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Genetics

# Diastrophic dysplasia gene maps to the distal long arm of chromosome 5

(osteochondrodysplasia/linkage analysis/restriction fragment length polymorphism)

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ABSTRACT **We have used polymorphic DNA markers to map the gene for a clinically well-characterized form of osteochondrodysplasia, diastrophic dysplasia (DD), an autosomal recessive disorder of unknown pathogenesis. Linkage was analyzed in 13 families with two or three affected sibs comprising a total of 84 individuals. Positive two-point logarithm-of-odds (lod) scores were obtained between the *DD* locus and three polymorphic markers on chromosome 5. The highest pairwise lod score estimate of 7.37 with zero recombination to locus *D5S72* suggests very tight linkage. There was no evidence of heterogeneity. Multipoint linkage analysis against the published order of the three loci gave the result centromere–*D5S84*–(*DD*, *D5S72*)–*D5S61*–terminus with a four-point lod score of 9.11. The present findings place the *DD* locus distal to the gene for adenomatous polyposis coli on the distal part of the long arm of chromosome 5. Our results provide a basis for refining the map position of the *DD* locus followed by physical localization, isolation, and characterization of the gene.**

The osteochondrodysplasias are constitutional disorders of the skeletal system that result in disturbed growth and/or density of bone. They frequently cause severe physical handicap including short stature, deformities of the spine, precocious osteoarthrosis, and a variety of other symptoms. Several forms are lethal. More than 80 distinct osteochondrodysplasias have been described and classified (1). Although most of the forms are rare, collectively they constitute a substantial group of human disorders. With a few exceptions discussed below, the underlying biochemical defects are unknown and so the diagnosis is based on clinical and radiographic findings (2, 3).

We have chosen diastrophic dysplasia (DD) as a model for an etiopathogenetic study of the osteochondrodysplasias. DD (McKusick no. 22260; ref. 4) was first described in 1960 by Lamy and Maroteaux (5). It is a well-defined entity among the osteochondrodysplasias. The clinical findings include short-limbed short stature, kyphoscoliosis, generalized dysplasia of the joints, peculiar flexion limitation of the finger joints, hitchhiker thumbs, metatarsus adductus deformity of the feet, and often deformation of the ear lobes and cleft palate. The joint changes are progressive in nature; painful osteoarthroses and contractures frequently develop at an early age. Patients are severely physically handicapped and need repeated corrective orthopedic surgery. Intelligence and sexual development are normal, but psychosocial problems often occur. There is increased mortality in infancy due to respiratory difficulties and spine anomalies, but thereafter the life expectancy is not clearly shortened (6, 7). The severity of the disease varies widely even among siblings (8). The disease is inherited as an autosomal recessive trait. It appears to occur with low frequency in most populations but is highly prevalent in Finland.

As there are no firm clues regarding the pathogenesis of DD, one way of approaching the disease is to clone and characterize the gene (*DD*) that causes it. A first step is to determine its chromosomal location. We have performed a systematic search for linkage by using polymorphic DNA markers. In this paper, we present linkage results that localize *DD* to the distal part of the long arm of chromosome 5.

Table 1. Probes used in this study

| Locus | Probe | Enzyme | Alleles observed, kilobases | Allele designation used in Fig. 1 | Ref(s). |
|---|---|---|---|---|---|
| *D5S84* | pMC5.61 | *Msp* I | 5.5 | 1 | 12 |
| | | | 5.0 | 2 | |
| *D5S72* | CRI-P148 | *Taq* I | 12.3 | 1 | 11, 13 |
| | | | 11.5 | 2 | |
| | | | 9.5 | 3 | |
| *D5S61* | CRI-L45 | *Msp* I | 7.4 | 1 | 11, 13 |
| | | | 4.0, 3.0 | 2 | |
| | | | 3.8, 3.2 | 3 | |
| | | *Taq* I | 12.9 | 1 | 11, 13 |
| | | | 8.1, 4.8 | 2 | |

## MATERIALS AND METHODS

**Patients.** The patients were examined and diagnosed by one of us (I.K.) at the Helsinki University Central Hospital or the Orthopedic Hospital of the Invalid Foundation (Helsinki). The diagnosis was based on clinical features, radiographic findings, and genetic evaluation that fulfill the diagnostic criteria for the disease (5–7, 9). The 13 families chosen for this study were the ones with two or more affected siblings who were willing to participate. The total number of individuals studied was 84, including 29 affected patients.

**Samples.** From each individual 20–40 ml of venous blood was collected. A lymphoblastoid cell line was established from part of the sample and stored in liquid nitrogen as a permanent source of DNA (10). Part of the sample was used as such for DNA extraction. High molecular weight DNA was extracted from leukocytes according to standard procedures.

