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Congenital and Idiopathic Scoliosis: Clinical and Genetic Aspects

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ABSTRACT

OBJECTIVE

Genetic and environmental factors influencing spinal development in lower vertebrates are likely to play a role in the abnormalities associated with human congenital scoliosis (CS) and idiopathic scoliosis (IS). An overview of the molecular embryology of spinal development and the clinical and genetic aspects of CS and IS are presented. Utilizing synteny analysis of the mouse and human genetic databases, likely candidate genes for human CS and IS were identified.

DESIGN

Review and synteny analysis.

METHODS

A search of the Mouse Genome Database was performed for "genes," "markers" and "phenotypes" in the categories *Neurological and neuromuscular, Skeleton,* and *Tail and other appendages*. The Online Mendelian Inheritance in Man was used to determine whether each mouse locus had a known human homologue. If so, the human homologue was assigned candidate gene status. Linkage maps of the chromosomes carrying loci with possibly relevant phenotypes, but without known human homologues, were examined and regions of documented synteny between the mouse and human genomes were identified.

RESULTS

Searching the Mouse Genome Database by phenotypic category yielded 100 mutants of which 66 had been mapped. The descriptions of each of these 66 loci were retrieved to determine which among these included phenotypes of scoliosis, kinky or bent tails, other vertebral abnormalities, or disturbances of axial skeletal development. Forty-five loci of interest remained, and for 27 of these the comparative linkage maps of mouse and human were used to identify human syntenic regions to which plausible candidate genes had been mapped.

CONCLUSION

Synteny analysis of mouse candidate genes for CS and IS holds promise due to the close evolutionary relationship between mice and human beings. With the identification of additional genes in animal model systems that contribute to different stages of spine development, the list of candidate genes for CS and IS will continue to grow.

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KEYWORDS:
Congenital scoliosis; Synteny; Idiopathic scoliosis; Spinal development; Developmental genes

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INTRODUCTION

Scoliosis may be broken down into two distinct categories, congenital scoliosis (CS) and idiopathic scoliosis (IS). CS is defined as a lateral curvature of the spine due to a developmental abnormality. Scoliosis present at birth that is not associated with an underlying developmental anomaly is referred to as infantile scoliosis. An incidence of approximately 0.5 to 1/1,000 births has been observed for CS.[1,2] This value was obtained by reviewing a series of 15,000 chest films in the state of Delaware (lumbar vertebral defects were not examined). Vertebral defects most commonly include hemivertebrae, block vertebrae, butterfly and wedged vertebra, and unsegmented bars (figures 1 and 2). Hemivertebrae usually represent an extra vertebral segment. Vertebral malformations that result in CS may be associated with genetic syndromes such as Alagille syndrome,[3] spondylocostal dysostosis,[4] and Jarcho-Levin syndrome.[5] By definition, the curve in the frontal plane as viewed on a posteroanterior standing radiograph of the spine must be greater than 10 degrees.[6] Although a single plane is used to establish the diagnosis, scoliosis is in fact a three dimensional deformity. As such, it results in the alteration of the normal sagittal thoracic kyphosis and lumbar lordosis.

In contrast to this, IS is defined as a lateral curvature of the spine for which no cause can be determined. The incidence of IS in the general population ranges from 0.2% to 3%, depending on the magnitude of the curve.[7] Subclassification of IS is based on the age of presentation into infantile (birth to age 3 years), juvenile (age 3 to 11 years) and adolescent (11 years and older). Evidence for a genetic contribution to IS was obtained by determining the incidence of IS of 6.94, 3.69 and 1.55%, respectively in the first, second, and third degree relatives of 114 affected individuals.[8] Since the incidence of IS in the general population of Edinburgh at the time of the study was approximately 0.39%, these findings are consistent with either an autosomal dominant or multifactorial mode of inheritance. Recently, a large family with autosomal dominant IS has been identified, enabling a locus to be assigned at 17p11.[9]

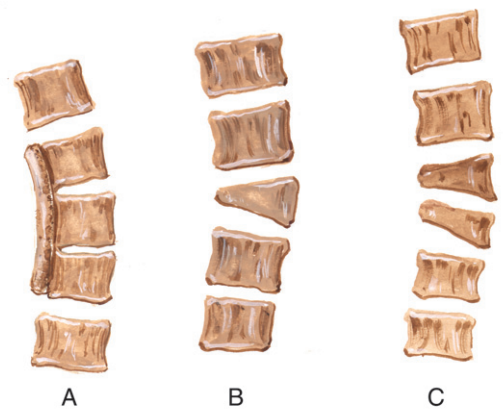

Figure 1. Commonly encountered vertebral malformations associated with CS including an unsegmented bar **(A)**, single hemivertebrae **(B)** and multiple hemivertebrae **(C)**.

Non-congenital scoliosis has many etiologies.[10] The hereditary musculoskeletal disorders, such as osteogenesis imperfecta,[11] Marfan syndrome,[12] Stickler syndrome,[13] Ehlers-Danlos syndrome,[14] and the muscular dystrophies,[15] can each include scoliosis as a manifestation. Neuromuscular diseases, such as cerebral palsy and myelomeningocele, are associated with the development of scoliosis secondary to muscle imbalance.[16,17] Paralytic disorders resulting from polio or spinal trauma may lead to a progressive scoliosis. Radiation therapy, tumors and syrinx formation have also been implicated as etiologies of scoliosis. CS may also be associated with myelodysplasia.

