Specific Gene Defects Leading to Osteoarthritis

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A number of genetic abnormalities in tissues that lie external to the joint may contribute to osteoarthritis (OA), e.g., the joint laxity in Ehlers-Danlos syndrome, in which patients exhibit hypermobility of the joints and may also have a problem with their articular cartilage or elastic tissue, leading to secondary OA. Malalignment of joints may occur on a congenital basis, as in some patients with dysplastic hips. In other cases, an abnormality may exist in the composition of the extracellular matrix of the articular cartilage, such as that due to the point mutation we discovered in the cDNA coding for Type II collagen, which resulted in an arginine-cysteine mutation at position 519 (Arg^{519}-Cys) in the molecule[1].

OA may be the result of abnormal forces acting on a normal joint or of normal forces acting on a joint in which the biomaterials, i.e., the articular cartilage or subchondral bone, are abnormal, perhaps on a genetic basis. In addition, certain genetic metabolic disorders (e.g., hemochromatosis, calcium pyrophosphate dihydrate deposition disease) may lead to secondary OA. A relationship exists, furthermore, between genetic and environmental factors, making it difficult at times to determine which is primarily operative.

In 1941, Stecher[2] described the hereditary aspects of nodal OA of the finger joints. If 100 men and 100 women live to be 100 years of age, about 30% of the women and 3% of the men will develop Heberden's nodes. Clearly, this disease exhibits a sex-linked difference and a hereditary pattern. If a patient has nodal OA, it is likely that other family members, particularly the women, will also have nodal OA.

A genetic abnormality may be not only the cause of the disease, i.e., result in a predisposition to OA, but may determine the age at which the disease will appear, the joint sites that will be involved (hips, knees, hands, or generalized OA), the severity, and how rapidly the disease will progress. As reported[3] (Figure 1), OA of the hands (defined by involvement of 3 hand joints) is associated with a high frequency of involvement also of the hip and/or knee.

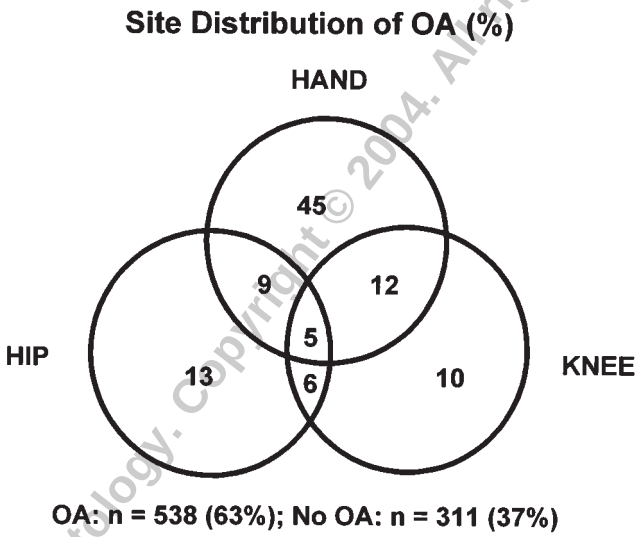

Figure 1. Patients with hand OA (defined as involvement of 3 or more joints) have a high frequency of hip and/or knee OA. From Huquenin, *et al*. Arthritis Rheum 2000;43 Suppl:S136 [abstract].

Therefore, people with hand OA are at risk for developing generalized OA. This is the type of information that is needed to help us elucidate the phenotypes needed to examine OA in genetic studies.

Prominent evidence exists of an increase in the relative risk of OA within families. Stecher[2], for example, showed a 3-fold increase in the prevalence of Heberden's nodes in the sisters, and a 2-fold increase in the mothers, of women with Heberden's nodes. Kellgren, *et al*[4] found a 2-fold increase in the risk of OA in siblings of probands, as did Lindberg[5], who examined hip OA specifically. A similar association was seen by Chitnavis, *et al*[6], who showed a 2-fold increase in the risk of total joint arthroplasty among siblings of patients with OA who had undergone joint replacement surgery. A study of British sibling pairs with nodal OA by Wright, *et al*[7] showed a significant association of the disease with 2 markers, 2q23-32 and 2q33-35. Genes for fibronectin and for interleukin 8 are present in the same vicinity.

