

Progress in the Understanding of the Genetic Etiology of Vertebral Segmentation Disorders in Humans

Philip F. Giampietro,^a Sally L. Dunwoodie,^b Kenro Kusumi,^c Olivier Pourquié,^{d,e} Olivier Tassy,^e Amaka C. Offiah,^f Alberto S. Cornier,^g Benjamin A. Alman,^h Robert D. Blank,ⁱ Cathleen L. Raggio,^j Ingrid Glurich,^k and Peter D. Turnpenny^l

^a*Department of Medical Genetic Services, Marshfield Clinic, Marshfield, Wisconsin USA*

^b*The Victor Chang Cardiac Research Institute, University of New South Wales, Darlinghurst NSW, Australia*

^c*School of Life Sciences, Arizona State University, Tempe, Arizona, USA*

^d*Howard Hughes Medical Institute, Kansas City, Missouri, USA*

^e*Stowers Institute for Medical Research, Kansas City, Missouri, USA*

^f*Department of Radiology, Great Ormond Street Hospital for Children, London WC1N 3JH, United Kingdom*

^g*Genetic Division, Department of Biochemistry, Ponce School of Medicine, Ponce, Puerto Rico and Department of Molecular Medicine, La Concepción Hospital, San Germán, Puerto Rico*

^h*Division of Orthopaedic Surgery and Program in Developmental and Stem Cell Biology, Hospital for Sick Children, University of Toronto, Toronto Ontario, Canada*

ⁱ*Department of Medicine, University of Wisconsin-Madison School of Medicine and Public Health and Geriatrics Research, Education, and Clinical Center, William S. Middleton Veterans Administration Medical Center, Madison, Wisconsin, USA*

^j*Department of Pediatric Orthopedics, Hospital for Special Surgery, New York, New York, USA*

^k*Marshfield Clinic Research Foundation, Marshfield, Wisconsin, USA*

^l*Clinical Genetics, Royal Devon & Exeter Hospital, and Peninsula Medical School, Exeter, United Kingdom*

Vertebral malformations contribute substantially to the pathophysiology of kyphosis and scoliosis, common health problems associated with back and neck pain, disability, cosmetic disfigurement, and functional distress. This review explores (1) recent advances in the understanding of the molecular embryology underlying vertebral development and relevance to elucidation of etiologies of several known human vertebral malformation syndromes; (2) outcomes of molecular studies elucidating genetic contributions to congenital and sporadic vertebral malformation; and (3) complex interrelationships between genetic and environmental factors that contribute to the pathogenesis of isolated syndromic and non-syndromic congenital vertebral malformation. Discussion includes exploration of the importance of establishing improved classification systems for vertebral malformation, future directions in molecular and genetic research approaches

Address for correspondence: Philip F. Giampietro, MD, PhD, Department of Genetic Services, Marshfield Clinic, 1000 North Oak Avenue,

Marshfield, Wisconsin 54449, USA Voice: +715-221-7404; fax: +715-389-4399. giampietro.philip@marshfieldclinic.org.

to vertebral malformation, and translational value of research efforts to clinical management and genetic counseling of affected individuals and their families.

Key words: congenital vertebral malformation; spondylothoracic dysostosis; Agagille syndrome; congenital scoliosis; Klippel–Feil syndrome; VACTERL; CHARGE syndrome; teratogens; Jarcho–Levin syndrome; ICVAS; array-based CGH; *DLL3*, *Wnt3A*; *T(Brachyury)*; *Tbx6*; *SLC35A3*; *MESP2* *LFNG*, *PAX1*, *JAG1*, *NOTCH2*; *CHD7*

Overview: Epidemiology of Congenital Vertebral Malformations

Congenital vertebral malformations (CVM) in humans represent a significant health problem because they may cause kyphosis and/or scoliosis, resulting in back and neck pain, disability, cosmetic disfigurement, and functional distress. The true incidence of vertebral malformations is unknown, although estimates indicate a prevalence of approximately 0.5–1 per 1000 persons.^{1–4} Vertebral malformations may represent an isolated finding, occur in association with other renal, cardiac, or spinal cord malformations, or occur as part of an underlying chromosome abnormality or syndrome with an estimated frequency of 30 to 60%.⁵ These include: 1) hemifacial microsomia: associated with craniofacial anomalies, including microtia and attributable to abnormal branchial arch development. Goldenhar is a subset of hemifacial microsomia, of which epibulbar dermoids are a key component;⁶ 2) Alagille syndrome: includes bile duct abnormalities, pulmonic stenosis, vertebral malformations (predominantly butterfly vertebrae), and characteristic facial features;⁷ 3) spondylothoracic dysostosis: characterized by a short trunk, widespread vertebral segmentation abnormalities, and posterior rib fusion abnormalities;⁸ 4) Klippel–Feil: defined by the presence of cervical vertebral fusion abnormalities;⁹ 5) VACTERL (vertebral, anal atresia, cardiac, tracheoesophageal fistula, renal, limb anomalies) syndrome.¹⁰ This condition usually represents a sporadic occurrence within a given family. Table 1 presents a subset of syndromes that have been associated with vertebral malformations.

While heterogeneous in their clinical manifestation, a variety of morphological features of CVM is also encountered and includes hemivertebrae, vertebral bars, supernumerary vertebrae, and butterfly and wedge-shaped vertebrae.^{1–2} Genetic transmission in some cases of CVM has been documented with frequency of familial recurrence rate estimated at 3%. Thus, with a lack of evidence to suggest otherwise, the prevailing assumption is that the majority of cases encountered are clinically sporadic.⁴ However, a notably substantial sibling recurrence rate suggests that further underlying genetic susceptibility yet to be defined may contribute to the pathogenesis of a subset of CVM. Examples of vertebral malformation phenotypes are depicted in Fig. 1. Notably, this figure highlights that vertebral malformations may be categorically classified into defects of formation and proper segmentation.

Studies in model organisms are consistent with a genetic contribution to CVM pathogenesis, with CVM resulting from mutations of multiple genes in the mouse subject. This review focuses on summarizing recent evidence suggesting a further role for genetics and environmental factors in the development of vertebral malformations. This shift from the more simplistic paradigm that vertebral malformations are isolated sporadic events is partially attributable to an expanded understanding of the molecular embryology underlying vertebral development and how current understanding of these events has contributed to the unraveling of the pathogenic mechanisms contributing to several known human vertebral malformation syndromes. Thus it is fitting to begin with a brief review of our current understanding of human vertebral development.

Table 1. Some Syndromes That Include Segmentation Defects of the Vertebrae

Syndrome	OMIM Reference	Gene(s)
Acrofacial dysostosis*	263750	
Aicardi*	304050	
Alagille	118450	<i>JAGGED1, NOTCH2</i>
Anhalt*	601344	
Atelosteogenesis III	108721	<i>FLNB</i>
Campomelic dysplasia	211970	<i>SOX9</i>
Casamassima-Morton-Nance*	271520	
Caudal regression*	182940	
Cerebro-facio-thoracic dysplasia*	213980	
CHARGE	214800	<i>CHD7</i>
'Chromosomal'		
Currarino	176450	<i>HLXB9</i>
De La Chapelle*	256050	
DiGeorge/Sedláčková	188400	Microdeletion 22q11.2; 10p14-p13
Dysspondylochondromatosis*		
Femoral hypoplasia-unusual facies*	134780	
Fibrodysplasia ossificans progressiva	135100	<i>ACVR1</i>
Fryns-Moerman*		
Goldenhar* (Oculo-auriculo-vertebral spectrum)	164210	
Holmes-Schimke*	308310	
Incontinentia Pigmenti	308300	<i>NEMO</i>
Kabuki*	147920	
Kaufman-McKusick	236700	<i>MKKS</i>
KBG syndrome*	148050	
Klippel-Feil*	148900	? <i>PAX1, GDF6</i>
Larsen	150250	<i>FLNB</i>
Lower mesodermal agenesis*		
Maternal diabetes*		
MURCS Association*	601076	
Multiple pterygium syndrome	265000	<i>CHRNA</i>
OEIS syndrome*	258040	
Phaver*	261575	
Rapadilino	266280	<i>RECQL4</i>
Robinow	268310	<i>ROR2</i>
Rolland-Desbuquois*	224400	
Rokitansky sequence*	277000	? <i>WNT4</i>
Silverman	224410	<i>HSPG2</i>
Simpson-Golabi-Behmel	312870	<i>GPC3</i>
Sirenomelia*	182940	
Spondylocarpotarsal synostosis	272460	<i>FLNB</i>
Spondylocostal dysostosis	277300	<i>DLL3, MESP2, LFNG</i>
Spondylothoracic dysostosis*	277300	
Thakker-Donnai*	227255	
Toriello*		
Urioste*		
VATER/VACTERL*	192350	
Verloove-Vanhorick*	215850	
Wildervanck*	314600	
Zimmer*	301090	

*Underlying cause not known

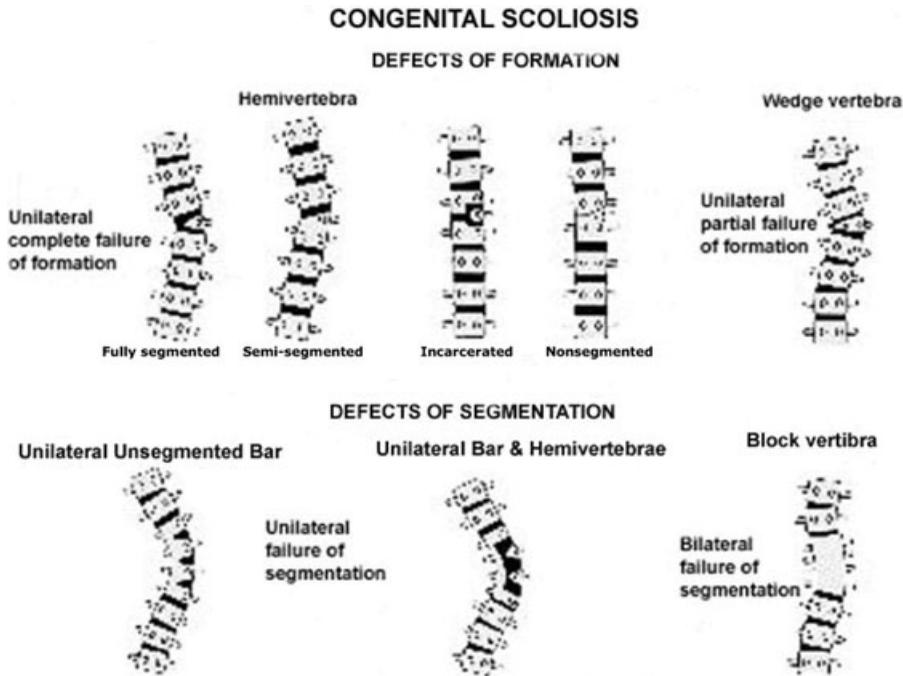


Figure 1. Different classifications of vertebral malformations are illustrated including: wedge shaped, hemivertebrae, and proper segmentation defects such as vertebral bars. (Originally published in *Congenital deformities of the spine*, JR McMaster, Coll. Surg. Edinb., April 2002, 47: 475–480. Reproduced with permission from the Royal College of Surgeons of Edinburgh.)

Overview of Human Vertebral Development and Some Major Genes Involved

In amniotes like humans, vertebrae derive from the paraxial mesoderm which forms initially from ingression of the superficial epiblast cells into the primitive streak during gastrulation with later participation from the tail bud. The newly produced cells of the posterior presomitic mesoderm (PSM) (the paraxial mesoderm) give rise to the segmental units of the somite in the vertebrate embryo (see Fig. 2). Following their segmentation at the rostral tip of the PSM, the somites subdivide into the ventral sclerotome containing vertebral precursors, and the dorsal dermomyotome. These in turn give rise to the corporal skeletal muscles in addition to the dermis of the back. Sclerotome induction from the somite is regulated by signals from the notochord and the floor plate of

the neural tube,¹¹ and involve the Sonic Hedgehog protein.¹² The sclerotome consists of rostral and a caudal compartmental subdivisions. Patterned anterior–posterior fusion of consecutive sclerotomes give rise to the vertebrae during a process termed “resegmentation.”¹³

Experimental evidence in model vertebrate species such as zebrafish, chick, and mouse embryos has demonstrated that vertebral segmentation involves a molecular oscillator. This oscillator, termed the segmentation clock, triggers cyclic activation of the Notch, Wnt, and FGF signaling pathways.¹⁴ Characterization in the mouse revealed that this oscillator prompts the periodic expression of 50 to 100 cyclic genes¹⁵ (see Fig. 3). For the most part, these genes belong to signaling pathways related to the development of vertebral precursors in the PSM. The segmentation clock is believed to control the rhythmic somitogenesis in the embryo and underlies production

