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 pelvic cavity and may present as a space-occupying lesion or meningitis due to ascending infection. All recurrences in families have been compatible with autosomal dominant inheritance except for those associated with the isomerism gene at Xq24–q27.1 (ref. 3). Several associated cytogenetic defects have been reported, including 7q deletions4. Previous studies failed to detect linkage to HLA markers4,6, but we now present evidence for a location on 7q36. The same region also contains a gene for holoprosencephaly, an early malformation of the extreme rostral end of the neural tube5.

In two previously reported sacral agenesis pedigrees8 we failed to detect linkage to markers at two other chromosomal regions before testing with distal 7q markers. Using a fully penetrant dominant inheritance model, maximum lod scores of over 4.0 at θ=0.00 were obtained with D7S559 and D7S594. Although there is no evidence for non-penetrance when using pelvic X-ray examination, we also employed an affecteds only analysis which also produced significant lod scores (see Table 1). In family A, crossovers in H-3 and H-5 indicate that the disease gene is distal to D7S567 and D7S396 (Fig. 1). As D7S637 physically maps to 7q36 (ref. 10), we conclude that a sacral agenesis gene maps to 7q36 between D7S396 and the telomere, an interval significantly less than 10 Mb. This represents the first localization of a human autosomal gene responsible for failure of development at the caudal end of the neural tube, a form of ‘spina bifida’.

The embryonic tail-bud comprises a population of mesenchymal cells remaining from the regressed primitive streak, which forms the caudal extremity of the four week embryo (Fig. 2a,b). All non-epidermal structures caudal to the first sacral vertebra derive from the tail-bud4,11. The spinal cord and hindgut develop by canalization of midline mesenchymal condensations, while the notochordal condensation remains solid. Somites develop from dorsolateral tail-bud cells and their sclerotomal derivatives migrate to surround the spinal cord and notochord, forming the vertebral elements. Several observations suggest a primary notochord defect in Currarino triad and isolated sacral agenesis (Figure 2c,d):

(i) diminished venous migration of sclerotomal cells, which depends on inductive signalling from the notochord10, produces anterior bony sacral defects; (ii) dorsal shift in position of the hindgut reduces contact with the ventral surface ectoderm, hampering development of the cloacal membrane and predisposing to anorectal and urogenital strictures; (iii) contact between the spinal cord and hindgut is facilitated, making fistulous connections likely; (iv) the notochord induces motor neurons12 and if this induction is diminished, motor function is more severely affected than sensory function, as in sacral agenesis; (v) persistence of undifferentiated tail-bud cells anterior to the spinal cord provides a source of teratomas. Tail-bud cells grafted to ectopic sites produce teratoma-like growths with restricted histological spectrum13, as found in Currarino triad cases; and (vi) tail-bud death and body truncations in the mouse can be caused by retinoic acid in the presence of a functioning gamma receptor14,15, while calcium malformations in Brachyury and Danforth's short tail are associated with failure to form a coherent notochord within the tail-bud16,17.

Holoprosencephaly, like sacral agenesis, is a midline embryonic defect. The forebrain neural plate is induced by mesoderm situated immediately rostral to the notochord. Impairment of this process results in failure of bilateral cleavage of the telencephalon18. The HPE-J holoprosencephaly gene maps in the same 7q36 region; deletion analysis suggests a critical 5 Mb region encompassing the markers D7S22 and D7S468 (ref. 21) and linkage analyses have provided large lod scores for genes, and involvement of different functional domains.

| Table 1 Two point lod scores for linkage of dominant sacral agenesis to markers on distal 7q |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Marker          | Lod scores for fully penetrant dominant model | Z_{max} at θ=0.00 | Z_{max} at θ=0.00 |
| D7S567          | 0.00            | -0.79           | 0.40            | 0.72            | 0.76            | 0.50            | 0.80            | 0.15            | 0.85            | 0.10            |
| D7S396          | 0.00            | -0.15           | 1.00            | 1.27            | 1.17            | 0.75            | 1.00            | 0.13            | 1.32            | 0.08            |
| D7S550          | 3.00            | 3.00            | 2.78            | 2.49            | 1.87            | 1.19            | 3.00            | 0.00            | 2.10            | 0.00            |
| D7S468          | 1.25            | 1.22            | 1.11            | 0.97            | 0.68            | 0.38            | 1.25            | 0.00            | 1.06            | 0.00            |
| D7S559          | 4.38            | 4.30            | 3.97            | 3.53            | 2.60            | 1.83            | 3.91            | 0.00            | 3.13            | 0.00            |
| D7S568          | 3.91            | 3.84            | 3.58            | 3.23            | 2.37            | 1.48            | 3.91            | 0.00            | 3.13            | 0.00            |

*Hypervariable minisatellite VNTR polymorphisms; other markers are (CA)7(TG)7 VNTR polymorphisms (see Methods).
could explain families with one or the other phenotype. 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\(^1\). 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.

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**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)/(TG) 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.

\(\text{PM} \quad \text{SA} \quad \text{AM} \quad \text{SB} \)

- Classical lumbar spina bifida
- Sacral agenesis
- Anorectal malformation
- Presacral mass = anterior meningocele and/or presacral teratoma

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

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**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 dermomyotome (dm) 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 meningocele 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 dorsalventral 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 elsewhere6. All affected individuals had radiological evidence of sacral agenesis. Six were asymptomatic, four had presacral teratomas 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 stenosis7.

Typing of genetic markers. Genomic DNA was extracted from teratomas and anterior sacral meningoceles. Three of the four had been described elsewhere6. All affected individuals had radiological anomalies. Marker loci which sacral agenesis was accompanied by anorectal stenosis1 were typed by Southern blot-hybridization following digestion of 5 μg genomic DNA samples using an appropriate restriction nuclease recognizing conserved restriction sites flanking the minisatellite.

Linkage and haplotype analyses. Using allele frequencies observed in control populations two point lod scores were calculated using the data management package LINKAGE in conjunction with the LINKAGE program package Version 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-D7S637-(7.6)-D7S550-(3.9)-D7S559-(2.6)-D7S22-(3.2)-D7S554-73 (ref. 11). In addition, the recent Cooperative Human Linkage Center (CHLC) map gives the following order: cen-D7S637-(3.2)-D7S554-(1.7)-D7S468-73 (ref. 11).

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