SHORT REPORT

A locus for asphyxiating thoracic dystrophy, ATD, maps to chromosome 15q13


Asphyxiating thoracic dystrophy (ATD), or Jeune syndrome, is a multisystem autosomal recessive disorder associated with a characteristic skeletal dysplasia and variable renal, hepatic, pancreatic, and retinal abnormalities. We have performed a genome wide linkage search using autozygosity mapping in a cohort of four consanguineous families with ATD, three of which originate from Pakistan, and one from southern Italy. In these families, as well as in a fifth consanguineous family from France, we localised a novel ATD locus (ATD) to chromosome 15q13, with a maximum cumulative two point lod score at D15S1031 (Zmax=3.77 at θ=0.00). Five consanguineous families shared a 1.2 cM region of homozygosity between D15S165 and D15S1010. Investigation of a further four European kindreds, with no known parental consanguinity, showed evidence of marker homozygosity across a similar interval. Families with both mild and severe forms of ATD mapped to 15q13, but mutation analysis of two candidate genes, GREMLIN and FORMIN, did not show pathogenic mutations.

Figure 1 Radiographs of probands C (left) and D (right), showing the typical skeletal abnormalities of ATD. C was stillborn at 30/40, and the radiograph of D is at 3 months of age. Both probands have short horizontal ribs, narrow, “bell shaped” thoraces, mesomelic shortening in the lower limbs, and abnormalities of the pelvis. Medial and lateral bony projections are present on the acetabular roof of C.

A material and methods

To determine the molecular basis of ATD we ascertained five consanguineous families containing a single affected subject and performed autozygosity mapping studies (fig 2). Informed consent was obtained from these families and the study was approved by the relevant Local Research Ethics Committees. Clinical notes and pedigrees indicated that the parents in families A to D are first cousins. In family E, there is anecdotal evidence of consanguinity (P Lebrune, personal communication), and the parents are thought to be first cousins. Three families originated from Pakistan (A-C) and are resident in the UK. Although families A-C originate from a relatively isolated region of Pakistan, Mirpur, the families are not known to be related. Family D and E originate from southern Italy and France, respectively. Clinical assessment supports a diagnosis in the probands as either severe (families...
B and C) or mild (families A, D and E) ATD. In all cases the diagnosis of ATD was confirmed by a perinatal pathologist and/or a radiologist specialising in skeletal malformation syndromes and a neonatal skeletal survey showed typical features of ATD (short horizontal ribs, with small narrow thoraces; acetubular roofs were horizontal, with medial and lateral spurs) in all cases (fig 1). In the case of proband B, a therapeutic termination of pregnancy was performed following an ultrasound diagnosis of ATD during the second trimester.

Affected fetus C was stillborn at 30 weeks. Probands A and D were aged 36 and 30 months, respectively, at the time of the study, and have normal renal function and no evidence of liver disease. Full details of proband E have been published elsewhere (case 3 in Lebrune et al). He developed biliary cirrhosis at 25 months, but subsequent cholestasis was controlled by treatment with ursodeoxycholic acid, with biliary acid and serum concentrations in the normal range, and no evidence of impaired renal function at age 11 years. The relevant clinical and radiographic findings for probands A to E are summarised in table 1.

In the first instance we investigated whether ATD was linked to previously suggested candidate regions (the EVC gene, 12p11-p12, and 6p21, 6q25-27, and 16p13.3 that are syntenic to the 7 cM interval on mouse chromosome 17 to which srt has been mapped). Analysis of haplotypes in families A-D excluded linkage to all of these regions (data not shown). To map a locus for ATD, we performed a genome wide linkage search in the four affected subjects, using an autozygosity mapping approach. Four hundred microsatellite markers, spaced at 10 cM intervals, from the Research Genetics linkage mapping set version 10, were amplified by PCR as described previously. PCR products were electrophoresed on an ABI 377 DNA Analyzer, and were analysed with Genescan v3.1.2 and Genotyper v2.5.2 software (Applied Biosystems). ATD was modelled as a fully penetrant autosomal recessive condition, with a disease allele frequency of 0.001. Alleles for marker loci were assumed to be codominant and to occur at equal frequencies, because population allele frequencies were not available. Two point lod scores were calculated with the MLINK program in the LINKAGE (version 5.1) software package. In addition to the proband and parents, the input files that defined the pedigree structure also included additional family members to create a first cousin consanguineous “loop”.