**Probes.** In our screening for linkage we have mainly used probes from the chromosomal mapping panels developed at Collaborative Research (11). The probes used in this study are described in Table 1.

**Southern Hybridization.** DNA samples were digested with restriction enzymes under conditions recommended by the suppliers (Promega and New England Biolabs). The digests

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Abbreviations: DD, diastrophic dysplasia; cM, centimorgan(s); lod, logarithm of odds.

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Table 2. Pairwise lod scores for *DD*

| Locus | Probe | lod score 0.00 | 0.01 | 0.05 | 0.10 | 0.15 | 0.20 | 0.25 | 0.30 | 0.35 | $Z_{max}$ | $\theta_{max}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *D5S84* | pMC5.61 | $-\infty$ | −3.94 | −0.84 | 0.15 | 0.52 | 0.62 | 0.58 | 0.47 | 0.31 | 0.62 | 0.20 (0.07–0.43) |
| *D5S72* | CRI-P148 | 7.37 | 7.19 | 6.46 | 5.52 | 4.59 | 3.66 | 2.75 | 1.90 | 1.14 | 7.37 | 0.00 (0.00–0.05) |
| *D5S61* | CRI-L45 | $-\infty$ | 1.80 | 3.80 | 3.98 | 3.65 | 3.11 | 2.45 | 1.76 | 1.10 | 4.01 | 0.09 (0.02–0.19) |

(5 μg of genomic DNA per lane) were electrophoresed in 0.8% agarose gels and transferred to nylon membranes (Zeta-Probe, Bio-Rad). The membranes were hybridized at 42°C with probes [radiolabeled by random priming (14)] in 0.75 M