CS is classified by orthopedists as a failure of segmentation (partial or completely fused vertebrae), failure of formation (such as hemivertebrae) and mixed defects. Each of these may cause development of a spinal curve based on asymmetric growth. The severity of the curve is related to the type of defect and whether or not the primary problem is accompanied by any compensatory developmental changes.

As the spinal curvature progresses, pulmonary function may be compromised if the deformity occurs in the thoracic region. Restrictive lung function is associated with spinal curvature that approaches 90 degrees.[18] The most significant pulmonary compromise is associated with severe scoliotic

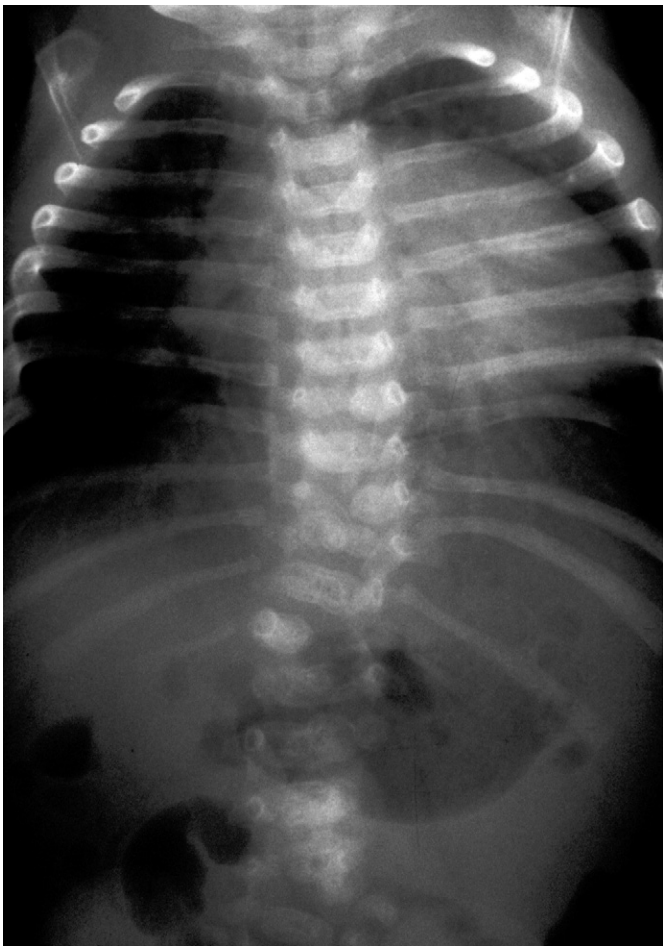

Figure 2. Radiograph illustrating multiple segmentation abnormalities of the thoracic spine.

curves in young children associated with multiple thoracic vertebral anomalies. Surgical treatment at an early age has been recommended in this group of patients.

The progression and ultimate prognosis are dependent upon the specific vertebral anomaly and anatomic location.[19,20] Hemivertebrae will usually result in a curve that exceeds 40 degrees by 10 years, except in instances where two hemivertebrae occupy an adjacent and opposite orientation on the spinal column. Multiple hemivertebrae on the same side are associated with a more rapid progression and usually require treatment before 5 years. A unilateral unsegmented bar has a very high likelihood of resulting in severe progressive scoliosis. An unsegmented bar with a hemivertebrae is associated with the most severe prognosis, with the possibility of a spinal curve exceeding 50 degrees by 3 years of age. Not all congenital vertebral malformations result in CS. For instance, two hemivertebrae may occupy an adjacent and opposite orientation on the spinal column. In this situation progressive deformity would not occur because the vertebral malformations are balanced and together do not result in increased spinal curvature.

ASSOCIATION OF CS WITH CONGENITAL MALFORMATIONS

Because CS arises from significant developmental disruptions, involvement of other organ systems is common. Spinal cord anomalies are particularly widespread, occurring in up to 20% of CS cases.[10] Common associated abnormalities are found in the nervous, urogenital, gastrointestinal, and cardiovascular systems. Specific abnormalities include esophageal atresia, tracheoesophageal fistula, diastematomyelia and other congenital spinal anomalies, anal atresia, Sprengel's deformity, facial asymmetry and bladder and cloacal exstrophy.[21-23] Additional associations include the Klippel-Feil syndrome (short neck, low posterior hairline, fusion of cervical vertebrae), Goldenhar's syndrome (associated with craniofacial anomalies, including microtia and epibulbar dermoids due to abnormal branchial arch development), incontinentia pigmenti (hyperpigmented whorls and streaks associated with eye, skin, hair, nail, teeth and central nervous system abnormalities), other recognizable syndromes, or the VACTERL association (**V**ertebral malformations, **A**nal atresia, **C**ardiac malformations, **T**racheo**E**sophageal fistula, **R**enal and **R**adial anomalies and **L**imb defects).

Genitourinary abnormalities have been reported to occur in 37 of 85 (43%) of patients with CS.[24] Since the genitourinary system and vertebral column are both mesoderm in origin and develop during the fifth week of embryonic life, insults to the embryo during this period could result in both CS and genitourinary tract abnormalities. A 13% incidence of renal and ureteral abnormalities in patients with CS has been reported.[25] Renal ectopia was observed to occur in conjunction with scoliosis with a 10-fold increase in chicks with CS induced by surgical technique.[26] Several mechanisms have been proposed to explain the association between CS and renal anomalies, including a failure of spine growth interfering with normal renal ascent, teratogenic agents and intrinsic genetic defects.