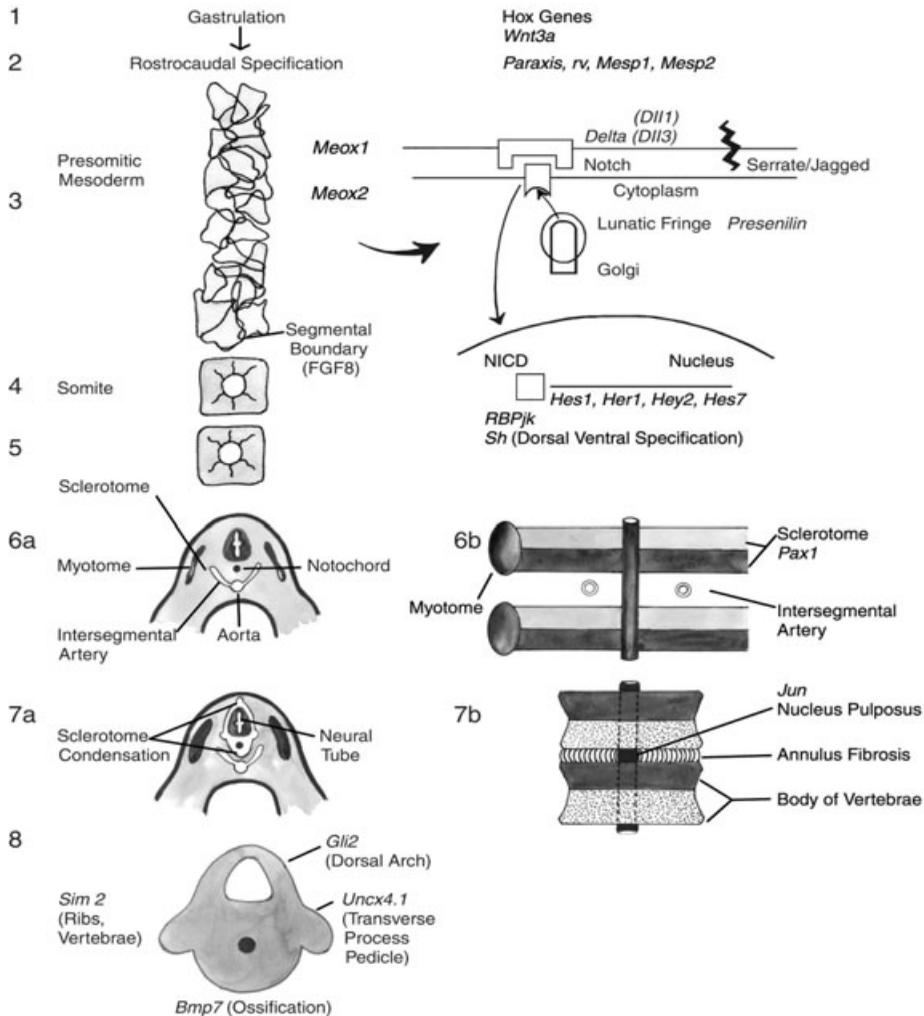


Figure 2. A schematic diagram of murine embryologic development of somite, sclerotome, and vertebral body with some of the murine genes involved at different stages. Segmentation is mediated by a molecular segmentation clock operating through the Notch signaling pathway. Segmental boundary formation is mediated by decreased concentrations of fibroblast growth factor 8. *Pax1* and *Meox1* 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*, *Uncx4.1*, *BMP-7*, and *Jun* are involved in vertebrae differentiation and ossification. (Reprinted with permission: P Giampietro, *et al.* 2003. Congenital and idiopathic scoliosis: clinical and genetic aspects. *Clin. Med. Res.* **1**: 125–136.)

of the characteristic segmental pattern observed in the later-stage, periodic organization of the spine. A key output of the segmentation clock oscillations is the periodic production of “stripes” of the *MESP2* transcription factor. *MESP2* is believed to control formation

of somite boundaries and define the rostrocaudal specification of the sclerotome. This template thus defines how vertebrae will form. As further described below, mutations affecting genes associated to the function of this molecular oscillator—genes including lunatic

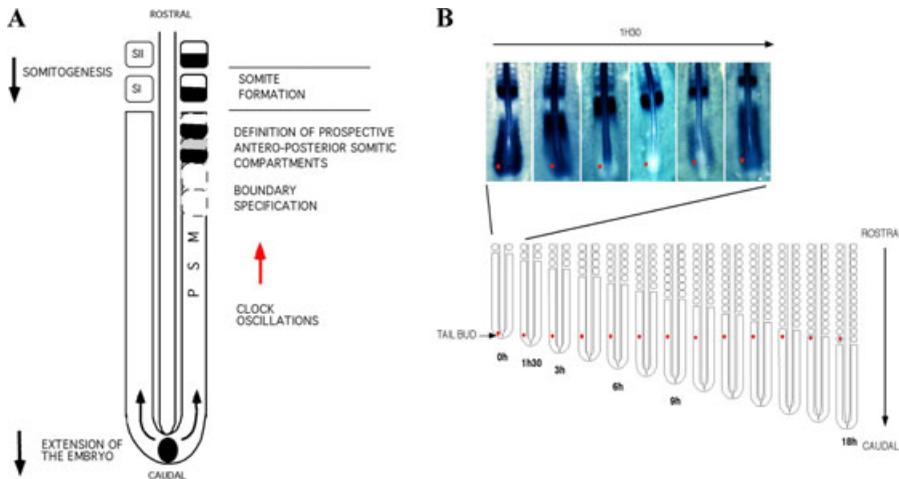


Figure 3. (A) Schematic representation of the caudal part of the chick embryo. The major steps leading to somite formation are indicated. (B) Dynamic and periodic expression of the cyclic genes in the PSM identifies a molecular clock linked to segmentation. Top panel: Sequence of expression of the lunatic fringe mRNA in the PSM of 17-somite-old chick embryos visualized by *in situ* hybridization. This expression appears as a wave sweeping across the whole PSM once during each somite formation, i.e., every 1.5 hours in the chick embryo. PSM cells as exemplified by the red dot will undergo a phase of upregulation of the cycling genes followed by a phase of downregulation of these genes each time a somite forms. Bottom panel: As shown in this schematic representation of the progression of somitogenesis in the embryo, the cycles of expression of the cyclic genes will last while the cells remain in the PSM, which corresponds approximately to the time to form 12 somites in the chick embryo. These PSM cells undergo 12 oscillations of the expression of cycling genes. (Reprinted with permission: Pourquie, O. & K. Kusumi. 2001. When body segmentation goes wrong. *Clin. Genet.* **60**: 409–16.)

fringe (*LFNG*), *DLL3*, or *MESP2*—contribute to CVM phenotypes in humans. These observations support the likelihood that a human segmentation clock is also involved in human somitogenesis.

The identification of three Mendelian forms of SCD enables distinct genetic and to some extent phenotypic classification of SCD due to mutation of *DLL3* (SCD1), *MESP2* (SCD2), or *LFNG* (SCD3).

Vertebral Malformation Syndromes with Known Genetic Etiology

Spondylocostal Dysostosis

Major defining features of spondylocostal dysostosis (SCD) include a loss of normal vertebral morphology throughout the entire spine, with a symmetric thoracic cage and nonprogressive scoliosis. Mutations in Notch signaling pathway genes [*delta-like 1* (*DLL3*); *mesoderm posterior 2* (*MESP2*); and *lunatic fringe* (*LFNG*)] have been found to cause SCD, a heterogeneous group of vertebral malformation syndromes.

SCD1 Is Caused by Mutation of *DLL3*

SCD1 (OMIM 277300) was localized to 19q13.1 using autozygosity mapping in a large consanguineous Arab kindred with seven affected individuals.^{16,17} The pudgy mouse phenotype, which is similarly characterized by severe vertebral and rib defects,¹⁸ has been shown to be due to mutations in *DLL3*.^{19,20} Synteny conversion indicated that the human orthologue for *DLL3* localized to 19q13.1.²¹ Turnpenny and colleagues subsequently identified mutations in *DLL3*.²² This finding represented a watershed in the genetic analysis of vertebral

TABLE 2. *DLL3* Mutations

Protein domain	Truncating mutations/References	Missense mutations
Amino-terminus	215delG, ²³ 395delG ²³	
DSL	602delG, ²⁴ 603ins5, ²² 614ins13, ²⁴ 615del, ²² C207× ²⁴	
EGF-like repeats 1–6	712C>T, ²⁵ 868del11, ²⁴ 945delAT, ²² 948delTG, ²⁴ Q360X, ²³ C362X, ²⁴ 1256ins18, ²³ 1285–1301dup17, ²⁵ 1365del17 ²⁴	C309Y, ²⁶ C309R, ²³ G325S, ²³ G385D, ²² G404C ²³
Juxtamembrane	1418delC ²⁴	
Transmembrane	1440delG ²⁷	G504D ²⁷

malformation, and defined spondylocostal dysostosis type I (SCD1), an autosomal recessive condition associated with severe axial skeletal malformations, including malformed vertebrae and ribs. Numerous mutations in *DLL3* have since been reported (Table 2),^{22–27} mutations that result in truncating and missense mutations. With the one exception of one mutation in the transmembrane domain, all mutations affect the extracellular domain of the gene and are clustered in exons 4–8. SCD1 is characterized by abnormal vertebral segmentation throughout the entire spine. Vertebral bodies appear smooth and rounded with a “characteristic pebble beach sign” on X-ray in early childhood.²⁴ Ribs are malaligned with points of fusion along their length varying in number (Fig. 4). There is an overall symmetry of the thoracic cage, as well as minor nonprogressive scoliosis. Spinal cord compression and associated neurological features are lacking in SCD1, and normal intelligence and cognitive performance are noted.

The Role of *DLL3* in Notch Signaling

DLL3 is a DSL ligand of Notch that was originally isolated following recognition of its expression in the mesoderm and primitive streak during mouse gastrulation.²⁸ Of the three delta-like ligands in mammals, *DLL3* is the most divergent and it lacks EGF-like repeat 2 that is otherwise highly conserved across mammalian DSL ligands.²⁹ A number of other highly conserved residues present in the DSL domains of all other DSL ligands are also absent. Moreover, the intracellular domain of

DLL3 is shorter and highly dissimilar to other DSL ligands.²⁸ Notably, the assumption that *DLL3* activates Notch signaling in trans by binding ligand on the cell surface was found to be erroneous. Instead, a cis mechanism of interaction with Notch was demonstrated, one that results in inhibition of Notch signaling.^{30,31} Further *DLL3* is predominantly expressed in the Golgi apparatus in contrast to other DSL ligands which are expressed on the cell surface.³⁰

Phenotypic Analysis of *DLL3*-Null Mice Defines the Developmental Origin of SCD1

Three *DLL3* mutant alleles have been described: *DLL3^{bu}*, *DLL3^{neo}*, and *DLL3^{oma}*. The *DLL3^{bu}* allele was originally described by Gruneberg.¹⁸ A mutation, identified by Kusumi and colleagues,²⁰ was characterized as a 4-nucleotide deletion from the full length *DLL3* transcript generated from the *DLL3^{bu}* allele in the third exon. This deletion results in a frameshift, and truncation of the *DLL3* protein amino-terminal to the DSL domain.^{18,20} Gene targeting generated the *DLL3^{neo}* null allele in which the DSL, EGF-like repeats, and transmembrane domain were deleted.¹⁹ *DLL3^{oma}* is a spontaneously arising allele whereby a single nucleotide substitution in EGF-like repeat-5 results in a glycine to cysteine conversion at amino acid 409.³² *DLL3*-null mice are easily identified during gestation by virtue of the vertebral column that is drastically shortened along its entire length and exhibits a mixture of irregular fused vertebrae and incompletely developed vertebral bodies interspersed with occasional normal vertebrae (Fig. 5). The adult body

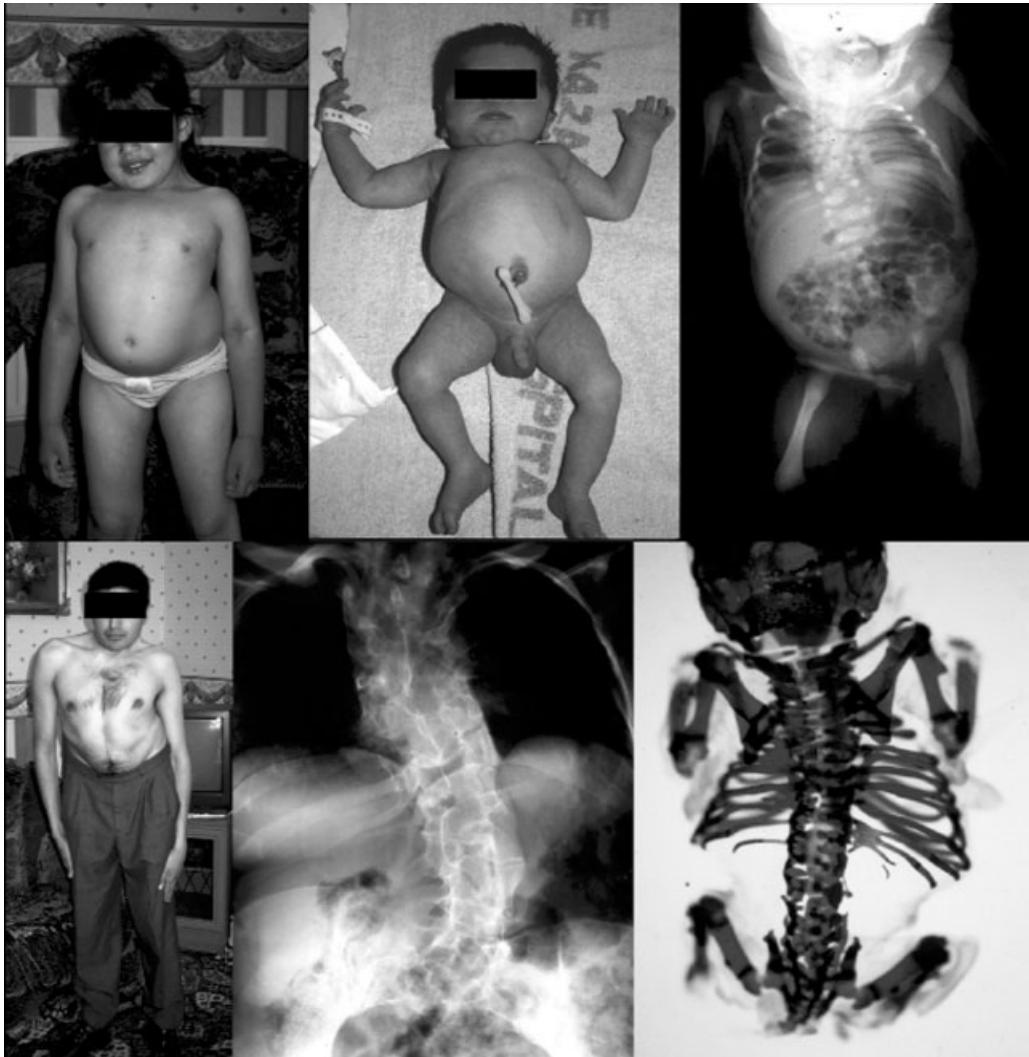


Figure 4. Clinical features of spondylocostal dysostosis. a) An affected child showing short trunk and short stature, with abdominal protrusion. b) An affected newborn showing truncal and neck shortening with secondary abdominal distension. c) Radiograph of an affected infant. The vertebral dysgenesis is most marked in the thoracic region with multiple misaligned ribs. d) Affected homozygous SD adult. e) Radiograph of an affected adult. Individual vertebrae cannot be readily distinguished within a spinal column demonstrating fixed curvatures and restricted movement. f) Vertebral and rib malformations as revealed in neonatal *DLL3^{pu/pu}* mouse. There is no loss of segments, but compressions of the vertebral bodies are evident in the lumbosacral region. Skeletal defects also encompass other sclerotomal derivatives, including bifurcations and fusions of ribs and delayed ossification of the occipital plate. (Reprinted and adapted by permission: Macmillan Publishers Ltd. Bulman, M.P., K. Kusumi, T.M. Frayling, et al. 2000. Mutations in the human Delta homologue, *DLL3*, cause axial skeletal defects in spondylocostal dysostosis. *Nat. Genet.* **24**: 438–41)

is considerably shortened and the tail lacks approximately 20 coccygeal vertebrae. On a mixed 129: C57BL/6 genetic background, approximately 60% of *DLL3* null mice die before weaning. Mortality increases to 100% when the

background is isogenic for C57BL/6 (unpublished results). In *DLL3*-null mutant embryos, somite boundary formation and somite patterning into rostral and caudal compartments is impaired.^{19,20} Lack of somite patterning results

