Chapter 3
Environmental Factors and Axial Skeletal Dysmorphogenesis

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Introduction

Axial skeletal development is part of the complex, inclusive process of axial or midline development. It involves the interaction of many tissues including the embryonic notochord, neural tube, somite compartments, intersomitic angiopotent cells, and neural crest cells. These tissues give rise to the axial skeleton, intervertebral discs, spinal cord, trunk musculature and dorsal dermis, intervertebral arteries, and spinal ganglia. Development of these tissues occurs in an interdependent and hierarchical manner over an extended period of time. These characteristics may make the axial skeleton disproportionately susceptible to environmental influence, accounting for the high incidence of axial skeletal defects among live and stillbirths. It may also account for the many manifestations of axial skeletal defects observed.

Data show that the axial skeleton is one of several organ systems with a high frequency of abnormality, 1 in 1,000 live births (Brent and Fawcett 2007, Cohen 1997, Dias 2007, Erol et al. 2002, Jaskwhich et al. 2000, O'Rahilly and Müller 1996, Oskouian et al. 2007) and a very low heritable component, estimated to be between 0.5 and 2%. Congenital axial skeletal defects may occur in isolation or as a component of more widespread syndromes or sequences (Cohen 1997, Dias, 2007, Erol et al. 2002, Jaskwhich et al. 2000, Oskouian et al. 2007) (Table 3.1 and Chapter 7). It is estimated that the skeletal defect is accompanied by an intra-spinal neural defect in 40% of cases. In addition, approximately 50–60% cases of congenital scoliosis suffer additional congenital defects in other organ systems including urogenital and cardiovascular systems (approximately 20% and 10–12%, respectively), gastrointestinal and limb defects (2–5%). These combinations of congenital defects and their frequencies are reflective of the degree of concurrent development of the different organ systems.

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Table 3.1 Genetic syndromes that are characterized by scoliosis

Syndrome	Features
Alagille syndrome (autosomal dominant)	Neonatal jaundice, cholestasis, peripheral pulmonic stenosis, occasional septal defects and patent ductus arteriosus, accompanied by abnormal facies, ocular, *vertebral*, and nervous system abnormalities
Bertolotti syndrome	*Sacralization of the fifth lumbar vertebrae* with sciatica and *scoliosis*
Caudal dysgenesis (agenesis, regression) syndrome	Failure *to form part or all of the coccygeal, sacral, and lumbar vertebrae* and corresponding spinal segments with malformation and dysfunction of the bowel and bladder
Cerebrocostomandibular syndrome (autosomal recessive)	Severe micrognathia, *severe costovertebral anomalies* including bell-shaped thorax, incompletely ossified, aberrant rib structure, abnormal rib connection to the vertebral body, accompanied by palatal defects, glossoptosis, pre- and post-natal growth deficiencies, mental retardation
Coffin-Siris syndrome	Hypoplasia of the fifth fingers and toes associated with mental and growth retardation, coarse facies, mild microcephaly, hypotonia, lax joints, mild hirsutism, and occasionally accompanied by cardiac, *vertebral,* and gastrointestinal abnormalities
Oculocerebral hypopigmentation syndrome (autosomal recessive)	Oculocutaneous albinism, microphthalmus, opaque corneas, oligophrenia with spasticity, high-arched palate, gingival atrophy, *scoliosis*
Kabuki make-up syndrome	Mental retardation, dwarfism, *scoliosis*, cardiovascular abnormalities, and facies reminiscent of a Japanese Kabuki actor
King's syndrome (malignant hyperthermia)	Short stature, *kyphoscoliosis*, pectus carinatum, cryptorchidism, delayed motor development, progressive myopathy, structural cardiovascular defects
Klippel–Feil syndrome	Reduced number of cervical vertebrae, *cervical hemivertebrae*, low hair-line, reduced neck mobility
Lenz's syndrome (X-linked)	Microphthalmia, anophthalmia, digital anomalies, narrow shoulders, double thumbs, *vertebral abnormalities*, dental, urogenital, and cardiovascular defects may occur
Multiple pterygium syndrome (autosomal recessive)	Pterygia of the neck, axillae, popliteal, antecubital, and intercrural areas, accompanied by hypertelorism, cleft palate, micrognathia, ptosis, short stature, and a wealth of skeletal anomalies including camptodactyly, syndactyly, equinovarus, rocker-bottom feet, *vertebral fusions, and rib abnormalities*

Table 3.1 (continued)

Syndrome	Features
Oculoauricularvertebral syndrome (Goldenhar syndrome)	Colobomas of the upper eye lids, bilateral accessory auricular appendages, *vertebral anomalies*, facial bossing, asymmetrical skull, low hair-line, mandibular hypoplasia, low-set ears, and sometimes hemifacial microsomia
Rubenstein–Taybi syndrome	Mental and motor retardation, broad thumbs and big toes, short stature, high-arched palate, straight, beaked nose, various eye abnormalities, pulmonary stenosis, keloid formation at surgical scars, large foramen magnum, *vertebral and sternal abnormalities*
Spondylothoracic dysplasia (Jarcho–Levin syndrome) (autosomal recessive)	*Multiple vertebral defects*, short thorax, rib abnormalities, camptodactyly, syndactyly, and accompanied by urogenital anomalies and respiratory dysfunction
VATER-VACTERL sequence	*Vertebral anomalies,* anal atresia, (cardiac abnormalities), tracheal fistula with esophageal atresia, renal defects, (limb abnormalities)

A selected list of recognized genetic syndromes that may include vertebral anomalies. Genetic syndromes associated with scoliosis are further discussed in Chapter 7.

While dramatic axial skeletal defects do occur in the context of syndromes and other anomalies, the majority of congenital spinal anomalies involve single structural defects of the spine and frequently few obvious coincident malformations or functional deficits (Erol et al. 2002, Jaskwhich et al. 2000, Oskouian et al. 2007), indicating that a time-dependent, tissue-specific insult may be involved. The complexity of axial skeletal development and the variety of axial skeletal defects suggest a variety of loci and mechanisms through which environmental factors may cause axial skeletal dysmorphogenesis.

Faced with the high social costs of resultant morbidity, it is critical to determine the possible impact any environmental factor may have on the embryo. Although many of the known human teratogens can produce axial skeletal defects, the etiology of over half of observed axial skeletal defects is unknown and is assumed to be multi-factorial, a combination of genetic susceptibility and environmental insult (Cohen 1997, Jaskwhich et al. 2000). This fact highlights the need for investigating the role of environmental factors, alone or in combination, in the production of this particular class of defects. Such study requires the convergence of at least two broad fields of study. The first is developmental biology, to understand the details of normal development and identify new markers, loci, and perhaps possible mechanisms of teratogenesis. The second field is teratology, a discipline closely related to reproductive toxicology that involves assessing the impact of environmental factors on the new biological markers, loci, and mechanisms discovered and characterized in developmental biology.

Vertebral Dysmorphogenesis in Human Congenital Scoliosis

Clinically, congenital scoliosis is defined as a spinal curvature of over 10% caused by a structural vertebral defect (Dias 2007, Erol et al. 2002, Oskouian et al. 2007). The abnormal spinal curvature is further defined by its anterior–posterior location and the plane of curvature as coronal for scoliosis and sagittal for kyphosis. The characteristic feature of congenital axial skeletal defects is the malformation of vertebral bodies or processes evident at birth. Broadly, these vertebral defects are clinically classified as failures in formation and morphogenesis represented by hemivertebrae, wedge vertebrae, open vertebral arches, bifid vertebrae, and vertebral agenesis or failures in segmentation represented by unilateral unsegmented bars or block vertebrae bilateral fusions (Fig. 3.1) (Dias 2007, Erol et al. 2002, Jaskwhich et al. 2000, Oskouian et al. 2007). Developmentally, however, all of these defects have their origin in somitogenesis, the initial manifestation of the vertebral column's metameric segmentation.

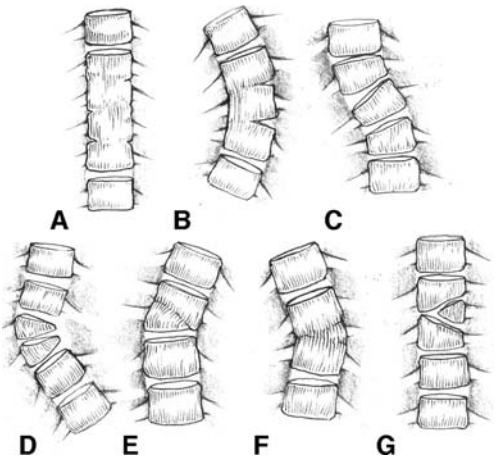


Fig. 3.1 Different forms of congenital scoliosis: block vertebrae (**a**), unilateral bar, (**b**), wedge vertebrae, (**c**), multiple hemivertebrae, (**d**), single, semi-segmented vertebrae, (**e**), non-segmented hemivertebrae, (**f**), incarcerated hemivertebrae, (**g**), defects in segmentation can produce these defects

Normal Development of the Axial Skeleton

As discussed in previous chapters, the axial skeleton is derived from the paraxial mesoderm, a primary germ layer, which undergoes the molecularly timed process of somitogenesis to produce blocks of tissue symmetrically arranged on either side of the midline neural tube and notochord (Table 3.2, Fig. 3.1) (Christ et al. 2000, Stockdale et al. 2000, Gridley 2006). The somite is a transient embryonic structure that plays an important role in the patterning of the axial skeleton (comprised of vertebral bodies, ribs, and intervertebral discs) and its associated tissues: the hypaxial and epaxial muscles of the spine, the dorsal dermis of the trunk, and the intervertebral arteries. The morphogenic description of somitogenesis can be conceptually

Table 3.2 Developmental timing of the axial skeleton in the human embryo

Developmental feature	Day of gestation	Other notable occurrences
Gastrulation	15	Neural plate formation
Notochord formation	17–19	Neural tube folding
First somite	19	Heart tube formation
Onset of neural tube fusion	22	Heart tube folding, optic and otic vesicle formation begins
Anterior neuropore closure	23–26	Embryonic circulation
Posterior neuropore closure	26–30	Forelimb bud
Sclerotomal segmentation	24–35	
Notochordal segmentation	28–30	
Last (30th) somite formed	32	Hind limb bud, optic cup formed
All rib primordia evident	42–44	
Chondrification of centra	36–42	
Chondrification of ribs and laminae	40–44	
Chondrification complete/onset of ossification	56–60	

divided into several phases: patterning, morphogenesis, differentiation, and growth and maturation (Tam and Trainor 1994, Christ et al. 2000, Alexander et al. 2007a). These are helpful classifications when characterizing and studying birth defects and their causes.

