



Volume 15 Spring 2002
Pages 37-42

Etiology of Congenital Scoliosis

B. Erol, M.D.,¹ K. Kusumi, Ph.D.,² J. Lou, B.A.,¹ and J. P. Dormans, M.D.¹

From ¹Department of Orthopaedic Surgery and ²Department of Human Genetics & Molecular Biology, Children's Hospital of Philadelphia, Philadelphia, PA.

Address correspondence to: John P. Dormans, Chief, Division of Orthopaedic Surgery, The Children's Hospital of Philadelphia, 2nd Floor Wood Center, 34th & Civic Center Blvd., Philadelphia, PA 19104-4.

Abstract: Congenital scoliosis is an abnormal lateral curvature of the spine, resulting from disruption of normal vertebral development. Although there are many types of defects observed in congenital scoliosis result from abnormal formation and segmentation of the vertebral precursors, called somites. Developmental studies in animal models have identified many genes regulating somite formation and segmentation. The interaction of environmental factors and these genes, is thought to be disrupted resulting in deformities such as congenital scoliosis. A basic science research program and a large, multi-center clinical genetic study congenital scoliosis and kyphosis have been established at our institution. Studying the developmental mechanisms in vertebral patterning may aid in the identification of some protective factors for normal spine development, towards the prevention of disfiguring congenital scoliosis.

Introduction

Congenital scoliosis is a lateral curvature of the spine that is due to the presence of vertebral anomalies that cause an imbalance in the longitudinal growth of the spine. While congenital scoliosis is often recognized at birth, more subtle spinal defects can remain undetected. A key feature of congenital scoliosis is the presence of one or more abnormally formed vertebrae. When these anomalies are identified, the curve should be classified as congenital, even if the deformity is not apparent until adolescence.

The vertebrae of the spine are formed during development by segmentation of the precursor spine tissue, a process called somitogenesis. In this process, segments of tissue called somites are formed in pairs

surrounding what will eventually become the spinal cord. When somitogenesis is disrupted even slightly has been done in animal models, congenital vertebral defects identical to those in congenital scoliosis have resulted. Developmental studies in animal models have identified many genes regulating somite formation and segmentation. Recently, genes in the "notch" family have been shown to regulate development of vertebral precursors in the mouse [2,6,10,12,15,18,20,38,42] and defects in human notch genes have been associated with congenital vertebral defects [5,21,31].

Congenital vertebral anomalies have been produced in newborn animals experimentally by transient hypoxia and transient exposure to toxic elements during fetal period [16,22,28,29,35,37,39]. In these studies, major gross vertebral and associated skeletal defects have been induced, including hemivertebrae, vertebral fusion, fragmented vertebral bodies, bifid ribs or junctions of two or more ribs. The nature and extent of skeletal malformations induced have been dependent upon the precise stage of somite differentiation at the time when maternal stress has been induced.

Currently, researchers hypothesize that the environmental factors affect the delivery of the genetic instructions during development, so it is the close interaction of genes and environment that produce the normal spine. This interaction is thought to be disrupted in deformities such as congenital scoliosis. We have initiated a large, multi-center clinical genetic study of congenital vertebral defects at our institution. Work centers on identifying genetic factors underlying these vertebral anomalies and using animal models to examine the developmental origins of these defects. Studying the developmental mechanisms in vertebral patterning will aid in the identification of protective factors for normal spinal development, toward the prevention of disfiguring congenital scoliosis.

Classification

The congenital vertebral anomalies are classified based on failure of formation, failure of segmentation or a combination of the two (mixed) (Fig. 1). The most common type of failure of formation anomaly is a hemivertebra. This is where a portion of the vertebra is missing resulting in a small, triangular shaped "half vertebra" or hemivertebra. Hemivertebrae can be subclassified based on their relationship to the adjacent spine (segmented, semisegmented, nonsegmented). When several vertebral segments fail to separate bilaterally, a block vertebra results producing fused vertebral bones. Unilateral unsegmented vertebral bars are caused by the failure of segmentation only on the left or right side of the spine.