There is some evidence in the literature to support the development of organ malformations at the body segment at which vertebral malformations occur.[4] In a series of 26 patients with multiple vertebral segmentation defects, all patients with congenital heart disease had thoracic involvement, 4 of 6 patients with renal anomalies were found to have lumbar involvement, and all 5 patients with imperforate anus were found to have lumbar involvement.

The developmental field concept described by Opitz is useful in providing a framework for understanding multisystem involvement. Developmental field defects are defined as "any dysmorphogenetically reactive unit of the developing organism that leads to final structure."[27] If a specific pattern of malformation can be attributable to different causes, then a developmental field defect is identified. For example, the association of segmentation anomalies of the spine and ribs in a series of 110 patients with multiple etiologies, including monogenic syndromes, chromosomal abnormalities, environmental agents, and unknown causes, provides evidence that vertebral and rib anomalies constitute a developmental field defect.[28] Through observation of a variable pattern of congenital malformations within the VACTERL complex associated with different etiologies, evidence has been provided for VACTERL complex representing a primary polytopic field defect.[29] Identification of genes associated with CS and malformations involving the genitourinary system, central nervous system and digestive system would extend this hypothesis.

MANAGEMENT OF PATIENTS WITH CS AND IS

Once CS is identified on clinical exam, x-ray studies and evaluation by a pediatric orthopedic surgeon are indicated. Approximately 50% of patients with CS ultimately require surgical correction because of curve progression.[10] Computerized tomography or magnetic resonance imaging scans may be required for further delineation of underlying vertebral and spinal cord anomalies. An evaluation for associated cardiac and renal anomalies should be performed. Early identification of CS and treatment are important for maintaining maximal pulmonary function in patients with thoracic deformities.

Infantile idiopathic scoliosis most often spontaneously regresses, whereas juvenile onset scoliosis is more likely to progress.[30] Treatment includes casting and bracing when there is progression of scoliosis.[31] If these conservative measures fail, surgical management with rodding for stabilization and fusion (arthrodesis) of vertebral levels to prevent growth is recommended during late adolescence.

GENETIC ASPECTS OF CS AND IS

While the absolute numbers are not large, CS is relatively common among congenital malformations. Using chromosomal deletion mapping,[32] a total of 114 cases of scoliosis among 1,753 patients with multiple congenital anomalies associated with various chromosome deletions were identified. Significant associations with haploinsufficiency for chromosomal loci 2p13-15, 6q13, or 15q12 were found, implying that genes mapping to these regions play a role in the development of CS and IS.

Two studies have provided evidence for a sporadic occurrence of CS.[2,33] In both studies, a sibling risk for neural tube defects of approximately 5% was reported, suggesting an etiological association between vertebral malformations and neural tube defects.

CS has also been observed in identical twins.[34-37] Different mechanisms have been postulated including non-genetic factors,[38] abnormalities of cleavage and somite formation,[36] or abnormalities in blood supply, or to a failure of cartilage formation in the mesenchymal tissues.[37] Monozygotic twin females concordant for the presence of right thoracic scoliosis and supravalvular pulmonic stenosis have been reported.[35] The first twin had a lumbar hemivertebrae and absent left kidney. The second twin had no congenital vertebral malformations. This finding provides evidence for multiple genetic loci and/or modifying genes that contribute toward the development of CS and IS.

In addition, occasional familial clusters, often with associated fused ribs and segmentation defects, have been noted.[39-44] These include spondylocostal dysostosis, a sporadic autosomal dominant or autosomal recessive short-trunk, short-stature syndrome that is characterized by non-progressive kyphoscoliosis, multiple hemivertebrae and rib fusion abnormalities, and spondylothoracic dysostosis, a severe and frequently lethal skeletal dysplasia which presents with a crab-like chest on x-ray. A gene for autosomal recessive spondylocostal dysostosis has been localized to chromosome 19q13.1-q13.3.[44] Three mutations in three different families with autosomal recessive spondylocostal dysostosis have been described.[45] These mutations include two protein truncating mutations and a missense mutation.

An autosomal recessive form of CS observed in male and female siblings of Iranian ancestry has been described.[46] The children's parents were first cousins. The spinal x-rays were consistent for thoracic scoliosis, lack of segmentation of thoracic vertebrae, and multiple rib fusions. The female child had a small, abnormally shaped left kidney. Their curve patterns differed from those encountered in both spondylocostal dysplasia and spondylothoracic dysplasia.

Unlike CS, several reports in the literature support the existence of a strong hereditary component in the development of IS, but there is uncertainty regarding its mode of inheritance. Evidence for autosomal dominant with variable penetrance, multifactorial and X-linked dominant modes of inheritance has been reported.[8] A locus for autosomal dominant IS has been identified corresponding to 17p11 in a three generation family of Italian ancestry with 11 affected individuals.[9] Although mutations in heparin sulfotransferase genes, *HS3ST3A1* and *HS3ST3B1*, are prevalent in this region, no mutations have been identified in these genes in two affected family members.

Interestingly, among 237 families who had at least one case of known CS, 17.3% reported having members with IS.[47] This observation could be due to chance. Alternatively it is possible that CS and IS share an underlying genetic mechanism, and that a single genetic defect can result in a predisposition to different types of spinal deformities.

Because CS is usually a sporadic condition, it is generally not possible to use conventional genetic linkage studies to identify chromosome regions that contribute to the development of this disorder. Data obtained from cytogenetic studies or genetic mapping studies will have to be supplemented, either by candidate genes identified by other strategies or by a broad-based mutation screen.

