MOLECULAR GENETIC STUDIES IN ACHONDROPLASIA

Clair A. Francomano, Phan-Lan Le and Reed E. Pyeritz
Division of Medical Pediatrics and Genetics
Johns Hopkins University, School of Medicine
Baltimore, MD (USA)

The clinical phenotype of achondroplasia has been extensively covered by other speakers in this symposium. In summary, the condition is a dominantly inherited form of rhizomelic dwarfism with an incidence estimated between 1/20,000 and 1/50,000 live births (1). The mutation presents as a primary disorder of bone growth; manifestations in other systems, such as the neuroaxis (2), appear to result from bony impingement. Because the achondroplasia gene acts principally at the growth plate, those gene products which are known to be structurally crucial to cartilage have naturally come under investigation in the search for the cause of achondroplasia.

The important structural components of cartilage include collagen type II, a fibrillar collagen (3), and types IX and X, less abundant short chain collagens which are cartilage specific (4). Proteoglycans, chondroitin sulfate and keratin sulfate are also important structural components of the growth plate (5).

Type II collagen is a homotrimer, synthesized from three constituent precursor chains called pro-alpha 1 type II collagen chains. Thus, unlike type I collagen, which is a heterotrimer (6), type II collagen is encoded by a single structural gene, designated COL2A1.

Chondroitin sulfate is the principle proteoglycan of the growth plate. This very large molecule is composed of a hyaluronic acid backbone. Side chains of chondroitin sulfate proteoglycan core protein, with glycosaminoglycan branches, are attached to the hyaluronate backbone by means of a much smaller protein called link protein (7).

The study of the growth plate and gene products acting on it has proven technically difficult for several reasons. Specimens are difficult to obtain, and chondrocytes have proven difficult to grow in culture, as they do not maintain their phenotype but rather dedifferentiate into fibroblast-like cells. These problems have limited studies of the gene products, that is RNA and proteins, synthesized in human cartilage.
For this reason investigation at the level of the DNA is technically much simpler than studies of gene products expressed at the growth plate. Techniques to culture human chondrocytes and to study the gene products themselves are under active development (8,9) but even with currently available technology we are capable of answering certain questions concerning the genes known to be important in the structural integrity of the growth plate.

Of the human genes encoding important structural components of cartilage, only COL2A1 has been cloned (10,11). Chick and rat clones for genes encoding collagen types IX and X, and rat clones for chondroitin sulfate proteoglycan core protein and link protein have been identified. The search for homologous human clones is in progress in several labs.

So far, however, we have had only the gene encoding type II collagen or COL2A1, of the important cartilage structural proteins, to study in the human skeletal dysplasias. For this reason, the remainder of this report will focus on the question of whether mutations at the COL2A1 locus play a role in the etiology of human achondroplasia.

The type II collagen gene is a relatively large one. It is 30-35 kilobases in length and comprised of approximately 50 coding regions, or exons. It has been localized to human chromosome 12, band q14.3 (12).

Three different approaches have been applied to answer the question: do mutations in COL2A1 cause achondroplasia? First, by studying the COL2A1 gene on Southern blots, we have looked for gross alterations (deletions, insertions, or rearrangements) in the gene. These would be manifest by observable changes in the size of the bands hybridizing to single copy fragments from COL2A1 employed as probes. Secondly, we have studied the distribution of alleles for COL2A1, as identified by the presence or absence of polymorphic restriction sites, to see if that distribution is the same in control populations and the population of individuals with achondroplasia. Finally, in families with multiple members with achondroplasia, one may ask whether the achondroplasia phenotype is inherited along with a particular allele; i.e., do the disease and the allele cosegregate in families and can we thereby establish evidence for or against linkage of the disease to COL2A1 (13)?

Individuals with achondroplasia were ascertained through the Medical Genetics Clinic at Johns Hopkins Hospital and with the assistance of the Little People of America, Inc. The diagnosis of achondroplasia was established by physical examination by one of the authors (CAF or REP).

Peripheral blood was obtained from affected persons, their unaffected relatives, and control individuals of Western European ancestry. The blood was collected in EDTA, and DNA isolated by previous published methods (14). DNA was digested with restriction enzymes under standard conditions and Southern blots prepared as previously described (15). Gene probes were prepared from the 5' and 3' ends of COL2A1.

No gross alterations in the type II collagen gene were observed when DNA from these 49 individuals was digested with a panel of restriction enzymes and subjected to Southern blot analysis. The patterns observed when such blots were hybridized to probes from both the 5' and 3' ends of COL2A1 were indistinguishable from those observed in control DNA samples.