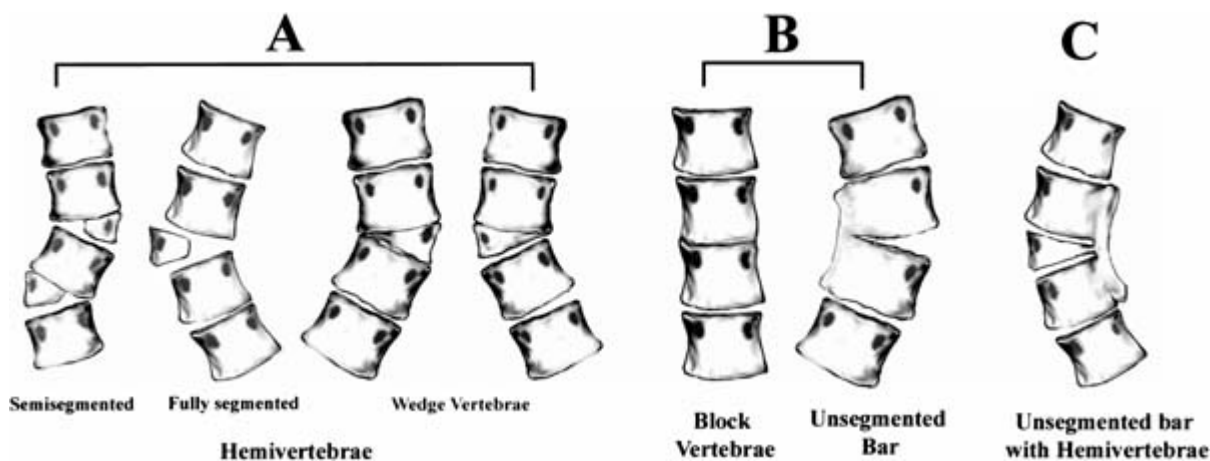


Fig. 1. Diagrammatic representation of classification system of congenital scoliosis. (A) Failures of formation; various

types of hemivertebrae (semisegmented, fully segmented, wedge vertebra). **(B)** Failures of segmentation; block vertebra, unsegmented bar. **(C)** Mixed; combination of hemivertebra and unsegmented bar.

In the involved area of the spine there is absent or abnormal growth potential due to an area of missing b (formation defect) or missing growth plates (segmentation defect). This results in an area of absent grow potential in the vertebral ring, and the growth in the remainder of the vertebral ring disrupts the normal alignment of the spine, producing different types of deformities. Failures of formation or segmentation n occur on either the right or left side of the body resulting in "pure" scoliosis (Fig. 2), or in the anterior or posterior elements resulting in "pure" kyphosis or lordosis, respectively. Combined deformities are most common, producing scoliosis and sagittal plane deformity.



Fig. 2. Plain radiographs of a pediatric patient with congenital scoliosis. **(A)** Posterior--anterior and **(B)** lateral views of the spine, with multiple congenital vertebral anomalies including hemivertebrae at thoracic and

lumbar spine and block vertebrae at lumbar spine.

Natural History and Treatment

The formation and segmentation defects usually have serious consequences in spinal growth during childhood. The severity of the congenital deformity depends on the type of anomaly, the site at which it occurred, and the overall growth potential of the individual [27]. The thoracic curves have the poorest prognosis, with the worst anomaly being a unilateral unsegmented bar combined with single or multiple convex hemivertebrae, followed by a unilateral unsegmented bar, double convex hemivertebrae, and a single convex hemivertebra, with the block vertebra having the best prognosis [25].

Children with congenital scoliosis tend to have their curvature noted much earlier in life than the typical child with idiopathic scoliosis or neuromuscular scoliosis. Congenital curves tend to be very rigid and resistant to correction. They are frequently progressive, and usually cause large deformities [27]. For this reason, the natural history of the individual curve should be understood, and deformities with relentless progression should not be allowed to worsen. In those with a known tendency for progression, early surgical intervention, such as spinal fusion, is essential and preferable to allowing severe curves to develop. Early surgical intervention for children with congenital deformities that have a poor prognosis allows for additional growth in the involved areas of the spine.

