Identification of a locus for a form of spondyloepiphyseal dysplasia on chromosome 15q26.1: exclusion of aggrecan as a candidate gene

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We have investigated a family with an autosomal dominant form of spondyloepiphyseal dysplasia (SED) characterised by short stature and severe premature degenerative arthropathy. Previous studies have excluded linkage between this condition and the locus for the II collagen gene. Here we report the identification of linkage between this disorder and a locus on the long arm of chromosome 15 between markers D15S979 and D15S1004. According to current linkage maps and sequence data, this locus includes that of the aggrecan gene (AGC1). Our linkage data from the SED family show, however, that AGC1 maps to a locus that is proximal to D15S979. This proximal location for AGC1 is further supported by linkage data from a second family with an autosomal recessive form of multiple epiphyseal dysplasia that also maps to the SED locus. In both families AGC1 is therefore excluded as a candidate gene.

The spondyloepiphyseal dysplasias (SED) are a heterogeneous group of conditions with predominant involvement of the vertebral bodies and the proximal epiphyses of the long bones. SED is characterised by short stature, flattening of the vertebral bodies, a barrel shaped chest, kyphosis, and lumbar lordosis. Cleft palate, myopia, retinal detachment, hearing loss, club foot, and pectus carinatum are also variable manifestations. In SED congenita (MIM 183900) dwarfism is pronounced, whereas SED tarda (MIM 313400 and MIM 184100) is characterised by mild stunting of stature with truncal shortening. Autosomal dominant, autosomal recessive, and X linked forms of inheritance have been reported. In most instances, the autosomal forms of SED are caused by mutations in the gene encoding type II collagen at Xp22.2-p22.1. Preliminary studies to locate the gene responsible for SED-K led to the exclusion of linkage to the COL2A1 locus. We therefore performed a genome wide scan to map the disease gene. We report here that SED-K maps to a locus on chromosome 15q26.1 that excludes the aggrecan gene. This locus is similar to that which has been previously described for an autosomal recessive (AR) syndrome of macrocephaly, multiple epiphyseal dysplasia (MED), and distinctive facies.

PATIENTS AND METHODS

The clinical and radiographic manifestations of SED-K have been described in detail previously (fig 1). Briefly, the phenotype of the affected subjects is of proportionate short stature (below the 5th centile for age), with a stocky habitus and progressive osteoarthropathy of the weight bearing joints. Radiographically there is prominent end plate irregularity and sclerosis of the vertebral bodies. Generalised epiphyseal changes are mild and variable. The phenotype of the family with the AR-MED syndrome has also been described previously. Genotyping

DNA samples from 14 subjects from the SED-K kindred (fig 2) including nine affected subjects, three unaffected related subjects, and two unrelated spouses were available for genotyping from the previously reported linkage study. The DNA samples from the SED-K family were typed using a standard set of fluorescently labelled microsatellite markers that spanned the genome and 10 additional markers spanning a 40 cM region of chromosome 15q26.1. DNA samples were available from the AR-MED family as described in Bayoumi et al and genotyped for markers D15S979 and D15S202. Genotyping was performed using an ABI 373 sequencer and GENESCAN 1.2.2-1 and GENOTYPER 1.1.1 software. Two point lod score (Z) values were computed by the LINKAGE 5.1 MLINK program for various recombination fraction (θ) values at a penetrance of 100% and a disease frequency of 0.0001. Multipoint linkage analysis was performed using the GENEHUNTER plus program. The order of the markers was obtained from online genetic mapping data at the Centre for Medical Genetics, Marshfield Medical Research Foundation Website.

Genotyping of AGC1 polymorphisms

The variable number of tandem repeats (VNTR) polymorphism within exon 12 of the AGC1 gene was amplified using the primers described by Doege et al. To type the VNTR, P”dCTP was included in the PCR reaction, the PCR products

Abbreviations: SED, spondyloepiphyseal dysplasia; SED-K, spondyloepiphyseal dysplasia type Kimberley; AR, autosomal recessive; MED, multiple epiphyseal dysplasia; VNTR, variable number of tandem repeats; SSCP, single stranded conformational polymorphism analysis
separated by PAGE (6% w/v), and visualised by autoradiography. Four alleles for the VNTR were identified containing from 27 to 30 repeats of the 57 bp sequence. The restriction fragment length polymorphism (RFLP) identified in exon 18 (see Results) was amplified by PCR using the primers: 5′ GTCATCCCAGGAGACCCTATG and 5′ TAACCCTGTGCAGCGAGAT. The resultant 270 bp fragment was restriction enzyme digested with DdeI and the products separated on a 2% (w/v) agarose gel. In the absence of the DdeI RFLP, three bands of 212 bp, 48 bp, and 14 bp were generated, whereas in the presence of the DdeI RFLP, four bands of 167 bp, 48 bp, 45 bp, and 14 bp were generated. The accuracy of the genotyping of the DdeI RFLP was confirmed by sequence analysis of the PCR products using the BigDye Terminator Sequencing Ready Reaction Kit (Perkin Elmer Co, Foster City, CA, USA) on an ABI 377 sequencer.

Screening of the AGC1 gene
Primers were designed to available intronic sequence in order to generate PCR fragments that spanned each of the 19 exons of the AGC1 gene and included the intron/exon boundaries. The PCR fragments generated from affected and unaffected subjects from the SED-K family were screened for mutations by single stranded conformational polymorphism analysis (SSCP) using previously published methods and by sequence analysis.

Bioinformatics
Human genome sequence data were analysed by BLAST (version 2.0.6 and the NCBI advanced version 2.1), GENESCAN, and “Electronic PCR”.