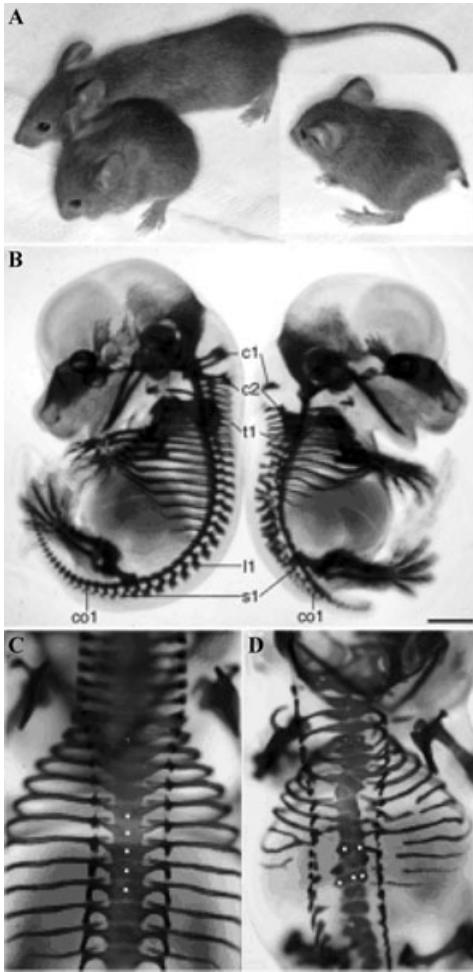


Figure 5. *DLL3^{neo}/DLL3^{neo}* mutants have a truncated body axis and skeletal dysplasia. **(A)** *DLL3^{neo}/DLL3^{neo}* mutants have a shortened body and tail compared with *DLL3/DLL3^{neo}* mice. **(B)** Lateral view of Alcian Blue-stained embryos (14.5 dpc). The positions of vertebrae: cervical (c1 and c2), thoracic (t1), lumbar (l1), sacral (s1), and coccygeal (co1) are indicated. **(C,D)** Dorsal view of developing skeleton. **(C)** *DLL3/DLL3^{neo}* embryo from left in **(B)**. **(D)** *DLL3^{neo}/DLL3^{neo}* embryo from right in **B**. Red dots indicate centrum corresponding to the position of t1, white dots indicate centrum of thoracic vertebrae. Note that in *DLL3^{neo}/DLL3^{neo}* embryos, ossification centers lie two and three in a row instead of lying in column as seen in *DLL3/DLL3^{neo}* embryo **(C)**. Scale bar: 1.35 mm in **B**; 675 μ m in **C**. (Reprinted with permission: Dunwoodie S.L., M. Clements, D.B. Sparrow, *et al.* 2002. Axial skeletal defects caused by mutation in the spondylocostal dysplasia/pudgy gene *DLL3* are associated with disruption of the segmentation clock within the presomitic mesoderm. *Development* **129**: 1795–806.)

in a disorganized arrangement of dorsal root ganglia and spinal nerve axons.^{19,33}

SCD2 Is Caused by Mutation of *MESP2*

SCD2 (OMIM 608681) was localized to 15q using autozygosity mapping in a consanguineous family of Lebanese Arab origin with two affected offspring. Linkage to a 36.6 Mb region on 15q21.3–15q26.1 was demonstrated following fine mapping studies.³⁴ This region contains in excess of 50 genes and exhibits synteny with mouse chromosome 7 in the region containing the *MESP2* gene. The *MESP2* gene, also a target of Notch signaling,²⁶ altered somite polarity and resulted in vertebral column defects.³⁵ Following sequencing of *MESP2* in the two affected siblings, a homozygous 4-bp (ACCG) duplication mutation in exon 1 was detected [*MESP2*, 4-BP, NT500].³⁴ This insertion overlaps the predicted 5' splice site, thus interfering with splicing, and resulting in a frameshift in *MESP2*. The parents were heterozygous for the ACCG duplication, and it was not present in the unaffected sibling. In this family the radiological phenotype is subtly different from SCD1. Unlike SCD1, where all the vertebrae are equally affected, the thoracic vertebrae are severely affected in SCD2, with the lumbar vertebrae only mildly affected (Fig. 6).³⁴ However, as with SCD1, no additional phenotypic manifestations other than those caused by abnormal vertebral segmentation are noted.

The Role of *MESP2* in Notch Signaling

MESP2 is a bHLH-type transcription factor^{35,36} and a direct target gene of Notch signaling. Transcription requires binding and activity of both the Notch-dependent CSL and T-box transcription factor Tbx6.²⁶ *MESP2* expression defines the formation of the somite border by suppressing Notch signaling. Suppression of Notch signaling is accomplished via activation of the glycosyltransferase *LFNG* and the suppression of another Notch ligand, delta-like 1 (*DLL1*).^{37,38} *MESP2* also defines



Figure 6. Anteroposterior MRI of the spine of an individual with SCD2. The morphology of the thoracic spine and the upper cervical spine is severely disrupted, with multiple hemivertebrae, whereas the remaining regions show less severe disruption. (Reprinted with permission: Whittock N.V., D.B. Sparrow, M.A. Wouters, et al. 2004. Mutated *MESP2* causes spondylocostal dysostosis in humans. *Am. J. Hum. Genet.* **74**: 1249–54.)

the anterior identity of the somites by suppressing the expression of *DLL1* and the paired type homeodomain-containing protein *Uncx4.1*, which is essential to development of their posterior identity.^{39,40} Thus, *MESP2* is of central importance in the rostral presomitic mesoderm during somitogenic processes.

Phenotypic Analysis of *MESP2*-Null Mice

MESP1 is separated from *MESP2* by 16kb and is co-expressed with *MESP2* in the rostral presomitic mesoderm.³⁵ Gene targeting generated a null allele of *MESP2* in mice, and those which were homozygous for the mutation do not form epithelial somites. Further, rostrocau-

dal patterning of the somitic mesoderm is disrupted with the loss of rostral properties.^{35,38,41} More recently, another *MESP2* null allele was generated, but this allele disrupted somite formation and patterning only mildly.⁴² The original null allele reduced the expression of *MESP1* gene, and thus the phenotype produced, was due to loss of *MESP2* and subsequent reduction in *MESP1*.⁴² Notably, the impact of the second mutation of *MESP2* in SCD2, also characterized as a null mutation, was limited to the lumbar vertebrae, which appear to be only mildly affected and did not impact *MESP1*.³⁴ That loss of only *MESP2* expression results in less severe vertebral defects has parity with the SCD2 phenotype. However, only two SCD2 patients have been identified to date and conclusions regarding the extent of the vertebral defects must await evaluation of further cases.

SCD3 Is Caused by Mutation of *LFNG*

A common defining feature of SCD1 and SCD2 is that these mutations occur in genes associated with the Notch signaling pathway. It was postulated that mutations affecting other genes associated with Notch signaling might cause SCD. *LFNG* encodes for a glycosyl transferase that modifies the Notch family of cell surface receptors,^{43,44} and it was considered as a good potential candidate gene for causing SCD in the absence of other *DLL3* and *MESP2* mutations. An SCD patient of Lebanese Arab origin had multiple vertebral segmentation defects in the cervical and the thoracic spine with kyphoscoliosis. The thoraco-lumbar spine presented with multiple hemivertebrae and multiple rib anomalies were noted. A homozygous missense mutation (c.564C>A) in exon 3 was detected, which resulted in the substitution of a leucine for phenylalanine (F188L).³³ The proband's consanguineous parents had normal spinal anatomy and were both heterozygous for the mutant allele. The phenylalanine substituted residue is highly conserved⁴⁵ and close to the active site of the enzyme.

The Role of *LFNG* in Notch Signaling

LFNG is a fucose-specific β -1,3-*N*-acetylglucosaminyltransferase that adds *N*-acetylglucosamine (GlcNAc) residues to *O*-fucose on the EGF-like repeats of Notch receptors.^{43,44} *LFNG* localizes to the Golgi, where modification of Notch receptors is believed to occur.⁴⁶ Studies *in vitro* demonstrated that *LFNG* enhances *DLL1*-dependent activation of Notch1, and reduces Notch1 signaling when Jagged1 is the activating ligand.⁴⁷ Paradoxically, in mouse, *LFNG* reportedly acts as a negative regulator of *DLL1*-activated Notch1 signaling.³⁷

Rather than being directly involved in UDP-*N*-acetylglucosamine or protein binding, the conserved phenylalanine (F188) that is substituted by leucine resides in a helix that packs against the strand containing Mn²⁺-ligating residues. This region is in contact with the enzymatic site that undergoes a conformational change following binding of UDP-*N*-acetylglucosamine. The amino acid substitution may interfere with this conformational change.³³ Functional assays demonstrated that the F188L (F187L in mouse) mutant protein did not localize to the Golgi like the wild type protein. Further, it was enzymatically inactive, and was unable to enhance *DLL1*-induced Notch1 signaling *in vitro*.³³ Taken together these data demonstrate that F188L in *LFNG* likely represents a null mutation, and its identification defines SCD3 (OMIM 609813).

Phenotypic Analysis of *LFNG*-Null Mice

LFNG is the only mammalian Fringe protein required for normal somitogenesis.^{48,49} In *LFNG*-null mouse embryos, somite formation is abnormal, anterior-posterior somite identity is disorganized, and severe axial skeletal defects are noted including reduced numbers of caudal vertebrae. These defects are reminiscent of those present in *DLL3*-null embryos.⁴⁹ Loss of *LFNG* function in mouse has also been

associated with defects in the inner ear,⁵⁰ and the ovary.⁵¹

The identification of mutations in three components of the Notch signaling pathway demonstrates the importance of Notch in vertebral column formation in humans. Targeted mutation in mouse, resulting in loss of gene function, has produced mouse models of SCD. Mice that lack *DLL3*, *MESP2*, or *LFNG* display abnormal somite formation and patterning. These deficiencies in somitogenesis represent the origin of the defects in vertebral segmentation. Vertebral column anomalies vary with SCD type, promoting the possibility of an existent correlation between the genotype and phenotype. There are currently insufficient data in humans to support comparison, since only single cases for SCD type 2 and SCD type 3 have been reported.

Spondylothoracic Dysostosis

Jarcho and Levin⁸ initially described a pair of siblings with a form of SCD characterized by widespread vertebral segmentation abnormalities, short trunks, and malalignment and points of fusion of the ribs. Early death occurred, apparently due to pulmonary insufficiency secondary to the small thoracic cage. Since this initial description, the eponym has been applied variously, either as an entity in keeping with Jarcho and Levin's original description or as an umbrella term encompassing a broad range of conditions. For example, subdivision of JLS into SCD and spondylothoracic dysostosis (STD) has been proposed.⁵² Others have subdivided the "Jarcho–Levin phenotype" into SCD, STD, JLS, and Casamassima–Morton–Nance syndrome.^{53–55} The term "Jarcho–Levin syndrome" (JLS) has also commonly been applied to any condition of short trunk dwarfism in which the radiological phenotype includes vertebral segmentation/formation defects and rib abnormalities.^{56–58} This incorrect and



Figure 7. X ray from a subject diagnosed with spondylocostal dysostosis type 1 (SCD1). Ribs are malaligned with a variable number of points of fusion along their length. Note “tram-like” appearance of spinal column, with vertebral segmentation defects distributed throughout the spinal column. (Courtesy of Alberto Santiago Cornier, MD, PhD)

inconsistent application of nomenclature has introduced ambiguity to genetic counseling of families. Solomon and colleagues⁵⁹ proposed usage of the term “STD” when fusion of all ribs at the costovertebral junctions is present bilaterally with a crab-like appearance, and vertebral segmentation and formation defects throughout were noted along the entire length of the spine (Fig. 7). This phenotype contrasts with SCD, where asymmetry of rib length and alignment occurs, and points of intercostal fusion are seen variably along the length of the ribs. STD-associated anomalies include imperforate anus, genitourinary abnormalities, diaphragmatic hernia, choanal stenosis and cleft palate.⁵² Computed tomography (CT) reconstruction studies have demonstrated malformed vertebral bodies with increased coronal diameter and decreased sagittal diameter closely approximating a “sickle-shape” configuration.⁶⁰ A high degree of consanguinity along with a founder effect has been reported pointing to a high prevalence in Puerto Rico. A homozygous recessive nonsense mutation in *MESP2*, E103X has been identified in 11 patients of Puerto Rican ancestry, providing fur-

ther evidence of a founder effect in STD among individuals of Puerto Rican descent.⁶¹ Mortality reached 44% in a series of 27 cases, in which death occurred within the first 6 months of life, secondary to pulmonary complications. Patients surviving beyond this period reportedly had minimal medical complications.