Associated Findings

Patients with congenital scoliosis frequently have other associated anomalies. Absent or fused ribs are a common associated defect, since the ribs derive from the same embryonic origins as the vertebrae. When number of ribs on the right or left side do not match, congenital vertebral anomalies should be suspected. Associated congenital anomalies can also involve non-skeletal organs [3]. The most frequently associated defect is found in the genitourinary system. About 20% to 40% of patients with congenital vertebral malformations have renal anomalies, including unilateral kidney, ureteric duplication or obstruction [14]. All patients diagnosed with a congenital spine anomaly must have a renal tract evaluation by a renal ultrasound. If an obstructive uropathy is detected during the ultrasound, appropriate urologic procedures should be done before instituting orthopaedic treatment of the scoliosis. A second area of concern is the detection of cardiac anomalies. About 10--15% of patients with congenital scoliosis have congenital heart defects [33]. A careful cardiovascular system evaluation is essential for these children. In approximately 20% of patients with congenital scoliosis, spinal cord developmental defects have been observed, including tethered cord, fibrous dural bands, diastematomyelia, or intradural lipoma [26]. These spinal cord abnormalities are frequently associated with cutaneous changes (hair patches, dimples, pigmentation) and various abnormalities of the lower extremities, including flat feet, cavus feet, vertical tali, clubfeet, or asymmetric reflexes. All these children should have an MRI scan to evaluate the spinal canal. These associated defects may reflect genetic or developmental commonalities with the formation of the spinal column.

Embryology and the Formation of the Spine

The spine is formed during a process called somitogenesis. This formation is a very early event, and take place between 20 and 30 days of gestation, during the first month of pregnancy, and in the mouse between 10 and 12 days. In this process, segments of mesodermal tissue called somites are formed in pairs surrounding what will eventually become the spinal cord (Figs. 3 and 4). These somites are regularly sized and spaced and this careful organization is essential for the normal patterning of the spine. The segmentation is associated with drastic cellular cytoskeletal rearrangements and biomechanical changes [19]. When somitogenesis is disrupted even slightly, as has been done in animal models, congenital vertebral defects similar to those in congenital scoliosis have resulted. The somites eventually mature, producing sclerotome cells that migrate and surround the developing spinal cord to form the bones of the vertebrae. These same somites also form the axial muscles that connect the vertebral segments, and the ribs associated with the thoracic vertebrae. Many other organs and tissues are being made during this important time in development including the heart, kidneys, brain, limbs and other organs.

