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


A asphyxiating thoracic dystrophy (ATD, MIM 208500), also known as Jeune syndrome, is an autosomal recessive multisystem developmental disorder, characterised by abnormal skeletal development, with typical radiographical findings (fig 1) that include a long, narrow, “bell shaped” thorax with short, abnormal ribs, metaphyseal irregularities, and short long bones (involving predominately the ulnae, radii, fibulae, and tibiae). Clavicles can be abnormal (“bicycle handlebar shaped”) and cone shaped epiphyses of the hands and abnormalities of the pelvis are considered to be diagnostic. Features of the latter, in the neonatal period, comprise small ilia and irregularity of the acetabulum (“trident shaped”), from which a medial and lateral bony projection is visible. Renal, hepatic, pancreatic, and retinal abnormalities are common features of ATD and polydactyly of both hands and/or feet has been reported.

ATD shows wide phenotypic variability and cases have been classified into lethal, severe, mild, and latent forms. Most patients are severely affected and die from asphyxia caused by a small thorax and hypoplastic lungs, in the perinatal period. However, approximately one-fifth of children with ATD survive beyond the neonatal period, only to develop significant renal impairment, with cystic changes and periglomerular fibrosis leading to chronic renal failure. Liver involvement may be severe and biliary cirrhosis can cause early morbidity. While ophthalmological involvement is not a presenting symptom, retinal dystrophy is an occasional feature. The molecular basis of ATD is at present unknown, with few clues to the location of genes likely to contribute to pathogenesis. A similar phenotype occurs in Ellis-van Creveld syndrome (EVC, MIM 225500), and has been reported in one case with a de novo deletion of chromosome 12p11-p12. A mouse model of ATD has been proposed, known as the shorty (srt) mutant, identified through a screen of recessive developmental mutations. The human chromosomal regions syntenic to the srt locus are chromosome 6p21, 6q25-27, and 16p13.3.

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...
been published elsewhere (case 3 in Lebrune et al). 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.

In the first instance we investigated whether 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; acetabular 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.

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.

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 D15S1030) (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 D15S1030, and they showed extended regions of homozygosity in probands A and B (31 cM, D15S976 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 D15S1030, and they showed extended regions of homozygosity in probands A and B (31 cM, D15S976 to D15S1507).

To map a locus for ATD, we performed a genome wide linkage search in the four affected subjects, using an autosomal recessive model. The data from the original genome wide linkage search was controlled by treatment with ursodeoxycholic acid, a common 1.2 cM region of homozygosity between markers D15S165 and D15S1010. DNA was not available from the parents of proband B or the father of proband E. (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 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 Marshfield mapping panels (Marshfield Medical Research Foundation; http://research.marshfieldclinic.org/genetics/Map_Markers/). Figure 2 shows the 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. The 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.
Table 1 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</th>
<th>Respiratory problems in neonatal period</th>
<th>Normal liver function</th>
<th>Renal function</th>
<th>Other 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>+/+</td>
<td>+/+</td>
<td>–/–</td>
<td>+/–</td>
<td>Normal</td>
<td>Normal</td>
<td>Handlebar shaped clavicles</td>
</tr>
<tr>
<td>B</td>
<td>Pakistan</td>
<td>Male</td>
<td>21+/40</td>
<td>643</td>
<td>+/–</td>
<td>+/–</td>
<td>–/–</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Hypoplastic middle phalanges of hands</td>
</tr>
<tr>
<td>C</td>
<td>Pakistan</td>
<td>Female</td>
<td>20/40</td>
<td>242</td>
<td>+/–</td>
<td>+/–</td>
<td>–/–</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Italy</td>
<td>Male</td>
<td>33</td>
<td>2490</td>
<td>+/–</td>
<td>+/–</td>
<td>–/–</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>France</td>
<td>Male</td>
<td>41</td>
<td>3310</td>
<td>+/–</td>
<td>+/–</td>
<td>–/–</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Italy</td>
<td>Female</td>
<td>46</td>
<td>3560</td>
<td>+/–</td>
<td>+/–</td>
<td>–/–</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Italy</td>
<td>Female</td>
<td>51⁄2 years</td>
<td>3150</td>
<td>+/–</td>
<td>+/–</td>
<td>–/–</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Belgium</td>
<td>Female</td>
<td>46</td>
<td>3200</td>
<td>+/–</td>
<td>+/–</td>
<td>–/–</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
</tr>
</tbody>
</table>

D15S165 and D15S1010 was detected in all five probands. A maximum cumulative two point lod score was detected at D15S1031 (Zmax = 3.77 at θ = 0.00) (table 2).

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 53 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[18] 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 renal dystrophy at the age of 5½ years.[19] 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, F, 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 2 Maximum cumulative two point lod scores for the ATD locus and markers from chromosome 15q13 in consanguineous families A to E

<table>
<thead>
<tr>
<th>Marker</th>
<th>Lod score at θ= 0.000</th>
<th>0.050</th>
<th>0.100</th>
<th>0.200</th>
<th>0.300</th>
</tr>
</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>0.483</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>
thought to be a morphoregulatory gene that regulates vertebrate limb. Hedgehog (SHH) during outgrowth and patterning of the embryo therefore represented an excellent candidate gene, since it encodes a protein that is a regulator during early development with respect to signalling in embryonic explants, *Xenopus* Gremlin was shown to act as an antagonist of BMP (A to C).

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).

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.cse.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 CKTSF1B (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)β family, the Norrie disease protein, the mucins, and von Willebrand factor. A role in early development and tissue specific differentiation for gremlin is inferred from the highly conserved homologues in *Xenopus* and rat. *Xenopus* Gremlin was shown to act as an antagonist of BMP signalling in embryonic explants, presumably by binding 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 terminal 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 kidney, 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 sequencing of the single coding exon and exon/intron boundaries in all probands from families A to H. The only sequence change found was a SNP in the 3′UTR of the gene, *+40 C>A*. Probands C and E were homozygous for the A allele, and G was heterozygous for the SNP. All of the remaining probands were homozygous for the C allele. The heterozygous SNP in proband G therefore reduces the region of homozygosity in this affected subject to between markers D15S165 and D15S1031 (fig 3). The *FORMIN* gene spans 380 kb of genomic DNA and contains 18 coding exons. We designed primers to PCR the coding region and splice site boundaries (primer sequences can be supplied on request). Direct sequencing of the PCR products in all probands did not show any pathogenic mutations, although numerous homozygous SNPs were found in both coding and intronic sequences. Putative missense mutations were excluded as pathogenic by one or more of the following three criteria: the presence of homoyogotes in normal controls, absence of segregation with disease phenotype in ATD families and/or lack of conservation of the mutated amino acid in the Formin sequences from other euakaryotic species. Thus, although both *FORMIN* and *GREMLIN* appeared to represent excellent candidate genes, we could not detect evidence of a pathogenic mutation in either gene. However, a mutation in a regulatory region or deep within an intron cannot be excluded. Studies of additional ATD families will refine the localisation of the gene and provide a basis for further candidate gene analysis. Genotyping additional markers within the candidate interval will confirm if a common ancestral haplotype exists in the cohort of European patients, as suggested by the block of shared homozygosity in probands D, F, G, and H centred on markers D15S976 and D15S1013 (fig 3). It remains to be determined whether other families with similar or overlapping phenotypes also show linkage to the same region. Preliminary work on further ATD families (both consanguineous and non-consanguineous) suggest that not all of them are linked to chromosome 15, indicating that ATD is likely to be genetically heterogeneous.

The identification of the ATD gene(s) will enable the development of molecular diagnostic tests to facilitate genetic
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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