Among the tissues of the spine, the axial skeleton and its composite tissues undergo multiple rounds of patterning, differentiation, and growth events, including somitogenesis, resegmentation, and ossification, among other processes. Briefly, the axial skeleton is derived from the sclerotome, the ventromedial quadrant of each somite. Cells of sclerotome are initially part of the epithelial somite. Shortly after expressing the paired-box gene *Pax1* (Wallin et al. 1994, Barnes et al. 1996a), the cells de-epithelialize and relocate themselves to surround the notochord. These cells then begin expressing *Sox9*, a chondrocyte-specific transcription factor, and producing prodigious amounts of cartilage matrix to form the cartilage anlage of the vertebral body (Healy et al. 1999). There is a distinct polarity to the somite as it matures (Tam and Trainor 1994) that is consequential in the course of resegmentation, in which the posterior half of one somite merges with the anterior half of the posterior somite (Christ et al. 2004). Together, these halves combine to form a vertebral body out of phase with the other tissue, characteristic of the vertebral motor unit.

Development of the axial skeleton and the surrounding tissues occurs in an interdependent and hierarchical manner over an extended period of time. This may make the axial skeleton disproportionately susceptible to environmental influence, accounting for the high incidence of axial skeletal defects among live and stillbirths. It may also account for the many manifestations of axial skeletal defects

observed. Understanding these processes (the normal development of the spine) and their effects upon the surrounding tissues is important in deciphering the etiology of various forms of congenital scoliosis and the mechanisms by which environmental agents may initiate abnormal development.

Experimental Axial Skeletal Teratology

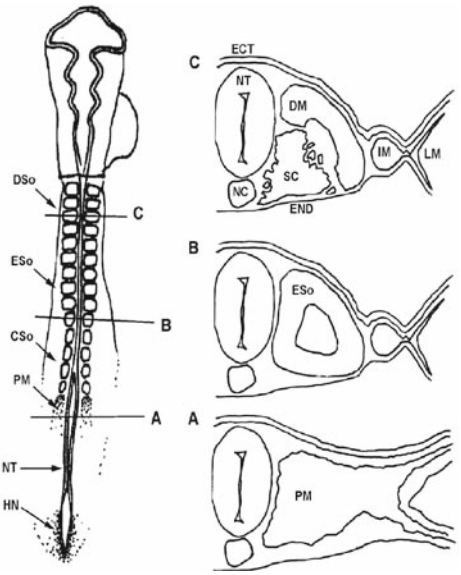
Given that the majority of axial defects have no known genetic cause (Cohen 1997, Dias 2007, Erol et al. 2002, Jaskwhich et al. 2000, Oskouian et al. 2007), the assumption must be made that there is an environmental component. The principles that helped define environmental teratogical agents were popularized in the wake of the "thalidomide experience" and with some modification remain applicable today (Wilson 1977, Sulik 1997, Sadler and Hunter 1994). In establishing the role of an environmental agent in inducing a congenital axial skeletal defect, we know that it must first affect the development and function of axial tissues and those that influence their differentiation including the notochord, neural tube, paraxial mesoderm, and overlying ectoderm. Second, the exposure must occur somewhere between the 4th and 10th week of human gestation, or organogenesis, during which time gastrulation, neurulation, and somitogenesis occur (Table 3.2) (Nishimura et al. 1974, O'Rahilley and Müller 1996). Third, the target of the teratogen must play a necessary role in the affected developmental process (e.g., somitogenesis) by acting via a specific mechanism. Finally, we must observe the dose-response effect of the environmental agent on embryonic development in both frequency and degree of malformation, including the graded manifestations of abnormal development: death, dysmorphogenesis, inhibition of growth or developmental delay, and functional deficit.

In the etiology of scoliosis, target organs may include the paraxial mesoderm and somites, the neural tube and notochord, and the overlying ectoderm. In particular, the patterning of the somite boundaries and the subsequent boundaries of differentiation defined by integrated signaling pathways under the influence of the surrounding tissues figures prominently. Morphological processes that may be affected include somitogenesis, neurulation, and gastrulation, which involve cell migration, epithelialization, and laminar fusion, as well as proliferation and apoptosis. Finally the differentiation, growth, and maturation of the axial skeletal elements may also play an important role (Table 3.3).

Pathogenesis of Abnormal Axial Development

The identification of the structural defect in congenital scoliosis in the fetus or neonate remains an analysis conducted long after the initial pathogenic events inducing the malformation. Identifying and understanding the initial pathogenic event is a critical step in characterizing the mechanisms of teratogenesis, which can

Table 3.3 Phases of somitogenesis in a stage 12 chick embryo and possible causal links between teratogen target tissues and hypothesized mature dysmorphogenesis

Stage 12 Chick Embryo	Transverse section/time of teratogenic insult	Target tissue	Possible resultant dysmorphogenes
		1. Notochord	Cleft vertebrae
		2.Ectoderm/ neural tube	Vertebral element agenesis
		3. Sclerotome	Vertebral disc anomalies
			Abnormal bone metabolism
		1. Notochord	Cleft vertebrae
		2. Ectoderm/ Neural tube	Vertebral agenesis
		3. Somitic mesoderm	Hemivertebrae
			Block vertebrae
		4. Lateral plate mesoderm	Bifid ribs
		1. Chordomesoderm/ notochord	Vertebral disk anomalies
			Caudal agenesis
		2. Paraxial mesoderm	Vertebral agenesis
			Hemiblock Vertebrae
		3. Ectoderm	Block or hemivertebrae
			Bifid or fused ribs vertebral agenesis

Phases of somitogenesis at three anterioposterior locations (A, B, and C) in a stage 12 chick embryo. Labels: DSo, differentiated somite; ESo, epithelial somite; CSo, condensed somite; PM, paraxial mesoderm; NT, neural tube; HN, Hensen's node; ECT, ectoderm; END, endoderm, NC, notochord; DM, dermomyotome; SC, sclerotome; IM, intermediate mesoderm; LM, lateral plate mesoderm.

then lead to the development of appropriate interventions. Environmental insults to a developing organism occur at molecular or sub-cellular levels. While the list of possible environmental insults is very large, the insults may be translated into types of cellular responses that result in recognizable patterns of dysfunction of dysmorphogenesis among tissues and organs (Table 3.4) (Wilson 1977, Sulik 1997).

Although teratogens are often discrete in nature (e.g., of known structure/composition and chemical characteristic), the determination of teratogenic mechanism is complicated. The main reasons for this are as follows. First, not all the possible targets of a teratogen have been identified, since many potentially affected targets remain unknown, i.e., normal developmental mechanisms still need identification and characterization. Related to this is the fact that it is highly unlikely that most teratogens act upon a single molecule or even a cellular pathway.

Table 3.4 Potential mechanisms, routes of pathogenesis, and ultimate morphogenetic outcome used by environmental teratogens in the induction of congenital malformation. Adapted from Wilson (1977)

Mechanisms	Pathogenesis	Final tissue outcome
Genetic mutation	Increased or decreased cell death	Reduction of cells to allow proper morphogenesis or tissue maturation
Chromosomal damage	Failed cell-cell interactions	
Epigenetic alteration	Reduced matrix biosynthesis	Imbalances in differentiation
Mitotic interference	Impeded morphogenic movements	Imbalances in growth
Nucleic acid synthesis/balance	Mechanical disruption of tissues	
Altered enzymatic substrates, co-factors, etc.		
Altered energy source		
Altered redox status		
Disrupted membrane or cytoskeletal integrity		
Altered signal transduction		

Multiple mechanistic pathways may combine to produce a single pathogenic mechanism contributing to the resultant congenital defect. Second, our ability to monitor the effect of the teratogen on the biochemistry of individual cellular targets is limited. Specifically, probes with sufficient sensitivity and specificity are unavailable for many processes and applications. Contributing to these issues is the fact that the amount of tissue available for study is usually very limited. Intertwined within these shortcomings is the difficulty of experimental interpretation, which varies with probe, detection, and particularly the endpoint chosen.

Despite these complications, we can hypothesize several intracellular processes that may be targets of teratogens. The teratogen generates its effects on the embryo often through a mode of molecular mimicry co-opting or undermining normal cellular processes such that they are activated, inactivated, or diverted in a manner inconsistent with developmental timing. Such processes include mitotic interference (mutagenesis and carcinogenesis), epigenetic changes (methylation and acetylation state), altered membrane function (composition or porosity), altered signal transduction, altered/inhibited energy metabolism, inhibition of waste (intermediary) metabolism, changed redox status, specific or general enzyme inhibition, and disturbances in nucleic acid synthesis, among other possibilities. The cellular responses to these insults may be grouped into several common outcomes, including necrosis or apoptosis, reduced biosynthesis, failed cell/cell or cell/matrix interactions, impeded morphogenetic movement, and mechanical disruption of tissues. Ultimately, the final defect may be manifested via loss of cells or tissue or imbalances in growth and differentiation.