On the other hand, the answers are not always as clear as we wish: In a Tasmanian population, Stankovich, *et al*[8] found no significant linkage of chromosome 2q to OA. In an investigation by Warman and Moskowitz of our study group to be published we defined our phenotype as a person who had a total hip or total knee arthroplasty for OA, and examined the prevalence of OA in the siblings. As controls, we used spouses of the proband and siblings of the spouses. We found the relative risk of having a joint replacement was

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significantly increased if a genetically related member of the family had had an arthroplasty. We had previously thought hip OA was much less likely than knee OA to have a hereditary component. It may, in fact, be the other way around. However, hereditary influences on hip OA may relate to dysplasia, a slipped femoral capital epiphysis, or Legg-Perthes disease. One or more subtle hereditary defects, rather than a single primary defect, may underlie primary OA of the hip.

Figure 2 is the radiograph of a patient who was referred to me when he was 39 years old with severe OA of the right hip, moderate OA of the left hip, and widespread arthritis involving also the knees, hands, and shoulders. In collaboration with investigators from Thomas Jefferson University it was demonstrated that the members of his family had a point mutation that resulted in coding for cysteine, rather than arginine, in Type II collagen[1]. This led to replacement of a single base, cytidine, by thymidine, which resulted in diffuse arthritis in 100% of the affected members of the kindred — if you had the mutation, you got the disease (Figure 3). Figure 4 shows the pedigree of this family, in which members of 3 generations were affected.

About a year after we published our description of the family described above, a second family from Bangor, in the northernmost part of the State of Maine, was referred to us who had the same Arg[519]-Cys mutation. Five months later, we identified what appeared to be a third family — from Portland, in the southern part of Maine — with the same mutation. As it turned out, the families from Bangor and Portland were the same family with a common founder. We have since identified a total of 11 families, referred to us from around the world, with the same Arg[519]-Cys mutation and phenotype[9-12].

In an attempt to ascertain whether these families were truly different or had a common ancestry, we identified 2 sets of exons and introns that were fairly well separated from each other and attempted to ascertain whether these were identical in each of the families[12]. If so, this would have suggested that they all came from the same founder. Indeed, there were essentially identical gene sequences in 3 of the families — one from the US, one from New Zealand, and one from Iceland. We were able to trace the Icelandic family back through multiple generations in Iceland, the New Zealand family back to ancestors from Ireland, and the US family to ancestors from Belgium. Interestingly, some 2000 years ago a Celtic migration occurred to Iceland, Ireland, and Belgium. It may well be that the founder in whom the mutation originally occurred was part of that migration story.

In addition to the 519 mutation, Arg-Cys point mutations have also been identified at amino acid loci 789[13] and 75[14–16] in Type II collagen. We are currently studying a large Icelandic kindred with familial OA that does not appear to have any of the mutations that have been identified previously. Using various polymorphic markers we have investigated various candidate genes, e.g., collagen genes, cartilage oligomeric matrix protein, link protein, decorin, but have found no linkages. Our Icelandic family presents considerable difficulty in sophisticated genetic analysis, however, because of the considerable degree of intermarriage.

One of our areas of interest is the downstream defect produced by the mutation. In collaborative studies with Dr. Brian Johnstone in the Department of Orthopaedics at Case Western Reserve University (unpublished data), we obtained mesenchymal stem cells from the bone marrow of humans, grew them in culture, and added various factors to drive them to differentiate into one cell type or another. For example, the addition of dexamethasone and transforming growth factor-ß pushes the stem cells into a chondrocyte line; with other growth factors they may exhibit an osteogenic lineage; with yet other additives, they become adipose cells.