RESULTS

The data from the original genome wide linkage search showed extended regions of homozygosity in probands A (29 cM from D15S128 to GATA50C03) and B (54 cM, D15S128 to D15S1507) (fig 2, markers shown in bold). No other regions of homozygosity, that were common to the four probands A to D, were found in the genome wide linkage search. These subjects were homozygous for the same allele at D15S822 and D15S165, and all Pakistani probands (A to C) were homozygous for the same allele at GATA50C03. The Italian proband, D, was homozygous at GATA50C03 and D15S659. This suggested that the gene for ATD was located between D15S822 and D15S165, an interval of 31 cM, on chromosome 15q13. To fine map this interval, we genotyped an additional seven microsatellite markers in all four affected probands (A to D) and their parents, as well as proband E and his mother (fig 2, markers shown in plain text). DNA was not available from the parents of proband B or the father of proband E. Suitable markers were identified from the Marshfield mapping panels (Marshfield Medical Research Foundation; http://research.marshfieldclinic.org/genetics/Map_Markers/) and their physical and genetic locations determined from both the Ensembl Genome Browser database (http://www.ensembl.org/Homo_sapiens/) and the deCODE Genetics high resolution genetic map. The order and distance between these markers was based on the deCODE map. The five probands were homozygous for markers D15S976, D15S1013, and D15S1031. The Pakistani probands A and C were homozygous for the same alleles at marker D15S1013, whereas B and C were identical at D15S1031 (fig 2). A common 1.2 cM region of homozygosity between markers

Figure 2 Haplotypes for 14 markers from chromosome 15q13 in four consanguineous families A to E with ATD. The origin of each family is indicated at the top, and affected probands are shown by filled symbols. The genetic distance of each marker is taken from the high resolution deCODE genetic map and listed on the left. The physical position of the forward strand start of the microsatellite sequence is also listed. Markers included in the original genome wide linkage search are shown in bold. Boxes around marker alleles indicate the regions of homozygosity. The minimal candidate interval that encompasses the ATD locus is the region between D15S165 and D15S1010.
ATD maps to 15p13

Radiographic and clinical features of probands A to H

<table>
<thead>
<tr>
<th>Proband</th>
<th>Country of origin</th>
<th>Sex</th>
<th>Age (months)</th>
<th>Birth weight (g)</th>
<th>Short horizontal ribs &amp; narrow thorax</th>
<th>Short limbs (upper/lower)</th>
<th>Trident acetabulum, (with medial/lateral spurs)</th>
<th>Bilateral postaxial polydactyly (hands/feet)</th>
<th>Respiratory problems in neonatal period</th>
<th>Normal conjugated Liver function</th>
<th>Renal function</th>
<th>Ophthalmological findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Pakistan</td>
<td>Female</td>
<td>39</td>
<td>1965 (at 34/40)</td>
<td>+/– hypoplastic right thorax; left side only mildly affected</td>
<td>+/+ +/– mild bowing of –/+ +/+ +/– humeri &amp; femora</td>
<td>Not known</td>
<td>–/– –/– –/– –/– –/– +/+ +/–</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Rod/cone dystrophy</td>
</tr>
<tr>
<td>B</td>
<td>Pakistan</td>
<td>Female</td>
<td>55</td>
<td>242</td>
<td>+/– +/– +/–</td>
<td>+/– +/–</td>
<td>+/–</td>
<td>–/– –/– –/– –/– –/– +/+</td>
<td>Normal</td>
<td>No abnormalities detected</td>
<td>Normal</td>
<td>Short middle phalanges</td>
</tr>
<tr>
<td>C</td>
<td>Pakistan</td>
<td>Male</td>
<td>33</td>
<td>2490</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
<td>–/– –/– –/– –/– –/– +/+</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>No abnormalities detected</td>
</tr>
<tr>
<td>D</td>
<td>Pakistan</td>
<td>Female</td>
<td>40</td>
<td>3310</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
<td>–/– –/– –/– –/– –/– +/+</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Cone shaped epiphyses of phalanges</td>
</tr>
<tr>
<td>E</td>
<td>France</td>
<td>Male</td>
<td>41</td>
<td>3560</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
<td>–/– –/– –/– –/– –/– +/+</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Ophthalmological findings</td>
</tr>
<tr>
<td>F</td>
<td>Italy</td>
<td>Male</td>
<td>46</td>
<td>3150</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
<td>–/– –/– –/– –/– –/– +/+</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Ophthalmological findings</td>
</tr>
<tr>
<td>G</td>
<td>Italy</td>
<td>Male</td>
<td>50</td>
<td>3200</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
<td>–/– –/– –/– –/– –/– +/+</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Ophthalmological findings</td>
</tr>
<tr>
<td>H</td>
<td>Belgium</td>
<td>Female</td>
<td>51⁄2</td>
<td>464</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
<td>–/– –/– –/– –/– –/– +/+</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Ophthalmological findings</td>
</tr>
</tbody>
</table>