RESULTS
Analysis of the genotype data generated from the SED-K family identified one marker on chromosome 15, D15S127, with a maximum Z (Zmax) value of 2.93 at θ=0.00. Further analysis with markers in this region of chromosome 15 identified a two point Zmax value of 3.01 for marker D15S116 (table 1). Multipoint analysis was performed using the marker order: cen - D15S131 - 4.57 cM - D15S211 - 2.45 cM - D15S206 - 0.62 cM - D15S205 - 4.48 cM - D15S979 - 2.24 cM - D15S202 - 0.01 cM - D15S116 - 1.17 cM - D15S127 - 0.01 cM - D15S158 - 11.63 cM - D15S1004 - 2.15 cM - D15S1038 - 0.01 cM - D15S130 - 11.99 cM - D15S120 - tel (fig 3). This analysis mapped the SED-K locus to between markers D15S979 and D15S1004 with a maximum multipoint lod score of 3.3. No other markers in the screen showed suggestive evidence of linkage. However, the presence of a cosegregating locus could not be excluded formally because of the relatively sparse marker density and small family size.
AGC1 genotyping and screening

As the AGC1 gene was purported to reside within the SED-K locus, we genotyped the members of the SED-K family with the AGC1 VNTR. This VNTR was not fully informative in this family but no recombination events were detected (see AGC1.1 in fig 2 and table 1). We therefore began the screening of AGC1 for mutations in genomic DNA from affected members of the SED-K family. The repeat region of exon 12 proved intractable to analysis but all other exonic sequence of the AGC1 gene was screened for mutations. This analysis did not identify any potential mutations but detected a previously identified SNP (NCBI dbSNP 2280467) in exon 18 (see sequence accession J05062; nucleotides 1349 to 1366) that would lead to the substitution of a glutamine with an arginine residue and created a DdeI restriction site. The genotypes of the members of the SED-K family for this RFLP are indicated in fig 2 (AGC1.2, where alleles 1 and 2 represent the presence and absence of the DdeI restriction site, respectively). The genotypes were also confirmed by sequence analysis. Examination of the haplotypes of AGC1.1 and AGC1.2 indicated that an intragenic recombination event had occurred between these two markers in IV.1 (fig 2), which was reflected by a comparison of the two point lod scores for the AGC1 markers (table 1). This recombination event placed AGC1 between markers D15S211 and D15S206 (fig 2).

Genotype analysis of the AR-MED family

As the position of AGC1 that we had identified differed from that on the GeneMap'99 available at that time, we genotyped DNA from the AR-MED family where the AGC1 locus had been shown to define the proximal limit of linkage to the disorder. All family members were genotyped with the markers D15S979 and D15S206 and D15S202 purported to flank the AGC1 locus.
Since this was contrary to the linkage data, we sought to establish the way in which the large NT_010356 contig had been assembled. We therefore looked for contigs that were derived. As the sequencing of chromosome 15 is not yet complete, almost all of the markers occurred on unordered contigs identified were unordered and contained many repeated sequences, we could not find overlaps simply by alignment with other sequences in the databases. Instead, the gene prediction program GENSCAN was run on each contig to identify any genes present. Further BLAST analyses determined which other clones or contigs contained each of the predicted genes. When the same group of genes was predicted on two different contigs, a potential region of overlap was indicated. After these investigations, a rough gene map of the whole area was constructed (data available on request). The order of this map was further verified using the program “Electronic PCR” to identify all other marker sequences on each of the potentially overlapping contigs. The sequence map constructed in this way was consistent with the working draft map assembly but identified gaps in the linked region and multiple repeat sequences. One of the largest gaps occurred in a location directly proximal to AGC1.

DISCUSSION

We have identified linkage between the SED-K phenotype and a locus on the long arm of chromosome 15 between markers D15S979 and D15S1004. According to linkage maps available at the time of this finding and now more current linkage maps, this locus spans that of AGC1, which is located between markers D15S1046 (a marker distal to D15S979) and D15S5202.

AGC1 (MIM 155760) was considered a likely candidate gene for SED-K as it encodes the major proteoglycan of the extracellular matrix of hyaline cartilage and plays an important role in cartilage biology and limb development. Mutations within AGC1 have been identified to cause forms of chondrodysplasia in both the mouse and chick but not as yet in humans. In the chick, nanomelia is a lethal disorder characterised by shortened and malformed limbs that is caused by homozygosity for a premature stop codon within AGC1 leading to the truncation of the core protein which is neither processed nor secreted from the chondrocyte. Similarly, mouse cartilage matrix deficiency (cmd), which is characterised by cleft palate and short limbs, is caused by homozygosity for a 7 bp deletion in exon 5 of AGC1 and premature chain termination. Examination of the heterozygote cmd mice has shown that they appear normal at birth but dwarfism and spinal degeneration are age related changes, a phenotype which is comparable with that of the SED-K family.

However, our genotype analysis of all the SED-K and AR-MED families using microsatellite markers spanning the purported AGC1 locus and polymorphic markers within the AGC1 gene support a locus for AGC1 that is proximal to AGC1.
D15S979. This locus for AGCI is inconsistent with available sequence data. Our detailed analysis of the available sequence data did not resolve this inconsistency. However, as yet the human genome sequence is a dynamic structure, still containing gaps and ambiguities, which are being resolved and updated continuously. Indeed the repeat sequences and gaps we identified in the chromosome 15 linked region indicate at least the potential for errors in contig assembly. Equally, the repeat sequences contained within this region may also lead to an increased frequency of recombination or double recombination events. It appears though that AGCI is an unlikely candidate gene for the SED-K and AR-MED phenotypes. However, the inconsistencies between the linkage and sequence data clearly need to be resolved before the targeted analysis of other genes within the linked region can be systematically performed.

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