OVERVIEW OF VERTEBRAL DEVELOPMENT AND SOME OF THE MAJOR GENES INVOLVED

Within the past two decades a great deal of information has been learned about the molecular embryology of spine development through the study of mouse and chick embryos. Positional information along the rostrocaudal and anterior posterior axes is laid down during gastrulation.[48] The discovery of homeobox genes in Drosophila (*HOM-C*)[49] and their counterparts in vertebrates (*Hox)* provide strong evidence for similarities in the segmentation process in metazoa, plants and fungi.[50] *Hox* genes are transcription factors that are involved in the specification of positional information along the rostrocaudal axis. They represent a subgroup of the homeobox gene family, which contains a 180 bp DNA sequence encoding a DNA-binding domain as part of a "homeoprotein."[49] The *Hox* family has been studied in great detail in multiple organisms, and homeotic mutations which resemble human dysmorphic syndromes include human synpolydactyly, associated with an in-frame insertion of polyalanine stretches in *HOXD13,* and human hand-foot-genital syndrome, which is due to a nonsense mutation in *HOXA13*.[50] *Hox* genes are expressed in mesodermally and ectodermally derived cells along the body axis. A specific "Hox code" defined by Kessel and Gruss[51] determines vertebral anatomy. There are four gene clusters, *Hox A, B, C* and *D*, containing 39 genes that are located on 4 different chromosomes. In general, genes at the 3' end of a particular cluster are expressed earlier and, with the exception of the second genes in the cluster, occupy more anterior expression domains.[49] Specification of vertebral position information is thought to be achieved by an interplay of *Hox* genes that are expressed at a given axial level.

In the initial stage of vertebral development (figure 3), cells from the epiblast ingress through the primitive streak and spread internally to form the endoderm followed by the

mesoderm, notably the notochord which is located in the midline, and the paraxial mesoderm. Spherical clusters of mesenchymal cells called somitomeres[52] precede the development of somites that in turn develop into vertebrae, ribs, skeletal muscle and dermis.[53] In addition, the *paraxis* gene has been shown to be responsible for normal somite formation in mice.[54] *Paraxis* codes for a basic helix-loop-helix transcription factor which is expressed in paraxial mesoderm and somites. Mice that are homozygous for a paraxis null mutation do not form somites and develop an improperly patterned axial skeleton and musculature.

As vertebrate development progresses, a molecular segmentation clock operating through the Notch signaling pathways is postulated to be responsible for coordinated vertebrate segmentation.[55] Hairy 1 oscillator regulates lunatic fringe expression, which in turn mediates the binding of Notch, a large transmembrane receptor, to Delta and Serrate, two transmembrane ligands. When Notch is proteolytically cleaved, it is translocated to the nucleus. In conjunction with the transcription factor Su(H)*/RBP/jk,* a series of genes of the *hairy* and *enhancer of split* family are transcribed. These genes include *c-hairy 1*, *c-hairy 2*, *Hes1*, *Hey2*, *Her1* and