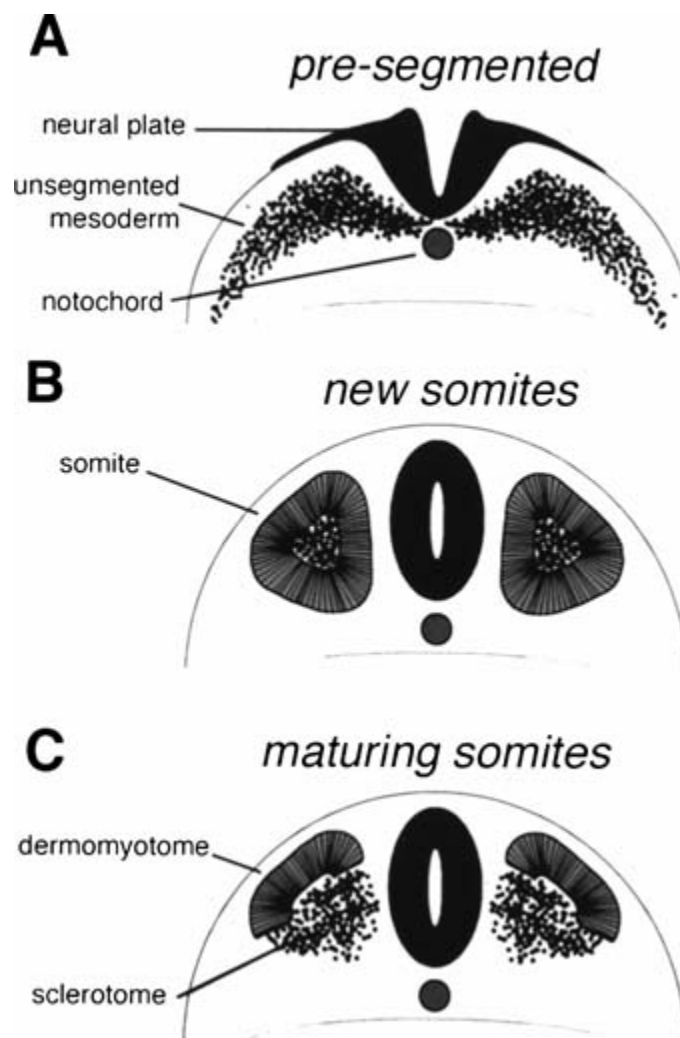


Fig. 3. Somitogenesis in the embryo. Cross-sections of the developing embryo are shown, representing the different stages of patterning in somitogenesis, which takes place between 20 and 30 days of gestation. (A) Prior to formation of the somite segments, the neural plate is folding to eventually form the neural tube, which is the precursor to the adult spinal cord. The

tissue that will form somites is unsegmented, and lies to the side of the notochord, which contributes to the intervertebral discs in the adult. (B) Upon segmentation, the somites are formed as a pair of spherical ball of cells on either side of the future spinal cord. (C) As the somites mature, the sections nearest the gut migrate away to form the sclerotome, the precursors to the vertebral bones. The remaining dermomyotome forms the muscles of the spine, and the dermis in the skin.

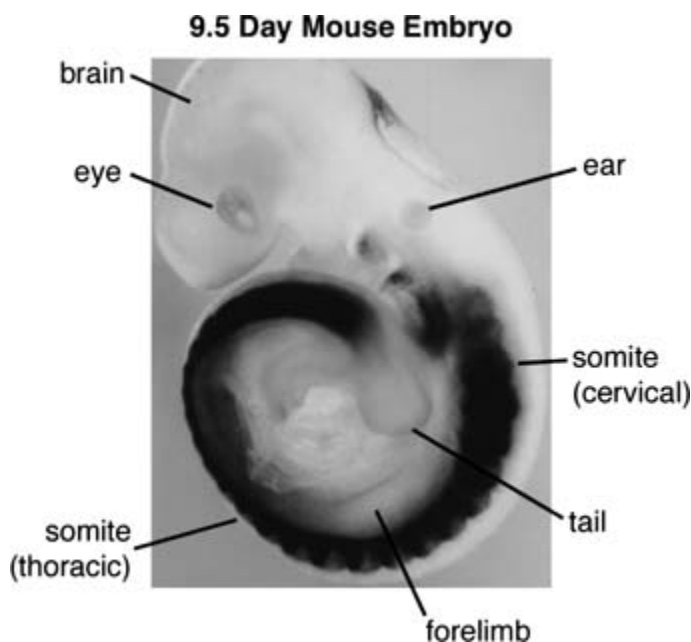


Fig. 4. Somites in a 9.5-day mouse embryo. A mouse embryo at 9.5 days of gestation (equivalent to a human embryo at 25 days of gestation) prepared to highlight the somites with a dark stain. The somites are the embryonic precursors to the adult vertebrae. The embryonic precursors to the adult eye, inner ear, brain (midbrain), forelimbs (equivalent of arms in human), and somites have been indicated. The *Meox 1* gene was used for whole mount in situ hybridization of the 9.5-day embryo.

What controls the regular production of somites, and the resulting highly organized spine? Studies from number of animal models have shown that certain families of developmental genes are essential for regulating somitogenesis, and when those genes are disrupted, vertebral deformities can result. In addition strong evidence has been accumulated that environmental factors can also disrupt somitogenesis. Current researchers hypothesize that these environmental factors affect the delivery of these genetic instructions during development, so it is the close interaction of genes and environment that produce the normal spine.

Genes and Spinal Development

With the completion of sequencing of the human genome, we know that there are approximately 30,000-40,000 genes encoded within human genome [17]. Human and mouse genes share high degree of homology due to the relatively recent evolutionary divergence of these mammals. By studying what happens to the axial skeleton when these genetic instructions are disrupted in the mouse, we have learned a great deal about the most important genes in spinal development. To date, some abnormalities of genes involved in mouse somitogenesis have been found to cause human spinal deformities.