While specific mechanisms of many teratogenic insults remain largely unknown, the characterization of cellular responses has been more successful. One particularly well-characterized outcome is the correlation of tissue-specific patterns of cell death and impending malformation (Sadler and Hunter 1994, Sulik et al. 1988, Sulik 1997, Zakeri and Ahuja 1997). This correlation has highlighted several characteristics of teratogenic action including the principles that different cell populations are sensitive to teratogenic insults at different time points, different agents target different tissues, and many teratogens expand areas of normal, developmentally regulated cell death. The observed changes in normal cell death patterns indicate the target tissue often plays a role in the subsequent dysmorphogenesis; however, the apoptotic cells do not participate in subsequent tissue formation – thus the effect of the teratogen on the surviving cells is important and presumed to be related to the cause of cell death. Nonetheless, the increase in cell death serves as an early marker for the teratogenic action. As we learn more about development and toxicological responses on the molecular level, we will create more sensitive cell response markers that will allow greater resolution of the teratological action.

Overview of Agents and Conditions Associated with Axial Skeletal Teratogenesis

As stated above, axial skeletal malformations are often linked to exposure to teratogenic conditions. The following summarizes the types of teratogens and teratogenic conditions associated with spinal malformations (Schardein 2000). Detailed descriptions of some of these factors will be presented in the following sections.

Recreational teratogens: Recreational drugs such as alcohol, cocaine, and cigarettes are known to significantly reduce fetal and post-natal growth, increase infant mortality, and cause congenital malformations of various types and severity.

Pharmaceutical teratogens: Most embryonic organs and the central nervous system are extremely sensitive to the teratogenic affects of pharmaceuticals such as thalidomide, diethylstilbestrol, retinoic acid, valproic acid, warfarin, chemotherapy, lithium, and nicotinic acid.

Industrial and environmental teratogens: Industrial processes required to provide for growing populations worldwide release a substantial amount of waste products into the environment, with the toxicologic and teratogenic effect of many species as yet uncharacterized. Among the chemicals with known teratogenic effects are organic solvents; arsenic, cadmium, and lead anesthetic gases; and organic mercury.

Agricultural teratogens: Insecticides and herbicides are critical to providing nutrition to growing populations. Studies have determined that organochlorine insecticides such as DTT, parathion, and malathion may interfere with fertility and reproduction by mimicking estrogen-like compounds. Among herbicides, the byproduct of Agent Orange, 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD), is highly teratogenic causing cleft palate and congenital renal abnormalities.

Infectious diseases: Microbial chemicals may act as teratogens. Microbes such as syphilis, cytomegalovirus, rubella, herpes, toxoplasma, and fifth disease affect 1–5% of all live births. These infections may cause a group of associated malformation known as the TORCH complex, as well as isolated structural defects and functional deficits.

Metabolic conditions: Some metabolic disorders, most prominently diabetes and hyperthermia also induce congenital malformations in the embryos. Diabetic pregnancy increases the frequency of a wide variety of congenital defects over background including cardiac defects, eye and ear defects, renal defects, and functional deficits in addition to a high rate of congenital scoliosis, in addition to increased embryonic death and life-long metabolic disorders.

Non-genetically Linked Conditions Characterized by Axial Skeletal Defects: VATER Association

The VATER spectrum is a non-random association characterized by vertebral anomalies (V), anal atresia (A), tracheoesophageal (TE) fistula, and renal (R) anomalies (Botto et al. 1997, Cohen 1997, Martínez-Frías and Frías 1997). This spectrum may also be associated with cardiovascular (C) anomalies and limb (L) anomalies (VACTERL). The incidence of VATER in diabetic mothers is 200× higher than in the general population, which occurs at a rate of 16 per 100,000 births (Pauli 1994, Cohen 1997, Martínez-Frías et al. 1998a). Vertebral defects in this association can involve agenesis, hypoplasia, and hemivertebrae, often afflicting many contiguous vertebral units. As the acronym suggests, many associated tissues are affected. The association of these different mesenchymally derived tissues to the vertebral column and the timing of their development are critical to hypothesizing the origin and mechanism of the defect(s). Analyses of the frequency and co-occurrence of the features of VACTERL and other syndromes suggest that the anomalies can extend to various cranio-caudal levels suggesting a time dependency and critical period through a defect in a common mechanism of dysmorphogenesis (Stewart et al. 1993). The VACTERL sequence can be conceptually included in a group of progressively severe spectrums of which it may be the most severe (Table 3.5). This broad spectrum of malformations has been coined the axial mesodermal dysplasia complex (AMDC) (Stewart et al. 1993). Some confounding features to any hypothesis are the broad range of defects sometimes involving tissues derived from all germ layers, its largely spontaneous occurrence, and low rate of subsequent inheritance.

There are two related, non-exclusive models currently employed to explain the etiology of AMDC suggesting that the collection of defects may arise from a single environmental insult at a time early in post-implantation development. In the first theory, the embryo at the time of early gastrulation is comprised of a single morphogenetic field, the primary developmental field (Optiz et al. 2002, Martinez-Frias et al. 1998b). At this time the embryo responds essentially as a single, homogeneous

Table 3.5 Common features of different associations within the axial mesodermal dysplasia complex spectrum

Malformation	VACTERL	VATER	OAV	PIV	PHS
Vertebral	**X**	**X**	**X**	**X**	
Imperforate anus	**X**	**X**		**X**	**X**
Craniofacial	**X**		**X**		
Tracheal–esophageal fistula	**X**	**X**			
Renal abnormalities	**X**	**X**			**X**
Limb anomalies	**X**			**X**	**X**

(OAV) Oculo-auriculo-vertebral dysplasia, (PIV) Polyoligodactyly-imperforate anus-vertebral anomalies syndrome, (PHS) Pallister-Hall syndrome.

entity. The primary effect of the insult at this time is to affect growth (proliferation) within the embryo, drastically affecting the existence and position of organizing centers and tissue morphogenesis throughout the embryo as this primary developmental field subdivides into secondary developmental fields that will give rise to the various organs and structures of the embryo including the axial skeleton (Martinez-Frias and Frias 1999). If the insult occurs at this time, there is necessarily a wide range of structures affected (polytopic defects) often of mesenchymal origin, but involving ectodermal and endodermal germ layers as well.

A second variant on the theory holds that the broad spectrum of defects reflects a common mechanistic cause in many tissues of a more heterogeneous entity comprised of multiple secondary developmental fields, such that different tissues of the embryo respond in specific manners to produce the wide spectrum of observed defects (Martínez-Frías and Frías 1997, Bohring et al. 1999). The defect then is thought to lie more in mechanisms of patterning or morphogenesis as the insult or defect occurs slightly later in development. This latter variation on developmental field defects and the etiology of multiple congenital anomalies such as VATER appears to more easily explain the wide spectrum of cranio-caudal positions of the defects and the wide degree of severity observed in several multiple congenital defect associations by allowing for a longer critical period. Both of these theories have been characterized theoretically and statistically to the range of defects observed in infants born to diabetic mothers, one of the most frequently recognized "causative" factors of the VATER spectrum (Martínez-Frías et al. 1998a). The high incidence of the VATER and other AMDC variants in diabetic mothers suggests an etiology that involves a fundamental metabolic imbalance in energy production or a dysfunction in a critical component of the embryonic stress response. Some investigators have suggested that the defects may arise from malformation or dysfunction of the notochord, which is critical to the establishment and maintenance of embryonic axes and the patterning and differentiation of many mesenchymal tissues (Gilbert-Barness et al. 2001). It has been suggested that notochord mutants such as brachyury (*T*) or sonic hedgehog (*SHH*) knock-outs could be used as models for VATER and AMDC (Arsic et al. 2002). We discuss the potential for energy metabolism dysregulation as the locus affected resulting in VATER in the context of diabetes-induced congenital scoliosis below.

Environmental Factors That Cause Axial Skeletal Dysmorphogenesis

Valproic Acid

Valproic acid (VPA) is an anti-epileptic drug that is associated with a 20-fold increased incidence of spina bifida, a neural tube defect, in children born to pregnant mothers undergoing VPA treatment (Lammer et al. 1987, Nau et al. 1991). Experimentally, VPA has been shown to be teratogenic in mouse, rat, chick, hamster, rabbit, and rhesus monkeys (Ehlers et al. 1992, Menegola et al. 1996, Vorhees et al. 1987, Barnes et al. 1996b, Basu and Wezeman 2000, Hendrickx et al. 1988). Skeletal abnormalities in these models were most commonly observed, involving vertebrae, ribs, digits, and craniofacial bones. These frequently occur in the context of other cardiovascular, urogenital, and neurological anomalies that together comprise the fetal valproate syndrome. The axial skeletal defects can include presacral vertebrae, cervical and thoracic ribs, indicating possible homeotic transformations. The defects may also include structural vertebral defects, indicating segmentation defects.

In general, the primary locus of teratogens causing spina bifida including VPA is believed to be the neural tube, resulting in failure of neural tube closure (Turner et al. 1990). Subsequently, the neural arches are unable to fuse. However, vertebral defects such as block vertebrae and hemivertebrae sometimes coincide with a neural tube defect have also been observed following VPA exposure (Barnes et al. 1996b). More detailed studies have shown that important patterning genes, such as *Pax1* and paraxis (*Tcf15*), are down-regulated by the administration of VPA in chick embryos (Barnes et al. 1996b, Barnes et al. 1997). The malformations produced by VPA can be mimicked through the administration of anti-sense deoxynucleotides during somitogenesis (Barnes et al. 1996b, Barnes et al. 1997). This type of data confirms that dysregulation of these genes can be teratogenic, but does not indicate a specific mechanism for how this may occur. The down-regulation of these genes may be caused by decreased signaling or reduced or delayed differentiation caused by increased ROS production or altered nucleic acid metabolism (Fantel 1996, Nau et al. 1991), as suggested by studies showing that folic acid administration can significantly reduce the incidence of experimentally induced VPA axial skeletal defects (Green and Copp 2005). More recently, VPA has been shown to also inhibit histone deacetylase activity at therapeutic levels, and that this activity is correlated with axial skeletal defects and exencephaly (Menegola et al. 2006). In a comparison of the teratogenicity and changes in gene expression by VPA and TSA (Trichostatin A), many of the shared genetic effects were specific to skeletal and cardiac muscle, assigning a more specific mechanistic action of VPA to dysregulating epigenetic control which leads to altered gene expression.

