A gene for autosomal dominant sacral agenesis maps to the holoprosencephaly region at 7q36

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Sacral agenesis is a rare disorder of uncertain incidence1 that has been reported in diverse populations. Although usually sporadic and most commonly associated with maternal diabetes, there is a hereditary form which may occur in isolation or with a presacral mass (anterior meningocele and/or presacral teratoma) and anorectal abnormalities, which constitute the Currarino triad2 (MIM 176450). The radiological hallmark of hereditary sacral agenesis is a hemi-sacrum (sickle-shaped sacrum) with intact first sacral vertebra. Bowel obstruction is the usual neonatal presentation, but, unlike other neural tube defects, adult presentation is not uncommon. The major pathology is confined to the usual neonatal presentation, but, unlike other neural tube defects, adult presentation is not uncommon. The major pathology is confined to the

The same region also contains a gene for autosomal dominant sacral agenesis due to mutation in the HOXD13 gene that maps to 7q36 (ref. 10). We conclude that a sacral agenesis gene maps to the first sacral vertebra derive from the tail-bud11,12. The radiological hallmark of hereditary sacral agenesis is a hemi-sacrum (sickle-shaped sacrum) with intact first sacral vertebra. Bowel obstruction is the usual neonatal presentation, but, unlike other neural tube defects, adult presentation is not uncommon. The major pathology is confined to the

In two previously reported sacral agenesis pedigrees13 we failed to detect linkage to HLA markers14, but we now present evidence for a location on 7q36. The same region also contains a gene for holoprosencephaly, an early malformation of the extremal rostral end of the neurotube15.

Table 1 Two point lod scores for linkage of dominant sacral agenesis to markers on distal 7q

<table>
<thead>
<tr>
<th>Marker</th>
<th>Lod scores for fully penetrant dominant model</th>
<th>Affecteds only analysis</th>
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<tbody>
<tr>
<td></td>
<td>Zmax at 0 = 0.00</td>
<td>0.05</td>
</tr>
<tr>
<td>D7S537</td>
<td>0.00</td>
<td>-0.26</td>
</tr>
<tr>
<td>D7S396</td>
<td>-0.05</td>
<td>-0.03</td>
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<tr>
<td>D7S539</td>
<td>3.00</td>
<td>-0.01</td>
</tr>
<tr>
<td>D7S637</td>
<td>1.25</td>
<td>1.22</td>
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<tr>
<td>D7S559</td>
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<td>D7S560</td>
<td>3.91</td>
<td>3.64</td>
</tr>
<tr>
<td>D7S584</td>
<td>4.24</td>
<td>4.16</td>
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*Hypervariable minisatellite VNTR polymorphisms; other markers are (CA)(TG) VNTR polymorphisms (see Methods).
Also, variable expression and early miscarriage may obscure the high frequency of coexistence of the two defects. The classical neural tube defects, anencephaly and spina bifida, may occur in sibships and so are regarded as alternate expressions of the same predisposition. I-3 in family B has classical spina bifida. Although no DNA sample was available for analysis, I-3 is the sister of I-2, an individual who has transmitted the disease haplotype to the second generation. This raises the possibility that the disease gene predisposes to other neural tube defects as is suggested by several other families, notably one with five individuals having sacral agenesis and classical spina bifida. It is likely, therefore, that the gene or genes at 7q36 play a critical role in differentiation of midline mesoderm at both ends of the developing notochord.

<table>
<thead>
<tr>
<th>Markers</th>
<th>D7S396</th>
<th>D7S550</th>
<th>D7S594</th>
<th>D7S637</th>
<th>D7S22*</th>
<th>D7S396*</th>
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<td>SA</td>
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<tr>
<td>D7S559*</td>
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<tr>
<td>D7S594</td>
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</table>

Affected individuals were subject to pelvic X-ray examination except for those indicated by diagonal stripes in the bottom right quadrant. Thus, for example:

- Sacral agenesis without other abnormalities
- Pelvic X-ray not available for review
- Clinically normal; normal pelvic X-ray
- Normal by report

Fig. 1 Haplotype analysis of sacral agenesis families A and B. Panels a and b represent members of families A and B respectively, and are derived from published pedigrees as described in Methods. Asterisked markers represent hypervariable minisatellite VNTR polymorphisms. Other markers are (CA)ITG) VNTR polymorphisms (see Methods). Boxes indicate haplotypes associated with disease. Note crossovers in unaffected individual II-3 and affected individual II-5 in Family A.

