Multiple osteochondromas

INTRODUCTION

Multiple osteochondromas (MO, diaphyseal aclasis, multiple osteocartilaginous exostoses, osteochondromatosis , hereditary multiple exostoses) is a metabolic disease from the group of O-xylosylglycan disorders of glycosylation with autosomal dominant inheritance and almost complete penetrance.Approximately 10% of affected individuals have MO as a result of a de novo mutation . The reported prevalence ranges from as high as one in 100 in a small population in Guam to approximately one in 100 000 in the European population(4,11).

Two main genes are known to be associated with hereditary multiple osteochondromas : EXT1 localized at 8q24 for approximately 56-78% of MO cases and EXT2 localized at 11p11 –mutated in 21-44% of MO cases. EXT1 and EXT2 encode transmembrane glycoproteins -glycosyltransferases that interact as heterooligomeric glycosyltransferases which add GlcAc and GlcNAc to heparan sulphate in the Golgi apparatus and participate in cell signaling and chondrocyte proliferation and differentiation .Mutations in EXT1 and EXT2 cause cytoskeletal abnormalities including actin accumulation , excessive bundling by alpha-actinin , and abnormal presence of muscle specific alpha-actin.Some evidence suggest that EXT1 and EXT2 may have tumor suppressor activity.

The clinical diagnosis of MO is established in individuals with : 1.Multiple osteochondromas arising from the area of the growth plate in the juxtaphyseal region of long bones or the surface of flat bones such as scapula .The key radiographic and anatomic feature is the uninterrupted flow of cortex and medullary bone from the host bone into the osteochondroma.Osteochondromas possess the equivalent of a growth plate that ossifies and closes with the onset of skeletal maturity.The family history is consistent with autosomal dominant inheritance and /or proven mutation of EXT gene .

The osteochondromas are present at birth , but usually they are diagnosed later in infancy when they grow in size.They gradually ossify during skeletal development and stop growing in most of the cases with skeletal maturity.By adulthood 2/3 of the individuals have a clinically evident bony deformity with functional impairement of cardiovascular, nerve and pulmonary systems. There is a risk of sarcomatous degeneration in 1-5 % of the cases. (4).

Presently most clinically diagnosed patients haven`t proceeded to genetic analysis and consultation.The aim of our work was to perform a molecular analysis of Bulgarian patients with clinically diagnosed MO, to include the disease in the national and international register for rare diseases,to analyse the results and search a genotype-phenotype correlation, to make a genetic consultation and propose a prenatal diagnosis and preimplantation genetic diagnosis according to the choice of the parents.

MATERIAL AND METHODS

Material and methods : 19 patients ( 9 boys and 10 girls ) from 7-28 year`s old diagnosed at University Hospital of Orthopedics and Traumatology “Prof. Boicho Boichev “ .There were used anthropometry, family history, history of disease , orthopedical status , rentgenography , CAT, angiography, biochemical investigations ( blood count, coagulation status, total protein, albumin, glucose, urea, creatinine, uric acid, ASAT, ALAT, AP), histological investigation.Patients had consultations with a pediatrician, neurologist and cardiologist. DNA analysis from peripheral blood was proceeded in 5 patients for a first time at the Department of Medical Genetics , University of Antwerp, Belgium.DNA analysis was performed by PCR amplification and direct sequencing of all coding regions (exon 1-11) of the EXT1 and all coding exons (exon 2-14) of the EXT2 gene.Numbering and sequence analysis are based on Genbank file NM_000401 with A of start ATGposition +1 for EXT1 and Genbank file sequence NM_000127 with A of start ATG position +1 according to Clines et al ( Genome Res.7(4):359-67, 1997 ) for EXT2.In addition MLPA (Multiplex Ligation-dependent Probe Amplification ) analysis ( Salsa MLPA P215) was performed. FISH (fluorescent in situ hybridization) analysis was performed with EXT1 cosmid probes 46F10 and 90D8 (Ahn et al, Nature Genet 11:137-143, 1995).