Hypoxia

Congenital vertebral anomalies have been produced in newborn animals experimentally by transient hypoxia and transient exposure during the embryonic period

(Grabowski and Paar 1958, Ingalls and Philbrook 1958, Rivard 1986, Webster and Abela 2007). In these studies, many gross vertebral and associated skeletal defects have been induced, including hemivertebrae, vertebral fusions, fragmented vertebral bodies, bifid ribs, or junctions of two or more ribs. The nature and extent of skeletal malformations induced have been dependent upon the precise stage of somite formation at the time when maternal stress has been induced. Hypoxia is thought to affect the early embryo through the induction of increased reactive oxygen species (ROS) homotopically (where ROS are already prominent; Fantel 1996) and later through altered vascularization (Grabowski 1961, Danielson et al. 1992, Webster and Abela 2007). Less well defined is the idea that hypoxia itself or its management is important in and of itself for morphogenic process or cell function during embryonic development (Chen et al. 1999, Semenza 1999).

During early organogenesis as the embryonic circulation develops, the embryo is known to undergo a transition from anaerobic respiration to aerobic respiration (Hunter and Sadler 1989, Hunter and Tugman 1995, Mackler et al. 1975, Miki et al. 1988a). Recent studies have confirmed that oxygenation and the cellular response to oxygenation as interpreted through expression patterns of heat shock proteins (protective chaparones) (Edwards et al. 1997, Mirkes 1997), antioxidant (superoxide dismutases) (Wells and Winn 1996, Ornoy 2007, Forsberg et al. 1996, Yon et al. 2008, Zaken et al. 2000), and HIF1alpha expression (Iyer et al. 1998, Maltepe et al. 1997, Jain et al. 1998, Minet et al. 2000) vary between different tissues of the embryo over time. Some of these variations have been correlated to periods of teratogenic susceptibility (Ornoy et al. 1999, Forsberg et al. 1996). In this transition, mitochondrial respiration may be inefficient producing higher-than-usual amounts of ROS at a time when embryonic defenses against ROS damage are not well developed. This combination can lead to excess ROS-induced cell stress and cell death (Dennery 2007, Dumollard et al. 2007, Burton et al. 2003). One hypothesis is that those tissues undergoing energetically demanding process such as morphogenesis are most susceptible to the oxygenation transition, a hypothesis furthered in diabetic embryopathy. The neural tube and somatic mesoderm have been shown to have a higher metabolic activity (Raddatz and Kucera 1983, Miki et al. 1988a, b, Mackler et al. 1971, Mackler et al. 1975) than surrounding tissues during early organogenesis, the time of greatest susceptibility to environmentally induced axial skeletal defects.

Carbon Monoxide

Early work studying the effects of hypoxia utilized carbon monoxide as a chemical hypoxic agent. Carbon monoxide (CO) is an odorless, colorless, nonirritating gas produced by the incomplete combustion of carbon containing materials. There have been no epidemiological studies of the direct effect of CO on human pregnancies (Schardein 2000). However, there are a number of case reports and anecdotes suggesting that CO may be a teratogen in humans (Robkin 1997, Longo 1977). Anecdotal accounts were given in Brander (Robkin 1997) and reported congenital malformations, such as microcephaly, micrognathia, and limb defects

including hip dysplasia, tetraplegia, equinovarus, and limb reduction. Indirect epidemiological information can be obtained from the observations of pregnancy outcomes among women who smoke. Maternal smoking is associated with various adverse outcomes including low birth weight, decreases in successful births (Fichtner et al. 1990), and various behavioral defects that can be mimicked by CO alone in animal models (Bnait and Seller 1995).

There are a limited number of studies linking CO to congenital malformations. Early studies in chick, rabbit, and rat showed a causative relationship (Baker and Tumasonis 1972, Murray et al. 1979); however, later studies failed to confirm this connection (Astrup et al. 1975). More recent studies exploring threshold levels and critical periods related to CO-induced effects upon the embryo have documented CO-induced dysmorphogenesis (Bailey et al. 1994, Daughtrey et al. 1983, Loder et al. 2000, Alexander and Tuan 2003). CO exposures during early organogenesis, the critical period, resulted in vertebral anomalies, microphthalmia, and a phenotype similar to caudal dysgenesis syndrome. Such malformations have been reported with CO exposures administered during organogenesis in the context of other teratogens at sub-teratogenic levels (Singh et al. 1993, Singh 2006). This may be a significant problem worldwide since acute carbon monoxide exposures may be higher and more frequent than often reported (Fichtner et al. 1990, Ralston and Hampson 2000).

CO does impair oxygen delivery to and into cells by binding hemoglobin, myoglobin, and other porphyrins; however, it may also function as a signaling molecule in the context of nitric oxide (NO) signaling (Maines 1997). When administered after the vascular system is developed, the axial defects caused by CO are attributed to vascular leakage and subsequent mechanical disruption of developing tissue (Baker and Tumasonis 1972). However, during early organogenesis, axial defects involve the reduction of important segmentation genes including *Pax1* and paraxis (*Tcf15*) (Alexander and Tuan 2003), resulting possibly in the impaired inductive interaction of the neural tube with the paraxial mesoderm with CO acting as a signaling molecule. Nitric oxide is known to regulate neurulation and other early embryonic processes (Lee and Juchau, 1994), and CO can alter the production of NO in axial tissues (Alexander et al. 2007a). The impaired interaction is likely due to a loss of cell function characterized or indicated by increased neural tube apoptosis and loss of neural tube-derived somite epithelialization signals.

Diabetes

Maternal diabetes is known to have many teratogenic effects (Finnell and Dansky 1991, Aberg et al. 2001). Malformations including neural tube defects, caudal dysgenesis, vertebral defects, congenital heart defects, femoral hypoplasia, renal, and craniofacial anomalies are described in infants of diabetic mothers. Caudal regression syndrome is a severe condition characterized by agenesis, regression, and or disorganization of the posterior (sacral–lumbar) vertebrae and the malformation of the soft tissue at that level and below (Bohring et al. 1999, Martinez-Frias

et al. 1998b). It occurs 200 times more frequently in diabetic than in nondiabetic pregnancies. Other major malformations of the midline are also much more frequent including VATER, OAV, and other major malformations. Together, these can be placed in a related and progressive spectrum of syndromes and non-random associations belonging to the ADMC.

Mouse models utilizing "diabetic environments" or hyperglycemia report various anomalies encompassing the full spectrum of embryonic embryopathy (Akazawa 1995, Ornoy et al. 1999). These models together reveal that hyperglycemia is sufficient to cause most of the defects observed in diabetic embryopathy including neural tube defects, axial skeletal defects, heart and craniofacial abnormalities, rib and renal defects – although no individual model phenocopies the condition completely. At physiological levels of hyperglycemia or ketosis, the most consistent outcome is a failure of anterior and posterior neuropore closure (Sadler et al. 1988, Sadler and Horton 1983, Horton and Sadler 1983, Ornoy et al. 1986, Sadler et al. 1989). Researchers have determined that the diabetic environment increases ROS production in these regions of the neural tube and in the primordia of the organs listed above including craniofacial region, otic, and optic cups, Hensen's node, and the notochord, caused by the diabetic environment. Coincident with the high ROS is an increase in cell death and a decrease in *Pax3*, a factor critical in neural tube closure (Fine et al. 1999, Loeken 2005). Application of folic acid and other antioxidants greatly reduced the incidence of ROS production (Ornoy 2007), insipient cell death and the reduction of *Pax3* expression.

The caudal agenesis/dysgenesis syndrome can be phenocopied by prolonged exposure to hyperglycemia, hyperketonemia, and streptozotocin. The collection of defects in these severely affected animals indicates an early patterning event is disturbed. The notochord is laid down during gastrulation and is responsible for dorsoventral and mediolateral patterning as well as survival of the mesoderm during axis elongation. High levels of cell death in the notochord are observed in severely affected animals, suggesting the notochord function is likely to be compromised, and mutations in the T-box gene brachyury (Rashbass et al. 1994) and disruption of *Shh* function (Kim et al. 2001) have been presented as possible models of caudal dysgenesis and other manifestations of ADMC.

The incidence and severity of malformations in diabetic pregnancies are correlated with poor glycemic control in the first trimester and can be reduced by instituting tight glycemic control prior to conception, and the evidence presented above of various antioxidants and insulin provides hope that a cocktail can be developed and delivered harmlessly to prevent the initiation of the diabetic embryopathic condition. While prevention of the condition appears at hand, the initial biochemical imbalance presents us with an interesting pattern. The condition of hyperglycemia provides a "free" energy source that is readily available to the mitochondrion for ATP production, a condition opposite to hypoxia, in which ATP production in greatly decreased. A reasonable hypothesis incorporating these two opposite conditions is that molecular regulation of any developmental process can be disturbed by abnormal maternal fuel metabolism, and the timing of specific episodes of poor glycemic control determines which organ systems are affected.

Retinoic Acid

Retinoic acid (RA) is an analog of vitamin A commonly used to treat acne and other skin conditions. In humans, prenatal exposure results in a characteristic pattern of defects including abnormalities in the ears, mandibles, palates, aortic arch, and central nervous system. In animal models, many similar defects are observed (Gudas 1994). At higher doses delivered during organogenesis, RA can induce axial skeletal defects as well as including homeotic transformations (Kessel and Gruss 1991, Rubin and LaMantia 1999, Kawanishi et al. 2003), and at higher doses axial skeletal truncations (Padmanabhan 1998).