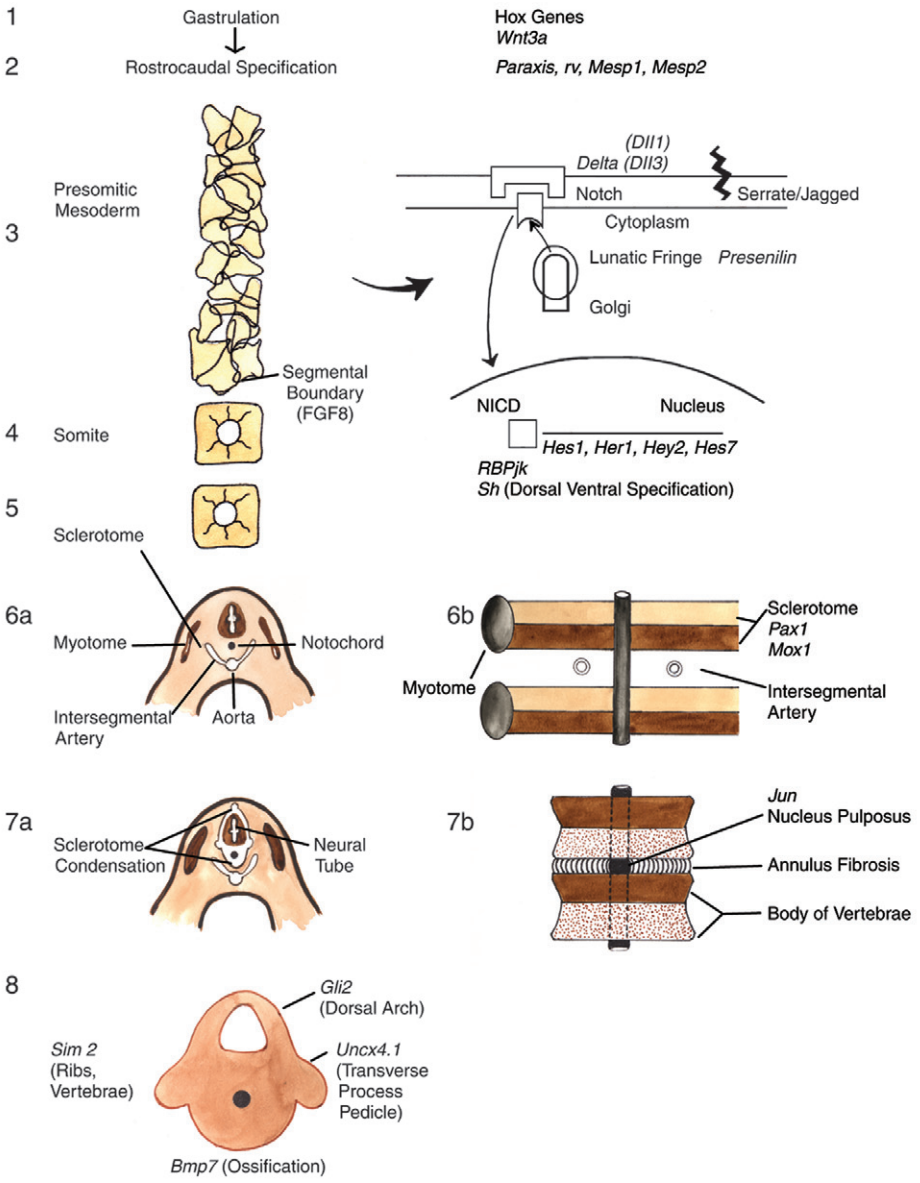

Figure 3. A schematic diagram of murine embryologic development of somite, sclerotome and vertebral body with some of the murine genes involved at different stages. Segmentation is mediated by a molecular segmentation clock operating through the Notch signaling pathway. Segmental boundary formation is mediated by decreased concentrations of fibroblast growth factor 8. *Pax1* and *Mox1* are involved in sclerotome condensation and subdivision into anterior and posterior halves. Transverse embryo sections and corresponding frontal embryo sections are shown. Other genes such as *Gli2, Unex4.1, BMP-7* and *Jun* are involved in vertebrae differentiation and ossification.

Hes7, the Notch ligand Delta C and the glycosyl-transferase, *Lunatic Fringe.*[56] *Presenilin 1* is required for Notch signaling mediated in the paraxial mesoderm.[57] Periodic pulsations of mRNA mediated by Notch signaling are responsible for the establishment of a regular array of somitic boundaries. Decreased concentrations of fibroblast growth factor 8 have been associated with segmental boundary formation in the rostral portion of developing chick embryos.[58]

MesP1 and MesP2 are beta-helix-loop-helix transcription factors that show segmental expression in the presomitic mesoderm.[59] Studies performed in zebrafish[60] have provided evidence that these genes are involved in the development of the anterior-posterior polarity within the developing somite through interaction with the fibroblast growth factor R and Delta-Notch signaling pathways.

The somite subsequently undergoes differentiation to the ventromedial and dorsomedial regions. The ventromedial region gives rise to the sclerotome (figure 3). The dorsolateral region gives rise to the dermomyotome. The posterior half of one sclerotome and the anterior half of its caudal neighbor fuse to form the nascent vertebral body. Sonic hedgehog protein[61,62] has been hypothesized to play a role in sclerotome formation. Experimental evidence suggests that in the platelet-derived growth factor-a receptor is involved with normal patterning of somites. *Pdgfra* null mutant embryos display predominantly cervical segmentation defects, rib fusions, spina bifida occulta and truncated acromion. This phenotype has similarities to Klippel-Feil syndrome.[63]

Cells within the posterior half of the lateral sclerotome are destined to form the neural arch. Densely packed cells located at the anterior sclerotomal center give rise to the intervertebral disc. The less cell dense areas between the intervertebral disc regions form the anlage of the vertebral body. The *Pax1* gene has been shown to be active during sclerotome formation and differentiation. *Pax1* mutations have been identified in undulated mice, suggesting that sclerotome condensation is a *Pax1* dependent process.[64] In the mouse mutant undulated, medial sclerotome condensation does not occur at the lumbosacral level, thus preventing the formation of intervertebral discs and vertebral bodies as a result of faulty sclerotome condensation. *Mox1* is also involved in the subdivision of sclerotomes into a posterior and anterior half.[65] A mouse mutant "rib-vertebrae" (*rv*) has recently been described by Nacke et al.[66] Homozygous *rv* mice have phenotypic features characterized by vertebral and rib defects and urogenital malformations. Somites are irregular in size. Both *Pax1* and *Mox1* are abnormally expressed. Based on these observations, the *rv* mutation results in elongation of the presomitic mesoderm and disruption of the anterior-posterior polarization of somites.

As vertebral development progresses, the prevertebrae are subsequently converted into cartilage during the sixth week of gestation. Endochondral ossification begins during the ninth week. Bone morphogenic protein (BMP) molecules are related to transforming growth factor-β and represent a group of osteoinductive cytokines from the bone matrix. Mice that are homozygous for the BMP-7 null allele display various skeletal defects, including lack of fusion of the neural spines of the atlas, twelfth thoracic, and first sacral vertebrae, openings on the side(s) of the neural arches of the third and fourth thoracic vertebrae, and absence of a lumbar vertebrae.[67] Intervertebral disks have unequal thickness, resulting in tail kinks. Mutant mice have small or nonexistent ossification centers. It has been postulated that the BMP-7 protein is required for the normal ossification process to occur.

The notochord eventually regresses to form the nucleus pulposus. *Jun*, a major component of the heterodimeric transcription factor AP-1 is required for axial skeletogenesis through the regulation of notochord survival.[68] *Jun* deficient mice die during midgestation and develop scoliosis due to fusion of vertebral bodies.