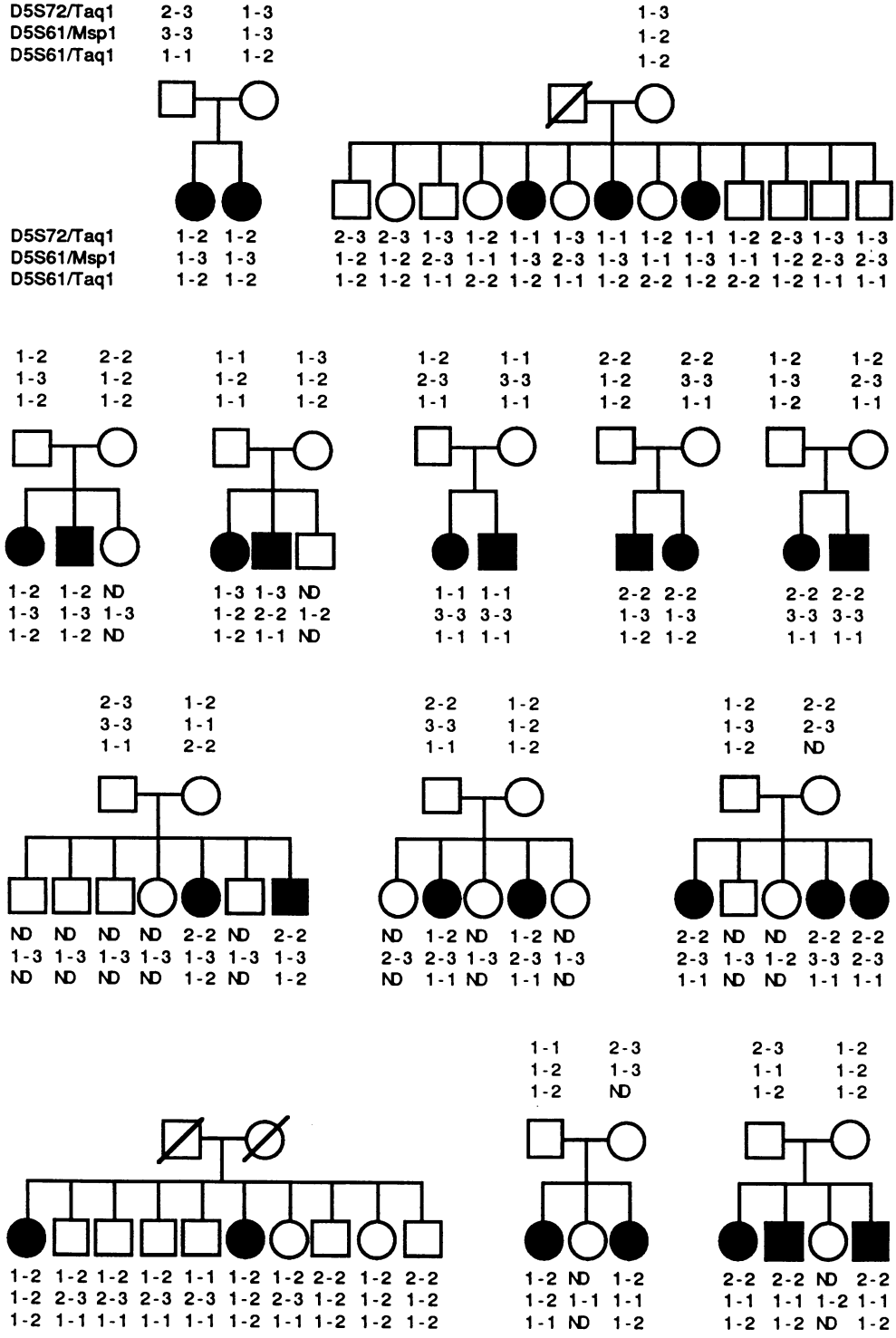


FIG. 1. Pedigrees of the families. Open symbols, unaffected; filled symbols, affected with DD. Above or below the symbols of the individuals studied are the alleles for the *Taq* I polymorphism at locus *D5S72*, the *Msp* I polymorphism at locus *D5S61*, and the *Taq* I polymorphism at locus *D5S61*, in that order. Alleles are designated as indicated in Table 1. ND, not determined.

NaCl/0.075 M sodium citrate, pH 7/20 mM sodium phosphate, pH 6.5/0.04% bovine serum albumin/0.04% Ficoll/0.04% polyvinylpyrrolidone/10% (wt/vol) dextran sulfate/50% (vol/vol) formamide containing sonicated herring sperm DNA at 0.1 mg/ml. The membranes were washed at appropriate stringencies and autoradiographed with intensifying screens for 1–7 days.

**Linkage Analysis.** The linkage analyses were performed using the computer programs MLINK and LINKMAP of the LINKAGE package (15). The confidence limits were calculated with power transformation using the program VACO3 written by Ott (16).

## RESULTS

In the absence of obvious candidate genes for DD we started a blind search in the 13 families by using markers spanning the autosomal genome (11, 17). After ≈60% of the genome had been excluded, linkage was detected with probe CRI-L45 (locus *D5S61*). The alleles for two of the markers, *D5S72* and *D5S61*, detected in the DD families are shown in Fig. 1. Pairwise logarithm-of-odds (lod) scores between *DD* and three markers on chromosome 5 are shown in Table 2. No recombination was observed between DD and *D5S72*, with a lod score of 7.37 suggesting very close linkage. All the families except 1 were informative for this marker; in 7 families both parents were informative (Fig. 1). With the remaining two loci recombination was observed, the maximum lod score between *DD* and locus *D5S61* being 4.01 at a recombination fraction of 0.09 and between *DD* and *D5S84* being 0.62 at a recombination fraction of 0.20.

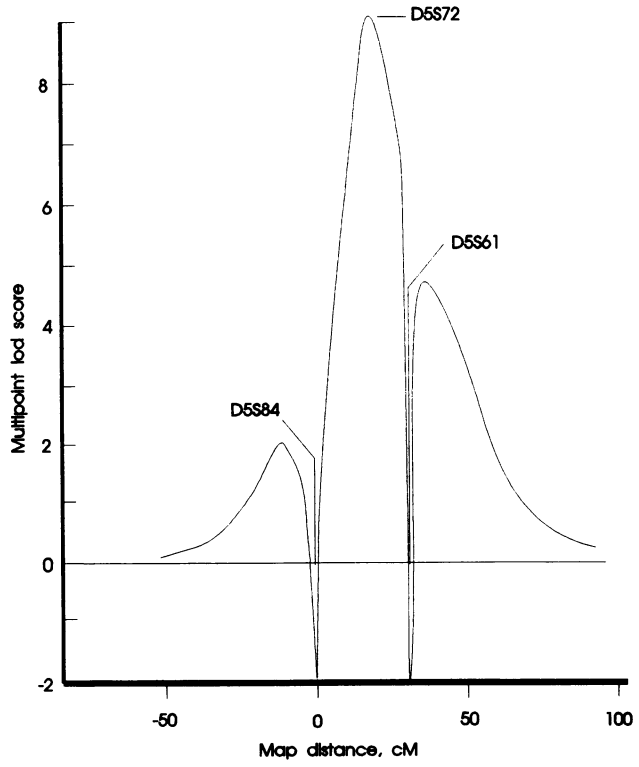


FIG. 2. Multipoint linkage analysis of the *DD* disease locus with three DNA marker loci spanning about 30 cM in the male map. A four-point linkage analysis was performed using the LINKMAP program of the LINKAGE package with a constant sex difference option (15). Multipoint lod scores were calculated as $\log_{10}[L(x)/L(\infty)]$, where $L(x)$ is the multipoint likelihood for the location of the disease locus on a fixed map of marker loci and $L(\infty)$ is the likelihood that the disease locus is unlinked to the group of marker loci (see text). The maximum multipoint lod score estimates for the peaks shown were 2.11 at −10 cM, 9.11 at 19 cM, and 4.75 at 35 cM, respectively.

