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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 incidence7 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 meningocoele and/or presacral teratoma) and anorectal abnormalities, which constitute the Currarino triad8 (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 deletions9. Previous studies failed to detect linkage to HLA markers5,6, 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 tube10.

In two previously reported sacral agenesis pedigrees11 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 D7S22 and D7S39612. Though 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 D7S396 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-bud13,14. 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 ventromedial migration of sclerotomal cells, which depends on inductive signalling from the notochord15, 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 neurons16 and if this induction is diminished, motor function is more severely affected than sensory function, as in sacral agenesis17; (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 spectrum18, 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 receptor19, while caudal malformations in Brachyury and Danforth's short tail are associated with failure to form a coherent notochord within the tail-bud20,21.

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 telencephalon22. The HPE5 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

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<th>Table 1 Two point lod scores for linkage of dominant sacral agenesis to markers on distal 7q</th>
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*Hypervariable minisatellite VNTR polymorphisms; other markers are (CA)(TG) VNTR polymorphisms (see Methods).
...and 1-2, an obscure the high frequency of coexistence of the two defects. The classical neural tube defects, as outlined in the definition, play a critical role in differentiation of midline mesoderm and develop a classical spina bifida. Although no DNA sample was available for analysis, 1-3 is the sister of 1-2, an unaffected Individual II-3 and affected individual II-5 in Family A. As such, it is likely that the disease gene predisposes to other neural tube defects as well as alternate expressions of the same predisposition. In Family B has classical spina bifida, as demonstrated by clinical and radiographic findings. The classical spina bifida is shown to be inherited as a mendelian trait, with the potential for presacral teratomatous development; and (iv) lack of notochordal development at both ends of the developing notochord.

Also, variable expression and early miscarriage may obscure the high frequency of concordance of the two defects. The classical neural tube defects, as outlined in the definition, play a critical role in differentiation of midline mesoderm and develop a classical spina bifida. Although no DNA sample was available for analysis, 1-3 is the sister of 1-2, an unaffected Individual II-3 and affected individual II-5 in Family A. As such, it is likely that the disease gene predisposes to other neural tube defects as well as alternate expressions of the same predisposition. In Family B has classical spina bifida, as demonstrated by clinical and radiographic findings. The classical spina bifida is shown to be inherited as a mendelian trait, with the potential for presacral teratomatous development; and (iv) lack of notochordal development at both ends of the developing notochord.
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. All affected individuals had radiological evidence of sacral agenesis. Six were asymptomatic, four had presacral teratomas and anterior sacral meningocoeles. 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 anorctal stenosis.

Typing of genetic markers. Genomic DNA was extracted from venous blood of indicated family members by standard methods. Marker loci D7S22, D7S530, D7S592 and D7S594 (ref. 31) constituted minisatellite VNTR polymorphisms. They were typed by PGR reaction. Amplification was carried out using 1–2 pmol primer, 0.15 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 performed using 30 cycles of denaturation (1 min, 94°C), annealing (1 min, 55°C) and extension (1 min, 72°C). Possible amplification products were separated by standard methods on a conventional 6% acrylamide gel and submitted to autoradiography. Marker loci D7S396, D7S468 and D7S222 (ref. 32) constituted minisatellite VNTR polymorphisms. They 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 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–D7S596–(5.6)-(3.8)-D7S468–(4.1)-D7S222–cen(11), while the most recent chromosome 7 workshop report lists the following order for five of the markers used: cen–D7S637–(7.0)-D7S550–(3.9)-D7S559–(2.6)-D7S222–(3.2)-D7S594–cen(11). In addition, the recent Cooperative Human Linkage Center (CHLC) map gives the following order: cen–D7S396–(3.2)-D7S550–(1.7)-D7S468–cen(11).

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