Figure 5 illustrates stem cells that have developed a

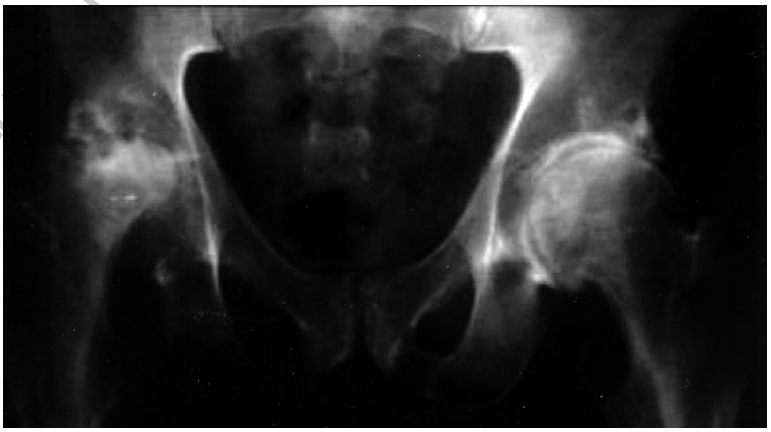

Figure 2. Radiograph of proband in the first family identified with the Arg[519]-Cys mutation in COL2A1, showing bilateral hip OA.

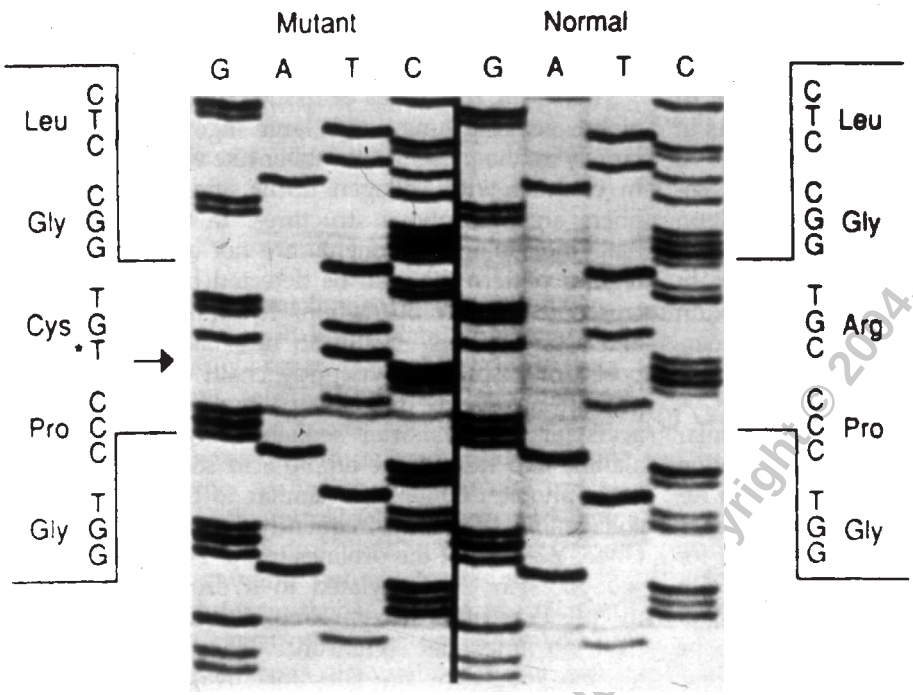

Figure 3. The genetic abnormality in the family of the proband shown in Figure 2 was a point mutation in the CDNA coding for cartilage collagen, resulting in substitution of thymidine for cytidine at position 519, resulting in substitution of arginine[519] by cysteine.