Key: Clinical status of family members is indicated by shading of different quadrants as below, with components of the Currarino triad represented by boxed acronyms.

- SB - classical lumbar spina bifida
- SA - sacral agenesis
- AM - anorectal malformation
- PM - presacral mass = anterior meningocele and/or presacral teratoma

Fig. 2 Proposed embryonic basis of the Currarino triad. a,c Diagrammatic transverse sections. b,d Diagrammatic mid-sagittal longitudinal sections through the caudal region of the embryo at approximately five weeks post-fertilization. Sloping lines in b and d show the levels from which sections a and c are taken. a,b, Normal development in which the secondary neural tube (nt), the notochord (no) and the hindgut (hg) all derive from a progenitor cell population at the caudal extremity, the tail-bud (tb). Sclerotomal cells migrate (arrows in a) from a position ventromedial to the somatopleure (sm) to surround the notochord, later giving rise to the skeletal elements of the sacral and coccygeal regions. Close apposition of the hindgut to the ventral surface ectoderm forms the cloacal membrane (cm), which is the forerunner of the anal and urogenital orifices. c,d, An abnormal situation, which we suggest represents early development of the Currarino Triad. Notochordal development is incomplete in the caudal region. The effects of this abnormality are severalfold: (i) sclerotomal migration, which is known to be induced in part by the notochord, is diminished leading to sacral skeletal defects; (ii) hindgut development takes place more dorsally than normal, leading to possible fistulous connections between spinal cord and gut (arrows in c), yielding ventral meningoceles and enteric cysts. Diminished contact between the hindgut and the ventral surface ectoderm reduces cloacal membrane size, predisposing to anorectal malformations; (iii) undifferentiated tail bud cells persist, in place of notochord, between spinal cord and hindgut (asterisk in d), with the potential for presacral teratomatous development; and (iv) lack of notochordal influence leads to lack of dorsoventral polarization of the neural tube and diminished development of ventral motor neurons, as described in cases of sacral agenesis.
Methods

Patients and families. Members of family A are illustrated in Fig. 1a and were drawn from a large Irish sacral agenesis family which has been described elsewhere.19 All affected individuals had radiological evidence of sacral agenesis. Six were asymptomatic, four had presacral teratoma and anterior sacral meningoceles. Three of the four had the full Currarino triad requiring permanent colostomies. Members of family B are illustrated in Fig. 1b and were drawn from a family in which sacral agenesis was accompanied by anorectal stenosis.20

Typing of genetic markers. Genomic DNA was extracted from blood of indicated family members by standard methods. Marker loci D7S537, D7S550 and D7S597 were identified (ref. 31) and used by conventional PCR methods. Amplification reactions included 1–2 pmol primer, 100 U of Taq polymerase (Promega) and 100–500 ng genomic DNA. The forward primer was kinase-labelled with γ-32P ATP prior to the PCR amplification. Amplification was carried out using 30 cycles of denaturation (1 min, 94°C), annealing (1 min, 55°C) and extension (1 min, 72°C), followed by thermal incubation of 10 min. Digested samples were visualized by autoradiography and were typed by Southern blot-hybridization. Following digestion of genomic DNA samples using an appropriate restriction nuclease recognizing polymorphic restriction sites, digested samples were electrophoresed on agarose gels and transferred to nylon membranes. Membranes were hybridized with specific probes at 5°C (ref. 32) and were visualized by autoradiography. Markers D7S468 and D7S522 (ref. 32) constituted minisatellite VNTR polymorphisms. They were typed by Southern blot-hybridization, following digestion of genomic DNA samples using an appropriate restriction nuclease recognizing conserved restriction sites flanking the minisatellites.

Linkage and haplotype analyses. Using allele frequencies observed in control populations two point lod scores were calculated using the data management package LINKSYS in conjunction with the LINKAGE programme Ver. 5.1 (ref. 33). Two models were considered: fully penetrant dominant inheritance and an affected only analysis. Marker order was inferred from recent maps. The order of the minisatellite markers used in the present study, with genetic intervals in brackets, has been reported to be cen-07S396–(5.6)–17S468–(4.1)–D7S22–(2.1)–17S3594–(2.2)–D7S22–(3.2)–D7S3594–(6.6)–17S468–(1.8)–D7S22 (ref. 31). In addition, the recent Cooperative Human Linkage Center (CHLC) map gives the following order: cen–17S5390–(3.2)–17S5398–(1.7)–17S468–(6.6)–D7S22 (ref. 31).

Acknowledgements

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