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9. We measured 220 notes sampled from 59 different syllables recorded from 45 individuals in Dutchess County, New York. Notes were digitized at a sampling rate of 25 kHz at 12-bit precision with a PDP-11/23 minicomputer [Engineering Design, *SIGNAL Software Manual* (Engineering Design, Belmont, MA, 1987)]. Measurement methods are described in D. A. Nelson, *Condor* **91**, 120 (1989). A *k*-means cluster analysis [L. Engelman and J. A. Hartigan, in *BMDP Statistical Software Manual*, W. J. Dixon, Ed. (Univ. of California Press, Berkeley, 1985), pp. 464–473] with four variables (maximal and minimal frequencies, note duration, FM rate) was used to identify 70 category 1 notes and 52 category 6 notes.
10. Durations were chosen to represent approximately equal intervals on a log scale ($\bar{x}$ = 0.307, SD = 0.030). All notes were frequency downsweeps taken from natural songs.
11. Notes were stored as files in a digital computer and manipulated using a synthesis program [see Engineering Design in (*9*)]. A constant 13-ms interval was used after notes in first and second positions, with a 34-ms intersyllable interval, which is similar to the species means for three-note syllables. Song durations ranged from 2212 to 2670 ms.
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13. The observer was ignorant of stimulus identity, and the order of presentation was randomized. Field trials were completed between 3 June and 10 July 1988 at three sites in Dutchess County under procedures described in (*12*).
14. We counted the number of 5-s intervals within each playback block in which at least one wing-wave occurred (36 maximum possible). To correct for individual variation, the number of wing-waves in the block prior to song shift was subtracted from the number of wing-waves in the first block after song shift. A Kruskal-Wallis analysis of variance was used to test for heterogeneity in difference scores among groups, followed by pair-wise comparisons at $P < 0.05$ [W. J. Conover, *Practical Nonparametric Statistics* (Wiley, New York, 1980)].
15. The number of blocks to reach the habituation criterion did not differ among groups (grand mean = 4.8 ± 2.29 blocks, Kruskal-Wallis χ^2 = 1.48, $P > 0.60$).
16. We thank D. Kroodsma for loaning tape recordings. The Institute of Ecosystem Studies at the Mary Flagler Cary Arboretum, Millbrook School, and Dutchess County Infirmary gave permission to work on their properties. Supported by grant MH 14561 to P.M., Biomedical Research Support grant BRSG S07 RR07065 to The Rockefeller University, and a fellowship from the Charles H. Revson Foundation to D.A.N.

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Identification of the Molecular Defect in a Family with Spondyloepiphyseal Dysplasia

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Spondyloepiphyseal dysplasias (SED) are a heterogeneous group of inherited disorders characterized by disproportionate short stature and pleiotropic involvement of the skeletal and ocular systems. Evidence has suggested that SED may result from structural defects in type II collagen. To confirm the validity of this hypothesis, the structure of the "candidate" type II collagen gene (COL2A1) has been directly examined in a relatively large SED family. Coarse scanning of the gene by Southern blot hybridization identified an abnormal restriction pattern in one of the affected members of the kindred. Analysis of selected genomic fragments, amplified by the polymerase chain reaction, precisely localized the molecular defect and demonstrated that all affected family members carried the same heterozygous single-exon deletion. As a consequence of the mutation, nearly 90 percent of the assembled type II collagen homotrimers are expected to contain one or more procollagen subunits harboring an interstitial deletion of 36 amino acids in the triple helical domain.