DISCUSSION

In certain recessive disorders, the identification of the disease gene has been expedited by the detection of allelic homozygosity in apparently non-consanguineous families. Thus in the search for NPHP4, homozygosity within the critical interval was detected in affected subjects from a family initially thought to be non-consanguineous, but in which distant consanguinity was eventually shown. To determine whether such an approach might be useful in sublocalising the ATD gene on chromosome 15q13, we ascertained three additional non-consanguineous European families with ATD (families F to H, fig 2). Families F and G originated from southern Italy. There is anecdotal evidence of distant consanguinity in family F, and both grandmothers of the proband originate from the same village in southern Italy (M Silengo, personal communication). Proband F had severe respiratory distress at birth, which eventually required tracheostomy, because of an extremely hypoplastic, short thorax. At the age of 33 months there is no evidence of renal disease. Proband G was noted to have postaxial polydactyly of both hands and feet. Skeletal x rays for both probands F and G were diagnostic for ATD, showing shortening of the long bones and typical acetalubar spurs (M Silengo, personal communication). Proband H also had a typical ATD phenotype and the family originates from Belgium. The affected child appeared to have a mild form of ATD, with typical features that include bilateral postaxial polydactyly, but presented with retinal dystrophy at the age of 5½ years. There was no evidence of renal or liver disease. Clinical and radiographic findings for probands F to H are summarised in table 1.

Families F to H were genotyped for the 14 microsatellite markers from the chromosome 15q interval that defined the haplotypes of families A to E (fig 3). Although the parents in families F to H are not known to be related, the haplotypes of the affected children contain small regions of homozygosity within the D15S165 to D15S1010 interval. The unaffected sib of proband F was heterozygous throughout this interval. Homozygosity in the affected children F to H may have arisen from distant consanguinity in these families. Probands D to H all share identical homozygous alleles at markers D15S1013 and D15S231 (fig 3), although the heterozygosity h of these markers is 0.53 and 0.50, respectively. Marker D15S976 (h=0.63) shares alleles for probands D, E, G, and H, D15S1010 (h=0.80) has identical homozygous alleles for D and E and different but homozygous alleles for G and H. These data support the previous conclusion that the ATD locus maps within a 1.2 cM interval from D15S165 to D15S1010, and may reduce the candidate interval to between D15S165 and D15S1031. Although homozygosity could also indicate that alleles for the

<table>
<thead>
<tr>
<th>Marker</th>
<th>Lod score at θ=0.00</th>
<th>Lod score at θ=0.05</th>
<th>Lod score at θ=0.10</th>
<th>Lod score at θ=0.20</th>
<th>Lod score at θ=0.30</th>
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</thead>
<tbody>
<tr>
<td>D15S1048</td>
<td>1.561</td>
<td>2.659</td>
<td>2.420</td>
<td>1.696</td>
<td>0.985</td>
</tr>
<tr>
<td>D15S165</td>
<td>0.938</td>
<td>2.130</td>
<td>1.939</td>
<td>1.326</td>
<td>0.744</td>
</tr>
<tr>
<td>D15S976</td>
<td>0.433</td>
<td>1.744</td>
<td>1.556</td>
<td>0.993</td>
<td>0.507</td>
</tr>
<tr>
<td>D15S1013</td>
<td>2.963</td>
<td>2.468</td>
<td>1.997</td>
<td>1.175</td>
<td>0.568</td>
</tr>
<tr>
<td>D15S1031</td>
<td>3.774</td>
<td>3.225</td>
<td>2.690</td>
<td>1.705</td>
<td>0.916</td>
</tr>
<tr>
<td>D15S1010</td>
<td>2.819</td>
<td>2.088</td>
<td>1.870</td>
<td>1.211</td>
<td>0.604</td>
</tr>
<tr>
<td>D15S231</td>
<td>-0.464</td>
<td>1.203</td>
<td>1.091</td>
<td>0.682</td>
<td>0.337</td>
</tr>
</tbody>
</table>
markers in this region are identical by state, the alleles at most of the markers in this interval for the European cohort (D to H) differ from those of the consanguineous Pakistani cohort (A to C).