Alagille Syndrome

Alagille syndrome is an autosomal dominant condition that is associated with bile duct paucity and abnormalities of the heart, eye, kidney, pancreas, and skeleton, and in facial dysmorphism.⁷ The most common cardiac abnormality observed is pulmonic stenosis.⁶² Facial features noted include broad forehead with deeply set eyes, moderate hypertelorism, pointed chin, and straight or saddle-shaped nose with broadened nasal tip.⁶³ Ophthalmologic abnormalities commonly include anterior chamber defects such as the persistence of posterior embryotoxon, Axenfeld anomaly, Reiger anomaly, and retinal pigment changes.⁶⁴ Butterfly vertebral anomalies, which appear as vertebral clefts, represent errors in somitogenesis and are observed in 22–87% of affected individuals.

Mutations in *JAG1*⁶⁵ have been identified in approximately 70% of patients with Alagille syndrome. Mutations in a second gene, *NOTCH2* have been observed in patients with Alagille syndrome with severe renal manifestations.⁶⁶ These mutations include missense, truncating, small and entire gene deletions, and nonsense and splicing mutations. *JAG1* is a ligand of the Notch receptor and is central to developmental regulation. Upon ligand binding, Notch undergoes proteolysis and releases a C-terminal fragment that translocates to the nucleus resulting in target gene transcription. Interaction between Jagged1 and Notch2 is supported by the observation that double heterozygous mice for the *Jag1* null allele and a *Notch2* hypomorphic allele show phenotypic abnormalities consistent with Alagille syndrome.⁶⁷

Teratogenic Causes of Vertebral Malformations

Human vertebral malformations have been reported in association with alcohol,^{68,69} anticonvulsant medications including valproic acid⁷⁰ and dilantin,⁷¹ hyperthermia,⁷² and maternal insulin-dependent diabetes mellitus.⁷³ To date, no epidemiological studies have been performed to evaluate the nature of the association between environmental teratogens and vertebral malformations.

The similarities between spinal cord anomalies in humans and neural tube defects in mice with congenital scoliosis (CS) described in the preceding sections promote the premise that similar etiological mechanisms may be involved in the emergence of these malformations. Folic acid use has been associated with the prevention of neural tube defects.⁷⁴ Further studies are required to determine the possible effects antenatal folic acid use may have on prevention of the development of CVM.

Vertebral malformations have been reported in Sprague–Dawley rat fetuses exposed to I(Kr)-blockers (class III antiarrhythmic agent) *in utero*.⁷⁵ 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.⁷⁶ Possible mechanisms for vertebral malformation include alteration of expression of homeobox genes or Sonic hedgehog by carbon monoxide; or direct action of carbon monoxide on the cartilaginous skeleton.

Rats exposed to boric acid on day 9 of gestation develop 6 cervical vertebrae, rather than the normal number, 7.⁷⁷ This malformation, presumably mediated by boric acid, has been associated with an alteration of *HOX* gene expression pattern.

The influence of various teratogens on the expression of genes that influence vertebral development has not been well-studied. One pos-

sible model for the development of vertebral malformations is multifactorial. An improved understanding of possible environmental influences on vertebral development may enable more informed genetic counseling for families.

Progress in the Understanding Etiology of other Syndromic and Non-syndromic Vertebral Malformations

Congenital Scoliosis

Congenital scoliosis represents a spinal curvature of 10° or greater, detected by radiograph,⁷⁸ that is caused by vertebral segmentation defects. Classification of CS by orthopedists is based on the underlying segmentation defects, which include fused vertebrae, vertebral body formation defects such as hemivertebrae, and mixed defects in which both types of lesions are encountered. These defects underlie development of a spinal curve resulting from asymmetric growth. The severity of the curve is related to the type of defect, and any compensatory developmental changes that may have arisen in response to the primary defect.

Once CS is identified on clinical and radiological examination, evaluation by a pediatric orthopedic surgeon is indicated. Approximately 50% of patients with CS ultimately require surgical correction because of curve progression.⁷⁹ 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.

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.⁷⁹ Commonly associated abnormalities are noted in the nervous, urogenital, gastrointestinal, and cardiovascular

systems. Specific abnormalities that have been found in association with CS include: esophageal atresia, tracheoesophageal fistula, diastematomyelia and other congenital spinal anomalies, anal atresia, Sprengel deformity, facial asymmetry, and bladder and cloacal exstrophy.^{80–82} Additional associated conditions include: 1) Klippel–Feil syndrome (phenotype: short neck, low posterior hairline, fusion of cervical vertebrae); 2) Goldenhar syndrome (phenotype: associated with craniofacial anomalies, including microtia and epibulbar dermoids due to abnormal branchial arch development); 3) incontinentia pigmenti (phenotype: hyperpigmented whorls and streaks associated with eye, skin, hair, nail, teeth, and central nervous system abnormalities); 4) VACTERL association (phenotype: vertebral malformations, anal atresia, cardiac malformations, tracheoesophageal fistula, renal and radial anomalies, and limb defects); or 5) other recognizable syndromes.

Genitourinary abnormalities have been reported to occur in 37 of 85 (43%) patients with CS.⁸³ Since the genitourinary system and vertebral column both share a mesodermal 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 rate of renal and ureteral abnormalities in patients with CS has been reported.⁸⁴ A tenfold increase in renal ectopia was observed in an animal model following induction of CS by a surgical technique in chicks.⁸⁵ Mechanisms postulated to explain the association between CS and renal anomalies include: 1) failure of spinal growth which interferes with normal renal ascent, 2) teratogenic agents, and 3) intrinsic genetic defects involving gene(s), which may interfere with pathways involved in renal and vertebral embryogenesis.

Some evidence suggests that the development of organ malformations occur at the level of the body segment at which vertebral malformations occur.⁵⁵ 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.

Congenital scoliosis cases have been analyzed for mutations in the *SCD1* gene: *DLL3*.⁸⁶ Examination of *DLL3* in 46 patients with congenital scoliosis identified a novel missense variant, S225N, which was highly conserved among human, mouse, rat, and zebrafish *Delta* genes. This study suggested that while heterozygous mutations in *DLL3* were not a major cause of CS, the presence of novel variant alleles such as S225N may be associated as a minor contributing factor. Analysis of associations with other-organ involvement in a cohort of 31 non-syndromic patients confirmed that other organ systems were affected during somitogenesis, a finding based on observation of the following frequency of occurrence: 19% cardiac, 16% renal/genitourinary, and 10% neurological associations.

There is evidence that congenital and idiopathic scoliosis (IS) may be attributable to different alleles at the same genetic locus. Interestingly, among 237 families who had at least one case of known CS, 17.3% reported having members with IS⁸⁷ (Fig. 8). This report was further supported in a case series of subjects with CS, where 3 of 31 congenital scoliosis cases confirmed having first degree relatives with diagnosed idiopathic scoliosis.⁸⁶ These findings point to the possibility 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. Since idiopathic scoliosis is defined by exclusion of neuromuscular or congenital scoliosis, this category likely represents a heterogeneous group. One possible explanation for CS and IS cases within a family may be presence of undetected anomalies in the IS cases. Congenital scoliosis is diagnosed when an ossified tissue malformation is observed in radiographs of infants. Cartilaginous or soft-tissue defects would not be easily detected.

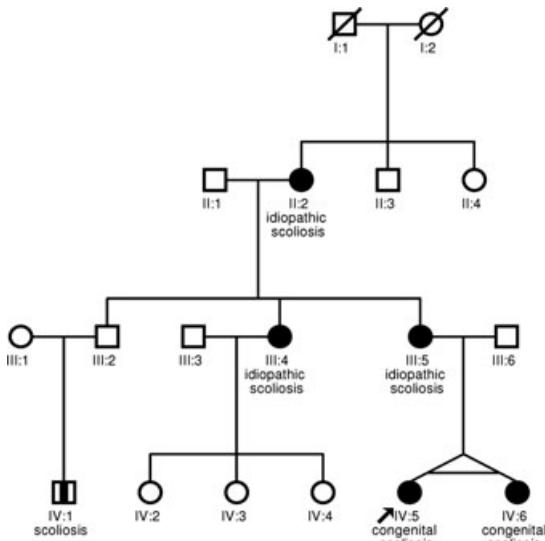


Figure 8. Family pedigree of members affected with congenital and idiopathic scoliosis. (Reprinted with permission: Purkiss S.B., B. Driscoll, W.G. Cole, *et al.* 2002. Idiopathic scoliosis in families of children with congenital scoliosis. *Clin. Orthop. Relat. Res.* **401**: 27–31.)

Recent genetic studies of idiopathic scoliosis also point to another possible shared underlying mechanism, i.e., IS may result from gene expression changes in a number of developmental genes. A role for genetic factors has been established in idiopathic scoliosis, and a number of studies have been carried out using candidate gene and genome-wide linkage analysis approaches (reviewed in Miller).⁸⁸ A recent study of 52 multiplex families with idiopathic scoliosis has refined a susceptibility locus to 8q12, centering over exons 2–4 of the chromodomain helicase DNA-binding protein 7.⁸⁹ Resequencing of *CHD7* in these families identified functional polymorphisms of the *cdx* (caudal type). A transcription factor-binding site that are significantly over-transmitted to affected progeny.⁸⁹ The *CHD7* gene is also associated with the CHARGE (coloboma, heart anomalies, choanal atresia, retardation, genital, and ear anomalies) syndrome, which is characterized by defects in a number of organ systems. In addition, CHARGE patients display a high rate (> 60%) of late-onset idiopathic scoliosis.⁹⁰ CHD proteins bind to promoter elements, reg-



Figure 9. Lateral neck radiograph of patient demonstrating cervical segmentation abnormalities.

ulating chromatin structure and gene expression. Thus, the IS associated polymorphisms observed in regulatory sites of the *CHD7* gene may affect a number of developmental genes, including those regulating the formation and growth of the musculoskeletal elements of the spine, and may potentially include genes associated with congenital scoliosis.

Klippel–Feil Syndrome

Klippel–Feil syndrome is a disorder of the cervical spine characterized by faulty segmentation of vertebrae in the cervical region of the spinal column. This condition was first reported in 1894,⁹¹ followed by Klippel and Feil's description of a French tailor with massive cervical fusion who died of renal disease.⁹² Phenotypically, the patient presented with a short neck, a low posterior hairline, and limited range of motion of the neck (Fig. 9). Klippel–Feil syndrome is clinically heterogeneous. The majority of cases represent sporadic occurrences within a family. However, Klippel–Feil syndrome may

represent a familial occurrence with multiple family members affected. Autosomal dominant, autosomal recessive, and X-linked forms of Klippel–Feil syndrome have been reported as described in Table 3.^{9,92–96} Multiple classification schemes have been proposed and are described in Table 3. Klippel–Feil syndrome may also be associated with additional multi-organ system malformations, including neural tube defects, thoracic cage abnormalities, pulmonary, cardiovascular, and other skeletal anomalies, genitourinary abnormalities, myopathy, neuropathy, and cognitive disorders (reviewed in Tracy *et al.*).⁹⁷ Various chromosomal abnormalities have been reported to occur in association with Klippel–Feil syndrome as indicated in Table 3.

The findings reported in Table 4^{98–101} are important because they may help to facilitate the identification of candidate genes for Klippel–Feil syndrome within the region of the inversion or translocation of the involved chromosome(s). For example, located within the breakpoints of 5q35 and 8p21.1 lie genes expressed in skeletal tissues, which include *SLIT3* (slit homolog 3); *FBXW11* (F-box and WD-40 domain protein 11); *DUSP1* (dual specificity phosphatase 1); *FGF18* (fibroblast growth factor 18); and *DC-UbP* (dendritic cell-derived ubiquitin-like protein) on 5q35; and *CDCA2* (cell division cycle associated 2) on 8p21.¹⁰¹

PAX1 has been studied as a candidate gene for Klippel–Feil syndrome.¹⁰² The *Pax1* gene was shown to be active during sclerotome formation and differentiation. *Pax1* mutations have been identified in the mouse *undulated*, suggesting that sclerotome condensation is a *Pax1*-dependent process.¹⁰³ In the mouse mutant *undulated*, medial sclerotome condensation fails to occur at the lumbosacral level, thus preventing formation of intervertebral discs and vertebral bodies. Among 63 patients diagnosed with Klippel–Feil syndrome who were studied for mutations in *PAX1*, 3 patients who exhibited significant physiological impact were detected. Of these three patients, one patient and the asymptomatic mother of the patient revealed a

Pro224Ala missense mutation. A mode of inheritance involving reduced penetrance could be postulated if this mutation can be shown to promote causation.

Candidate Gene Approach to Understand Genetic Etiology of Isolated Vertebral Malformation Disorders

Since isolated CVM most often represents a sporadic occurrence within a particular family, traditional linkage analysis is not a viable approach to identifying causative genes. Instead, candidate gene analyses offer a reasonable alternative method of study. Based on mouse–human synteny analysis, 27 eligible loci, 21 of which cause vertebral malformations in the mouse, have been identified.^{21,104}

Extrapolating from the murine models, six candidate genes have been chosen for analysis to date in a phenotypically well-defined cohort of patients with CVM.^{105–108} The vertebral malformations represented among this cohort spanned the length of the entire spine and are described in greater detail in an earlier paper.¹⁰⁸

The six candidate genes studied are briefly described below.