CHONDRODYSPLASIAS, A HIGHLY HETerogeneous group of disorders involving endochondral ossification and displaying abnormal skeletal growth, are believed to result from mutations affecting either the structural integrity of cartilage matrix components or the regulatory pathway of chondrogenesis (*1*). For example, COL2A1 has been linked to the Stickler syndrome by genetic analysis (*2*). However, an understanding of the pathogenesis of chondrodysplasias has been greatly hampered by the difficulty in obtaining sufficient amounts of biological material (cartilage) as well as by the inability to maintain differentiated human chondrocytes in culture. Despite these obstacles, biochemical analysis of small cartilage samples from chondrodysplastic individuals has recently suggested that some of these conditions, such as the spondyloepiphyseal dysplasias, Kniest dysplasia, and type II achondrogenesis-hypochondrogenesis, may be associated with type II collagen defects (*3*). Type II collagen, extracted from cartilage of patients affected by sporadic forms of SED, exhibits abnormalities in SDS–polyacrylamide gel electrophoretic (SDS-PAGE) mobility when compared to normal controls. When cyanogen bromide (CNBr)–cleaved type II collagen molecules from affected cartilage were further examined on SDS-PAGE, decreased electrophoretic mobility of some but not all CNBr peptides suggested an increased degree of hydroxylation and glycosylation progressively NH_2-terminal to the putative site of the mutation. Such a pattern is reminiscent of the metabolic consequences of type I collagen mutations in fibroblasts from osteogenesis imperfecta pa-

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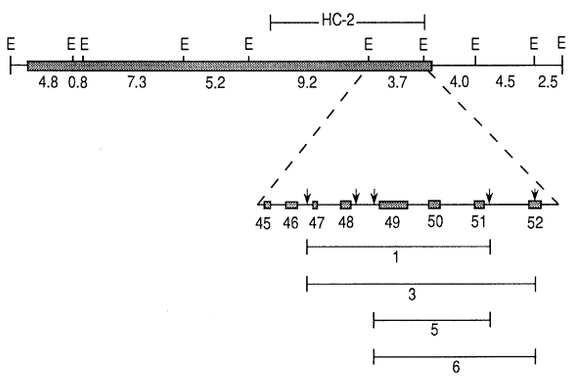

Fig. 1. Eco RI (E) restriction map of COL2A1. Relative positions of exons 45–52, the region spanned by the cDNA clone HC-2, and the oligonucleotides (arrows) used for PCR amplification and sequencing are shown. Below are the respective fragments produced in PCR analysis of the 3.7-kb Eco RI fragment shown in Fig. 2B. Primer sequences, direction of priming (in relation to the coding strand), and positions [referred to the first nucleotide's number in (*10*)] are as follows: intron 46, nucleotide 1401, forward direction, GAATATAGATAGATATGTCTGTGCTGACCG; intron 48, nucleotide 2127, reverse direction, AAGAGCTCAAGCCTCC; intron 48, nucleotide 2386, forward direction, ACAATCCTGGCTGATCTCT; intron 51, nucleotide 3984, reverse direction, AGGCAGTTTGGGCACAGGCAGC; and exon 52, nucleotide 4654, reverse direction, TTGCAACGGATTGTGTTGTTTCTG.