USE OF SYNTENY ANALYSIS TO IDENTIFY CANDIDATE GENES FOR HUMAN CS AND IS

The first major goal of the "genome project" was to identify and map thousands of markers in the human and mouse genomes. This task has been completed.[69,70] Work to date has made it apparent that family relationships among genes have been conserved over evolution. One practical result of these findings is that genes harboring functionally important mutations in model organisms become candidates for corresponding human diseases, sharing features of the mutant phenotypes. Moreover, we have learned that chromosomal organization is highly conserved in these two species, with the preservation of linkage arrangements among groups of neighboring genes over evolution. Only about 200 chromosome rearrangements are thought to have occurred since the human and murine lineages diverged.[71,72] A second practical consequence of the genome project's progress, therefore, is that genetic mapping data from the mouse can be used to predict the locations of corresponding human genes. An extensive collection of conserved synteny data is conveniently accessible via the National Center for Biotechnology Information's Human/Mouse Homology Relationships Web site.[73]

Synteny conservation was used as the basis to identify potential human candidate genes by map position. We therefore undertook a systematic review of mouse mutations with skeletal, tail, or neuromuscular phenotypes with the goal of identifying potential human candidate genes for CS and IS.[74] When the human homologue of the mouse mutant gene was known, this became a candidate. When such a homologue was not known, the established syntenic relationships between the two species were used to identify additional human candidate genes by their chromosomal locations. We report the results of this review, which have subsequently been updated, yielding a relatively small number of human candidate genes meeting our criteria for inclusion.

METHODS

A search of the Mouse Genome Database on the Mouse Genome Informatics Web site[75] was performed for "genes," "markers" and "phenotypes" in the categories *Neurological and neuromuscular, Skeleton,* and *Tail and other appendages*. Retrieved loci were reviewed for possible scoliotic phenotypes, choosing at this point to err in being more, rather than less inclusive. Either the Online Mendelian Inheritance in Man Web site,[76] or the Genome Database Web site was used.[77] The Online Mendelian Inheritance in Man site was used to determine whether each mouse locus has a known human homologue. If so, the human homologue was assigned candidate gene status. Linkage maps of the chromosomes carrying loci with possibly relevant phenotypes, but without known human homologues, were examined and regions of documented synteny between the mouse and human genomes were identified. Mouse loci mapping to regions in which synteny is not well-conserved or mapping close to syntenic region endpoints were excluded from further analysis. The Genome Database Web site[77] was then used to identify human genes mapping within ~5% of the map position specified by the synteny map. The descriptions of the identified human genes were reviewed, and once again liberal criteria was used for assignment of candidate status.

RESULTS

Searching the Mouse Genome Database by phenotypic category yielded 100 mutants of which 66 had been mapped. Next the descriptions of each of the 66 loci were retrieved to determine which among these included phenotypes of scoliosis, kinky or bent tails, other vertebral abnormalities, or

Table 1. Synteny-Defined Candidates for IS and CS.

Mouse mutant or locus	Map position (chromosome, cM)	Human syntenic region	Human candidate(s)	Human syndrome(s)
Dbf (Pax3, Ihh)	1, 40	2q35	*PAX3, IHH*	Waardenburg, CFDH
Gli2	1, 63	2q14	*GLI2*	
Lmx1a	1, 88.2	1q21-q23	*LMX1.1*	
Ltap	1, 93.4	1q21-q23	*VANGL2*	
us and *Lmx1b*	2, 14 and 21	9q34	*LMXIB*	NP
rh and *Hoxd*	2, 38 and 45	2q31	*HOXD* cluster	
Pax1 and *dm*	2, 82 and 80	20p11	*PAX1*	
Jun	4, 44.6	1p31-32	*JUN*	
sks and *sno*	4, 54.6 and 58.3	1p33-p32.2	*COL9A2*	MED type 2
ct	4, 69	1p35	*PAX7, CRTM*	
lx	5, 22	4p16.1	*MSX1*	Wolf-Hirschorn
hop	6, 13	7q22-qter	*PTN, PAX4*	
tc	6, 35.6	2p13-pII	*TGFA*	
Dll3	7, 10	19q13.2-q13.3	*DLL3*	
Tks	9, 9	llq22-q24 or 19p13.3-PI3.2	*MMP* cluster or *ACP5*	
lu	9, 23	llq22-q24	*MMP* cluster	
Aft	9, 32	15q23-q25	*CSK, PML*	
tk	9, 48	6q12-q13	*COL12A1*	
Ky	9, 56	3q21	*MYLK*	
Wnt3a	11, 32	1q42	*WNT3A*	
Ts	11, 73.5	17q25	*TIMP2*	
Rbt	11, 74	17q25	*TIMP2, CBX2*	
Bst	16, 31.5	3q13.2	*COL8A1*	
Sim2	16, 67.6	21q22.2	*SIM2*	
mctl	17, 18.5	6p21.3	*COL11A2, RXRB*	type 2 Stickler, OSMED
Fbn-2	18, 29	5q23.3-q31	*FBN2*	CCA
ocd	19, 6	llql3	*LTBP3*	

CFDH, craniofacial deafness-hand syndrome; NP, nail-patella syndrome; MED, multiple epiphyseal dysplasia; OSMED, otospondylomegaepiphyseal dysplasia syndrome; CCA, congenital contractural arachnodactyly.

disturbances of axial skeletal development. Forty-five loci of interest remained at this point, and for 27 of these the comparative linkage maps of mouse and human were used to identify human syntenic regions to which plausible candidate genes had been mapped (table 1). For each of these 'synteny candidates,' the mouse locus symbol, locus name, map position, corresponding human map position, and human candidate gene(s) symbol are listed. Thus, by reading across each entry in the table, the process used for assessing each locus is briefly recapitulated.