One family of these somite genes is those in the "notch" pathway. Genes in the notch pathway regulate cell fate determination and embryonic patterning in animals. Recent experiments on notch pathway in the mouse and chick embryo have uncovered a mechanism that regulates and maintains the repeated production of somites. The mechanism involves the "clock and wave-front" model of somitogenesis, which involves an internal oscillator or clock underlying the regular periodicity of patterning events [7,8,32]. Until recently there was no significant supporting biological evidence for this phenomenon. However, the identification of genes expressed in an oscillatory manner, in synchrony with each somite cycle, including notch pathway genes *Lfng*, *Hes1*, *Hes7*, *Hey2* in the mouse, *hairy1* and *hairy2* in the chick, has revived interest in this model [4, 18]. The cycling of these genes is thought to regulate the periodic activation of the notch signaling pathway, which would be required for the segmentation process.

Disrupting many genes in the notch pathway produces somite segmentation defects and vertebral anomalies in mice. Mouse mutations in genes of the notch pathway produce severe somite segmentation phenotype including *Notch1*, *Notch2*, *Dll1*, *Dll3*, *Psen1*, and *Lfng* [2,6,10,12,15,18,20,38,42]. Recently in humans, mutations in two genes in the notch family have been identified with human defects of vertebral deformity: delta-like3 in spondylocostal dysostosis (MIM277300) [5], and jagged 1 in Alagille syndrome (MIM118210) [21,31]. Spondylocostal dysostosis is a severe vertebral defect syndrome that is characterized by general vertebral anomalies, rib fusions, and congenital kyphoscoliosis. Alagille syndrome is a congenital, multi-organ disorder that is also associated with multiple vertebral anomalies and liver and heart problems.

Somite genes that control or influence somitogenesis, including those in the notch family, are currently being examined in human spinal defects. We have established a basic science research program and a large multicenter clinical genetic study of congenital scoliosis and kyphosis centered at our institution. We are working to find and better understand the factors underlying vertebral anomalies, by combining results from our molecular genetic studies with information from our growing patient database of congenital scoliosis and kyphosis cases. Study also centers using animal models to examine the developmental origins of these defects.

Inherited congenital vertebral malformations, such as spondylocostal dysostosis, are rare. The genetic etiology for congenital scoliosis is still unclear. Wynn-Davis reported a series of patients in which there was a definite correlation between mixed, multilevel defects, and spina bifida cystica, but she found no genetic implication for isolated lesions such as hemivertebrae [41]. In one study, only 13 out of 1,250 patients with congenital spinal deformities had a first- or second-degree relative with vertebral defect [40]. However, spinal defects are not necessarily binary, i.e., there may be some genetic lesions that do not produce a malformation normally, but might make the fetus susceptible to environmental causes of congenital scoliosis. This interaction between genes and environment has been seen in complex human diseases such as diabetes or heart disease, and researchers expect that this may also underlie congenital scoliosis.

Environmental Factors Associated with Congenital Scoliosis

Congenital vertebral defects have been produced in newborn animals experimentally by transient hypoxia

exposure to toxic elements during fetal period. The first reported experiments on the harmful effect of oxygen deficiency on embryonic development were performed in the mouse animal model [16,28,35]. Hypoxia produced many gross skeletal defects including hemivertebrae, vertebral fusions, fragmented vertebral bodies, bifid ribs or junctions of two or more ribs. The location of the vertebral defects depends on the time during fetal development--the affected structures were derived from the somites being formed at the time.

Carbon monoxide has been shown to induce fetal hypoxia, causing congenital vertebral anomalies in mice. Studies investigating maternal carbon monoxide exposure during somite formation in the developing mouse produced vertebral and other skeletal anomalies in the offspring of mice and rabbit [22,29,37,39]. The incidence and severity of congenital spinal deformities reported in these studies were also directly related to dose and time of exposure. Carbon monoxide exposure at 9.5 days of gestation in the mouse resulted in the highest incidence of vertebral malformations [22,34].

Carbon monoxide is one of the most commonly encountered occupational and environmental pollutants. Approximately 28--30% of the female population smoke during their child bearing years [13], thus making cigarette smoking by pregnant women a common source of high concentrations of carbon monoxide exposure to the developing fetus. In human studies, significant associations were reported between cigarette smoking and spontaneous abortions and low birth weight [1,24]. In addition, severe, acute exposure to carbon monoxide has been shown to cause fetal deaths and anatomical malformations such as Mongoloid type features, missing and deformed limbs or low-set ears [30].