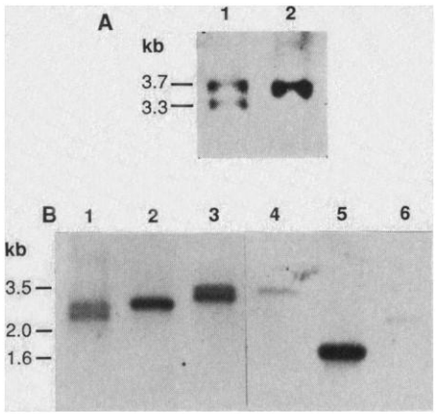

Fig. 2. COL2A1 analysis by Southern blot hybridizations of genomic and PCR-amplified DNA. (**A**) genomic blot of proband (lane 1) and control (lane 2) DNA digested with Eco RI and hybridized to the COL2A1 3.7-kb Eco RI fragment. (**B**) Southern blot of the amplified products from proband and control genomic DNA hybridized with the 3.7-kb probe. In lanes 1, 3, 5, and 6 are the hybridized PCR fragments containing exons 47–51 (2.6 kb), 47–52 (3.2 kb), 49–51 (1.6 kb), and 49–52 (2.3 kb), respectively, from proband DNA. In lanes 2 and 4 are the hybridized PCR fragments containing exons 47–51 and 47–52, respectively, from a control sample. Southern blot hybridization analyses were carried out as in (*8*). Genomic DNA (1 μg) was amplified for 40 cycles with 2.5 U of Taq polymerase (Perkin-Elmer Cetus) and 5 pmol of each primer in a total volume of 100 μl (*12*). Denaturation was for 1.5 min at 94°C. Annealing was at 55°C for 2.5 min. Extension was at 72°C for 4.5 min. The last extension was for 7 min. One-tenth of the product was electrophoresed on a 0.8% agarose gel at 10 V/cm and transferred onto nitrocellulose filters as described (*8*). Oligonucleotides were produced with the model 8600 Biosearch DNA synthesizer.

tients (*4*) and type III collagen mutations in Ehlers-Danlos syndrome type IV fibroblasts (*5*). In these disorders, structural mutations in the type I and type III collagen subunits are believed to decrease the rate of helical assembly and expose greater regions of unassembled chains to overmodification; the relative location of a defect within the helical domain is believed to directly affect the overall degree of collagen modification (*6*).

We have examined the structure of the "candidate" gene, COL2A1 (*7*), in a relatively large SED family in which evidence of biochemical defects was not yet available. The proband was a 3½-year-old girl, apparently normal at birth, who had a history of ear infections, slowed growth, and genu valgum. Family history revealed the clinical manifestations of SED (kyphoscoliosis, retinal detachment, myopia, genu valgum, cervical instability, and dwarfism) in the father, four paternal aunts, and two nieces. Physical examination of the proband revealed short stature, lordosis, mild kyphosis, and rhizomelic shortening of extremities. Radiologic examination showed hypoplastic epiphyseal

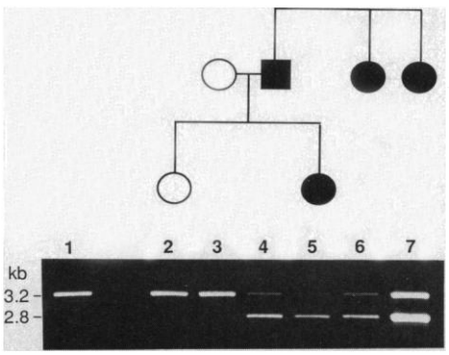

Fig. 3. Partial pedigree of the SED family and PCR analysis of some of its members. DNA fragments containing exons 47–52 of COL2A1 were amplified from genomic DNA of family members. Lane 1, normal, unrelated individual; lane 2, unaffected sister; lane 3, unaffected mother; lane 4, affected father; lane 5, affected proband; lane 6, affected paternal aunt; and lane 7, affected paternal aunt.

centers with no metaphyseal involvement, ovoid thoracic vertebrae, and odontoid hypoplasia. Her father was dwarfed and severely deformed with kyphoscoliosis and enlarged joints.

Initial Southern blot hybridization (*8*) of the proband's DNA with a cDNA spanning from exon 22 to exon 52 of COL2A1 [exons are numbered in the 5′ to 3′ direction (*9*)] revealed the presence of a novel 3.3-kb Eco RI fragment not seen in control samples (*10*). Hybridizations with genomic subclones of COL2A1 identified the 3.3-kb band as an allelic deletion of the normal 3.7-kb Eco RI fragment (Figs. 1 and 2A). This segment of COL2A1 contains exons 45–52, which code for the COOH-terminal propeptide and the last 123 amino acid residues of the triple helical domain (Fig. 1) (*11*). Further Southern blot hybridization analysis of the 5′ portion of COL2A1 failed to detect any additional rearrangement (*10*).

