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Short Communication

KARYOTYPING ANALYSIS OF A MULTIPLE HEREDITARY EXOSTOSES AFFECTED PROBAND

Vichare V. V*[1, 2], Kokane. K[1], Kutty. B.C[1], Gangawane A K[3]

[1]Department of Biotechnology, Pillai's College of Arts Commerce and Science, Dr. K.M.Vasudevan Pillai's Campus, Plot 10, Sector 16, New Panvel, Navi Mumbai- 410206.

[2]CMJ University, Modrina Mansion, Laitumkhrah, Shillong, Meghalaya

[3]Departmentof Biotechnology, Rizvi College of Engineering, Bandra (W), Mumbai-400 050

ABSTRACT

Multiple Hereditary Exostoses (MHE) is an autosomal dominant orthopaedic disorder most frequently caused by mutations in the *ext*1 gene. It is a very rare kind of disorder having occurrence as 1/50000 individuals. It is a condition in which people develop multiple benign (noncancerous) bone tumors called exostoses. Exostosin protein is found to be mutated in such affected patients. This exostosin gene locus has been identified at three different chromosomes i.e. *ext1* at chromosome 8q23-24, 5; *ext2* to chromosome 11p11-p12, 6 and *ext3* to chromosome 19p. This present study was an attempt to identify the chromosomal aberrations of *ext*1, *ext*2 and *ext*3 genes at chromosomes 8, 11 and 19 through karyotyping analysis. The chromosomal analysis reveals a normal karyotype and hence further molecular techniques like FISH, or MLPA or dHPLC has to be used to determine the mutations.

KEY WORDS: Hereditary Exostoses, Exostosin, *ext*1, *ext*2, and *ext*3 and Karyotype

INTRODUCTION

Hereditary Multiple Exostoses (MHE) is a rare medical condition in which multiple bony spurs or lumps (also known as exostoses, or Osteochondromas) develop on the bones of affected patient. MHE is characterized by the growth of cartilage-capped benign bone tumors around areas of active bone growth, particularly the metaphysic of the long bones. [2] HME can lead to the shortening and bowing of bones; affected individuals often have a short stature. MHE can cause pain to people of all ages but for children with such disorder has a lot of pain. During exercise, it can cause a lot of pain. Depending on their location the exostoses it can cause the following problems: pain or numbness from nerve compression, vascular compromise, inequality of limb length, irritation of tendon and muscle, as well as a limited range of motion at the joints upon which they encroach. MHE can lead to the shortening and bowing of bones and affected individuals often have a short stature. The proportion of individuals with multiple hereditary exostoses who have clinical findings increases from approximately 5% at birth to 96% at age 12 years. By adulthood,

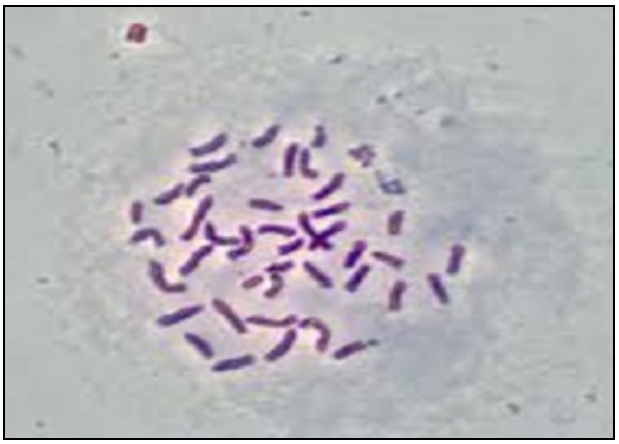
75% of affected individuals have a clinically evident bony deformity. Males tend to be more severely affected than females. Most commonly involved bones are the femur (30%), radius and ulna (13%), tibia (20%), and fibula (13%). Hand deformity resulting from shortened metacarpals is common. [6] Surgery, physical therapy and pain management are currently the only options available to MHE patients, but success varies from patient to patient and many struggle with pain, fatigue and mobility problems throughout their lives. It is not uncommon for MHE patients to undergo numerous surgical procedures throughout their lives to remove painful or deforming exostoses, correct limb length discrepancies or improve range of motion. MHE is an autosomal dominant hereditary disorder with occurrence rate as 1 in 50,000.[1,4] Multiple hereditary Exostoses is genetically heterogeneous and at present, two genes, *ext*1 and *ext*2 located on 8q24 and 11p11–p12, respectively, have been cloned. [3] There are different types of mutation observed on the said locus of the chromosome. Frame shift mutations caused because of deletion or insertion are very common in exon 1 and exon 6 of the *ext*1 gene causing MHE type 1. Also deletion of exons is observed at exon 10 of ext1 and exon 2 of ext2 genes. [5, 7] This study is a comparative analysis of a normal karyotype and karyotype of the affected proband of MHE.