Research has uncovered additional environmental factors associated with vertebral anomalies. For example, patients with fetal alcohol syndrome have an increased incidence of Klippel-Feil syndrome [36], a related cervical vertebral defect characterized by congenital fusion of the cervical spine ranging from a two-segment fusion to involvement of the entire cervical spine [9]. Also, hyperthermia, or exposure to above normal body temperature, has been shown to disrupt vertebral development in animal models [11]. The severity of hyperthermia-induced spinal deformities has been correlated with the time and duration of exposure. Further epidemiological work will help to define how exactly these environmental variables interact with genetic factors, and what additional environmental factors are associated with vertebral birth defects.

Summary

Although there are many types of vertebral dysmorphology observed in congenital scoliosis, they result from abnormal segmentation of the vertebral precursors, called somites. The interaction of environmental factors and the genes that play a role in regulation of somite segmentation, is thought to be disrupted in congenital vertebral deformities such as congenital scoliosis. A large multi-center, clinical genetic study of congenital scoliosis and kyphosis has been established at our institution, and the origins of segmental defects are being examined in animal models. Studying the developmental mechanisms in vertebral patterning will aid in the identification of protective factors for normal spinal development, and ultimately will create a better understanding of ways to prevent and better treat those with congenital scoliosis.

References

1. Armstrong BG, McDonald AD, Sloan M. Cigarette, alcohol, and coffee consumption and spontaneous abortion. *Am J Public Health* 1992;82:85--87.
2. Barrantes IB, Elia AJ, Wunsch K, et al. Interaction between *Notch* signaling and *Lunatic fringe* during somite boundary formation in the mouse. *Curr Biol* 1999;9:470--480.
3. Beals RK, Robbins JR, Rolfe B. Anomalies associated with vertebral malformations. *Spine* 1993;18:1329--1332.
4. Bessho Y, Miyoshi G, Sakata R, Kageyama R. *Hes7*: a bHLH-type repressor gene regulated by Notch and expressed in presomitic mesoderm. *Genes Cells* 2001;6:175--185.
5. Bulman MP, Kusumi K, Frayling TM, et al. Mutations in the human delta homologue, *DLL3*, cause axial skeletal defects in spondylocostal dysostosis. *Nat Genet* 2000;24:438--441.
6. Conlon RA, Reaume AG, Rossant J. *Notch1* is required for the coordinate segmentation of somites. *Development* 1995;121:1533--1545.
7. Cooke J. A gene that resuscitates a theory-somitogenesis and a molecular oscillator. *Trends Genet* 1998;14:85--88.
8. Cooke J. The problem of periodic patterns in embryos. *Phil Trans R Soc Lond* 1981;B295:509--524.
9. Copley LA, Dormans JP. Cervical spine disorders in infants and children. *J Am Acad Orthop Surg* 1998;6:204--214.
10. De Angelis MH, McIntyre JI, Gossler A. Maintenance of somite borders in mice requires the *Delta* homologue *Dll1*. *Nature* 1997;386:717--721.
11. Edwards MJ. Hyperthermia as a teratogen: a review of experimental studies and their clinical significance. *Teratog Carcinog Mutagen* 1986;6:563--582.
12. Evrard YA, Lun Y, Aulehla A, Gan L, Johnson RL. *Lunatic fringe* is an essential mediator of somite segmentation and patterning. *Nature* 1998;394:377--381.
13. Fiore MC, Novotny TE, Pierce JP, et al. Trends in cigarette smoking in the United States. The changing influence of gender and age. *J Am Med Assoc* 1989;261:149--155.
14. Hensinger R, Lang JE, MacEwen GD. Klippel-Feil syndrome: a constellation of associated anomalies. *J Bone Joint Surg* 1974;56-A:1246--1253.
15. Huppert SS, Le A, Schroeter EH, et al. Embryonic lethality in mice homozygous for a processing-deficient allele of *Notch1*. *Nature* 2000;405:966--970.
16. Ingalls TH, Curley FJ. Principles governing the genesis of congenital malformations induced in mice by hypoxia. *N Engl J Med* 1957;257:1121--1127.
17. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* 2001;409:860--921.
18. Jiang YJ, Aerne BL, Smithers L, et al. Notch signalling and the synchronization of the somite segmentation clock. *Nature* 2000;408:475--479.
19. Keynes RJ, Stern CD. Mechanisms of vertebrate segmentation. *Development* 1988;103:413--429.
20. Kusumi K, Sun ES, Kerrebrock AW, et al. The mouse pudgy mutation disrupts *Delta* homologue *Dll3* and initiation of early somite boundaries. *Nat Genet* 1998;19:274--278.
21. Li L, Krantz ID, Deng Y, et al. Alagille syndrome is caused by mutations in human Jagged 1, which encodes a ligand of Notch 1. *Nat Genet* 1997;16:243--251.
22. Loder RT, Hernandez MJ, Lerner AL, et al. The induction of congenital spinal deformities in mice by maternal carbon monoxide exposure. *J Pediatr Orthop* 2000;20:662--666.
23. MacEwen GD, Winter RB, Hardy JH. Evaluation of kidney anomalies in congenital scoliosis. *J Bone Joint Surg* 1972;54-A:1451--1454.
24. McDonald AD, Armstrong BG, Sloan M. Cigarette, alcohol, and coffee consumption and prematurity. *Am J Public Health* 1992;82:87--90.
25. McMaster MJ. Congenital scoliosis caused by a unilateral failure of vertebral segmentation with contralateral hemivertebrae. *Spine* 1998;23:998--1005.
26. McMaster MJ. Occult intraspinal anomalies and congenital scoliosis. *J Bone Joint Surg* 1984;66-A:558--562.
27. McMaster MJ, Ohtsuka K. The natural history of congenital scoliosis. A study of two hundred and fifty-one patients. *J Bone Joint Surg* 1982;64-A:1128--1147.
28. Murakami U, Kameyama Y. Vertebral malformations in the mouse fetus caused by maternal hypoxia during early stage pregnancy. *J Embryol Exp Morphol* 1963;11:107--118.
29. Murray FJ, Schwetz BA, Crawford AA, et al. Embryotoxicity of inhaled sulfur dioxide and carbon monoxide in mice.