To characterize and more finely map the deletion, four overlapping DNA fragments containing exons 49–52, 49–51, 47–52, and 47–51, respectively, were amplified from the proband's DNA by the polymerase chain reaction (PCR) (*12*) and hybridized to the normal 3.7-kb Eco RI subclone (Fig. 2B). Only two of the PCR products, those containing exons 47–52 and 47–51, hybridized with a pattern displaying a doublet, thus localizing the deletion around exons 47 and 48. Extensive restriction mapping of Bst EII–, Hinf I–, and Sac I–digested PCR products containing exons 47–52 suggested the presence of a heterozygous deletion of approximately 400 bp in the region surrounding exon 48. To correlate the mutation with the SED phenotype, DNA from affected and unaffected family members was similarly amplified and analyzed. Amplification of COL2A1 exons 47–52 from genomic DNA of unaffected family members produced a single 3.2-kb fragment, whereas analogous amplifications of DNA from affected family members produced the normal 3.2-kb fragment and a deleted 2.8-kb fragment. This finding established the segregation of the deleted COL2A1 allele with the abnormal SED phenotype (Fig. 3).

The breakpoints of the deletion were determined in the DNA of the proband and the affected father. The 2.8-kb PCR fragment containing exons 47–52 was kinased and subcloned into the Sma I restriction site of the pUC18 plasmid. Sequencing of the 5′ end of the insert revealed that both individuals exhibited a 390-bp deletion that spans

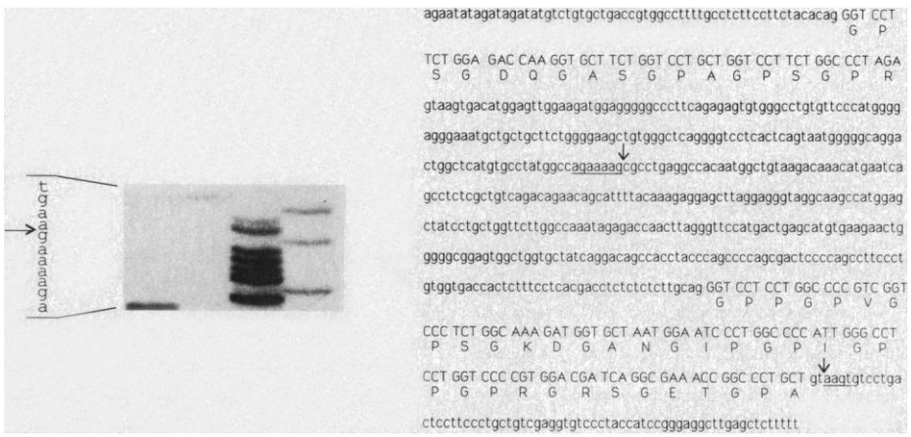

Fig. 4. Determination of the deletion breakpoints. On the left is the autoradiogram of the sequencing gel from proband DNA; on the right is the nucleotide sequence of the normal type II collagen gene (*10*). Arrows indicate the breakpoint of the deletion; nucleotides shown in the autoradiogram are underlined on the right. Lowercase letters signify intervening sequences; uppercase letters indicate coding sequences, with the single-letter amino acid translation shown underneath (*14*). Both strands were sequenced by the dideoxy chain termination method (*15*) with the use of double-stranded DNA as a template (*16*).

from the middle of intron 47 to the 5′ splice site of intron 48 (Fig. 4). The deletion eliminates the whole of exon 48 and, consequently, 36 amino acids (residues 964 to 999) of the type II triple helical domain. We therefore conclude that, based on the identity of the affected gene product, the nature of the mutation, and its mendelian segregation with the SED phenotype, the COL2A1 deletion is the genetic lesion responsible for this particular case of dwarfism.

No biochemical data on the cartilage of this family are available; therefore, we can speculate about the metabolic consequences of the deletion only by analogy to similar mutations in the homotrimeric type III collagen (*13*). The loss of exon 48 does not alter the reading frame of mRNA or the ability of the shortened chains to participate in trimer assembly. As a result of random assortment, equal proportions (one-eighth) of the type II procollagen molecules will be homotrimers composed of either three normal length or three shortened collagen chains. Both of these molecules are expected to have normal stability and to be efficiently secreted (*13*). The remaining proportion of procollagen trimers (three-fourths) are likely to be overmodified, unstable, and excluded from secretion since they will consist of both normal and shortened chains (*13*).