MATERIALS AND METHODS

The patients suffering from Multiple Hereditary Exostoses are identified and their consent forms were filled up as per the format and guidelines given by Medical Council of India. The ethical clearance for the present study is obtained from the Institutional Ethics Committee. Blood Sample of the identified patients was collected in BD vacutainer (Sodium heparin 68 USP units) with the help of a certified lab technician. About 2 ml blood sample was collected from five patients belonging to different family. Chromosomal analysis was done from the peripheral blood samples collected from the patients using GTG banding method. In this method the chilled slides with cell suspension (added drop by drop) were flamed gently, cooled and stored at 60^0C overnight. This slide was then incubated with Sorenson's buffer at 60^0C for 8 minutes in a water bath. After incubation the slides were kept in trypsin EDTA solution for 3-5 seconds and then transferred to 0.1M NaCl solution. The slides were further stained with Geimsa stain for 3 minutes, cleaned with water and observed under 100x of the light microscope.

RESULTS AND DISCUSSION

The different samples five in numbers of MHE affected probands were analyzed for chromosomal aberrations. In each sample 20 cells were observed and each sample was karyotyped thrice. The karyotype obtained of all five different samples appeared to be normal and no significant findings were reported. So it is stated that chromosomal aberrations observed in MHE affected probands in each of these cases may be very small and hence higher molecular biology techniques are to be applied for the chromosomal changes.