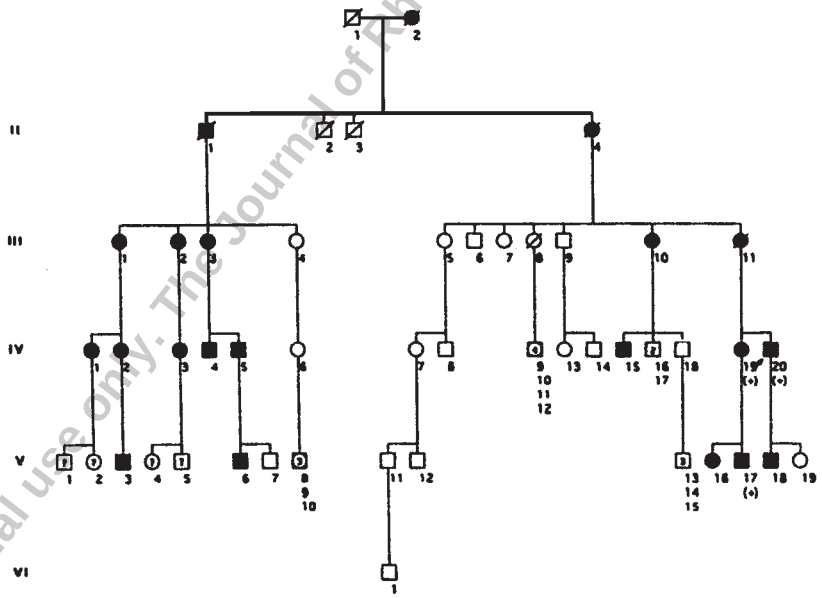

Figure 4. Pedigree of the first family identified with the Arg[519]-Cys mutation, showing transmission of the phenotype through 3 generations.

chondrogenic lineage. The cell pellets contain Type II collagen and proteoglycans. In a similar experiment, when we used mesenchymal stem cells from patients with the 519 mutation, it was apparent that the cells did not grow as floridly (Figure 6), did not form tight pellets, and produced Type I, rather than Type II, collagen, i.e., they did not synthesize a normal chondrogenic material. We are now attempting to see whether this abnormality is specific for the chondrogenic lineage or affects also differentiation into, e.g., osteocytes or adipocytes.

In addition to the Arg[519] mutation, an Arg-Cys mutation has been described at Arg[75] in Type II collagen[14,15]. This

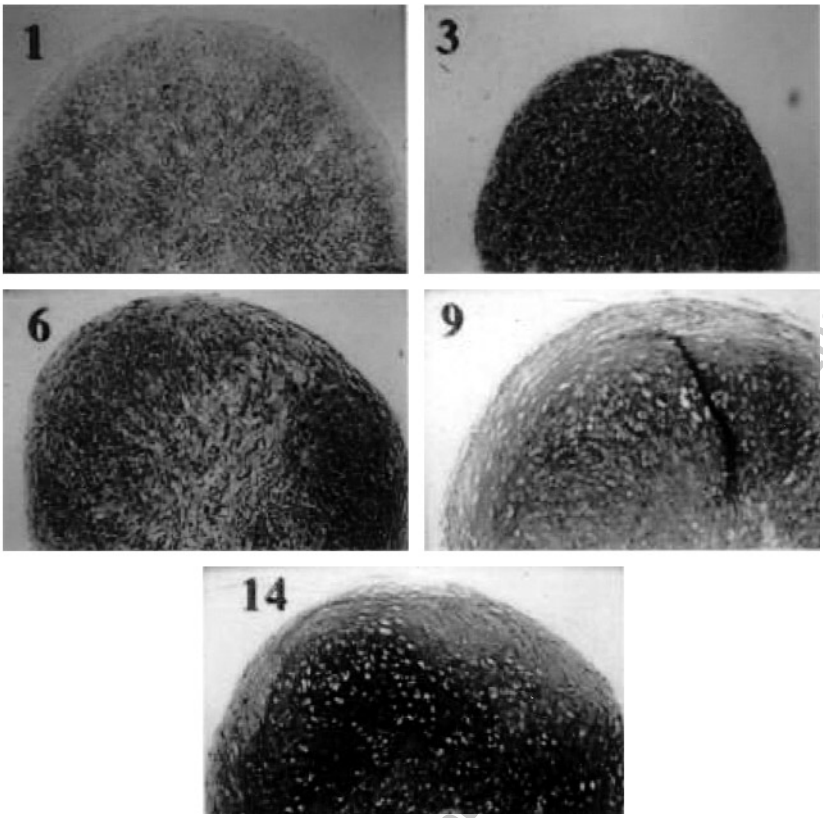

Figure 5. Development of chondrogenic lineage by human mesenchymal stem cells from bone marrow. Numbers indicate days in culture. A prominent extracellular matrix develops, containing Type II collagen and proteoglycans. (With permission, from Johnstone B, Barthel T, Yoo J. In vitro chondrogenesis with mammalian progenitor cells. In: Rosier RN, Evans CH, editors. Molecular biology in orthopaedics. Rosemont, IL: American Acadmy of Orthopaedic Surgeons; 2003:273-88.)