DISCUSSION

For further details regarding each mouse locus, the interested reader is referred to "mouse genome informatics." Specific lesions in cloned mouse genes have been shown to cause scoliosis. Seven of these are briefly discussed below:

Pax1

Three mutant alleles (*undulated, undulated-extreme and undulated-short tail*) of *Pax1* have been described in the mouse. All three mutations reduce or eliminate *Pax1* gene expression and cause deficient development of the anterior vertebral element.[78] Scoliosis, split and fused vertebrae, and hypomorphic intervertebral discs, more prominently in the more posterior regions of the body, result. *Pax1* appears to be important in specifying ventromedial differentiation of the sclerotome, thus accounting for the vertebral abnormalities seen in the various undulated mutant alleles. Murine *Pax1* maps to 80 cM on chromosome 2,[79] while the human homologue maps to 20p11 by somatic cell hybrid and fluorescence *in situ* hybridization analysis.[80] The mutant diminutive (*dm*) which causes decreased viability, decreased body size, macrocytic anemia, extra ribs, vertebral deformations, and short, kinked tails,[81] maps to 82 cM,[82] and may be an additional *Pax1* allele. However mutation analysis of *Pax1* in *dm/dm* mice failed to find any coding-region lesions.

Dll3

The *Dll3* gene is a homologue of the Drosophila Notch ligand Delta[83] and is located at 6 cM on mouse chromosome 7.[84] This locus was recently shown to harbor the pudgy (*pu*) gene.[83] The mutation disrupts the proper formation of morphological borders in early somite development and formation of rostral-caudal compartment boundaries within somites. The mutation may have arisen as a consequence of x-ray exposure and leads to multiple axial skeletal abnormalities in affected animals. The vertebral column, which is drastically shortened along its entire length, is composed of a mixture of irregular fused vertebrae and incompletely developed vertebral bodies interspersed with occasional normal vertebrae. Multiple rib fusions occur which involve the sternum, particularly in the caudal half of the thoracic region. In embryonic development, animals have indistinct, late-appearing, and shortened somites. Segmentation does not occur as far distally as in normal embryos. Adults have shortened, toad-like bodies and both viability and fertility are impaired.[85] An underlying abnormality in the process of segmentation appears to be the mechanism underlying the *pu/pu* phenotype. Crowe et al[86] demonstrated that the Notch and Delta ligands are involved in patterning development of feather buds in the chick embryo, highlighting their role in numerous cell fate decisions in different organisms. Alagille syndrome is associated with vertebral segmentation defects and mutations in the jagged gene (*JAG1*), a ligand of the Notch receptor.[87]

The human region syntenic to the mouse *Dll3* is 19q13.2-13.3.[73,88] On the basis of genome-wide scanning by homozygosity mapping in a large consanguineous Arab-Israeli family with six affected family members, a gene for autosomal recessive spondylocostal dysostosis has been localized to this region,[44] corresponding to a gene coding the Notch ligand Delta-like 3 (*Dll3).* Mutations in three families with autosomal recessive spondylocostal dysostosis have been identified, including two protein truncating mutations and a missense mutation.[45]

Wnt3a

This gene is a member of a moderate-sized multigene family comprised of at least 12 members in humans and the mouse. Genes in this family function both in establishing the body plan in development and as potential oncogenes.[89] The *Wnt* proteins are relatively insoluble, have affinity for extracellular proteoglycans, particularly heparin sulfate, and are secreted inefficiently.[90]

This affinity for extracellular matrix components coupled with their growth-promoting actions provides a simple mechanistic framework for understanding their role in directing establishment of the body plan by promoting proliferation of cells over a limited anatomic region. *Wnt3a* is necessary for generation of the posterior portion of the neuraxis, as knockout mice fail to develop a tailbud and are truncated from a point slightly anterior to the hindlimbs.[91] A spontaneous mouse mutant, vestigial tail (*vt*), has a similar but less severe phenotype. Homozygous (*vt/vt*) mice develop a severely hypomorphic tail, with either a small filament only, or a short stump-like tail. Usually, there is absence of caudal vertebrae, with the remaining vertebrae exhibiting morphologic abnormalities. In some instances, presacral vertebrae are absent and two ossification centers are present in the caudal, lumbar and sacral vertebrae. Caudal neural tube anomalies were observed in regions corresponding to defective somite formation. Embryos display a reduction in the ventral ectodermal ridge of the tail at the tenth day of gestation. Accessory neural tubes are also observed.[92] The *Wnt3a* gene has been localized to mouse chromosome 11 at 32cM. The human orthologue, *Wnt3a,* has been mapped to human chromosome 1q42 by fluorescence in situ hybridization analysis.[93] It is approximately 53.0 kb long and composed of 4 exons.

Ky

A degenerative myopathy precedes the development of kyphoscoliosis in the *ky* mouse.[94] An absence of muscle hypertrophy in response to increased demand is not observed in *ky* mice. Muscles obtained from *ky/ky* mice are smaller and have slower and weaker muscle contractions as compared to control mice. A shift to the expression of slow muscle contractile protein isoforms including, myosin heavy chains and myosin light chains, occurs. The *Ky* gene has been localized on chromosome 9 at 56cM. The encoded gene is a novel muscle specific protein, not one of the myosin chains. A GC deletion in codon 24 that leads to a premature stop codon at position 125 has been observed in *ky/ky* mice. The human *Ky* orthologue falls into a conserved synteny region on 3q21. Myosin light polypeptide kinase is localized within this region and could possibly be a candidate gene for CS and IS.