- 1) *PAX1*: *PAX1* has three mutant alleles (*undulated*, *undulated-extreme*, and *undulated-short tail*) that have been described in the mouse. Scoliosis, split, and fused vertebrae and hypomorphic intervertebral discs are features seen most prominently in the lumbar regions.¹⁰⁹ *PAX1* therefore appears to be important in specifying ventromedial differentiation of the sclerotome. In humans, *PAX1* mutations have been reported to occur in 6 of 63 patients with Klippel–Feil syndrome.¹⁰²
- 2) *WNT3A*: *WNT3A* is necessary for generation of the posterior portion of the neuraxis, since knockout mice fail to develop a tail bud and are truncated from a point slightly anterior to the hindlimbs.³² This gene is a member of a

TABLE 3. A Summary of Clinical Features Associated with Klippel–Feil Syndrome (with citation)

Reference	Cervical fusion abnormalities	Thoracic fusion abnormalities	Lumbar fusion abnormalities	Other malformations	Inheritance
Klippel ⁹²	Short neck, low posterior hairline. Absence of cervical vertebrae				Sporadic
Feil ⁹³	Group I: massive fusion of many vertebrae Group II: fusion of one or two cervical interspaces Group III: cervical	Groups I and III	Group III	Sprengel deformity Type II (failure of proper scapular descent)	
Heisenger ⁹⁴	Type I: C2–C3 fusion with occipitalization of the atlas Type II: Long cervical fusion with an abnormal occipitocervical junction Type III: Two blocked vertebral segments with a single open interspace KF1: C1 fusion is the most rostral	NA	NA	KF1-cardiac, urogenital, hearing, craniofacial, limb, digital, ocular defects KF2-craniofacial, hearing, otolaryngeal, skeletal and limb defects KF3-craniofacial abnormalities	KF1: Autosomal recessive KF2: Autosomal dominant KF3: Autosomal recessive and autosomal dominant
Clarke ⁹⁵	KF2: C2-C3 fusion is dominant and most rostral KF3: C3 (C2-3 or C3-4) most rostral fusion. Isolated fusions	NA	NA	KF4-Wildervank syndrome (Duane retraction syndrome, characterized by narrowed palpable fissure, globe retraction and failure of abduction of eye + sensorineural hearing loss)	KF4: X linked dominant
Manaligod ⁹⁶	Cervical fusion	Thoracic fusion (isolated)	NA		
Samartzis ⁹	Type I: Single congenital fused cervical segment Type II: Multiple noncontiguous congenitally fused segments Type III: Multiple contiguous, congenitally fused cervical segments	NA	NA		

TABLE 4. Chromosome Abnormalities Associated with Klippel–Feil Syndrome

Citation	Chromosome abnormality
Clarke <i>et al.</i> ⁹⁸	Pericentric inversion inv(8) (q22.2q22.3) Four generation family with autosomal dominant Klippel–Feil syndrome
Fukushima <i>et al.</i> ⁹⁹	De novo balanced reciprocal translocation t(5;17)(q11.2;q23)
Papagrigorakis <i>et al.</i> ¹⁰⁰	De novo pericentric inversion inv(2)(p12q34)
Goto <i>et al.</i> ¹⁰¹	Translocation t(5;8)(q35.1;p21.1)

moderate-sized multigene family comprising at least 12 members in humans and the mouse. Genes in this family have a dual function: they a) establish the body plan during development and b) are potentially oncogenes.³² *Wnt3a* has been proposed as a major regulatory gene in the oscillation of Notch signaling essential to segmentation processes.¹¹⁰

- 3) *DLL3*: Mutations in *DLL3* are responsible for the *pudgy* mouse phenotype, which is characterized by severe vertebral and rib defects described earlier.³²
- 4) *SLC35A3*: The naturally occurring complex vertebral malformation syndrome seen in cattle is a bovine model of human vertebral malformations and is caused by a mutation in the gene for *SLC35A3*.¹¹¹ *SLC35A3* encodes a UDP-N-acetylglucosamine (UDP-GlcNAc) transporter. A single base transversion of guanine to thymine at position 559 in the *SLC35A3* gene on bovine chromosome 3 results in a valine to phenylalanine substitution at residue 180 in affected cattle and carriers.¹¹²

Complex vertebral malformation is a recessive lethal condition occurring in both female and male calves.¹¹³ Stillborn, aborted, and preterm calves display shortened cervical and thoracic regions of the vertebral column, bilateral symmetrical contraction of the metacarpophalangeal and metatarsophalangeal joints, and symmetrical arthrogryposis. Vertebral malformations include fusion of the last two cervical vertebrae and distortion of the first three thoracic vertebrae, resulting in

scoliosis. Additional malformations noted in association with CVM include cardiac anomalies, cleft palate, epibulbar dermoids, micrognathia, and ventrally displaced ears.¹¹³

- 5) *T (Brachyury)*: The *T* gene is a transcription factor that plays an essential role in mesodermal development.¹¹⁴ Mutations of *T* have been observed in mice for 80 years and identified as causes of dominant brachyury and recessive lethality at approximately 10 days gestation.^{115,116} *T* is located within a complex chromosomal rearrangement with several *T* alleles representing duplications and deletions, a location that facilitated its cloning.¹¹⁷ The *T* protein is transiently expressed in the nuclei of nascent mesodermal cells, which migrate from the primitive streak, gut endoderm, and neuroectoderm, but the *T* protein is persistently expressed only in the notochord and tail bud.¹¹⁸ The protein is a transcription factor with its DNA-binding domain lying within a conserved protein domain, the T-box.¹¹⁹ The T-box is located within the N-terminal of the protein and is highly conserved during evolution in both vertebrates and invertebrates. Although the C-terminal half of *T* displays far less sequence conservation than does the T-box, it nevertheless maintains functional conservation in mediating transcriptional activation in both *Xenopus* and *Danio*.¹²⁰
- 6) *TBX6*: Homologies within the T-box have defined a family of related proteins. One of these, *Tbx6*, is associated with rib

TABLE 5. Mutations Identified in Candidate Genes for CVM

Gene	Phenotypic features	DNA	Amino acid	Frequency
<i>PAX1</i>	T 11 wedge	c.1229C>T	Pro410Leu	1/170
	Multiple thoracic and lumbar CVM, VSD, polydactyly	c.1238C>T	Pro413Leu	6/2312
<i>DLL3</i>	T5–T6 Block, VACTERL	c.805G>A	Gly269Arg	0/174
<i>WNT3A</i>	Supernumerary hemivertebra between T12 and L1	c.400G>A	Ala134Thr	3/890
<i>SLC35A3</i>		None		
<i>TBX6</i>		None		
<i>T</i>	Sacral agenesis	c.1013C>T	A338V	0/886
<i>T</i>	Klippel–Feil			
<i>T</i>	Multiple cervical and thoracic vertebral malformations			

fusions, spinal arch fusions, and vertebral body irregularities in mice homozygous for the *Tbx6^{rv}* allele.¹²¹

DNA sequence analysis was undertaken and spanned the coding sequence, splice junctions, and promoter region of each candidate gene described above. Potentially pathogenic DNA sequence variants detected and corresponding phenotypic information are summarized in Table 5. No mutations in *TBX6* were observed in the patient cohort studied by Ghebranious and colleagues.¹²²

Of the sequence variants observed, the A338V allele of *T* displayed the most compelling evidence of pathogenicity, because of its statistically significant association with CVM ($P = 9.87 \times 10^{-4}$, Fisher's exact test). Its presence in three unrelated individuals, its absence in a large reference population, and evolutionary conservation of the altered amino acid all support its biological importance.¹²² This variant lies within the second transactivation domain.¹²² The vertebral malformation phenotypes differed among the patients and in each family, and, notably, in a phenotypically unaffected parent also harbored the mutant allele.¹²²

Papapetrou and colleagues previously identified the c.1013C>T mutation in a patient with sacral agenesis and in a clinically unaffected parent. These investigators did not encounter the mutation in their reference population, which included 60 individuals.¹²³ These

observations suggest a widened clinical spectrum for *T* mutations.

Each of the observed sequence variants in the other candidate genes studied, *PAX1*, *DLL3*, and *WNT3A*, was additionally observed in one of the proband's clinically asymptomatic parents, in whom no radiographic abnormalities were detected. The presence of the c.1013C>T variant in 3 of 50 unrelated subjects with CVM, in conjunction with its absence in a large reference sample, is strongly suggestive that mutations of *T* substantially increase the risk of CVM. The presence of the c.1013C>T alteration in affected subjects' phenotypically normal parents demonstrates that the change alone is not sufficient by itself to cause CVM. Since expression of *T* is distributed throughout the notochord, it is possible for the phenotypic manifestation of a mutation to occur anywhere along the vertebral column. This is exemplified by the observation that *T* mutations in mice affect not only the tail, but also more anterior vertebral segments,^{124,125} spinal cord,^{124,126} genitourinary system,^{124,127} and heart.^{128,129} An association between a C to T transition in intron 7 of *T* and increased risk of neural tube defects^{130–132} has been reported by some investigators, but this finding has not been uniformly reproduced.^{133–135} The potential range of mutant *T* phenotypes should be broadened to include neural tube defects, as well as CVM. A “multihit” kinetic hypothesis for CVM¹³⁶ would account for the lack of phenotypic expression in the parents harboring the

TABLE 6. Classification of SDV according to Mortier et al.⁵⁵

Nomenclature	Definition
Jarcho–Levin syndrome	Autosomal recessive Symmetrical crablike chest, lethal
Spondylothoracic dysostosis	Autosomal recessive Intrafamilial variability, severe/lethal Associated anomalies uncommon
Spondylocostal dysostosis	Autosomal dominant Benign
Heterogeneous group	Sporadic Associated anomalies common

c.1013C>T mutation and explain why most CVM represent a sporadic occurrence. An environmental or additional genetic or epigenetic alternation could represent the second event. The locus affected by a putative second genetic lesion, or the time at which a putative environmental second event occurred, could determine the level and severity of the resulting phenotype. The observation in zebrafish that morpholino-induced knockdown of Hsp90 during early development altered the phenotypic consequences of various eye mutants supports this hypothesis.¹³⁷ Phenotypic expression of mutations in other genes active during somitogenesis, which would normally be buffered by normal T protein, could be mediated by decreased T function.

Radiographic Classification of Syndromic and Non-syndromic Vertebral Malformations

A systematic classification scheme of vertebral malformation disorders is necessary in order to facilitate further advances in the discovery of mutations in genes associated with the development of vertebral malformations. Several classification systems have been proposed during the past two decades. As illustrated in Table 6, an early classification scheme by Mortier and colleagues,⁵⁵ which was based on a review of 26 patients with radiographic

data, recognized 3 familial clinical entities, including JLS, spondylothoracic dysostosis, and spondylothoracic dysostosis. A heterogeneous fourth group of patients with nonfamilial vertebral malformations, some of whom had malformations in related organ systems, was included. McMaster and Singh's² classification scheme, shown in Table 7, addressed disorders of vertebral formation and segmentation. It is possible for individuals to present with mixed types of defects.

There has been inconsistency in the literature regarding the use of the term “Jarcho–Levin syndrome” (JLS). Initially described in 1938 referencing two siblings with short trunks, asymmetric rib abnormalities, and vertebral segmentation defects, JLS has been variably applied. In some instances JLS has been subdivided into SCD and STD.^{52,138} Other reports documented in the literature further subclassified into the “Jarcho–Levin phenotype” into SCD, STD, JLS, and Casamassima–Morton–Nance (crab-like thoracic cage in association with urogenital abnormalities) syndrome.^{53–55} The “most-widely inclusive” use of JLS has been to describe any condition involving short trunk dwarfism and radiographic anomalies that include vertebral formation and segmentation defects and rib abnormalities.^{57,58} The terms “Jarcho–Levin syndrome,” costovertebral/spondylocostal/spondylothoracic, and dysostosis/dysplasia have all been used interchangeably to describe segmentation abnormalities of the spine and ribs. These conditions are more appropriately referred to as “dysostoses” rather than “dysplasias”^{139–141} since a dysostosis represents a morphological error in blastogenesis, whereas a dysplasia represents a developmental (prenatal) and ongoing (postnatal) abnormality of chondro-osseous tissues.

Figure 10 summarizes the proposed classification system by the International Consortium for Vertebral Anomalies and Scoliosis (ICVAS). Discontinuation of use of the term “Jarcho–Levin syndrome” is recommended because of its indiscriminate usage. However,

TABLE 7. Classification (Surgical/Anatomical) of Congenital Kyphosis/Kyphoscoliosis, according to McMaster²

Type	Anatomical deformity	Anomalies
I	Anterior failure of vertebral body formation	Posterolateral quadrant vertebrae – single vertebra – two adjacent vertebrae Posterior hemivertebrae – single vertebra – two adjacent vertebrae Butterfly (sagittal cleft) vertebrae Anterior or anterolateral wedged vertebrae – single vertebra – two adjacent vertebrae
II	Anterior failure of vertebral body segmentation	Anterior unsegmented bar
III	Mixed	Anterolateral unsegmented bar
IV	Unclassifiable	Anterolateral unsegmented bar contralateral posterolateral quadrant vertebrae

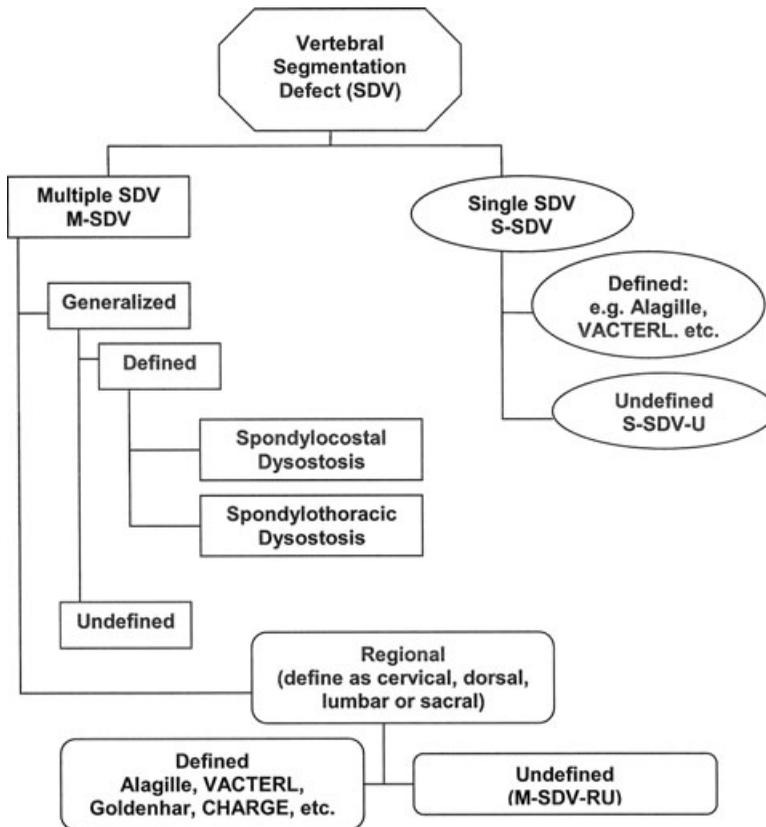


Figure 10. ICVAS proposed clinical classification system.

well-established eponymous syndromic associations that refer to a well-described phenotype such as Alagille syndrome should be sustained. Vertebral malformation(s) may occur singly or in multiples. A single vertebral malformation may be categorized as “defined” if the patient meets clinical criteria for a given syndrome. If clinical criteria for a particular syndrome are not met, an alternative designation of “single segmentation defect of vertebra-undefined” (S-SDV-U) should be applied. For multiple vertebral segmentation defects an assessment needs to be made in order to determine whether the corresponding pattern is “generalized” (10 or greater contiguous vertebral segmentation defects) or represents a more regional focal spinal distribution. Generalized contiguous multiple segmentation defects may be defined and fulfill diagnostic criteria for SCD or STD, or may be “undefined” and designated as M-SDV-GU. Multiple regional vertebral defects may, in association with clinical features, fulfill diagnostic criterion for a known syndrome or may be “undefined” and designated as M-SDV-RU.

