Asphyxiating thoracic dystrophy (ATD), or Jeune syndrome, is a multisystem autosomal recessive disorder associated with 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.

**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 A-D) or mild (family E) ATD.
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; 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. 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 probes were homozygous for markers D15S976, D15S1013, and D15S1031. The Pakistani probands A and C were homozygous for the same alleles at marker D15S1031, whereas B and C were identical at D15S1031 (fig 2). A common 1.2 cM region of homozygosity between markers...
ATD maps to 15p13

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, (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>Normal Renal function</th>
<th>Chest circumference below 3rd centile at 9 months</th>
<th>Ophthalmological findings</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>+/– hypoplastic right thorax; left side only mildly affected</td>
<td>+/+ +/+ +/+ +/+</td>
<td>+/−</td>
<td>–/+</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Italy</td>
<td>Male</td>
<td>21+/40</td>
<td>643</td>
<td>+/−</td>
<td>+/+ +/+ +/+ +/+ +/+ +/+</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Pakistan</td>
<td>Female</td>
<td>Termination 21+/40</td>
<td>242</td>
<td>–/–</td>
<td>–/+</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Pakistan</td>
<td>Male</td>
<td>20/40</td>
<td>3310</td>
<td>+/−</td>
<td>+/+ +/+ +/+ +/+ +/+ +/+</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Italy</td>
<td>Male</td>
<td>33</td>
<td>3560</td>
<td>+/−</td>
<td>+/+ +/+ +/+ +/+ +/+ +/+</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Italy</td>
<td>Female</td>
<td>132</td>
<td>3150</td>
<td>+/−</td>
<td>+/+ +/+ +/+ +/+ +/+ +/+</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Italy</td>
<td>Female</td>
<td>41</td>
<td>3200</td>
<td>+/−</td>
<td>+/+ +/+ +/+ +/+ +/+ +/+</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Belgium</td>
<td>Male</td>
<td>46</td>
<td>46</td>
<td>+/−</td>
<td>+/+ +/+ +/+ +/+ +/+ +/+</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>Normal</td>
<td></td>
</tr>
</tbody>
</table>

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 = 0.00</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>0.433</td>
<td>2.088</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>3.734</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>

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 acetabular 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 candidate interval to between D15S165 and D15S1031. Although homozygosity could also indicate that alleles for the
thought to be a morphoregulatory gene that regulates noggin that of the proteins encoded by the pattern-inducing genes hedgehog (SHH) during outgrowth and patterning of the vertebrae limb of all four limbs, and a renal defect that consists of either renal agenesis or failure of ventral metanephric kidney development. Gremlin was shown to act as an antagonist of BMP signalling in embryonic explants, and therefore represented an excellent candidate gene, since 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 GREMLIN maps to a position proximal but adjacent to D15S1010, and 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. For 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 D15S231 (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 homozygotes 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 eukaryotic 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

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**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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