AN ARGinine–GLYCine MUTATION IN FRZB IN A FAMILY WITH A NEWLY DESCRIBED HEREDITARY CHONDROLYSIS SYNDROME CARRIED BY AFFECTED AND UNAFFECTED INDIVIDUALS

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Introduction
Chromosome 2q has been suggested as a locus associated with familial osteoarthritis(1,2). Among candidate genes in this region is the gene for secreted frizzled related protein-3 (SFRP3 or FRZB). Two structural domains comprise this protein. The N-terminal of these domains has been shown to have considerable (>50%) homology with the developmental protein, frizzled, in Drosophila(3). The C-terminal domain shares homology with several proteins central to skeletal development and tissue remodeling(4). The purpose of the current investigation was to determine whether changes in FRZB gene sequence accompanied osteoarthritis or other arthropathies.

Materials and Methods
EDTA blood samples were obtained with patient consent in a manner approved by our institutional review boards. Genomic DNA was purified from the whole blood either with Stratagen® DNA Extraction Kit or with Qiagen® DNA Blood Maxi Kit. In all, 29 individuals from seven families were tested. Six of the families had been diagnosed with apparent familial osteoarthritis. The family of interest in the present study had been diagnosed over a period of years with a range of anatomical irregulaties, including but not confined to: Osgood-Schlatter, Legg-Calvé-Perthes, spondyloepiphyseal dysplasia, multiple epiphyseal dysplasia and metaphyseal dysplasia. Hips, shoulders and knees were affected at various times in different members of the family resulting in numerous arthroscopies and arthroplasties. These individuals are of normal to above average height, thin habitus, generally seeking medical attention for joint pain in the late first or early second decade of life. This family, whom a diagnosis of hereditary chondrolysis syndrome was made based on arthroscopic findings, and in whom there appeared to be a related FRZB mutation, was studied in further detail.

Genomic DNA from all subjects was sequenced to identify single nucleotide polymorphisms (SNP’s) in the FRZB gene. PCR primers were designed and conditions optimized for the six FRZB exons and the 5’ and 3’ untranslated regions (UTR). PCR products were sequenced in both directions with the ABI PRISM® Big Dye® Terminator Cycle Sequencing Ready Reaction Kit. Data assembly and analysis were performed using SEQUENCER 3.1 software.

Results
Laboratory findings:
Two unique SNP’s were identified in patients with hereditary chondrolysis syndrome not seen in any of the familial osteoarthritis patients. One SNP was found in the 3’ UTR, a G to A transition; a non-synonymous polymorphism was found in exon 6. This latter, unusual C to G mutation would result in an Arg to Gly substitution in the C-terminal region of the protein. The proband (father) and 3/3 of his affected children but not his unaffected wife carried the UTR and ORF SNP’s, suggesting a possible association of the phenotype with the mutation. Further examination of the family demonstrated that both SNP’s were present in the proband’s unaffected mother and two of three unaffected siblings of the proband.

Clinical findings:
Radiologic findings of the middle and eldest offspring included significant deformity of the femoral head with marked flattening of the femoral head and significant coxa valga. The father had been diagnosed with “a rare form of possible Perthes disease” and at various times had been told that he might have spondyloepiphyseal dysplasia. He had undergone nailing of his left hip with a Smith-Petersen nail. He subsequently developed severe hip deformity and underwent total hip arthroplasty. Prior to arthroplasty severe degenerative changes were noted along with osteocartilaginous loose bodies. Thus, the father, son and elder daughter all have radiologic changes in the hip showing femoral head flattening and secondary degenerative arthritis.

Arthroscopic findings in all four of the affected individuals showed striking similarities. Of note were effusions, large loose bodies, and bubbling and delamination of the cartilage with exposure of subchondral bone. The youngest sibling underwent several arthroscopic procedures beginning at age 16. “Debonding” of articular cartilage from subchondral bone involving essentially the entire weight-bearing portion of the lateral tibial plateau was noted in one of the knee procedures. This same individual underwent hip arthroscopy in which “a large chondral flap”3.5 x 2.5 cm in dimension was noted and removed from the femoral head.

The middle sibling underwent multiple arthroscopic procedures with removal of loose bodies and finally underwent total hip arthroplasty at age 26.

Fig. 1. Knee (left) and shoulder arthroscopies on youngest sibling. Knee at 18 years, note friability and delamination. Shoulder at 20 years, note multiple loose bodies and exposed subchondral bone.

Discussion
Pathogenic FRZB mutation(s) have not been reported in humans. The importance of the Wnt/Frizzled axis in embryonic development has long been appreciated in species as diverse as Drosophila, Xenopus, and mice. The non-conservative nature of the presumptive mutation described in the present study could provide insight into the mechanism of FRZB function in human skeletal development. A mutation resulting in the replacement of an Arg with a Gly residue could easily alter the structural arrangement of the protein as well as its charge, thereby changing the protein’s ability to interact with ligands important in its function. Since unaffected family members also have this change in presumptive amino acid sequence, it is clear that this mutation is, in and of itself, insufficient to cause the phenotype. Whether the mutation plays a role in what could be a polygenic trait responsible for the phenotype remains to be seen. It should be noted that these patients also have a unique G to A transition in the 3’ UTR of this gene. While this would not affect the amino acid sequence, it could conceivably change a regulatory function of the UTR in a way which might lead to loss of function.

The clinical findings reported here represent a newly defined clinical syndrome associated with chondrolysis and cartilage debonding. Previous laboratory studies conducted elsewhere have shown that FRZB appears in developing human limbs at about 6 weeks, with the highest level of expression seen at 13 weeks at the epiphyses of long bones (3). The phenotype described here is characterized by delamination of the cartilage from the underlying bone and exposure of subchondral bone at a very early age further supporting an anatomical correlate to the mutation(s) described here.

References

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