Advantages of utilizing this classification system include the ability to classify vertebral malformations in broader categories, obviating the need for a clinician to be overly concerned about identifying the exact spinal level of a particular vertebral malformation, which could vary from one observer to another. This system was built on the foundation of prior classification system and allows for the classification of patterns of vertebral malformations. Reviewer comments pertaining to vertebral and rib morphology, thoracic cage structure, and presence or absence of scoliosis may be recorded in the appropriate box beneath the corresponding classification category. This system should facilitate communication among clinicians including radiologists, orthopedic surgeons and clinical geneticists who provide medical management for a particular patient. The ICVAS system is simple and has flexibility for modification, in response to advances in the field.

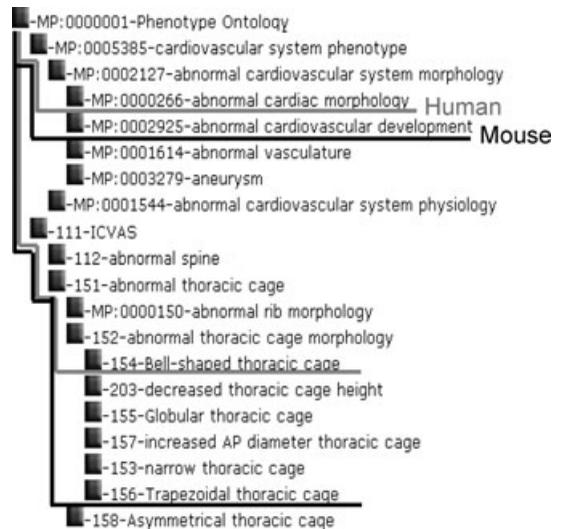


Figure 11. Interspecies phenotype comparisons.

In addition to its clinical application, the ICVAS system represents a useful research tool for predicting potential candidate genes for human vertebral malformations on the basis of ontological interspecies phenotype comparisons, as illustrated in Fig. 11. Through classification of diagnostic phenotypic groupings of vertebral malformations utilizing the ICVAS system, corresponding phenotypes for vertebral malformations in other species can be identified. Associated genes with a species-specific genotype can be studied in humans as potential vertebral candidates.

Pilot testing among 5 ICVAS members was undertaken using 10 radiographs of diverse phenotypes that were previously unseen and therefore not discussed by the group. In addition, the same 10 radiographs were submitted to 7 radiologists who had no involvement in developing the classification system. They initially provided descriptions and diagnoses without knowledge of the new system, and again after being shown the new system. The results (submitted) indicate that the system helped to significantly increase reporting consistency and, subjectively, the invited radiologists found the new system relatively easy to use.

Directions to Understanding Etiology of CVM

Microarray technologies have allowed for genome-wide analysis of changes in expression level during somitogenesis and in mouse mutant models. These functional genomic studies have identified genes that are highly expressed in the somites and presomitic mesoderm,¹⁴² and genes that display oscillatory expression in mouse embryos¹⁴³ and in mouse and human cell culture models.¹⁴⁴ These microarray studies have identified hundreds of potential oscillatory genes in multiple signaling pathways, including the Notch, Wnt, and Fgf pathways. In addition, the segmentation oscillatory period for human cells has finally been estimated at 5 hours.¹⁴⁴ There are many targeted mutations in developmental genes associated with somitogenesis, and microarray analysis of mutant embryos has also identified key genes that are disrupted.^{145,146} Altogether, the list of potential candidate genes for genetic studies of congenital and idiopathic scoliosis has been greatly expanded from these recent functional genomic studies.

Linkage analysis applied to large families with affected members who exhibit vertebral malformation phenotypes is another important approach for identification of loci associated with vertebral malformations. Unfortunately, large families with multiple affected individuals with vertebral malformations are difficult to identify in clinical practice. Array-based CGH is a technique that was developed in order to determine alterations in dosage distribution of small DNA segments throughout the entire genome.¹⁴⁷ Array-based CGH has also successfully applied to identify a major gene, CHD7, which is responsible for CHARGE syndrome described above.¹⁴⁸ Array-based CGH allows detection of regions exhibiting microaneuploidy in association with vertebral malformations across the entire length of the genome. Epigenetic modification, such as DNA methylation may also contribute towards the development of CVM. Additional studies ex-

amining the methylation patterns in genes associated with vertebral malformations in humans would be necessary to provide evidence for DNA methylation as a contributing factor in the development of vertebral malformations.

Translational Value of Expanded Understanding

Improved understanding of the factors contributing to CVM occurrence can contribute to improved management of children suffering from their consequences. A better means to associate patterns of vertebral malformations with a genetic marker could help to optimize management for children with vertebral defects.

There are often challenges in the management of children with CVM. For example, clinical circumstances that flag a need to screen for existence of concomitant birth defects have not been defined. If a genetic marker is identified which could differentiate children who suffer exclusively with CVM and have no other concomitant birth defects, this would represent a potential cost saving for the family because these children do not require an extensive work up. Screening could be limited to children with CVM and other birth defects. Orthopedic follow-up for children with CVM is often limited to watchful waiting, with the child and family subjected to multiple spinal X rays and visits to the orthopedic surgeon to assess status of spinal curvature.

The occurrence of CVM has been linked to environmental factors impacting the mother such as maternal alcohol, anticonvulsant medication, hyperthermia, and diabetes. Not all fetuses exposed to these environmental agents will develop CVM. If genetic factors associated with metabolism or interaction with environmental factors that are unique to mothers at high risk for pregnancy resulting in offspring with CVM can be identified, exposures may be minimized. For example discontinuing the use of a particular anticonvulsant medication

such as dilantin or valproic acid several months prior to conception in mothers genetically predisposed to high-risk pregnancies may decrease incidence of the CVM and related defects.

Presently, genetic counseling for families who have had a child with CVM is problematic. While isolated CVM and those other genes associated with birth defects in which an underlying syndrome has not been identified usually represent a sporadic occurrence within a particular family, there are some families with multiple affected members and recurrence risks can range from 25 to 50%, depending upon the particular mode of inheritance in that family.

Improved understanding of the genetic causes for CVM can lead to improved genetic counseling for families who have had children with this condition.

Conclusion

Recent advances in the understanding of molecular events underlying vertebral development and genetic involvement in these processes have contributed greatly towards an advanced understanding of congenital vertebral malformation. However, the emerging information has presented new challenges to both the clinician and researchers. Validation of emerging hypotheses related to causation of CVM and other anomalies will require a large cohort of phenotypically well-defined patients with sufficient numbers of a broad range of syndromic and non-syndromic CVM which will allow meaningful assessments of potential genetic contributions. For example, Tassabehji and colleagues have recently described a missense mutation in c.746C>A (p.Ala249Glu) of GDF6, a member of the BMP family that was identified in a 3 generation family with autosomal dominant Klippel–Feil syndrome.¹⁴⁹ These investigators were able to further demonstrate recurrent mutation in a highly conserved residue c.866T>C (p.Leu289Pro) of GDF6 that was identified in 2 of 121 sporadic cases of Klippel–Feil syndrome.¹⁴⁹ One of the spo-

radic cases was a female fetus with multiple vertebral segmentation abnormalities encompassing the entire spine, rocker bottom feet, and Arnold Chiari malformation. The occurrence of GDF6 mutations in only 2 of 121 patients studied provides evidence for genetic heterogeneity of Klippel–Feil syndrome. Availability of cases representing both familial and sporadic occurrence increases the understanding of disease transmission and clinical impact.

Further, determining functionality of mutations in *in vitro* models or animal models will be necessary. The maternal genotype for metabolizing genes of possible teratogenic substances such as anticonvulsant medications remains unexplored and requires rigorous study. The genetic factors that render infants of mothers with diabetes or infants exposed to alcohol susceptible to the development of CVM as well as other malformations remain largely unexplored. Epigenetic modifications in the DNA, such as DNA methylation, which may alter expression of key regulatory genes involved in somitogenesis, also remain to be studied. Emergence of the newly formed consortium, ICVAS (International Consortium for Vertebral Anomalies and Scoliosis, www.icvas.org), whose expressed goal is to foster collaborative research between clinician researchers and basic scientists, will be central to expediting elucidation of answers to these important questions in the future.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

1. McMaster, M. & K. Ohtsuka. 1982. The natural history of congenital scoliosis: a study of two

- hundred and fifty-one patients. *J. Bone Joint Surg. Am.* **64**: 1128–1147.
2. McMaster, M. & H. Singh. 1999. Natural history of congenital kyphosis and kyphoscoliosis: a study of one hundred and twelve patients. *J. Bone Joint Surg. Am.* **81**: 1367–1383.
 3. Shands, A. & H. Eisberg. 1955. The incidence of scoliosis in the state of Delaware: a study of 50,000 minifilms of the chest made during a survey for tuberculosis. *J. Bone Joint Surg.* **37A**: 1243.
 4. Wynne-Davies, R. 1975. Congenital vertebral anomalies: aetiology and relationship to spina bifida cystica. *J. Med. Genet.* **12**: 280–288.
 5. Jaskwlich, D., R. Ali, T. Patel, *et al.* 2000. Congenital scoliosis. *Curr. Opin. Pediatr.* **12**: 61–66.
 6. Rollnick, B., C. Kaye, K. Nagatoshi, *et al.* 1987. Oculoauriculovertebral dysplasia and variants: phenotypic characteristics of 294 patients. *Am. J. Med. Genet.* **26**: 361–375.
 7. Alagille, D., M. Odievre, M. Gautier, *et al.* 1975. Hepatic ductular hypoplasia associated with characteristic facies, vertebral malformations, retarded physical, mental, and sexual development, and cardiac murmur. *J. Pediatr.* **86**: 63–71.
 8. Jarcho, S. & P.M. Levin. 1938. Hereditary malformations of the vertebral bodies. *Johns Hopkins Med. J.* **62**: 216.
 9. Samartzis, D., J. Herman, J. Lubicky, *et al.* 2006. Classification of congenitally fused cervical patterns in Klippel-Feil patients. *Spine* **31**: 798–804.
 10. Botto, L., M. Khouy, P. Mastroiacovo, *et al.* 1997. The spectrum of congenital anomalies of the VATER association: an international study. *Am. J. Med. Genet.* **71**: 8–15.
 11. Pourquie, O., M. Coltey, M.A. Teillet, *et al.* 1993. Control of dorsoventral patterning of somitic derivatives by notochord and floor plate. *Proc. Natl. Acad. Sci. USA* **90**: 5242–5246.
 12. Fan, C.M., J.A. Porter, C. Chiang, *et al.* 1995. Long-range sclerotome induction by sonic hedgehog: direct role of the amino-terminal cleavage product and modulation by the cyclic AMP signaling pathway. *Cell* **81**: 457–465.
 13. Hirsinger, E., C. Jouve, J. Dubrulle, *et al.* 2000. Somite formation and patterning. *Int. Rev. Cytol.* **198**: 1–65.
 14. Dequeant, M. & O. Pourquie. 2008. Segmental patterning of the vertebrate embryonic axis. *Nat. Rev. Genet.* **9**: 370–382.
 15. Pourquie, O. & K. Kusumi. 2001. When body segmentation goes wrong. *Clin. Genet.* **60**: 409–416.
 16. Turnpenny, P.D., M.P. Bulman, T.M. Frayling, *et al.* 1999. A gene for autosomal recessive spondylocostal dysostosis maps to 19q13.1-q13.3. *Am. J. Hum. Genet.* **65**: 175–182.
 17. Turnpenny, P.D., R.J. Thwaites & F.N. Boulos. 1991. Evidence for variable gene expression in a large inbred kindred with autosomal recessive spondylocostal dysostosis. *J. Med. Genet.* **28**: 27–33.
 18. Gruneberg, H. 1961. Genetical studies on the skeleton of the mouse XIX: pudgy. *Genet. Res.* **2**: 384–393.
 19. Dunwoodie, S.L., M. Clements, D.B. Sparrow, *et al.* 2002. Axial skeletal defects caused by mutation in the spondylocostal dysplasia/pudgy gene *DLL3* are associated with disruption of the segmentation clock within the presomitic mesoderm. *Development* **129**: 1795–1806.
 20. Kusumi, K., E.S. Sun, A.W. Kerrebrock, *et al.* 1998. The mouse pudgy mutation disrupts delta homologue *DLL3* and initiation of early somite boundaries. *Nat. Genet.* **19**: 274–278.
 21. Giampietro, P.F., C.L. Raggio & R.D. Blank. 1999. Synteny-defined candidate genes for idiopathic and congenital scoliosis. *Am. J. Med. Genet.* **83**: 164–177.
 22. Bulman, M., K. Kusumi, T. Frayling, *et al.* 2000. Mutations in the human Delta homologue, *DLL3*, cause axial skeletal defects in spondylocostal dysostosis. *Nat. Genet.* **4**: 438–441.
 23. Turnpenny, P., B. Alman, A. Cornier, *et al.* 2007. Abnormal vertebral segmentation and the notch signaling pathway in man. *Dev. Dyn.* **236**: 1456–1474.
 24. Turnpenny, P., N. Whittock, J. Duncan, *et al.* 2003. Novel mutations in *DLL3*, a somitogenesis gene encoding a ligand for the Notch signaling pathway, cause a consistent pattern of abnormal vertebral segmentation in spondylocostal dysostosis. *J. Med. Genet.* **40**: 333–339.
 25. Bonafe, L., C. Giunta, M. Gassner, *et al.* 2003. A cluster of autosomal recessive spondylocostal dysostosis caused by three newly identified *DLL3* mutations segregating in a small village. *Clin. Genet.* **64**: 28–35.
 26. Yasuhiko, Y., S. Haraguchi, S. Kitajima, *et al.* 2006. Tbx6-mediated Notch signaling controls somite-specific *MESP2* expression. *Proc. Natl. Acad. Sci. USA* **103**: 3651–3656.
 27. Whittock, N.V., S. Ellard, J. Duncan, *et al.* 2004. Pseudo-dominant inheritance of spondylocostal dysostosis type 1 caused by two familial delta-like 3 mutations. *Clin. Genet.* **66**: 67–72.
 28. Dunwoodie, S.L., D. Henrique, S.M. Harrison, *et al.* 1997. Mouse *DLL3*: a novel divergent delta gene which may complement the function of other delta homologues during early pattern formation in the mouse embryo. *Development* **124**: 3065–3076.
 29. Maine, E.M., J.L. Lissemore & W.T. Starmer. 1995. A phylogenetic analysis of vertebrate and invertebrate Notch-related genes. *Mol. Phylogenet. Evol.* **4**: 139–149.