- rabbits. *J Environ Sci Health* 1979;13:233--250.
30. Norman CA, Halton DM. Is carbon monoxide a workplace teratogen? A review and evaluation of the literature. *Ann Occup Hyg* 1990;34:335--347.
 31. Oda T, Elkahloun AG, Pike BL, et al. Mutations in the human Jagged 1 gene are responsible for Alagille syndrome. *N Genet* 1997;16:235--242.
 32. Palmerim I, Henrique D, Ish-Horowicz D, Pourquié O. Avian hairy gene expression identifies a molecular clock linke vertebrate segmentation and somitogenesis. *Cell* 1997;91:639--648.
 33. Reckles LH, Peterson HA, Bianco AJ, Weidman WH. The association of scoliosis and congenital heart disease. *J Bon Joint Surg* 1975;57-A:449--455.
 34. Rivard CH. Effects of hypoxia on the embryogenesis of congenital vertebral malformations in the mouse. *Clin Orthop* 1986;208:126--130.
 35. Rivard CH, Narbaitz R, Uhthoff HK. Time of induction of congenital vertebral malformations in human and mouse embryo. *Orthop Rev* 1979;8:135--139.
 36. Schilgen M, Loeser H. Klippel-Feil anomaly combined with fetal alcohol syndrome. *Eur Spine J* 1994;3:289--290.
 37. Schwetz BA, Smith FA, Leony BKJ, et al. Teratogenic potential of inhaled carbon monoxide in mice and rabbits. *Teratology* 1979;19:385--392.
 38. Shen J, Bronson RT, Chen DF, et al. Skeletal and CNS defects in *Presenilin-1* deficient mice. *Cell* 1997;89:629--639.
 39. Singh J, Aggison L, Moore-Cheatum L. Teratogenicity and developmental toxicity of carbon monoxide in protein def mice. *Teratology* 1984;48:149--159.
 40. Winter RB. Congenital scoliosis. *Orthop Clin North Am* 1988;19:395--408.
 41. Wynne-Davies R. Congenital vertebral anomalies: etiology and relationship to spina bifida cystica. *J Med Genet* 1975;12:280--288.
 42. Zhang N, Gridley T. Defects in somite formation in *lunatic fringe*-deficient mice. *Nature* 1998;394:374--377.