Aside from being a well-characterized teratogen, retinoic is also a naturally occurring chemical involved in many aspects of embryonic patterning, including the patterning of the somites. The teratogenic effects of retinoic acid above are consistent with the in situ expression of RA receptors (Maden 1994, Cui et al. 2003, Iulianelle et al. 1999) and metabolic-transforming enzymes (Swindell et al. 1999, Niederreither et al. 2002, Reijntjes et al. 2004, Cammas et al. 2007) as well as the effect of knocking down these molecules in murine models. RA, its receptors, and *CYP26* are expressed in the paraxial mesoderm and act as critical regulators in the coordination of the somitogenesis clock and HOX gene expression (Duester 2007, reviewed in Sewell and Kusumi 2007). At higher levels, it is hypothesized that RA interrupts tissue morphogenesis, neural crest migration, and at highest doses causes cell death in morphogenically critical tissues, such as the neural tube, notochord, and paraxial mesoderm, resulting in a phenotype similar to caudal dysgenesis syndrome (Iulianelle et al. 1999).

Hyperthermia

Exposure of the human fetus to high temperatures (for example, 2°C over normal), as in the case of high fever or prolonged hot tub usage, is associated with neural tube defects, heart defects, microphthalmia, and functional deficits (Graham et al. 1998, Edwards et al. 1997). There is no epidemiological evidence suggesting heat shock causes axial skeletal defects. In studying the mechanisms of heat shock teratogenesis in animal models, vertebral defects were observed in many species including mice, rats, and chicks (Breen et al. 1999, Mirkes and Cornel 1992, Primmet et al. 1988). The severity of these defects is correlated to the time and duration of exposure. Experimental studies in chick embryos revealed that at moderate levels and exposure times (42°C for 20 minutes), one or two adjacent segments were fused into a single large somite. This effect was repeated every 7–8 somites separated by normal somitogenesis (Primmet et al. 1988). This result suggested a cell cycle-dependent mechanism to the defect and to somitogenesis itself, prompting the proposal of a clock and wave-front model for the patterning process of somitogenesis (Primmet et al. 1989).

The response of the embryo to hyperthermia is very dependent upon the degree of temperature increase, its duration, and the stage at which the heat shock is

experienced (Graham et al. 1998). There is a steep threshold for embryonic survival and resorption, which suggests the general outcome of hyperthermia is embryonic resorption. At levels of hyperthermia inducing embryonic survival and malformation, tissue-specific cell death is observed. Investigators identified the induction of heat shock proteins (HSP) as a prominent feature of the embryonic response. These molecular chaperones play important roles in regulating protein folding during normal cell function, but they also serve to protect cells from environmental insult. In the process, the HSP-bound proteins are not able to perform their function (Buckiova et al. 1998, Walsh et al. 1999). During teratological doses of hyperthermia, the cell cycle is slowed, suggesting a mechanism of the vertebral anomalies observed. Recently the mechanism of somitogenesis was shown to involve the tightly controlled, cyclic expression of a variety of proteins many belonging to the Notch/Delta signaling system (Shifley and Cole 2007). During heat shock, some of these proteins or their targets may be bound by HSP, and we can hypothesize that this would disrupt the somitogenic clock resulting in disrupted pattern and ultimately vertebral defects. An important feature of the protective heat shock response then and its relation to teratogenesis is that their activation and function may reduce or delay tissue development or morphogenic actions. In fact, many teratogenic insults induce HSP activity, and as such HSP activation may be an underlying commonality in teratogenic mechanisms along with ROS production and apoptosis.

Arsenic

Arsenic, a metal pollutant, is found naturally in groundwater and unnaturally in mine waste sites, industrial byproducts, and in agricultural runoff. It is toxic to humans and is known to cause birth defects including spina bifida, craniofacial defects, developmental retardation and to decrease birth weight and increase incidences of fetal mortality, miscarriage, and still birth (Willhite and Ferm 1984, DeSesso et al. 1998). In experimental in vitro models, arsenic is teratogenic in mice, rats, and chicks (Hood and Bishop 1972, Chaineau et al. 1994, Beaudoin 1974, Lindgren et al. 1984, Peterková and Puzanová 1976, DeSesso et al. 1998), with neural tube defects being common among all of them (Shalat et al. 1996, Takeuchi 1979). Its toxicity is greatly dependent on its redox state: arsenate vs. arsenite. The structure of arsenate can mimic that of phosphate groups, imparting arsenate with the ability to disrupt various cell processes including nucleic acid metabolism, lipid metabolism, and electron transport. Inefficient electron transport can lead to high production of ROS, which have documented cell destructive activities and teratogenic capacity (Hunter 2000, Kitchin and Ahmad 2003, Bernstam and Nriagu 2000). In addition, arsenate can reduce to arsenite. The effects of arsenite on disruption of cell cycle and cytoskeletal structure have been attributed to its reaction to sulfhydryl groups (Levinson et al. 1980), which may account for its strong induction of the heat shock response (German et al. 1986, Mirkes and Cornel 1992,

Bernstam and Nriagu 2000). In addition, arsenite can disrupt the citric acid cycle and electron transport via binding to thiol group enzymatic active sites (DeSesso et al. 1998). A disruption in the energy status of different tissues of the developing embryo is attributed to teratogenicity of arsenic causing similar malformations to those observed in hypoxic or hyperglycemic environment; however, arsenic has other distinctive effects on the embryo (DeSesso et al. 1998). Arsenic and other metal compounds are very effective inducers of the heat shock response (Mirkes and Cornel 1992, Bernstam and Nriagu 2000), which may protect cell from molecular damage, but induce birth defects in its own right via disruption of the cell cycle and other cyclic and time-dependent morphogenetic processes (Wlodarczyk et al. 1996). In addition, cells surviving the initial arsenic insult, may pass on genetic damage that contribute to subsequent carcinogenic transformation later in ontogeny (Bernstam and Nriagu 2000). These multiple, interacting mechanisms may account for the wide range of malformations observed following acute arsenic exposure.

Ethanol

Ethanol, widely consumed as a recreational drug, has long been strongly associated with teratogenesis as fetal alcohol syndrome (FAS). FAS is present in one in three children of alcoholic mothers, with an estimated 40,000 children born every year in the United States (Schardein 2000, Thackray and Tift 2001). FAS manifestations include growth deficiency, central nervous system problems, characteristic facial features, and organ malformations. Features of FAS have been observed in animal models exposed to ethanol in utero or in vitro, including mice, rat, chick, and others (Sulik et al. 1981, Becker et al. 1996, Fernandez et al. 1983, Sanders and Cheung 1990, Yelin et al. 2007, Schardein 2000, Chaudhuri 2000, Padmanabhan and Muawad 1985).

The mouse model has been a particularly effective model in elucidating the etiology of ethanol-induced birth defects. One mechanism, of ethanol-induced teratogenesis is through ethanol impaired placental blood flow to the fetus by constricting blood vessels and inducing embryonic/fetal hypoxia and malnutrition (Shibley et al. 1999). Since ethanol rapidly crosses the placenta into the fetus, there are other direct embryonic and fetal targets of ethanol. The mouse model has been a particularly effective model in elucidating the etiology of ethanol-induced birth defects. Ethanol has been shown to increase cell death in critical cell populations including anterior neural folds and neural crest cells (Sulik et al. 1988, Rovasio and Battiato 1995, Dunty et al. 2001), which play a critical role in the morphogenesis of the face. Neural crest cells are particularly vulnerable to ethanol, inducing delayed/altered migration and cell death (Rovasio and Battiato 1995). Correlations have been made to increased ROS production within the neural crest population (Kotch et al. 1995), mitochondrial dysfunction and cell death in the etiology of ethanol and other teratogens (Ornoy 2007). The anterior neural tube and cranial

neural crest have been the subject of intense scrutiny in the teratogenic mechanisms of ethanol, however, other tissues are also affected, including the eye, ear, heart, renal system, and axial skeleton (Kennedy and Elliott 1986, Parnell et al. 2006, Sulik 2005, Webster and Abela 1984, Assadi and Zajak 1992, Sanders and Cheung 1990, Carvan et al. 2004). With respect to the axial skeleton, investigators observed a misalignment or segmentation defect in ethanol-exposed embryos. Despite the substantial morphological difference with heat shock- treated embryos, the investigators suggested that the mechanism may be similar to heat shock (Carvan et al. 2004), involving the induction of the stress response by increased ROS production.

Methanol

Methanol is an alcohol encountered frequently during industrial processes. When the effects of inhaled ethanol and methanol were compared, the highest doses of methanol significantly increased the incidence of various defects including skeletal malformations (Nelson et al. 1985). Skeletal malformations were the most prevalent congenital defects observed and included vertebral abnormalities and an increased incidence of cervical ribs. Other skeletal abnormalities caused by methanol have been observed including holoprosencephaly, facial dysmorphogenesis, basicranial malformation, duplications of the atlas and axis and cervical vertebral abnormalities, and abnormal number of presacral vertebrae (Connelly and Rogers 1997, Rogers et al. 2004). Initial cellular responses appear similar to ethanol at the level of tissue-specific cell death (Abbott et al. 1995). In contrast to ethanol, many of the axial skeletal defects indicate homeotic shifts in segment identity.

Conclusion

Advances in cell and molecular biology with respect to normal development and somitogenesis and the pathogenesis and mechanisms of teratogenesis are occurring at a tremendous rate. This allows teratologists and developmental toxicologists the opportunity to revisit old problems with new tools. Despite the large number of cellular processes that may be disturbed by a teratogen, there are only a limited number of cellular and morphological outcomes. This has led investigators to strive for the identification of very defined critical periods and doses in a variety of model systems to aid in the identification of the initial targets of a teratogen and the true, hypothetically singular target molecule or process, as proposed by Wilson in 1956. Applying genomic and proteomic technologies to the problem of teratogenesis should begin to reveal the full spectrum of cellular processes affected, and elucidate links between variations in genotype and the effect of the environment on the phenotype that produce birth defects such as congenital scoliosis. The identification, at least in part, of this “holy grail” will aid in the development of new preventative treatments to a variety of teratogenic insults.

