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.

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).1–4 Clavicles can be abnormal (“bicycle handlebar shaped”) and cone shaped epiphyses of the hands and abnormalities of the pelvis are considered to be diagnostic.5 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.6 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 periapillary fibrosis leading to chronic renal failure.6 Liver involvement may be severe and biliary cirrhosis can cause early morbidity.7 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-p12.8 A mouse model of ATD has been proposed, known as the shorty (srt) mutant, identified through a screen of recessive developmental mutations.9 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 no evidence of liver disease. Full details of proband E have at the time of the study, and have normal renal function and Probands A and D were aged 36 and 30 months, respectively, trimester.
ing an ultrasound diagnosis of ATD during the second therapeutic termination of pregnancy was performed follow-
spurs) in all cases (fig 1). In the case of proband B, a acetubular roofs were horizontal, with medial and lateral features dromes and a neonatal skeletal survey showed typical features diagnosis of ATD was confirmed by a perinatal pathologist B and C) or mild (families A, D and E) ATD. In all cases the spurs) in all cases (fig 1). In the case of proband B, a therapeutic termination of pregnancy was performed follow-
affected probands are shown by filled symbols. The genetic distance of each marker is taken from the high resolution deCODE genet on April 5, 2022 by guest. Protected by copyright.http://jmg.bmj.com/ J Med Genet: first published as 10.1136/jmg.40.6.431 on 1 June 2003. Downloaded from

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 D15S976, D15S1013, and D15S1031. The Pakistani probands A and 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 D15S659, 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 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. 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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>Degree of parental consanguinity</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>Offspring?</td>
<td>Female</td>
<td>39</td>
<td>1965 (at 34/40)</td>
<td>+/+</td>
<td>+/–</td>
<td>−/+</td>
<td>−/–</td>
<td>+/–</td>
<td>Normal</td>
<td>Normal</td>
<td>Rod/cone dystrophy</td>
</tr>
<tr>
<td>B</td>
<td>Pakistan</td>
<td>Male</td>
<td>44</td>
<td>29</td>
<td>643</td>
<td>+/–</td>
<td>+/–</td>
<td>−/–</td>
<td>−/–</td>
<td>−/–</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>C</td>
<td>Pakistan</td>
<td>Male</td>
<td>242</td>
<td>242</td>
<td>2490</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>D</td>
<td>Pakistan</td>
<td>Male</td>
<td>49</td>
<td>49</td>
<td>3310</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>E</td>
<td>Italy</td>
<td>Male</td>
<td>50</td>
<td>50</td>
<td>3560</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
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<td>+/–</td>
<td>Normal</td>
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<tr>
<td>F</td>
<td>Italy</td>
<td>Male</td>
<td>46</td>
<td>46</td>
<td>3150</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
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<tr>
<td>G</td>
<td>France</td>
<td>Male</td>
<td>3200</td>
<td>3200</td>
<td>3200</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
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<td>3000</td>
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<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
<td>+/–</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</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 localising 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 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 consanguninity in these families. Probands D to H all share identical homoyzgous alleles at markers D15S1013 and D15S231 (fig 3), although the heterozygosity 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 homoyzgous alleles for D and E and different but homoyzgous 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 homoyzgosity 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 vertebrate limb.

...encodes a protein that is a regulator during early development therefore represented an excellent candidate gene, since it unilateral or bilateral renal dysplasia.

...The biochemical characterisation of rat Gremlin showed that a small, secreted protein of 184 amino acids that contains 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 cystine knot superfamily 1, mucins, and von Willebrand factor. A role in early 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.

ACKNOWLEDGEMENTS

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