Lmx1a

The phenotypic mutant Dr (dreher) falls within the *Lmx1a:* gene. Dreher mice have absence of interneurons in the dorsal spinal cord and granule neurons in the cerebellar cortex that result from a failure of roof plate development.[95] There is also a failure of formation of the dorsal neural arches. *Lmx1a:* serves an important function in the specification of dorsal cell fates in the central nervous system and in developing vertebrae. Using Northern analysis, *Lmx1a:* has been localized to the roof plate and immediate neighboring structures during early central nervous system development. *Lmx1a:* maps to a conserved synteny region, 1q22-q23, and stimulates transcription of insulin.[96]

Fbn-2

The shaker-with-syndactylism (*sy*) phenotype is associated with auditory and vestibular defects, fusion of digits and early lethality.[97] A reduction in the caliber of the shafts of long bones and decreased bone density of the long bones of the limbs, girdles, and vertebrae have been observed. Loss of function mutations in *Fbn-2* occurring outside of a "conserved neonatal region" has been observed in *sy,* sy^{fp} and $sy^{fp\text{-}2J}$.[98] In humans, *Fbn-2* maps to a conserved synteny region on 5q23.3-3.1. Mutations in *Fbn-2* are associated with congenital contractural arachnodactyly.[99] The association of scoliosis with congenital contractural arachnodactyly supports the candidacy of *Fbn-2* for IS and CS.

Sim2

Sim2 mice have CS resulting from unequal sizes of ribs and vertebrae.[100] They die shortly after birth because of lung atelectasis and breathing failure. They also have diaphragm hypoplasia, rib protrusions and abnormal intercostal muscle attachments. It has been suggested that rib overgrowth in *Sim2* mice is responsible for the development of CS. The expression of *Sim2* in the vertebral body, and not the somites, provides evidence for its contribution toward the later stages of vertebral development by regulating their growth. *Sim2* is localized to human chromosome 21q22.2 that shares synteny conservation with the 67.6 region of mouse chromosome 16.[101] The 21q22.2 region is associated with many of the features of Down's syndrome. *Sim2* belongs to a family of transcription factors characterized by a basic helix-loop-helix-PAS (PER, ARNT, SIM) domain.[102] *Sim* is responsible for midline central nervous system development in the fly.[103]

ENVIRONMENTAL FACTORS WHICH MAY BE ASSOCIATED WITH THE DEVELOPMENT OF CS

Vertebral malformations in humans have been reported to occur in association with alcohol,[104] anticonvulsant medications including valproic acid[105] and dilantin,[106] hyperthermia,[107] and maternal insulin dependent diabetes mellitus.[108] No epidemiologic studies have been performed to evaluate the association between environmental teratogens and vertebral malformations.

The association of spinal cord anomalies in humans and neural tube defects in mice with CS as described in the preceding sections, raises the possibility that similar etiologic mechanisms are involved in the development of these malformations. Folic acid use has been associated with the prevention of neural tube defects.[109] Further studies need to be performed to determine the possible effects antenatal folic acid use may have on the development of congenital vertebral malformations.

An association of vertebral malformations has been reported in Sprague-Dawley rat fetuses exposed to I(Kr)-blockers (class III antiarrhythmic agent) *in utero*.[110] A temporary induction of hypoxia and reoxygenation injury via the induction of embryonic cardiac arrhythmia has been proposed as the mechanism of teratogenicity.

Thoracic vertebral malformations have been induced in a dose dependent fashion in mice with maternal exposure to carbon monoxide at 9 days of gestation.[111] Possible mechanisms for vertebral malformation include alteration of expression of homeobox genes or sonic hedgehog by carbon monoxide, or through direct action of carbon monoxide on the cartilaginous skeleton.

Rats exposed to boric acid on day 9 of gestation develop 6, rather than the normal number of 7 cervical vertebrae.[112] This has been associated with an alteration of *hox* gene expression pattern, presumably mediated by boric acid.

The influence of various teratogens on the expression of genes that influence vertebrate development has not been well studied. One possible model for the development of CS is multifactorial. An understanding of possible environmental influences on vertebrate development may help to provide more complete genetic counseling for families.

DIRECTIONS FOR FUTURE WORK

It is likely that different approaches will enable an elucidation of the genetic and environmental basis of CS. Recent advances in understanding spine development provide evidence for a genetic basis in the determination of a particular

vertebrate identity during development. Since CS is due to localized alterations in vertebral body development as opposed to a more widespread distribution of vertebral malformations, a model needs to be developed that incorporates position identity and failure of segmentation. The association of renal, cardiac, skeletal and spinal cord malformations with CS may reflect the involvement of different genes that are associated with developmental pathways in several organs. The usual sporadic nature of CS could be due to tissue mosaicism. Synteny analysis of mouse candidate genes for CS holds some promise due to the close evolutionary relationship between mice and human beings. With the identification of additional genes in animal model systems that contribute to different stages of spine development, the list of candidate genes for CS will continue to grow. As genes are identified that contribute toward the development of IS, allelic mutations in these genes may also be responsible for the development of CS. Epidemiologic studies can provide assistance in determining whether various environmental factors contribute to CS. Microarray expression analysis in developing mice embryos may be used to investigate influences of various environmental factors on genes associated with spine development.

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