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References

Abbott, B.D., Ebron-McCoy, M., and Andrews, J.E. 1995. Cell death in rat and mouse embryos exposed to methanol in whole embryo culture. Toxicology 97:159–171.

Aberg, A., Westbom, L., and Källén, B. 2001. Congenital malformations among infants whose mothers had gestational diabetes or preexisting diabetes. Early Hum. Dev. 61:85–95.

Akazawa, S. 2005. Diabetic embryopathy: studies using a rat embryo culture system and an animal model. Cong. Anom. 45:73–79.

Alexander, P.G., Boyce, A.T., and Tuan, R.S. 2007a. Skeletal development. In Principles of Developmental Genetics, ed. S.A. Moody, pp. 866–905. New York, NY: Elsevier Academic Press

Alexander, P.G., Chau, L., and Tuan, R.S. 2007b. Role of nitric oxide in chick embryonic organogenesis and dysmorphogenesis. Birth Defects Res. A Clin. Mol. Teratol. 79:581–594.

Alexander, P.G. and Tuan, R.S. 2003. Carbon monoxide-induced axial skeletal dysmorphogenesis in the chick embryo. Birth Defect Res. Part A Clin. Mol. Teratol. 67:219–230.

Arsic, D., Qi, B.Q., and Beasley, S.W. 2002. Hedgehog in the human: a possible explanation for the VATER association. J. Paediatr. Child Health 38:117–121.

Assadi, F.K. and Zajac, C.S. 1992. Ultrastructural changes in the rat kidney following fetal exposure to ethanol. Alcohol 9:509–512.

Astrup, P., Trolle, D., Olsen, H.M. et al. 1975. Moderate hypoxia exposure and fetal development. Arch. Environ. Health 30:15–16.

Bailey, L.T., Johnston, M.C., and Billet, J. 1994. Effects of carbon monoxide and hypoxia on cleft lip in A/J mice. Cleft Palate-Craniofac. J. 32:14–19.

Baker, F.D. and Tumasonis, C.F. 1972. Carbon monoxide and avian embryogenesis. Arch. Environ. Health 24:53–61.

Barnes, G.L., Alexander, P.G., Hsu, C.W. et al. 1997. Cloning and characterization if chicken *Paraxis*: a regulator of paraxial mesoderm development and somite formation. Dev. Biol. 189:95–111.

Barnes, G.L., Hsu, C.W., Mariani, B.D. et al. 1996a. Chicken *Pax-1* gene: structure and expression during embryonic somite development. Differentiation 61:13–23.

Barnes, G.L., Mariani, B.D., and Tuan, R.S. 1996b. Valproic acid induced somite teratogenesis in the chick embryo: relationship with *Pax-1* gene expression. Teratology 54:93–102.

Basu, A. and Wezeman, F.H. 2000. Developmental toxicity of valproic acid during embryonic chick vertebral chondrogenesis. Spine 25:2158–2164.

Beaudoin, A.R. 1974. Teratogenicity of sodium arsenate in rats. Teratology 10:153–157.

Becker, H.C., Diaz-Granados, J.L., and Randall, C.L. 1996. Teratogenic actions of ethanol in the mouse: a minireview. Pharmacol. Biochem. Behav. 55:501–513.

Bernstam, L. and Nriagu, J. 2000. Molecular aspects of arsenic stress. J. Toxicol. Environ. Health B Crit. Rev. 3:293–322.

Bnait, K.S. and Seller, M.J. 1995. Ultrastructural changes in 9-day old mouse embryos following maternal tobacco smoke inhalation. Exp. Toxicol. Pathol. 47:453–461.

Bohring, A., Lewin, S.O., Reynolds, J.F. et al. 1999. Polytopic anomalies with agenesis of the lower vertebral column. Am. J. Med. Genet. 87:99–114.

Botto, L.D., Khoury, M.J., Mastroiacovo, P. et al. 1997. The spectrum of congenital anomalies of the VATER association: an international study. Am. J. Med. Genet. 71:8–15.

Breen, J.G., Claggett, T.W., Kimmel, G.L. et al. 1999. Heat shock during rat embryo development in vitro results in decreased mitosis and abundant cell death. Reprod. Toxicol. 13:31–39.

Brent, R.L. and Fawcett, L.B. 2007. Developmental toxicology, drugs, and fetal teratogenesis. In Clinical Obstetrics: The Tetus and Mother, 3rd Ed., eds. E.A. Reece and J.C. Hobbins, pp. 217–235. Malden, MA: Blackwell Publishing, Inc.

Buckiova, D., Kubinova, L., Soukup, A. et al. 1998. Hyperthermia in the chick embryo: HSP and possible mechanisms of developmental defects. Int. J. Dev. Biol. 42:737–740.

Burton, G.J., Hempstock, J., and Jauniaux, E. 2003. Oxygen, early embryonic metabolism and free radical-mediated embryopathies. Reprod. Biomed. Online 6:84–96.

Cammas, L., Romand, R., Fraulob, V. et al. 2007. Expression of the murine retinol dehydrogenase 10 (Rdh10) gene correlates with many sites of retinoid signalling during embryogenesis and organ differentiation. Dev. Dyn. 236:2899–2908.

Carvan, M.J. 3rd, Loucks, E., Weber, D.N. et al. 2004. Ethanol effects on the developing zebrafish: neurobehavior and skeletal morphogenesis. Neurotoxicol. Teratol. 26:757–768.

Chaineau, E., Binet, S., Pol, D. et al. 1994. Embryotoxic effects of sodium arsenite and sodium arsenate on mouse embryos in culture. Teratology 41:105–112.

Chaudhuri, J.D. 2000. Alcohol and the developing fetus–a review. Med. Sci. Monit. 6:1031–1041.

Chen, E.Y., Fujinaga, M., and Giaccia, A.J. 1999. Hypoxic microenvironment within an embryo induces apoptosis and is essential for proper morphological development. Teratology 60: 215–225.

Christ B., Huang R., Scaal M. (2004) Formation and differentiation of the avian sclerotome. Anat Embryol 208:333–350.

Christ, B., Huang, R., Wilting, J. 2000. The development of the avian vertebral column. Anat. Embryol. 202:179–194.

Cohen, M.M. 1997. The Child with Multiple Birth Defects. pp. 3–14. New York, NY: Oxford University Press

Connelly, L.E. and Rogers, J.M. 1997. Methanol causes posteriorization of cervical vertebrae in mice. Teratology 55:138–144.

Cui, J., Michaille, J.J., Jiang, W. et al. 2003. Retinoid receptors and vitamin A deficiency: differential patterns of transcription during early avian development and the rapid induction of RARs by retinoic acid. Dev. Biol. 260:496–511.

Danielson, M.K., Danielsson, B.R., Marchner, H. et al. 1992. Histopathological and hemodynamic studies supporting hypoxia and vascular disruption as explanation to phenytoin teratogenicity. Teratology 46:485–497.

Daughtrey, W.C., Newby-Schmidt, M.B., and Norton, S. 1983. Forebrain damage in chick embryos exposed to carbon monoxide. Teratology 28:83–89.

Dennery P.A. 2007. Effects of oxidative stress on embryonic development. Birth Defects Res. C Embryo Today 81:155–162.

DeSesso, J.M., Jacobson, C.F., Scialli, A.R. et al. 1998. An assessment of the developmental toxicity of inorganic arsenic. Reprod. Toxicol. 12:385–433.

Dias, M.S. 2007. Normal and abnormal development of the spine. Neurosurg. Clin. N. Am. 18:415–429.

Duester, G. 2007. Retinoic acid regulation of the somitogenesis clock. Birth Defects Res. Part C 81:84–92.

Dumollard, R., Duchen, M., and Carroll, J. 2007. The role of mitochondrial function in the oocyte and embryo. Curr. Top. Dev. Biol. 77:21–49.

Dunty, W.C. Jr., Chen, S.Y., Zucker, R.M. et al. 2001. Selective vulnerability of embryonic cell populations to ethanol-induced apoptosis: implications for alcohol-related birth defects and neurodevelopmental disorder. Alcohol Clin. Exp. Res. 25:1523–1535.

Edwards, M.J., Walsh, D.A., Li, Z. 1997. Hyperthermia, teratogenesis and the heat shock response in mammalian embryos in culture. Int. J. Dev. Biol. 41:345–358.

Ehlers, K., Stürje, H., Merker, H.J. et al. 1992. Valproic acid-induced spina bifida: a mouse model. Teratology 45:145–154.

Erol, B., Kusumi, K., Lou, J. et al. 2002. Etiology of congenital scoliosis. U. Penn. Orthoped. J. 15:37–42.

Fantel, A.G. 1996. Reactive oxygen species in developmental toxicity: review and hypotheses. Teratology 53:196–217.

Fernandez, K., Caul, W.F., Boyd, J.E. et al. 1983. Malformations and growth of rat fetuses exposed to brief periods of alcohol in utero. Teratog. Carcinog. Mutagen. 3:457–460.

Fichtner, R.R., Sullivan, K.M., Zyrkowski, C.L. et al. 1990. Racial/Ethnic differences in smoking, other risk factors and low birth weight among low-income pregnant women, 1978–1988. M.M.W.R. C.D.C. Surveill. Summ. 39(3):13–31.

Fine, E.L., Horal, M., Chang, T.I. et al. 1999. Evidence that elevated glucose causes altered gene expression, apoptosis, and neural tube defects in a mouse model of diabetic pregnancy. Diabetes 48:2454–2462.

Finnell, R.H. and Dansky, L.V. 1991. Parental epilepsy, anticonvulsant drugs, and reproductive outcome: epidemiological and experimental findings spanning three decades; 1: Animal Studies. Reprod. Toxicol. 5:281–299.

Forsberg, H., Borg, L.A., Cagliero, E. et al. 1996. Altered levels of scavenging enzymes in embryos subjected to a diabetic environment. Free Radic. Res. 24:451–459.