RESULTS

The family history of 18 children showed an autosomal dominant inheritance with more than 3 members affected in every family.In 2 of the cases the disease was diagnosed shortly after birth.The median age of diagnosis was 3 year`s old and in 90 % of the cases the disease have been already diagnosed to 12 year`s old.The orthopedic treatment started significantly later . Most commonly affected bones were femur (55%), tibia (45%), fibula (15%), humerus( 55%, radius (25%), scapula (25%), ribs(10%), metacarpal, metatarsal and phalangues (5%).Fig.1,2,3 Multiple osteochondromas of a patient with mosaic deletion of the EXT1 gene.

The following bone deformity have been described: valgus deformities (2), shortening of limbs-2 cm(2), decreased joint movements-knee (5.2%), elbow (5.2%), ankle (5.2%).Rapid growth and increasing pain was found in 36.4% of the cases.All the children were treated with at least one operative procedure and many have multiple procedures with simple surgical excision, corrective osteotomies.Only in one child there were complications after the operation-paresis of n.peroneus.

The DNA analysis of 5 patients with this disease is performed for the first time in Bulgarian patients . The first patient harbours a c.1468 del C(p.Leu490TrpfsX9 ) mutation in exon 6 of the EXT1 gene which causes a frameshift and premature stop codon , resulting most likely in the formation of an instable mRNA or a truncated , non-functional EXT1 protein.The molecular analysis of the second patient shows a mosaic deletion of the entire EXT1 gene.The third patient harbours a c.1057-2A>G(IVS2-2A>G)mutation in intron 2 of EXT1 , which affects most likely proper splicing of exon 3 , resulting in the formation of an unstable mRNA or a truncated , non-functional EXT1 protein.The fourth patient harbours c1079+1G>T mutation in intron 6 of the EXT2 gene that most likely affects proper splicing of exon 6 , resulting in the formation of an unstable mRNA or a truncated , non-functional EXT2 protein and the fifth mutation identified c.1065dupT(p.Leu356SerfsX2 ) in the EXT2 gene causes a frameshift and premature stop codon , resulting most likely in the formation of an instable mRNA or a truncated , non-functional EXT2 protein .

The patient with the mosaic deletion of the entire EXT1 gene shows the most severe clinical picture , followed by the patient with frameshift c.1065 dupT (p. Leu. 356 Serfs X2) in the EXT2 gene and the patient with the c. 1057-2A>G(IVS2-2A>G) of intron 2 in the EXT1gene.

The presence of osteochondromas was noticed in both of them at 2 years. The number of osteochondromas is from 10-20 with involvement of pelvic bone and scapula . The patient with the mosaic EXT1 deletion shows at the same age skeletal deformities with shortening of the lenghth of limbs, decreased joint movements and height at the lower range of normal.The patient with splicing of EXT1 presents with a lower number of osteochondromas, but skeletal deformations and tooth anomalies are present.The third female patient with a mutation of EXT1 shows a later beginning of the disease , lower number of osteochondromas (5-10) with involvement of the flat bones and abnormal tooth arrangement , but at present without skeletal deformities and normal height.The second patient with an EXT2 splice site mutation showed a later beginning of the disease at 10 year`s old, lower number of osteochondromas (2-5) and normal height .

The biochemical investigations , blood count, renal, liver investigations, calcium phosphorus status and coagulation status were in normal ranges.

The histology showed the presence of „ obese nucleus ” of the chondrocytes , cytoskeletal abnormalities including abnormal accumulation of actin, alfa-actinin and presence of muscle specific alfa-actinin.

*DISCUSSION*

The median age of diagnosis in our study is 3 year`s old .The disease has been diagnosed in 90 % of the cases to the age of 12 year`s old.These data are similar to the data in the literature. (4,8). The later beginning of orthopaedic treatment -3-10 years after the apparition of the cartilage capped bony growths follows the grow in size which causes functional deficit , pain and deformation due to compression , rapid growth after the closing of epyphyses.

