Clinical and Genetic Studies of Three Inherited Skeletal Disorders

Eva-Lena Stattin

Akademisk avhandling

som med vederbörligt tillstånd av Rektorsämbetet vid Umeå universitet för avläggande av Medicine Doktorsexamen vid Medicinska fakulteten framägs till offentligt förvar i sal E04, byggnad 6E, Norrlands Universitetssjukhus, Umeå, fredagen den 29 maj, kl. 09:00.
Avhandlingen kommer att förvaras på svenska.

Fakultetsopponent: Professor, Jaakko Ignatius, Institution of Clinical Medicine, department of Clinical Genetics, Oulu University Hospital, Oulu, Finland.
Clinical and genetic studies of three inherited skeletal disorders
Eva-Lena Stattin, Department of Medical Biosciences, Medical and Clinical Genetics, Umeå University, SE-901 85 Umeå, Sweden

Abstract

Mutations in genes of importance for cartilage development may lead to skeletal malformations, chondroskeletal dysfunction and increased susceptibility to degenerative joint disease. Characterization of these mutations and identification of molecular pathways for the corresponding gene products have contributed to our understanding of mechanisms regulating skeletal patterning, endochondral ossification and joint formation.

A five generation family segregating autosomal dominant osteochondritis dissecans (OCD) was identified. Affected family members presented with OCD in knees, hips and elbows, short stature, and early osteoarthritis. A genome wide scan and a multipoint linkage analysis identified aggrecan (ACAN) as a prime candidate gene. DNA sequence analysis of the ACAN-gene revealed heterozygosity for a missense mutation (c.6907G>A) in affected subjects, resulting in a p.V2303M substitution in the aggrecan G3 domain C-type lectin. This domain is important for the interaction with other proteins in the cartilage extracellular matrix. To determine the effect of the V2303M substitution on secretion and interaction, we performed binding studies with recombinant mutated and wild type G3 proteins. We found decreased affinity or complete loss of interaction between V2303M aggrecan and fibulin1, fibulin2 and tenacinR. Analysis of articular cartilage from an affected family member confirmed that V2303M aggrecan is produced and present.

In search for gene mutations associated with multiple epiphyseal dysplasia (MED) we considered the ACAN-gene a likely candidate. The ACAN-gene was analysed in 39 individuals with MED and screened negative for mutations in six previously known MED genes. Sequence analysis revealed a heterozygous missense mutation (c.1448G>T) in one adult male and compound heterozygous missense mutations (c.1366T>C and c.836G>A) in a 5 year old boy with healthy parents, each of them carrier for one of the mutations.

A large family segregating autosomal dominant brachymesophalangia and OCD in finger joints was characterised. The clinical presentation in six affected family members was consistent with the diagnosis Brachydactyly type A1, in this family characterized by shortening of the middle phalanges, short ulnar styloid process, flattening of the metacarpal heads and mild osteoarthritis. The condition may be caused by mutations in the Indian hedgehog gene (IHH) or a yet unidentified gene on chromosome 5p13. Sequence analysis of the IHH-gene in affected individuals revealed a novel C to T transition (c.472C>T) leading to a p.158Arg>Cys substitution. Residue 158 in IHH is highly conserved throughout evolution and molecular structure modelling of IHH suggests that the R158C substitution leads to a conformational change at the site of interaction with the IHH-receptor. This supports that the substitution causes Brachydactyly type A1 in this family.

In summary, we report on the clinical, radiological and molecular genetic characteristics of the three skeletal disorders OCD, MED and BDA1. Our results provide a novel molecular mechanism in the pathophysiology of familial osteochondritis dissecans confirming the importance of aggrecan C-type lectin for cartilage function. We also show that ACAN-gene mutations may be associated with MED extending the spectrum of skeletal dysplasias associated with the aggrecan gene. Finally, we report on a novel missense mutation in a conserved region of the IHH-gene associated with BDA1.

Keywords: Skeletal disorders, Osteochondritis dissecans, ACAN-gene, Multiple epiphyseal dysplasia, Brachydactyly type A1, IHH-gene