Four-point linkage analysis was carried out using the available published information on marker order and distances (17). As the distance between the most widely spaced markers in the interval studied by us measures 28 centimorgans (cM) in males and 60 cM in females, we used the distance ratio value of 2 in our calculations. The results of the multipoint linkage analysis are shown in Fig. 2. *DD* maps with locus *D5S72*, giving a maximum lod score of 9.11. The odds for this location as compared with the second best (between *D5S72* and *D5S61*) were of the order of $2 \times 10^4$ to 1. Our linkage data are in concordance with the published locus maps of the region (17, 18), allowing us to tentatively place *DD* at least 20 cM distal to locus *D5S84*. This locus has been extensively studied due to its proximity to the locus for adenomatous polyposis coli (*APC*). Available data (12) indicate that *D5S84* maps some 4 cM distal to *APC*, which is believed to reside in 5q21–22 (19), that is, in the distal half of the long arm of chromosome 5. Thus we conclude that *DD* is distal to 5q21–22.

## DISCUSSION

The gene mutations responsible for the numerous osteochondrodysplasias are mostly unidentified. Collagens are major structural proteins of bone and cartilage. Consequently the collagen genes have been considered to be prime candidate sites for the mutations in several osteochondrodysplasias. Indeed, mutations in the *COL1A1* and *COL1A2* genes of type I collagen have been found in osteogenesis imperfecta (20). Mutations in the *COL2A1* gene of type II collagen occur in some forms of spondyloepiphyseal dysplasia (21, 22). Genetic linkage has been established between *COL2A1* and Stickler syndrome in some families (23). All these osteochondrodysplasias are inherited as autosomal dominants. In DD attention has also been focused on type II collagen. Abnormalities of collagen II have been observed, e.g., by electron microscopy (2) and by peptide mapping (24); however, these changes seem not to be due to a defect in *COL2A1*. Southern blot analysis using gene probes from *COL2A1* failed to show any gross rearrangements (25, 26), and segregation analysis using *COL2A1* probes and flanking markers did not suggest linkage (26). In recessive disorders like DD, abnormalities in genes coding for structural proteins may be less likely than mutations in genes encoding proteins affecting connective-tissue metabolism. For instance, it has been suggested that an enzymatic deficiency in chondrocyte mucopolysaccharide and glucose metabolism might cause the disease (27). However, confirmative evidence has not been reported.

The region in which we have mapped the *DD* locus is known to contain a number of genes encoding proteins with a possible pathogenetic significance for the disease, including genes encoding growth factors, growth factor receptors, and hormone receptors (18). On distal 5q there also resides a gene for osteonectin, a major noncollagenous protein in bone (18). These and other genes in the region should now be probed for a pathogenetic role in DD. The map around the *DD* locus should be refined by further linkage studies using already existing polymorphic DNA probes (28) that map to the region. Moreover, physical mapping and sequencing will probably be needed in order to find, clone, and characterize the *DD* gene. Animal homologies should be sought to serve as model systems (29).

DD is a rare condition. Its occurrence and frequency in different populations are difficult to determine because diagnostic accuracy varies. Moreover, before DD was first described in 1960 (5) and characterized clinically in 1972 (6), DD patients were reported under various other designations. As suggested by McKusick (4), cases may have been placed in the "wastebasket of arthrogryposis multiplex congenita." Moreover, according to Rimoin (9) many DD patients have

simply been thought to represent achondroplasia. For these reasons DD may still be underdiagnosed in many populations. In contrast, Finland provides a situation in which the ascertainment of rare disorders is as complete as generally possible (30). Since the first description of DD in Finland (31), one of us (I.K.) has studied it extensively. Cases have been ascertained nationwide through collaboration with geneticists, pediatricians, orthopedic surgeons, and other members of the medical community. In 1980 at least 40 patients had been discovered (7). Presently 160 patients affected with DD are known in Finland (I.K., unpublished data).

As far as we know, descriptions of less than 300 patients with DD have been published in the rest of the world; thus the frequency of the *DD* gene is likely to be considerably higher in Finland than in other populations. That the maximum lod score of 7.37 occurred at a recombination fraction of 0.00 strongly suggests that *DD* is genetically homogeneous in Finland. It remains to be determined if DD families from elsewhere show evidence of mapping to the same region, which would suggest genetic homogeneity worldwide. When even closer markers than those described in this paper are identified, homozygosity and disequilibrium mapping (32) could be used to refine the map in the immediate vicinity of *DD*.

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