30. Geffers, I., K. Serth, G. Chapman, et al. 2007. Divergent functions and distinct localization of the Notch ligands *DLL1* and *DLL3* in vivo. *J. Cell Biol.* **178**: 465–476.
31. Ladi, E., J.T. Nichols, W. Ge, et al. 2005. The divergent DSL ligand *DLL3* does not activate Notch signaling but cell autonomously attenuates signaling induced by other DSL ligands. *J. Cell Biol.* **170**: 983–992.
32. Shinkai, Y., T. Tsuji, Y. Kawamoto, et al. 2004. New mutant mouse with skeletal deformities caused by mutation in delta like 3 (*DLL3*) gene. *Exp. Anim.* **53**: 129–136.
33. Sparrow, D., G. Chapman, M. Wouters, et al. 2006. Mutation of the lunatic fringe gene in humans causes spondylocostal dysostosis with a severe vertebral phenotype. *Am. J. Hum. Genet.* **78**: 28–37.
34. Whittock, N.V., D.B. Sparrow, M.A. Wouters, et al. 2004. Mutated *MESP2* causes spondylocostal dysostosis in humans. *Am. J. Hum. Genet.* **74**: 1249–1254.
35. Saga, Y., N. Hata, H. Koseki, et al. 1997. *MESP2*: a novel mouse gene expressed in the presegmented mesoderm and essential for segmentation initiation. *Genes Dev.* **11**: 1827–1839.
36. Saga, Y., N. Hata, S. Kobayashi, et al. 1996. *MESPI*: a novel basic helix-loop-helix protein expressed in the nascent mesodermal cells during mouse gastrulation. *Development* **122**: 2769–2778.
37. Morimoto, M., Y. Takahashi, M. Endo, et al. 2005. The *MESP2* transcription factor establishes segmental borders by suppressing Notch activity. *Nature* **435**: 354–359.
38. Takahashi, Y., K. Koizumi, A. Takagi, et al. 2000. *MESP2* initiates somite segmentation through the Notch signaling pathway. *Nat. Genet.* **25**: 390–396.
39. Leitges, M., L. Neidhardt, B. Haenig, et al. 2000. The paired homeobox gene *Uncx4.1* specifies pedicles, transverse processes and proximal ribs of the vertebral column. *Development* **127**: 2259–2267.
40. Takahashi, Y., T. Inoue, A. Gossler, et al. 2003. Feedback loops comprising *DLL1*, *DLL3* and *MESP2*, and differential involvement of *Psen1* are essential for rostrocaudal patterning of somites. *Development* **130**: 4259–4268.
41. Nomura-Kitabayashi, A., Y. Takahashi, S. Kitajima, et al. 2002. Hypomorphic *MESP* allele distinguishes establishment of rostrocaudal polarity and segment border formation in somitogenesis. *Development* **129**: 2473–2481.
42. Takahashi, Y., A. Takagi, S. Hiraoka, et al. 2007. Transcription factors *MESP2* and *Paraxis* have critical roles in axial musculoskeletal formation. *Dev. Dyn.* **236**: 1484–1494.
43. Bruckner, K., L. Perez, H. Clausen, et al. 2000. Glycosyltransferase activity of fringe modulates Notch-Delta interactions. *Nature* **406**: 411–415.
44. Moloney, D.J., L.H. Shair, F.M. Lu, et al. 2000. Mammalian Notch1 is modified with two unusual forms of O-linked glycosylation found on epidermal growth factor-like modules. *J. Biol. Chem.* **275**: 9604–9611.
45. Correia, T., V. Papayannopoulos, V. Panin, et al. 2003. Molecular genetic analysis of the glycosyltransferase Fringe in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **100**: 6404–6409.
46. Haines, N. & K.D. Irvine. 2003. Glycosylation regulates Notch signaling. *Nat. Rev. Mol. Cell Biol.* **4**: 786–797.
47. Hicks, C., S.H. Johnston, G. di Sibio, et al. 2000. Fringe differentially modulates Jagged1 and Delta1 signaling through Notch1 and Notch2. *Nat. Cell Biol.* **2**: 515–520.
48. Evrard, Y.A., Y. Lun, A. Aulehla, et al. 1998. Lunatic fringe is an essential mediator of somite segmentation and patterning. *Nature* **394**: 377–380.
49. Zhang, N., C.R. Norton & T. Gridley. 2002. Segmentation defects of Notch pathway mutants and absence of a synergistic phenotype in lunatic fringe/radical fringe double mutant mice. *Genesis* **33**: 21–28.
50. Zhang, N., G.V. Martin, M.W. Kelley, et al. 2000. A mutation in the Lunatic fringe gene suppresses the effects of a Jagged2 mutation on inner hair cell development in the cochlea. *Curr. Biol.* **10**: 659–662.
51. Hahn, K.L., J. Johnson, B.J. Beres, et al. 2005. Lunatic fringe null female mice are infertile due to defects in meiotic maturation. *Development* **132**: 817–828.
52. Cornier, A.S., N. Ramirez, S. Carlo, et al. 2003. Controversies surrounding Jarcho-Levin syndrome. *Curr. Opin. Pediatr.* **15**: 614–620.
53. Bannykh, S.I., S. Emery, J. Gerber, et al. 2003. Aberrant PAX1 and Pax9 expression in Jarcho-Levin syndrome: report of two Caucasian siblings and literature review. *Am. J. Med. Genet.* **120A**: 241–246.
54. Martinez-Frias, M.L. & M. Urioste. 1994. Segmentation anomalies of the vertebrae and ribs: a developmental field defect: epidemiologic evidence. *Am. J. Med. Genet.* **49**: 36–44.
55. Mortier, G.R., R.S. Lachman, M. Bocian, et al. 1996. Multiple vertebral segmentation defects: analysis of 26 new patients and review of the literature. *Am. J. Med. Genet.* **61**: 310–319.
56. Hatakeyama, K., S. Fuse, H. Tomita, et al. 2003. Jarcho-Levin syndrome associated with a complex congenital heart anomaly. *Pediatr. Cardiol.* **24**: 86–88.

57. Perez-Comas, A. & J.M. Garcia-Castro. 1974. Occipito-facial-cervico-thoracic-abdomino-digital dysplasia; Jarcho-Levin syndrome of vertebral anomalies. Report of six cases and review of the literature. *J. Pediatr.* **85**: 388–391.
58. Rastogi, D., E.B. Rosenzweig & A. Koumbourlis. 2002. Pulmonary hypertension in Jarcho-Levin syndrome. *Am. J. Med. Genet.* **107**: 250–252.
59. Solomon, L., R.B. Jimenez & L. Reiner. 1978. Spondylothoracic dysostosis: report of two cases and review of the literature. *Arch. Pathol. Lab. Med.* **102**: 201–205.
60. Cornier, A.S., N. Ramirez, S. Arroyo, *et al.* 2004. Phenotype characterization and natural history of spondylothoracic dysplasia syndrome: a series of 27 new cases. *Am. J. Med. Genet. [A]* **128**: 120–126.
61. Cornier, A.S., K. Staehling-Hampton, K.M. Delventhal, *et al.* 2008. Mutations in the *MESP2* gene cause spondylothoracic dysostosis/Jarcho-Levin syndrome. *Am. J. Hum. Genet.* **82**: 1334–1341.
62. Emerick, K.M., E.B. Rand, E. Goldmuntz, *et al.* 1999. Features of Alagille syndrome in 92 patients: frequency and relation to prognosis. *Hepatology* **29**: 822–829.
63. Kamath, B.M., K.M. Loomes, R.J. Oakey, *et al.* 2002. Facial features in Alagille syndrome: specific or cholestasis facies? *Am. J. Med. Genet.* **112**: 163–170.
64. Hingorani, M., K.K. Nischal, A. Davies, *et al.* 1999. Ocular abnormalities in Alagille syndrome. *Ophthalmology* **106**: 330–337.
65. Colliton, R.P., L. Bason, F.M. Lu, *et al.* 2001. Mutation analysis of Jagged1 (*JAG1*) in Alagille syndrome patients. *Hum. Mutat.* **17**: 151–152.
66. McDaniell, R., D.M. Warthen, P.A. Sanchez-Lara, *et al.* 2006. *NOTCH2* mutations cause Alagille syndrome, a heterogeneous disorder of the notch signaling pathway. *Am. J. Hum. Genet.* **79**: 169–173.
67. Xue, Y., X. Gao, C.E. Lindsell, *et al.* 1999. Embryonic lethality and vascular defects in mice lacking the Notch ligand Jagged1. *Hum. Mol. Genet.* **8**: 723–730.
68. Treadwell, S.J., D.F. Smith, P.J. Macleod, *et al.* 1982. Cervical spine anomalies in fetal alcohol syndrome. *Spine* **7**: 331–334.
69. Smith, D.F., G.G. Sandor, P.M. MacLeod, *et al.* 1981. Intrinsic defects in the fetal alcohol syndrome: studies on 76 cases from British Columbia and the Yukon Territory. *Neurobehav. Toxicol. Teratol.* **3**: 145–152.
70. Bantz, E.W. 1984. Valproic acid and congenital malformations. A case report. *Clin. Pediatr. (Phila)* **23**: 352–353.
71. Hanold, K.C. 1986. Teratogenic potential of valproic acid. *J. Obstet. Gynecol. Neonatal. Nurs.* **15**: 111–116.
72. Edwards, M.J. 1986. Hyperthermia as a teratogen: a review of experimental studies and their clinical significance. *Teratog. Carcinog. Mutagen.* **6**: 563–582.
73. Ewart-Toland, A., J. Yankowitz, A. Winder, *et al.* 2000. Oculoauriculovertebral abnormalities in children of diabetic mothers. *Am. J. Med. Genet.* **90**: 303–309.
74. Lewis, D.P., D.C. Van Dyke, P.J. Stumbo, *et al.* 1998. Drug and environmental factors associated with adverse pregnancy outcomes. Part II: improvement with folic acid. *Ann. Pharmacother.* **32**: 947–961.
75. Skold, A.C., K. Wellfelt & B.R. Danielsson. 2001. Stage-specific skeletal and visceral defects of the I(Kr)-blocker almokalant: further evidence for teratogenicity via a hypoxia-related mechanism. *Teratology* **2001**: 292–300.
76. Farley, F.A., R.T. Loder, B.T. Nolan, *et al.* 2001. Mouse model for thoracic congenital scoliosis. *J. Pediatr. Orthop.* **21**: 537–540.
77. Wery, N., M.G. Narotsky, N. Pacico, *et al.* 2003. Defects in cervical vertebrae in boric acid-exposed rat embryos are associated with anterior shifts of hox gene expression domains. *Birth. Defects. Res. A Clin. Mol. Terato.* **167**: 59–67.
78. Scoliosis Research Society. 1976. Glossary of scoliosis terms. *Spine* **1**: 57–58.
79. Winter, R. 1979. Posterior spinal fusion in scoliosis: indications, techniques, and results. *Orthoped. Clin. N Amer.* **10**: 787.
80. Chetcuti, P., D.R. Dickens & P.D. Phelan. 1989. Spinal deformity in patients born with oesophageal atresia and tracheo-oesophageal fistula. *Arch. Dis. Child.* **64**: 1427–1430.
81. Lonstein, J. 1977. Screening for spinal deformities in Minnesota schools. *Clin. Orthoped.* **126**: 33.
82. Winter, R., J. Lonstein & F. Denis. 1988. Pain patterns in adult scoliosis. *Orthoped. Clin. N Amer.* **19**: 339–345.
83. Vitko, R.J., A.S. Cass & R.B. Winter. 1972. Anomalies of the genitourinary tract associated with congenital scoliosis and congenital kyphosis. *J. Urol.* **108**: 655–659.
84. Cowell, H.R., G.D. MacEwen & C. Hubben. 1974. Incidence of abnormalities of the kidney and ureter in congenital scoliosis. *Birth Defects Orig. Artic. Ser.* **10**: 142–145.
85. Maizels, M. & F.D. Stephens. 1979. The induction of urologic malformations. Understanding the relationship of renal ectopia and congenital scoliosis. *Invest. Urol.* **17**: 209–217.
86. Maisenbacher, M., J. Han, M. O'Brien, *et al.* 2005. Molecular analysis of congenital scoliosis: a candidate gene approach. *Hum. Genet.* **116**: 416–419.