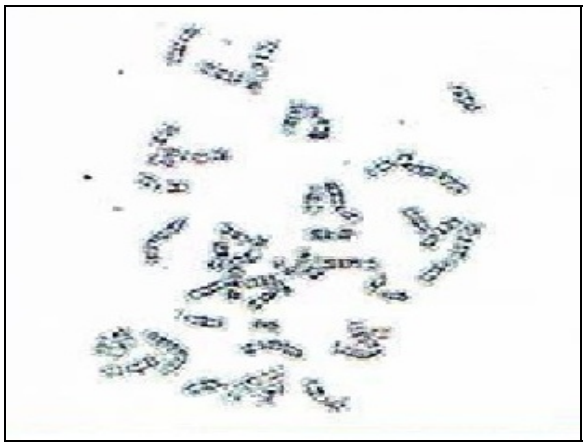


Figure 1 and 2: chromosomes of MHE probands in chromosomal analysis

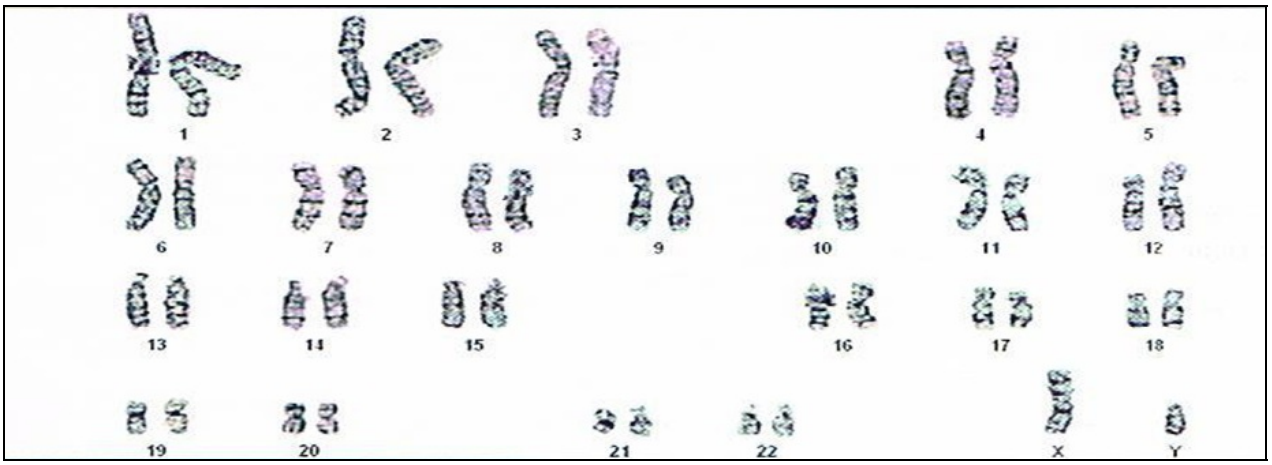


Figure 3: Karyogram of MHE affected proband

CONCLUSION:

It is concluded by the present study that no significant changes are observed in the karyotype of multiple hereditary exostoses affected probands and hence cannot be used as a tool for diagnostic method.

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REFERENCES

1. A Kivioja, H. Ervasti, J. Kinnunen, I. Kaitila, M. Wolf, T. Böhling, Chondrosarcoma in a family with multiple hereditary exostoses, *The Journal of Joint and Bone surgery* 2000 March Vol. 82-B, No. 2,.

2. Bove´e JVMG, Cleton-Jansen AM, Kuipers-DykshoornNJ, Van den Broek LJCM, Taminiau AHM, Cornelisse CJ, Hogendoorn PCW, Loss of heterozygosity and DNA ploidy point to a diverging genetic mechanism in the origin of peripheral and central chondrosarcoma, *Genes Chromosomes Cancer*, 1999 Nov;26 (3) :237-46

3. Geraldine R Vink, Stefan J White, Strelicija Gabelic, Pancras CW Hogendoorn, Martijn H Breuning and Egbert Bakker, Mutation screening of *ext*1 and *ext*2 by direct sequence analysis and MLPA in patients with multiple osteochondromas: splice site mutations and exonic deletions account for more than half of the mutations, *European Journal of Human Genetics* (2005) 13, 470–474

4. Ivy Jennes, Mark M. Entius, Els Van Hul, Alessandro Parra, Luca Sangiorgi, and Wim Wuyts, 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, *Journal of Molecular Diagnostics*, 2008 Jan,Vol. 10, No. 1,

5. Pedrini. E,De Luca. A, Valente. E.M., Maini. V, Capponcelli. S, Mordenti.M, Mingarelli. R, Sangiorgi. L, and Dallapiccola. B, Novel *ext*1 and *ext*2 Mutations Identified by DHPLC in Italian Patients with Multiple Osteochondromas. *Human Genome variation Society*, 2005 Sep, Vol.23 Issue3, Pg 280.

6. Porter, D. E., Lonie, L., Fraser, M., Dobson-Stone, C., Porter, J. R., Monaco, A. P. & Simpson, A. H. Severity of disease and risk of malignant changes in hereditary multiple exostoses. Agenotype-phenotype study. *J Bone Joint Surg Br*. (2004) 86, 1041–1046.

7. Wolfram Heinritz, Ulrike

Huffmeier, Sibylle Strenge, Bianca Miterski, Christiane Zweier, Steffen Leinung, Axel Bohring, Beate Mitulla, Usha Peters and Ursula G. Froster, New Mutations of *ext1* and *ext2* Genes in German Patients with Multiple Osteochondromas. *Annals of Human genetics* , 2009, 283-291

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