Molecular Exclusion of Mutations in EXT1 and EXT2 as the Cause of Metachondromatosis

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Introduction

Metachondromatosis (OMIM#156250) is a rare, autosomal dominant disorder characterized by multiple metaphyseal protuberances (exostoses) beginning in the second or third decade of life (Bernard et al. 2001, Stickens et al. 1996). Typical clinical features include pain and deformities of the extremities, which often require surgical intervention. Genes encoding the EXT proteins have been implicated as candidate genes in our 2 metachondromatosis families.

Patients and Methods

We have assayed 2 families with metachondromatosis at the Research Center at Shriners Hospitals for Children, St. Louis. The families are of Missouri and Indiana and are of European ancestry.

Methods

40 weeks and 45 cycles of PCR amplification at 30 sec at 94°C, 45 sec at 60°C, and 45 sec at 72°C, followed by a final extension of 10 min at 72°C. PCR products were labeled with fluorescent dyes and run on a 3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, CA). DNA sequencing reactions were performed in 20 ul reaction volumes that included 1X Big Dye Terminator Mix and run on a 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA).

Results

For Metachondromatosis Family 1, no polymorphisms were detected in the exons or the intronic sequence. For Metachondromatosis Family 2, all exons and adjacent mRNA splice junctions (including at least 20 bp of intronic sequence) of EXT1 were amplified by PCR using primer pairs (Table 1) designed by us. None of the amplified fragments showed any deviations from normal sequence. DNA sequencing reactions were performed in 20 ul reaction volumes that included 1X Big Dye Terminator Mix and run on a 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA). DNA sequencing text files were aligned to control sequence to identify any defects. No mutations in either gene (EXT1 or EXT2) were found for the patient from family 1, no polymorphisms were detected in the exons or the intronic sequence.

Discussion

Metachondromatosis and hereditary multiple exostoses (HME) are both inherited conditions that manifest in the second or third decade of life. Both are autosomal dominant. HME is characterized by a greater number of exostoses and a more aggressive phenotype, often requiring early surgical intervention. Metachondromatosis, on the other hand, is characterized by fewer exostoses and a milder phenotype. Given that these 2 conditions are characterized by exostoses, it is likely that EXT1 and EXT2 are the genes responsible for these conditions. Because of the clinical similarities between metachondromatosis and HME, we screened patients for mutations in the multiple exostoses gene family. We found no evidence of mutations in either EXT1 or EXT2 that would explain the bone disease in our families. The absence of mutations in EXT1 and EXT2 does not exclude the possibility of complex deletions of other genes.

References


Acknowledgments

Figure 1 Family 1 Individual M-1: a 27-year-old man with metachondromatosis who has multiple exostoses on both hands and feet. Figure 2 Family 2 Individual V-2: a 5-year-old boy with metachondromatosis who has multiple exostoses on both hands and feet.