87. Purkiss, S.B., B. Driscoll, W.G. Cole, *et al.* 2002. Idiopathic scoliosis in families of children with congenital scoliosis. *Clin. Orthop. Relat. Res.* **401**: 27–31.
88. Miller, N.H. 2007. Genetics of familial idiopathic scoliosis. *Clin. Orthop. Relat. Res.* **462**: 6–10.
89. Gao, X., D. Gordon, D. Zhang, *et al.* 2007. *CHD7* Gene polymorphisms are associated with susceptibility to idiopathic scoliosis. *Am. J. Hum. Genet.* **80**: 957–965.
90. Doyle, C. & K. Blake. 2005. Scoliosis in CHARGE: a prospective survey and two case reports. *Am. J. Med. Genet. A* **133**: 340–343.
91. Hutchinson, J. 1894. Deformity of shoulder girdle. *Br. Med. J.* **1**: 634.
92. Klippel, M. & A. Feil. 1912. Un cas d'absence des vertebres cervicales. *Nouv. Icon. Salpet.* **25**: 223.
93. Feil, A. 1919. L'absence et la diminution des vertebres cervicales (etude clinique et pathologique); le syndrome de recution numerique cervicale. Thesis de Paris.
94. Heisenger, R. & G. MacEwen. 1982. Congenital anomalies of the spine. In *The Spine*. R. Rothman & F. Simeone, Eds.: 188–315. WB Saunders. Philadelphia.
95. Clarke, R., G. Catalan, A. Diwan, *et al.* 1998. Heterogeneity in Klippel-Feil syndrome: a new classification. *Pediatr. Radiol.* **28**: 967–974.
96. Manaligod, J., A. Menezes, N. Bauman, *et al.* 1999. Cervical vertebral anomalies in patients with anomalies of the head and neck. *Ann. Otol. Rhinol. Larymol.* **108**: 925–933.
97. Tracy, M., J. Dormans & K. Kusumi. 2004. Klippel-Feil Syndrome. *Clin. Orthop. Relat. Res.* **424**: 183–190.
98. Clarke, R.A., S. Singh, H. McKenzie, *et al.* 1995. Familial Klippel-Feil syndrome and paracentric inversion inv(8)(q22.2q23.3). *Am. J. Hum. Genet.* **57**: 1364–1370.
99. Fukushima, Y., H. Ohashi, K. Wakui, *et al.* 1995. De novo apparently balanced reciprocal translocation between 5q11.2 and 17q23 associated with Klippel-Feil anomaly and type A1 brachydactyly. *Am. J. Med. Genet.* **57**: 447–449.
100. Papagrigorakis, M., P. Synodinos, C. Daliouris, *et al.* 2003. De novo inv(2)(p12q34) associated with Klippel-Feil anomaly and hypodontia. *Eur. J. Pediatr.* **162**: 594–597.
101. Goto, M., G. Nishimura, T. Nagai, *et al.* 2006. Familial Klippel-Feil anomaly and t(5;8)(q35.1;p21.1) translocation. *Am. J. Med. Genet.* **140A**: 1013–1015.
102. McGaughran, J., A. Oates, D. Donnai, *et al.* 2003. Mutations in *PAX1* may be associated with Klippel-Feil syndrome. *Eur. J. Hum. Genet.* **11**: 468–474.
103. Dietrich, S. & P. Gruss. 1995. Undulated phenotypes suggest a role of Pax-1 for the development of vertebral and extravertebral structures. *Dev. Biol.* **167**: 529–548.
104. Giampietro, P., R. Blank, C. Raggio, *et al.* 2003. Congenital and idiopathic scoliosis: clinical and genetic aspects. *Clin. Med. Res.* **1**: 125–136.
105. Ghebranious, N., J. Burmester, I. Glurich, *et al.* 2006. Evaluation of *SLC35A3* as a candidate gene for human vertebral malformations. *Am. J. Med. Genet.* **140A**: 1346–1348.
106. Ghebranious, N., C.L. Raggio, R.D. Blank, *et al.* 2007. Lack of evidence of *WNT3A* as a candidate gene for congenital vertebral malformations. *Scoliosis* **2**: 13.
107. Giampietro, P., C. Raggio, C. Reynolds, *et al.* 2006. *DLL3* as a candidate for vertebral malformations. *Am. J. Med. Genet.* **140A**: 2447–2453.
108. Giampietro, P., C. Raggio, C. Reynolds, *et al.* 2005. An analysis of *PAX1* in the development of vertebral malformations. *Clin. Genet.* **68**: 448–453.
109. Wallin, J., J. Wilting, H. Koseki, *et al.* 1994. The role of Pax-1 in axial skeleton development. *Dev. Suppl.* **120**: 1109–1121.
110. Aulehla, A., C. Wehrle, B. Brand-Saberi, *et al.* 2003. Wnt3a plays a major role in the segmentation clock controlling somitogenesis. *Dev. Cell* **4**: 395–406.
111. Thomsen, M., U. Schneider, M. Weber, *et al.* 1997. Scoliosis and congenital anomalies associated with Klippel-Feil syndrome types I–III. *Spine* **22**: 396–401.
112. Thomsen, B., P. Horn, F. Panitz, *et al.* 2006. A missense mutation in the bovine *SLC35A3* gene, encoding a UDP-N-acetylglucosamine transporter, causes complex vertebral malformation. *Genome Res.* **16**: 97–105.
113. Agerholm, J., C. Bendixen, O. Anderson, *et al.* 2001. Complex vertebral malformation in Holstein calves. *J. Vet. Diagn. Invest.* **13**: 283–289.
114. Kispert, A., B. Koschorz & B.G. Herrmann. 1995. The T protein encoded by *Brachyury* is a tissue-specific transcription factor. *EMBO J.* **14**: 4763–4772.
115. Dobrovolska-Zavadakaia, N. 1927. Sur la mortification spontanee de la queue chez la souris nouveaunee sur l'existence d'un caractere (facteur) Hereditaire “non-viable”. *CR Seances Soc. Biol. Fil.* **97**: 114–116.
116. Gluecksohn-Schoenheimer, S. 1944. The development of normal and homozygous *Brachy (T/T)* mouse embryos in the extraembryonic coelom of the chick. *Proc. Natl. Acad. Sci. USA* **30**: 134–140.
117. Herrmann, B., S. Labeit, A. Poustka, *et al.* 1990. Cloning of the T gene required in mesoderm formation in the mouse. *Nature* **343**: 617–622.
118. Kispert, A. & B.G. Herrmann. 1994. Immunohistochemical analysis of the *Brachyury* protein in

- wild-type and mutant mouse embryos. *Dev. Biol.* **161**: 179–193.
119. Bollag, R.J., Z. Siegfried, J.A. Cebra-Thomas, *et al.* 1994. An ancient family of embryonically expressed mouse genes sharing a conserved protein motif with the T locus. *Nat. Genet.* **7**: 383–389.
 120. Conlon, F.L., C.V. Wright & E.J. Robertson. 1995. Effects of the TWis mutation on notochord formation and mesodermal patterning. *Mech. Dev.* **49**: 201–209.
 121. Watabe-Rudolph, M., N. Schlautmann, V. Papaioannou, *et al.* 2002. The mouse rib-vertebrae mutation is a hypomorphic *Tbx6* allele. *Mech. Dev.* **119**: 251–256.
 122. Ghebranious, N., R.D. Blank, C.L. Raggio, *et al.* 2008. A missense *T(Brachyury)* mutation contributes to vertebral malformations. *J. Bone Miner. Res.* [DOI] 10.1359/jbmr.080503.
 123. Papapetrou, C., F. Drummond, W. Reardon, *et al.* 1999. Genetic study of the human *T* gene and its exclusion as a major candidate gene for sacral agenesis with anorectal atresia. *J. Med. Genet.* **36**: 208–213.
 124. Park, C.H., J.H. Pruitt & D. Bennett. 1989. A mouse model for neural tube defects: the curtailed (Tc) mutation produces spina bifida occulta in *Tc/+* animals and spina bifida with meningocele in *Tc/t*. *Teratology* **39**: 303–312.
 125. Searle, A.G. 1966. Curtailed, a new dominant T-allele in the house mouse. *Genet. Res.* **7**: 86–95.
 126. Bogani, D., N. Warr, P. Elms, *et al.* 2004. New semidominant mutations that affect mouse development. *Genesis* **40**: 109–117.
 127. Lyon, M.F. 1996. An additional type of male sterility and inherited urinary obstruction in mice with the t-haplotype th7. *Genet. Res.* **67**: 249–256.
 128. Abe, K., K. Yamamura & M. Suzuki. 2000. Molecular and embryological characterization of a new transgene-induced null allele of mouse Brachyury locus. *Mamm. Genome.* **11**: 238–240.
 129. Chesley, P. 1935. Development of the short-tailed mutant in the house mouse. *J. Exp. Zool.* **70**: 429–459.
 130. Jensen, L.E., S. Barbaux, K. Hoess, *et al.* 2004. The human *T* locus and spina bifida risk. *Hum. Genet.* **115**: 475–482.
 131. Morrison, K., C. Papapetrou, J. Attwood, *et al.* 1996. Genetic mapping of the human homologue (T) of mouse T (Brachyury) and a search for allele association between human *T* and spina bifida. *Hum. Molec. Genet.* **5**: 669–674.
 132. Shields, D.C., D. Ramsbottom, C. Donoghue, *et al.* 2000. Association between historically high frequencies of neural tube defects and the human T homologue of mouse *T* (Brachyury). *Am. J. Med. Genet.* **92**: 206–211.
 133. Richter, B., A.H. Schultealbert & M.C. Koch. 2002. Human *T* and risk for neural tube defects. *J. Med. Genet.* **39**: E14.
 134. Speer, M., E. Melvin, K. Viles, *et al.* 2002. Group NC. *T* locus shows no evidence for linkage disequilibrium or mutation in American Caucasian neural tube defect families. *Am. J. Med. Genet.* **110**: 215–218.
 135. Trembath, D., A.L. Sherbondy, D.C. Vandyke, *et al.* 1999. Analysis of select folate pathway genes, PAX3, and human *T* in a midwestern neural tube defect population. *Teratology* **59**: 331–341.
 136. Knudson, A.G. Jr. 1971. Mutation and cancer: statistical study of retinoblastoma. *Proc. Natl. Acad. Sci. USA* **68**: 820–823.
 137. Yeyati, P.L., R.M. Bancewicz, J. Maule, *et al.* 2007. Hsp90 selectively modulates phenotype in vertebrate development. *PLoS Genet.* **3**: e43.
 138. Vazquez-Lopez, M.E., M.I. Lopez-Conde, C. Somoza-Rubio, *et al.* 2005. Anomalies of vertebrae and ribs: Jarcho Levin syndrome. Description of a case and literature review. *Joint Bone Spine* **72**: 275–277.
 139. Cantu, J.M., J. Urrusti, G. Rosales, *et al.* 1971. Evidence for autosomal recessive inheritance of costovertebral dysplasia. *Clin. Genet.* **2**: 149–154.
 140. David, T.J. & A. Glass. 1983. Hereditary costovertebral dysplasia with malignant cerebral tumour. *J. Med. Genet.* **20**: 441–444.
 141. Norum, R. & V. McKusick. 1969. Costovertebral anomalies with apparent recessive inheritance. *Birth Defects Orig. Artic. Ser.* **18**: 326–329.
 142. Buttitta, L., T.S. Tanaka, A.E. Chen, *et al.* 2003. Microarray analysis of somitogenesis reveals novel targets of different WNT signaling pathways in the somitic mesoderm. *Dev. Biol.* **258**: 91–104.
 143. Dequeant, M., E. Glynn, K. Gaundenz, *et al.* 2006. A complex oscillating network of signaling genes underlies the mouse segmentation clock. *Science* **314**: 1595–1598.
 144. William, D.A., B. Saitta, J.D. Gibson, *et al.* 2007. Identification of oscillatory genes in somitogenesis from functional genomic analysis of a human mesenchymal stem cell model. *Dev. Biol.* **305**: 172–186.
 145. Loomes, K.M., S.A. Stevens, M.L. O'Brien, *et al.* 2007. DLL3 and Notch1 genetic interactions model axial segmental and craniofacial malformations of human birth defects. *Dev. Dyn.* **236**: 2943–2951.
 146. Machka, C., M. Kersten, M. Zobawa, *et al.* 2005. Identification of *DLL1* (Delta1) target genes during mouse embryogenesis using differential expression profiling. *Gene Expr. Patterns.* **6**: 94–101.

147. Pinkel, D., R. Seagraves, D. Sudar, et al. 1998. High resolution analysis of DNA copy number variation using comparative genomic hybridization microarrays. *Nat. Genet.* **20**: 207–211.
148. Vissers, L.E., C. van Ravenswaaij, R. Admiraal, et al. 2004. Mutations in a new member of the chromosome domain gene family cause CHARGE syndrome. *Nat. Genet.* **36**: 955–957.
149. Tassabehji, M., Z.M. Fang, E.N. Hilton, et al. 2008. Mutations in *GDF6* are associated with vertebral segmentation defects in Klippel-Feil syndrome. *Hum. Mutat. Apr* **18**: [Epub ahead of print] doi: 10.1253/circj.71.1485.

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