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A gene for smooth running joints

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Andrew Cope,[Aff1]

Aff1 Kennedy Institue of Rheumatology, London, UK

Keywords

Arthritis, caclification, chondrocalcinosis, pyrophosphate

Context

Although the genetic susceptibility to diseases such as rheumatoid arthritis (RA) has been well documented, twin studies indicate that the contribution of inherited genes to RA may be as little as 15%. The data indicate that, for OA and related conditions, the genetic contribution is likely to be much higher. Animal models have been useful for dissecting the molecular mechanisms that contribute to arthritis. Kingsley and co-workers have studied the *ank/ank* mouse, an autosomal recessive model of arthritis, characterised by an abnormal flat-footed gait and loss of joint mobility due to deposition of hydroxyapatite crystals in articular surfaces and synovial fluid. This process is accompanied by joint space narrowing, cartilage erosion and osteophyte formation, features resembling OA and ankylosing spondylitis in man. Until now, the genetic defect was not known. To identify the gene product encoded at the *ank* locus on mouse chromosome 15, and to define the mutation in *ank/ank* mice.

Significant findings

The *ank* gene product is a 492 amino acid protein with an expected MW of 54.3 kD, carrying three *N*-linked glycosylation sites and multiple putative phosphorylation sites. There are 9-12 hydrophobic stretches consistent with transmembrane domains of a multipass transmembrane protein. No other consensus sequences or motifs were identified. Northern blotting demonstrated the gene to be expressed in many nonskeletal tissues, but *in situ* hybridisation revealed strong signals in the cartilage of developing limbs in mice. Immunofluorescence detected protein expression at the cell surface, but also in the cytoplasm in 30-50% cells. *Ank/ank* mice carry a single nucleotide G to T substitution which leads to a nonsense mutation in the open reading frame. This leads to truncation of the carboxy-terminal region of the protein with greatly reduced activity. The characteristic metabolic defect in skin fibroblasts from mutant mice is a twofold increase in intracellular PPi, together with a three- to fivefold decrease in extracellular levels. Reconstitution of mutant fibroblasts with wild-type ANK reversed this defect, leading to a decrease in intracellular levels. This could be blocked with probenecid, indicating that ANK functions through a probenecid-sensitive anion transport mechanism.

Comments

This study provides significant insight into the biochemical basis of the arthritic process in *ank/ank* mice. To some extent it might also explain the ectopic calcification, mineral deposition, osteophyte formation and vertebral fusion characteristic of osteoarthritis (OA) and ankylosing spondylitis in man. In the mouse model, which perhaps most closely resembles familial chondrocalcinosis in man, defects in the *ank* gene (which encodes an anion transporter) lead to imbalances of intracellular and extracellular pyrophosphate (PPi) levels (raised levels intacellularly). As PPi is a potent inhibitor of calcification, the model predicts that the presence of mutant ANK protein leads to drastic reductions in the capacity to maintain extracellular tissue in an unmineralised state. Although the study provides an important link between crystal deposition and the development of arthritis, it should be noted that increased, rather than decreased, levels of PPi are commonly found in osteoarthritic joints. Other mechanisms must be playing a role in these patients.

Methods

The following methods were employed to identify and characterise the *ank* gene product: linkage analysis with microsatellite markers; isolation of the relevant interval in bacterial artificial chromosomes (BAC); *in vivo* complementation through the generation of BAC-transgenic mice; shotgun sequencing of BACs; expression of the gene product in COS-7 monkey kidney cells; immunofluorescence; northern blotting of murine tissues; assays for intracellular and extracellular PPi using skin fibroblasts from wild-type and mutant mice (+/- reconstitution); and cloning of the human orthologue using the murine exon/intron map and available human expressed sequence tag clones.

Additional information

Details of the *ank/ank* arthritis model can be found in these articles: Sweet *et al, J Hered* 1981, **72**:87; Hakim *et al, Arthritis Rheum* 1984, **27**:1411-1420 (PubMed abstract); and Hakim *et al, Arthritis Rheum* 1986, **29**:114-123 (PubMed abstract).

References

1. Ho AM, Johnson MD, Kingsley DM: Role of the mouse ank gene in control of tissue calcification and arthritis. Science. 2000, 289: 265-270.