German, J., Louie, E, and Banerjee, D. 1986. The heat-shock response in vivo: experimental induction during mammalian organogenesis. Teratog. Carcinog. Mutagen. 6:555–562.

Gilbert-Barness, E., Debich-Spicer, D. et al. 2001. Evidence for the "midline" hypothesis in associated defects of laterality formation and multiple midline anomalies. Am. J. Med. Genet. 101:382–387.

Grabowski, C.T. 1961. A quantitative study of the lethal and teratogenic effects of hypoxia on the three-day chick embryo. Am. J. Anat. 109:25–35.

Grabowski, C.T. and Paar, J.A. 1958. The teratogenic effects of graded doses of hypoxia on the chick embryo. Am. J. Physiol. 103:313–347.

Graham, J.M. Jr., Edwards, M.J., and Edwards, M.J. 1998. Teratogen update: gestational effects of maternal hyperthermia due to febrile illnesses and resultant patterns of defects in humans. Teratology 58:209–221.

Green, N.D.E. and Copp, A.J. 2005. Mouse models of neural tube defects: investigating preventative mechanisms. Am. J. Med. Genet. Part C 135:31–41.

Gridley, T. 2006. The long and short of it: somite formation in mice. Dev. Dyn. 235:2330–2336.

Gudas, L.J. 1994. Retinoids and vertebrate development. J. Biol. Chem. 269:15399–153402.

Healy, C., Uwanogho, D., and Sharpe, P.T. 1999. Regulation and role of Sox9 in cartilage formation. Dev. Dyn. 215:69–78.

Hendrickx, A.G., Nau, H., Binkerd, P. et al. 1988. Valproic acid developmental toxicity and pharmacokinetics in the rhesus monkey: an interspecies comparison. Teratology 38: 329–345.

Hood, R.D. and Bishop, S.L. 1972. Teratogenic effects of sodium arsenate in mice. Arch. Environ. Health 24:62–65.

Horton, W.E. Jr. Sadler, T.W. 1983. Effects of maternal diabetes on early embryogenesis. Alterations in morphogenesis produced by the ketone body, B-hydroxybutyrate. Diabetes 32:610–616.

Hunter, E.S. 3rd. 2000. Role of oxidative damage in arsenic-induced teratogenesis. Teratology 62:240.

Hunter, E.S. 3rd and Sadler, T.W. 1989. Fuel-mediated teratogenesis: biochemical effects of hypoglycemia during neurulation in mouse embryos in vitro. Am. J. Physiol. 257:E269–276.

Hunter, E.S. 3rd and Tugman, J.A. 1995. Inhibitors of glycolytic metabolism affect neurulation-staged mouse conceptuses in vitro. Teratology 52:317–323.

Ingalls, T.H. and Philbrook, F.R. 1958. Monstrosities induced by hypoxia. New Eng. J. Med. 259:558–564.

Iulianella, A., Beckett, B., Petkovich, M. et al. 1999. A molecular basis for retinoic acid-induced axial truncation. Dev. Biol. 205:33–48.

Iyer, N.V., Kotch, L.E., Agani, F. et al. 1998. Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev. 12:149–162.
Jain, S., Maltepe, E., Lu, M.M. et al. 1998. Expression of ARNT, ARNT2, HIF1 alpha, HIF2 alpha and Ah receptor mRNAs in the developing mouse. Mech. Dev. 73:117–123.
Jaskwhich, D., Ali, R.M. et al. 2000. Congenital scoliosis. Curr. Opin. Pediatr. 12:61–66.
Kawanishi, C.Y., Hartig, P., Bobseine, K.L. et al. 2003. Axial skeletal and hox expression domain alterations induced by retinoic acid, valproic acid, and bromoxynil during murine development. J. Biochem. Mol. Toxicol. 17:346–356.
Kennedy, L.A. and Elliott, M.J. 1986. Ocular changes in the mouse embryo following acute maternal ethanol intoxication. Int. J. Dev. Neurosci. 4:311–317.
Kessel, M. and Gruss, P. 1991. Homeotic transformations of murine vertebrae and concomitant alteration of Hox codes induced by retinoic acid. Cell 67:89–104.
Kim, P.C.W., Mo, R., Hui, C.-C. 2001. Murine models of VACTERL syndrome: role of sonic hedgehog signaling pathway. J. Pediatr. Surg. 36:381–384.
Kitchin, K.T. and Ahmad, S. 2003. Oxidative stress as a possible mode of action for arsenic carcinogenesis. Toxicol. Lett. 137:3–13.
Kotch, L.E., Chen, S.Y., and Sulik, K.K. 1995. Ethanol-induced teratogenesis: free radical damage as a possible mechanism. Teratology 52:128–136.
Lammer, E.J., Sever, L.E., and Oakley, G.P. 1987. Teratogen up date: Valproic acid. Teratology 35:465–473.
Lee, Q.P. and Juchau, M.R. 1994. Dysmorphogenic effects of nitric oxide (NO) and NO-synthase inhibition: studies with intra-amniotic injections of sodium nitroprusside and NG-monomethyl-L-arginine. Teratology 49:452–464.
Levinson, W., Oppermann, H., Jackson, J. 1980. Transition series metals and sulfhydryl reagents induce the synthesis of four proteins in eukaryotic cells. Biochim. Biophys. Acta. 606: 170–180.
Lindgren, A., Danielsson, B.R., Dencker, L. et al. 1984. Embryotoxicity of arsenite and arsenate: distribution in pregnant mice and monkeys and effects on embryonic cells in vitro. Acta Pharmacol. Toxicol. (Copenh.) 54:311–320.
Loder, R.T., Hernandez, M.J., Lerner, A.L. et al. 2000. The induction of congenital spinal deformities in mice by maternal carbon monoxide exposure. J. Pediatr. Orthoped. 20:662–666.
Loeken, M.R. 2005. Current perspectives on the causes of neural tube defects resulting from diabetic pregnancy. Am. J. Med. Genet. C Semin. Med. Genet. C 135:77–87.
Longo, L.D. 1977. The biological effects of carbon monoxide on the pregnant woman, fetus, and the newborn infant. Am. J. Obstetr. Gynecol. 129:69–103.
Mackler, B., Grace, R., Duncan, H.M. 1971. Studies of mitochondrial development during embryogenesis in the rat. Arch. Biochem. Biophys. 144:603–610.
Mackler, B., Grace, R., Tippit, D.F. et al. 1975. Studies of the development of congenital anomalies in rats. III. Effects of inhibition of mitochondrial energy systems on embryonic development. Teratology 12:291–296.
Maden, M. 1994. Distribution of cellular retinoic acid-binding proteins I and II in the chick embryo and their relationship to teratogenesis. Teratology 50(4):294–301.
Maines, M.D. 1997. The heme oxygenase system: a regulator of second messenger gases. Ann. Rev. Pharmacol. Toxicol. 37:517–554.
Maltepe, E., Schmidt, J.V., Baunoch, D. et al. 1997. Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. Nature 386:403–407.
Martínez-Frías, M.L., Bermejo, E., Rodríguez-Pinilla, E. et al. 1998a. Epidemiological analysis of outcomes of pregnancy in gestational diabetic mothers. Am. J. Med. Genet. 78:140–145.
Martínez-Frías, M.L., and Frías, J.L. 1997. Primary developmental field. III: Clinical and epidemiological study of blastogenetic anomalies and their relationship to different MCA patterns. Am. J. Med. Genet. 70:11–15.
Martinez-Frias, M.L., and Frias, J.L. 1999. VACTERL as primary, polytopic, developmental field defects. Am. J. Med. Genet. 83:13–16.

Martinez-Frias, M.L., Frias, J.L., and Opitz, J.M. 1998b. Errors of morphogenesis and developmental field theory. Am. J. Med. Genet. 76:291–296.

Menegola, E., Broccia, M.L., Nau, H. et al. 1996. Teratogenic effects of sodium valproate in mice and rats at midgestation and at term. Teratog. Carcinog. Mutagen. 16:97–108.

Menegola, E., Di Renzo, F., Broccia, M.L. et al. 2006. Inhibition of histone deacetylase as a new mechanism of teratogenesis. Birth Defects Res. C Embryo Today 78:345–353.

Miki, A., Fujimoto, E., Ohsaki, T. et al. 1988a. Effects of oxygen concentration on embryonic development in rats: a light and electron microscopic study using whole embryo culture techniques. Anat. Embryol. 178:337–343.

Miki, A., Mizoguchi, A., Mizoguti, H. 1988b. Histochemical studies of enzymes of the energy metabolism in post implantation rat embryos. Histochemistry 88:489–495.

Minet, E., Michel, G., Remacle, J. et al. 2000. Role of HIF-1 as a transcription factor involved in embryonic development, cancer progression, and apoptosis. Int. J. Mol. Med. 5: 253–259.

Mirkes, P.E. 1997. Molecular/cellular biology of the heat stress response and its role in agent-induced teratogenesis. Mutat. Res. 396:163–173.

Mirkes, P.E. and Cornel, L. 1992. A comparison of sodium arsenite- and hyperthermia-induced stress responses and abnormal development in cultured postimplantation rat embryos. Teratology 46:251–259.

Murray, F.J., Schwetz, B.A., Crawford, A.A. et al. 1979. Embryotoxicity of inhaled sulfur dioxide and carbon monoxide in mice and rabbits. J. Environ. Sci. Health C 13:233–250.

Nau, H., Hauck, R.S., and Ehlers, K. 1991. Valproic acid-induced neural tube defects in mouse and human: aspects of chirality, alternative drug development, pharmacokinetics and possible mechanisms. Pharmacol. Toxicol. 69:310–321.

Nelson, B.K., Brightwell, W.S., MacKenzie, D.R. et al. 1985. Teratological assessment of methanol and ethanol at high inhalation levels in rats. Fundam. Appl. Toxicol. 5:727–736.