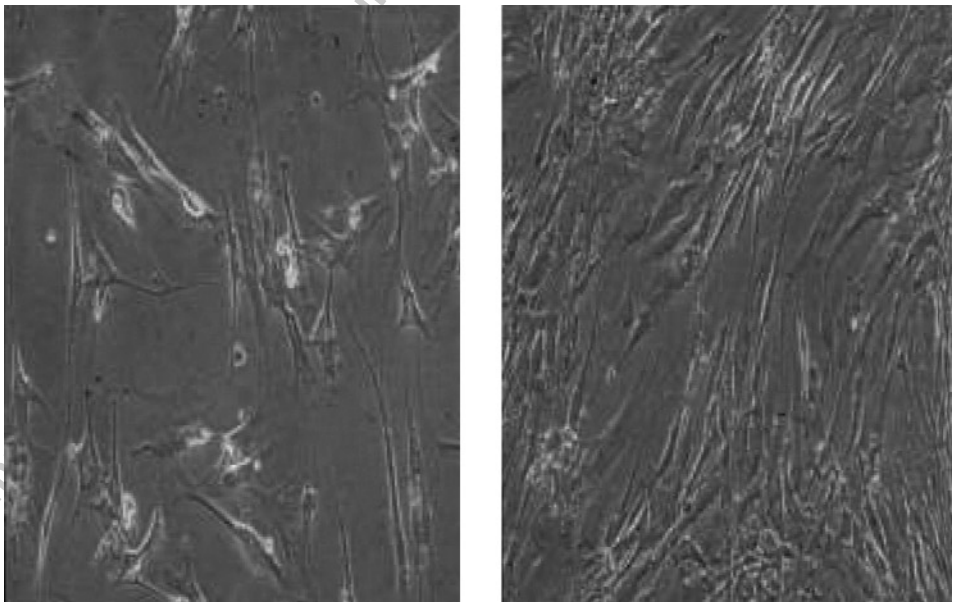

Figure 6. In an experiment similar to that shown in Figure 5, when mesenchymal stem cells from patients with the 519 mutation were cultured, cell growth was much slower than normal, and the cells did not form tight pellets and produced Type I rather than Type II collagen. Both panels show cells from passage 5, after 9 days of culture. Left panel shows cells from a patient with the mutation; right panel shows control cells.

base substitution occurred in exon 11 of COL2A1 and was identified in a Chilean family with precocious generalized OA, mild spondyloepiphyseal dysplasia, and short stature[14]. We have described a family with a similar mutation[15]. The proband was a 42-year-old man with mild epiphyseal and spinal chondrodysplasia and osteochondromatosis who had a daughter, age 20, with the same problems. Both the father and daughter were tall (about 182 cm), differing from the findings in the Chilean family, and they had moderate deafness. In both the father and daughter we found the Arg^{75}-Cys mutation.

A question arises, therefore, whether there are mutational "hot spots." We have 11 families with the Arg^{519}-Cys mutation and 3 have been reported with the Arg^{75}-Cys mutation. An additional 2 families have been described with an Arg^{789}-Cys mutation[13]. Hot spots such as these are not uncommon, e.g., CG dinucleotide hotspots have been described in patients with hemophilia A and B, thalassemia, phenylketonuria, and osteogenesis imperfecta. However, we have studied 35 other families with classical familial premature OA in which to date we have not been able to define the mutation (unpublished data).

All the subjects with familial OA have abnormal cartilage. If they were to spend their days in a sedentary occupation they might never get OA; i.e., in some patients the mutation may be silent. In others (Figure 7), if the mechanical stresses on the cartilage are great enough, OA will likely develop. Aging may represent yet another factor that will result in the eventual expression of OA.