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10. Proband and normal control genomic DNA (10 μg) were each digested with Eco RI and Hind III and electrophoresed overnight on a 0.8% agarose gel. Southern blot hybridization was carried out by using the cDNA HC-2 and the 4.8-kb, 7.2-kb, and 5.2-kb Eco RI subclones of COL2A1 as probes. A novel 3.3-kb Eco RI fragment was noted only in the proband DNA in the hybridization with HC-2. No differences in the pattern of hybridization between normal and proband DNA were observed with the genomic probes.
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A Pertussis Toxin–Sensitive G Protein in Hippocampal Long-Term Potentiation

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High-frequency (tetanic) stimulation of presynaptic nerve tracts in the hippocampal region of the brain can lead to long-term synaptic potentiation (LTP). Pertussis toxin prevented the development of tetanus-induced LTP in the stratum radiatum–CA1 synaptic system of rat hippocampal slices, indicating that a guanosine triphosphate–binding protein (G protein) may be required for the initiation of LTP. This G protein may be located at a site distinct from the postsynaptic neuron (that is, in presynaptic terminals or glial cells) since maximal activation of CA1 neuronal G proteins by intracellular injection of guanosine-5′-O-(3-thiotriphosphate), a nonhydrolyzable analog of guanosine 5′-triphosphate, did not occlude LTP.

LONG-TERM POTENTIATION (LTP) IS a simple form of synaptic plasticity which may be an underlying event in learning and memory (*1*). Protein phosphorylation mediated by protein kinase C (PKC) has been implicated in the induction and maintenance of LTP (*2–4*), but the steps leading to PKC activation during and after the induction of LTP are unclear. Activation of PKC can be regulated by neurohumoral receptors that activate certain types of G proteins. These G proteins regulate activity of the enzyme phospholipase C (PLC), and in this way control the production of diacylglycerol. Diacylglycerol promotes the translocation of PKC from a soluble form to a more active membrane-bound form (*5*). Tetanic stimulation of presynaptic nerve tracts may lead to activation of G protein–linked receptors and PLC, giving rise to an increase in diacylglycerol production and subsequent translocation and activation of PKC.

Pertussis toxin (PT) is the causative agent in pertussis vaccine encephalopathy. This syndrome is associated with major disturbances in learning and neuronal development (*6*). Certain G proteins, including some that couple neurohumoral receptors to PLC activation (*7*), become functionally uncoupled from receptors after treatment with PT. We now report the effects of PT on LTP. We studied the stratum radiatum–CA1 synaptic system of transversely sectioned rat hippocampal slices maintained in an in vitro tissue slice chamber (*8*). The PT was stereotaxically injected at two sites directly over the right hippocampus 2 to 4 days before the preparation of brain slices (*8*). Sham controls were injected with the vehicle alone. In some experiments brain slices from the contralateral hippocampus were used as controls. As there were no significant differences between results obtained with the two groups of control slices, the control data were pooled.

Tetanus-induced LTP of the stratum radiatum–CA1 population spike was absent in slices obtained from rats injected intracerebrally with PT (3 to 4 μg) 3 to 4 days beforehand (Fig. 1 and Table 1). However, LTP could be induced in slices taken 2 days after PT injection (Table 1). This observation is consistent with the suggestion that the activity of PT is dependent on a relatively slow uptake process, which is followed by intracellular release of the active A promoter (*9*). Thus, the minimum time required for PT to exert its effects appears to be 2 days.

The PT-induced uncoupling of G proteins from their receptors abolishes hippocampal neuronal responses to baclofen (*10*), an agonist of the B subtype of the γ-aminobutyric acid ($GABA_B$) receptor. By monitoring the effect of baclofen in controls and slices obtained from PT-treated rats, we ascertained whether PT was indeed uncoupling G proteins and receptors. Baclofen (10

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