The most common affected bones according to the medical literature are femur (70%), humerus(50%), radius and ulna (50%), scapula (50%) and the bones around the ankle (25%), which is similar to our results .The complication of MO are more often shortened stature, bone deformities, mechanical blocks to motion of joints , compression of nerves and vessels, and later joint degeneration, arthroses / 5,7,8,13,14,16/. Our results are similar to the literature data /1/.They are more pronounced with increase of durability of the disease and number of osteochondromas in the skeleton and of the single bone.

The apparition of pain and rapid growth of a known osteochondroma , after the closure of epyphyses is an alarming signal for sarcomatous degeneration but in our patients nobody developed chondrosarcoma during follow up of 7 years.

The surgical treatment was necessary for correction of the occurred skeletal deformation , lengthening procedures or simple resection of the osteochondromas which impair the joint movements , compress nerves or vessels and in cases of malignant transformation. The normal results of the biochemical investigations in all patients showed absence of a biochemical marker for this metabolic disease.

The most important investigation for confirmation of the diagnosis is the DNA analysis .The majority of the mutations are insertions and deletions that produce frameshifts resulting in premature termination and loss of function .Nonsense and splice site mutations have also been observed. Most of the reported missense mutations detected occurred in residues that are highly conserved evolutionarily and are thought to be crucial for the activity of the protein.Missense mutations affecting residues that are not as tightly conserved may be rare polymorphisms/17/.The majority of patients harbour inactivating mutations , mostly located in the first 6 exons of EXT1 or the first 8 exons of EXT2 /18/.The last observation is confirmed in the investigated Bulgarian patients.The described mutations of us are new for the medical literature.The number of investigated children with DNA analysis is too small for statistically significant genotype-phenotype correlations .Our observations are however in agreement with the tendency for larger number of osteochondromas , skeletal deformations , shortened stature and complications in children with EXT1 mutations.The patients with EXT2 showed a tendency for less severe clinical picture and better prognosis.For a first time the patients were given a genetic consultation and the possibility for prenatal diagnosis.A genetic consultation and DNA analysis is important to be given to the relatives of the proband because of the different expression of this which permits a big number of the relatives of the proband to be undiagnosed and transfer the disease to future generations.

The histological investigation found in the literature described cytoskeletal abnormalities (3,9,10).The presence of so called “obese” nuclei in the chondrocytes in adults could be interpreted as beginning of malignant transformation but in children it is an expression of intensive proliferation of chondrocytes in the cartilage cap of the osteochondromas.With increasing of age this activity diminishes and “obese” nuclei disappear.

The differential diagnosis of the MO includes solitary osteochondromas , juxtacortical osteosarcoma, soft tissue osteosarcoma , metachondromatosis and heterotopic calcification. Metachondromatosis is inherited in autosomal dominant manner too but in contrast to MO it is characterized by both osteochondromas and intraosseous enchondromas.The osteochondromas occur predominantly in the digits and unlike those of MO point toward the nearby joint and do not cause shortening or bowing of the long bone, joint deformity and subluxation. Affected individuals with osteochondromas , mental retardation and characteristic craniofacial and digital anomalies must be investigated for Langer-Giedeon syndrome and Potocki-Shaffer syndrome /11p deletion syndrome /OMIM 601224/.

The general prognosis of osteochondromas is good.They present a benign bone outgrowths and don`t affect the intelligence and the life span.However the risk of malignant transformation in the cartilage cap exists and is 1-5 %.These increased tendency for malignisation could be explained to be due to their large number and thus multiple risk for occurrence in every of them (1,6,7,19). The prognosis of the secondary chondrosarcomas depends of the histological stage : 10 years survival in 83% of patients in stage I and 29% in patients of stage III /15/.

CONCLUSIONS

1. We recommend the proceeding of genetic consultation , DNA analysis of the patients and their families for exact diagnosis, evaluation of genetic risk and prophylaxis of the disease.

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