Evidence against the structural gene encoding type II collagen (COL2A1) as the mutant locus in achondroplasia

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SUMMARY The structure of the locus encoding the major cartilage collagen gene (COL2A1) was studied in a total of 19 cases of achondroplasia. No gross rearrangements were seen. The segregation of COL2A1 was examined in three affected kindreds using restriction site and length variants as genetic markers. In two kindreds discordant segregation between the achondroplasia and COL2A1 loci was demonstrated. Paternity/maternity was confirmed using a 'minisatellite' core sequence probe which reveals cross hybridising polymorphic loci.

Achondroplasia, the most familiar of the chondrodystrophies, is a well defined clinical entity inherited as a fully penetrant autosomal dominant trait. The incidence at birth is about 1:30 000 and the mutation rate has been estimated at 14 ± 5 per million gametes with a paternal age effect.^{1 2} About 80% of cases are the result of new mutation and the prevalence is stabilised by selective elimination from the population due to a corresponding reduction in biological fitness.³

The characteristics of achondroplasia are rhizomelic dwarfism, bulging cranium, low nasal bridge, pronounced lumbar lordosis with a thoracolumbar gibbus, and caudal narrowing of the lumbar spinal canal. Biopsy of the chondro-osseous junction demonstrates regular and well organised endochondral ossification although this is often accompanied by an overgrowth of ossified periosteum. The relatively normal cellular architecture of the region has led to the suggestion that the basic defect in achondroplasia is a quantitative reduction in cartilage proliferation at the epiphyseal plate.⁴ The hormonal regulators of epiphyseal growth, growth hormone and somatomedin, have been shown to function normally in achondroplasia⁵ and recent attention has focused on disturbances of matrix synthesis.

Type II collagen is the major protein of the cartilage matrix and in man its subunit α chains are

encoded at a single locus (COL2A1) on chromosome 12.⁶ The few biochemical studies that have been reported in achondroplasia have not revealed any abnormality of type II collagen content, extractability, or cyanogen bromide fragmentation pattern.⁷⁻⁹ Mutation of collagen structural genes can affect extracellular matrix synthesis and two reports, from the same laboratory, have associated changes in COL2A1 with achondroplasia.^{10 11} In one patient the disorder was attributed to an insertion into COL2A1. In eight other cases large deletions at the same locus were inferred from gene dosage analysis.

Here we describe structural and segregation analyses to test COL2A1 as the achondroplasia locus.

Patients and methods

We studied 19 Caucasian patients with typical achondroplasia, of whom 15 had no previous family history. All were examined by at least one of the authors and all demonstrated the distinctive achondroplasia phenotype.

Extraction of DNA from peripheral blood, restriction enzyme digestion, electrophoresis, blotting, and hybridisation followed our usual versions of standard procedures. Gross rearrangements, that is, insertions or deletions of greater than about 100 bp, were screened with combinations of the restriction endonucleases *Bam*HI, *Eco*RI, *Hind*III, and *Pvu*II. Segregation analysis used two types of variation found at this locus: (1) restriction site dimorphisms

FIG 1 Restriction site and length variation at COL2A1: DNA from achondroplasia patients and their relatives digested with *Hind*III (tracks 1 to 6) and *Pvu*II (tracks 7 to 13) and probed with cosH col 1. A 14 kb *Hind*III fragment (allele 2) is cleaved at the variable site to give two comigrating 7 kb fragments (allele 1). Track 3 is an H 1-1 homozygote, the others are H 1-2 heterozygotes. A 3.3 kb *Pvu*II fragment (allele 1) is cleaved at the variable site to give two fragments at 1.6 and 1.7 kb (allele 2). Tracks 8, 11, and 12 are P 2-2 homozygotes, track 9 is a P 1-1 homozygote, and tracks 7, 10, and 13 are P 1-2 heterozygotes. Length variation beyond the 3' end of the gene is seen in track 13, an unaffected subject who is homozygous for an allele (L2) (arrowed) which is about 100 to 150 bp shorter than the major allele (L1). The L1 fragment comigrates with a non-variable *Pvu*II fragment. Tracks 7, 9, and 12 are L 1-2 heterozygotes and the others are L 1-1 homozygotes. The identity of these variable length fragments was confirmed by rehybridising the blot with fragment E7 alone.

FIG 2 Location of the *Pvu*II (P) and *Hind*III (H) restriction site dimorphisms and the E7' length polymorphism (L) on the map of COL2A1 (upper line). The coding region is indicated by the thicker line. The *Eco*RI map of the hybridisation probe cosH col 1 is shown on the lower line.

Results

Fig 1 shows the three markers used in this analysis and fig 2 shows their location on the gene map. From this and other autoradiographs we were unable to detect any gross rearrangement of type II collagen gene structure that could not be accounted for by normal variation. The hybridisation probe cosH col 1 spans the 30 kb gene and 7 kb of flanking sequences.^{17 18} Deletion of the entire gene from one chromosome could be discounted in 14 patients because they were heterozygous for at least one marker. The proportion (five of 19) of apparent homozygotes, who could conceivably be hemizygous at this locus, was within normal expectations.

Segregation analysis demonstrated discordant segregation of COL2A1 and the achondroplasia locus in kindreds a and b (fig 3).

revealed by *Hind*III (H) and *Pvu*II (P)^{12 13}; and (2) length polymorphism in a segment of DNA 1 to 2 kb downstream from the 3' end of the gene.^{14 15} Paternity/maternity was tested using a 'minisatellite' core sequence as a hybridisation probe for *Hinf*I digests of DNA.¹⁶

FIG 4 Confirmation of relationships in a kindred using *HinfI* digests probed with minisatellite 15.1.11.4. The autoradiograph shows fragments between 2.5 and 6.0 kb. The unlabelled track is from an unrelated subject.

hybridisation of 'minisatellite' sequences. This will clearly become a routine procedure in any comparable linkage analysis. Although heterogeneity can never be ruled out, if the clinical homogeneity of achondroplasia were a reflection of mutation at a single locus then these findings can be taken to exclude COL2A1 as that locus in most if not all cases. We offer no explanation of the conflicting results reported by Strom *et al.*^{10 11}

The major benefit to be derived from these results is to direct effort away from investigations of type II collagen in the search for the basic defect in achondroplasia.

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