I would like to conclude with another interesting finding: About a year ago, an orthopedic surgeon in Grand Rapids, Michigan, USA, referred a family to us in which the father, a son, and 2 daughters, all of whom were thin and of average height, had symptoms in hips, shoulders, and knees that began when they were teenagers, or even younger. Several had been given a diagnosis of osteochondritis or dysplasia. Radiographic findings included flattening of the femoral heads, coxa valgus, osteocartilaginous loose bodies, and secondary degenerative joint changes. Arthroscopic examination of these patients revealed joint effusions, multiple large loose bodies, and a "bubbling" or delamination of the articular cartilage, with exposure of subchondral bone and chondral flaps.

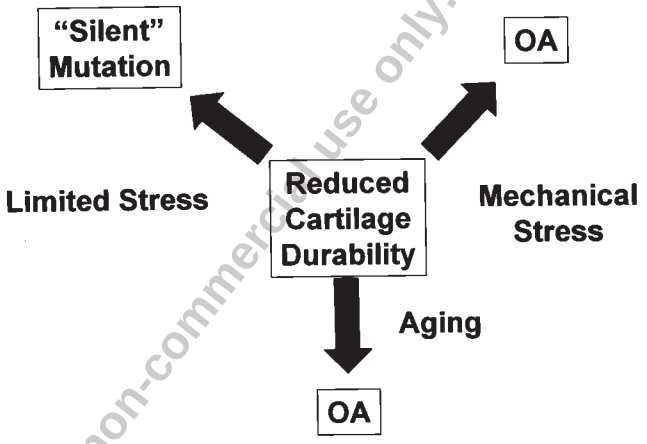

Figure 7. Whether mutations associated with OA lead to the disease may be influenced by the amount of mechanical stress on the relevant tissues within the joint. Aging may also influence the expression of OA in subjects with a mutation.

When we attempted to ascertain the pattern of inheritance and to identify a specific genetic defect, we went through the recognized collagen mutations — e.g., at positions 519, 789, and 75 — and did not find any of those in this family. However, in collaboration with Drs. Dennis Carson and Maripat Corr at the University of California, San Diego, USA, we targeted our study to genes for frizzled proteins, transmembrane receptors for the so-called Wnt proteins, signaling molecules that have been studied extensively in Drosophila[17]. It was suggested that the FRZB gene on chromosome 2q might be involved in connective tissue homeostasis and regulation of embryogenesis.

Gene analysis in this family revealed 2 unique single nucleotide polymorphisms[17]. One — a guanine to adenine transition — was in the 3'-untranslated region; the other — a cytidine to guanine mutation — was in exon 6, leading to a substitution of glycine for arginine in the protein. When we examined the family, we found that the father, son, and both daughters who had the disease exhibited the polymorphism. Both polymorphisms were, however, present also in the unaffected mother and 2 of 3 unaffected siblings of the proband. Because unaffected family members also carried the mutation, the mutation itself appears insufficient to have resulted in the phenotype. An interplay of mutations in a polygenic trait cannot be excluded. This study identified a previously undescribed syndrome that is familial and inherited with possible polygenic etiology. Better understanding of the clinical picture awaits identification of possible additional contributing genes.

Where does this leave us with respect to the genetics of OA? We hope that by learning about gene mutations we will eventually be able to define a therapeutic approach to these diseases, either by manipulating the gene itself or by downstream manipulation to modify the effects of the mutation. In the case of the 519 mutation this will be very difficult. It is more difficult to modify an existing mutation than to replace a missing gene. Perhaps, however, the gene can be inactivated with antisense technology or the response to the gene otherwise blocked. For example, we know that the substitution of arginine by cysteine adds sulfhydryl groups that are not normally present in cartilage collagen. Might blocking these sulfhydryl groups have a beneficial effect?

In summary, we have known for many years that a hereditary component is associated with OA. It is likely we will find polygenic influences in this disease. They may be strong; they may be weak; environmental input is likely to

play a significant role in manifestation of the phenotype. The more we know about these mutations and how they alter tissue biochemistry and physiology the better we will be able to define genetic factors and pathophysiologic mechanisms in "run-of-the-mill" OA. Application of such knowledge may open the door to definitive disease modification and prevention.