Scrutiny of the Ensembl Genome Browser database and the "Golden Path" June 2002 Build 30 human genome assembly at the UCSC Genome Browser (http://genome.ucsc.edu/) showed that the minimal critical interval of D15S165 to D15S1010 was 1.5 Mb in size and contains seven known genes and nine predicted genes. Two of the known genes, GREMLIN and FORMIN, appeared to be excellent candidates for ATD. GREMLIN maps to a position proximal but adjacent to D15S1010, and both D15S1010 and D15S231 are intragenic with respect to FORMIN (fig 3). FORMIN on the reverse strand is adjacent to GREMLIN on the forward strand. Gremlin protein, also known as CKTSF1B1 (cysteine knot superfamily 1, bone morphogenetic protein (BMP) antagonist 1), is predicted to be a small, secreted protein of 184 amino acids that contains a highly conserved cysteine rich repeat region, termed a cystine knot. This structural protein motif is shared by a superfamily that includes members of the transforming growth factor (TGF) family, the Norrie disease protein, the β-formin family, the Norrie disease protein, the β-formin family, and rat. Xenopus Gremlin was shown to act as an antagonist of BMPs and hence preventing their interaction with receptors. The biochemical characterisation of rat Gremlin showed that it could bind to BMP-4 in vitro. This mechanism is similar to that of the proteins encoded by the pattern-inducing genes noggin and chordin. The novel gene SOST encodes sclerostin, another member of the cysteine knot superfamily, and closely related to Gremlin. Mutations in SOST cause sclerosteosis (SOST, MIM 269500), a severe sclerosing skeletal dysplasia characterised by bone overgrowth and syndactyly. Gremlin therefore represented an excellent candidate gene, since it encodes a protein that is a regulator during early development of bones and terminally differentiated tissues.

BMP antagonism by Gremlin relays signalling by Sonic hedgehog (SHH) during outgrowth and patterning of the vertebrate limb. Mesenchymal Gremlin expression is lost in the limb buds of mouse embryos homozygous for recessive limb deformity (ld) mutations, which disrupt the Formin gene. The ld phenotype is characterised by synostoses and syndactyly of all four limbs, and a renal defect that consists of either unilateral or bilateral renal dysplasia. Formin is therefore thought to be a morphoregulatory gene that regulates epithelial-mesenchyme interactions during the patterning of limb skeletal elements and the induction of metanephric kidneys, and the human FORMIN gene is therefore a second candidate gene for ATD. None of the haplotypes for any family preclude FORMIN as a candidate, since both D15S1010 and D15S231 are intragenic. Proband A, for example, has an extended region of homozygosity that spans the region proximal to D15S1010.

We initially analysed the GREMLIN gene by direct sequenc-}

Figure 3  Haplotypes for the same 14 markers as shown in fig 2 in probands D, E, and three additional probands with ATD [F to H], who are children of non-consanguineous parents. The origin of each family is indicated at the top. Boxes around marker alleles indicate the regions of homozygosity, and grey shading indicates alleles that are both homozygous and identical to those in proband D. The approximate positions of the candidate genes, GREMLIN and FORMIN, are indicated (see text for details).
counselling, carrier testing, and prenatal diagnosis. Interestingly, both severe and mild forms of ATD mapped to 15q13, suggesting that phenotypic variation in ATD reflects allelic heterogeneity and not locus heterogeneity. Identification of the ATD gene(s) may provide important molecular insights into fundamental developmental pathways.

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