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REFERENCES

1. Ala-Kokko L, Baldwin CT, Moskowitz RW, Prockop DJ. Single base mutation in the type II procollagen gene (COL2A1) as a cause of primary osteoarthritis associated with a mild chondrodysplasia. Proc Nat Acad Sci USA 1990;87:6565-8.
2. Stecher R. Heredity in hypertrophic arthritis of the finger joints. Am J Med Sci 1941;201:801.
3. Huquenin T, Jordan JM, Luta G, et al. Hand osteoarthritis as a marker of knee and hip osteoarthritis severity [abstract]. Arthritis Rheum 2000;43 Suppl:S136.
4. Kellgren JH, Lawrence JS, Bier F. Genetic factors in generalized osteoarthritis. Ann Rheum Dis 1963;22:237-55.
5. Lindberg H. Prevalence of primary coxarthrosis in siblings of patients with primary coxarthrosis. Clin Orthop Rel Res 1986;203:273-5.
6. Chitnavis J, Sinsheimer JS, Clipsham K, et al. Genetic influences in end-stage osteoarthritis: sibling risks of hip and knee replacement for idiopathic osteoarthritis. J Bone Joint Surg Br 1997;79:660-4.
7. Wright GD, Hughes AE, Regan M, Doherty M. Association of two loci on chromosome 2q with nodal osteoarthritis. Ann Rheum Dis 1996;55:317-9.
8. Stankovich J, Sale MM, Bahlo M, et al. Investigation of chromosome 2q in osteoarthritis: no significant linkage in a Tasmanian population. Ann Rheum Dis 2002;61:1081-4.
9. Pun YL, Moskowitz RW, Lie S, et al. Clinical correlations of osteoarthritis associated with a single-base mutation (arginine 519 to cysteine) in type II procollagen gene. A newly defined pathogenesis. Arthritis Rheum 1994;37:264-9.
10. Williams CJ, Rock M, Considine E, et al. Three new point mutations in type II procollagen (COL2A1) and identification of a fourth family with the COL2A1 Arg519—>Cys base substitution using conformation sensitive gel electrophoresis. Hum Mol Genet 1995;4:309-12.
11. Holderbaum D, Bleasel JF, Jonsson H, et al. Arg519-Cys-associated haplotype in Icelandic family suggests early origin of this mutation. Matrix Biol 1996;15:178.
12. Bleasel JF, Holderbaum D, Brancolini V, et al. Five families with arginine 519-cysteine mutation in COL2A1: evidence for three distinct founders. Hum Mutat 1998;12:172-6.
13. Chan D, Taylor TKF, Cole WG. Characterization of an arginine 789 to cysteine substitution in α1 (II) collagen chains of a patient with spondyloepiphyseal dysplasia. J Biol Chem 1993;268:15238-45.
14. Williams CJ, Considine EL, Knowlton RG, et al. Spondyloepiphyseal dysplasia and precocious osteoarthritis in a family with an Arg75–Cys mutation in the procollagen type II gene (COL2A1). Hum Genet 1993;92:499-505.
15. Bleasel JF, Bisagni-Faure A, Holderbaum D, et al. Type II associated with spondyloepiphyseal dysplasia, tall stature and precocious osteoarthritis. J Rheumatol 1995;22:255-61.
16. Bleasel JF, Holderbaum D, Mallock V, Haqqi TM, Williams HJ, Moskowitz RW. Hereditary osteoarthritis with mild spondyloepiphyseal dysplasia — are there "hot spots" on COL2A1? J Rheumatol 1996;23:1594-8.
17. Holderbaum D, Ciesielski CJ, Moskowitz RW, Malvitz T, Carson D, Corr MP. An arginine-glycine mutation in a family with a newly described hereditary chondrolysis syndrome is carried by affected and unaffected individuals. To be presented, Orthopaedic Research Society, San Francisco, March 2004.