Niederreither, K., Fraulob, V., Garnier, J.M. et al. 2002. Differential expression of retinoic acid-synthesizing (RALDH) enzymes during fetal development and organ differentiation in the mouse. Mech. Dev. 110:165–171.

Nishimura, H., Tanimura, T., Semba, R. et al. 1974. Normal development of early human embryos: observation of 90 specimens at Carnegie stages 7 to 13. Teratology 10:1–5.

Opitz, J.M., Zanni, G., Reynolds, J.F. Jr. et al. 2002. Defects of blastogenesis. Am. J. Med. Genet. 115:269–286.

O'Rahilley, R., and Müller, F. 1996. Human Embryology and Teratology, 3rd Ed. New York, NY: Wiley-Liss Publishers

Ornoy, A. 2007. Embryonic oxidative stress as a mechanism of teratogenesis with special emphasis on diabetic embryopathy. Reprod. Toxicol. 24:31–41.

Ornoy, A., Zaken, V., and Kohen, R. 1999. Role of reactive oxygen species (ROS) in the diabetes-induced anomalies in rat embryos in vitro: reduction in antioxidant enzymes and low-molecular-weight antioxidants (LMWA) may be the causative factor for increased anomalies. Teratology 60:376–386

Ornoy, A., Zusman, I., Cohen, A.M. et al. 1986. Effects of sera from Cohen, genetically determined diabetic rats, streptozotocin diabetic rats and sucrose fed rats on in vitro development of early somite rat embryos. Diabetes Res. 3:43–51.

Oskouian, R.J., Sansur, C.A., and Shaffrey, C.I. 2007. Congenital abnormalities of the thoracic and lumbar spine. Neurosurg. Clin. N. Am. 18:479–498.

Padmanabhan, R. 1998. Retinoic acid-induced caudal regression syndrome in the mouse fetus. Reprod. Toxicol.12:139–511.

Padmanabhan, R. and Muawad, W.M. 1985. Exencephaly and axial skeletal dysmorphogenesis induced by acute doses of ethanol in mouse fetuses. Drug Alcohol Depend. 16:215–227.

Parnell, S.E., Dehart, D.B., Wills, T.A. et al. 2006. Maternal oral intake mouse model for fetal alcohol spectrum disorders: ocular defects as a measure of effect. Alcohol Clin. Exp. Res. 30:1791–1798.

Pauli, R.M. 1994. Lower mesodermal defects: a common cause of fetal and early neonatal death. Am. J. Med. Genet. 50:154–172.

Peterková, R., and Puzanová, L. 1976. Effect of trivalent and pentavalent arsenic on early developmental stages of the chick embryo. Folia Morphol. 24:5–13.

Primmett, D.R.N., Norris, W., Carlson, G. et al. 1989. Periodic segmental anomalies induced by heat-shock in the chick embryo are due to interference with the cell division cycle. Development 105:119–130.

Primmett, D.R.N., Stern, C.D., Keynes, R.J. 1988. Heat-shock causes repeated segmental anomalies in the chick embryo. Development 104:331–339.

Raddatz, E. and Kucera, P. 1983. Mapping of the oxygen consumption in the gastrulating chick embryo. Resp. Physiol. 51:153–166.

Ralston, J.D. and Hampson, N.B. 2000. Incidence of severe unintentional carbon monoxide poisoning differs across racial/ethnic categories. Public Health Rep. 115:46–51.

Rashbass, P., Wilson, V., Rosen, B. et al. 1994. Alterations in gene expression during mesoderm formation and axial patterning in Brachyury (T) embryos. Int. J. Dev. Biol. 38: 35–44.

Reijntjes, S., Gale, E., and Maden, M. 2004. Generating gradients of retinoic acid in the chick embryo: Cyp26C1 expression and a comparative analysis of the Cyp26 enzymes. Dev. Dyn. 230:509–517.

Rivard, C.H. 1986. Effects of hypoxia on the embryogenesis of congenital vertebral malformations in the mouse. Clin. Orthop. Relat. Res. 208:126–130.

Robkin, M.A. 1997. Carbon monoxide and the embryo. Int. J. Dev. Biol. 41:283–289.

Rogers, J.M., Brannen, K.C., Barbee, B.D. et al. 2004. Methanol exposure during gastrulation causes holoprosencephaly, facial dysgenesis, and cervical vertebral malformations in C57BL/6 J mice. Birth Defects Res. B Dev. Reprod. Toxicol. 71:80–88.

Rovasio, R.A. and Battiato, N.L. 1995. Role of early migratory neural crest cells in developmental anomalies induced by ethanol. Int. J. Dev. Biol. 39:421–422.

Rubin, W.W. and LaMantia, A.S. 1999. Age-dependent retinoic acid regulation of gene expression distinguishes the cervical, thoracic, lumbar, and sacral spinal cord regions during development. Dev. Neurosci. 21:113–125.

Sadler, T.W. and Horton, W.E. Jr. 1983. Effects of maternal diabetes on early embryogenesis. The role of insulin and insulin therapy. Diabetes 32:1070–1074.

Sadler, T.W., Hunter, E.S. 3rd, Balkan, W. et al. 1988. Effects of maternal diabetes on embryogenesis. Am. J. Perinatol. 5:319–326.

Sadler, T.W. and Hunter, E.S. III. 1994. Principles of abnormal development. In Developmental Toxicology, 2nd Ed., eds. C.A. Kimmel and J. Buelke-Sam, pp. 53–63. New York, NY: Raven Press, Ltd.

Sadler, T.W., Hunter, E.S. 3rd, Wynn, R.E. et al. 1989. Evidence for multifactorial origin of diabetes-induced embryopathies. Diabetes 38:70–74.

Sanders, E.J. and Cheung, E. 1990. Ethanol treatment induces a delayed segmentation anomaly in the chick embryo. Teratology 41:289–297.

Schardein, J.L. 2000. Chemically Induced Birth Defects, 3rd Ed., pp. 998–1000. New York, NY: Marcel Dekker, Inc.

Semenza, G.L. 1999. Perspectives on oxygen sensing. Cell 98:281–284.

Sewell, W. and Kusumi, K. 2007. Genetic analysis of molecular oscillators in mammalian somitogenesis: clues for studies of human vertebral disorders. Birth Defects Res. C Embryo Today 81:111–120.

Shalat, S.L., Walker, D.B., and Finnell, R.H. 1996. Role of arsenic as a reproductive toxin with particular attention to neural tube defects. J. Toxicol. Environ. Health 48:253–272.

Shibley, I.A. Jr., McIntyre, T.A., and Pennington, S.N. 1999. Experimental models used to measure direct and indirect ethanol teratogenicity. Alcohol 34:125–140.

Shifley, E.T. and Cole, S.E. 2007. The vertebrate segmentation clock and its role in skeletal birth defects. Birth Defects Res. C Embryo Today 81:121–133.

Singh, J. 2006. Interaction of maternal protein and carbon monoxide on pup mortality in mice: implications for global infant mortality. Birth Defects Res. B Dev. Reprod. Toxicol. 77: 216–226.
Singh, J., Aggison, L. Jr., and Moore-Cheatum, L. 1993. Teratogenicity and developmental toxicity of carbon monoxide in protein-deficient mice. Teratology 48:149–159.
Stewart, F.J., Nevin, N.C., and Brown, S. 1993. Axial mesodermal dysplasia spectrum. Am. J. Med. Genet. 45:426–429.
Stockdale, F.E., Nikovitis, W. Jr., Christ, B. 2000. Molecular and cellular biology of avian somite development. Dev. Dyn. 219:304–321.
Sulik, K.K. 1997. Pathogenesis of abnormal development. In Handbook of Developmental Toxicology, ed. R.D. Hood, pp. 43–60. New York, NY: CRC Press
Sulik, K.K. 2005. Genesis of alcohol-induced craniofacial dysmorphism. Exp. Biol. Med. 230:366–375.
Sulik, K.K., Cook, C.S., and Webster, W.S. 1988. Teratogens and craniofacial malformations: relationships to cell death. Development 103(Suppl):213–231.
Sulik, K.K., Johnston, M.C., and Webb, M.A. 1981. Fetal alcohol syndrome: embryogenesis in a mouse model. Science 214:936–938.
Swindell, E.C., Thaller, C., Sockanathan, S. et al. 1999. Complementary domains of retinoic acid production and degradation in the early chick embryo. Dev. Biol. 216:282–296.
Tam, P.P. and Trainor, P.A. 1994. Specification and segmentation of the paraxial mesoderm. Anat. Embryol. 189:275–305.
Takeuchi, I.K. 1979. Embryotoxicity of arsenic acid: light and electron microscopy of its effect on neurulation-stage rat embryo. J. Toxicol. Sci. 4:405–416.
Thackray, H. and Tifft, C. 2001. Fetal alcohol syndrome. Pediatr. Rev. 22:47–55.
Turner, S., Sucheston, M.E., De Philip, R.M. et al. 1990. Teratogenic effects on the neuroepithelium of the CD-1 mouse embryo exposed in utero to sodium valproate. Teratology 41:421–442.
Vorhees, C.V. 1987. Teratogenicity and developmental toxicity of valproic acid in rats. Teratology 35:195–202.
Wallin, J., Wilting, J., Koseki, H. et al. 1994. The role of *Pax-1* in axial skeleton development. Development 120:1109–1121.
Walsh, D., Grantham, J., Zhu, X.O. et al. 1999. The role of heat shock proteins in mammalian differentiation and development. Environ. Med. 43:79–87.
Webster, W.S. and Abela, D. 2007. The effect of hypoxia in development. Birth Defects Res. C Embryo Today 81:215–228.
Wells, P.G. and Winn, L.M. 1996. Biochemical toxicology of chemical teratogenesis. Crit. Rev. Biochem. Mol. Biol. 31:1–40.
Willhite, C.C. and Ferm, V.H. 1984. Prenatal and developmental toxicology of arsenicals. Adv. Exp. Med. Biol. 177:205–228.
Wilson, J.G. 1977. Current status of teratology: general principles and mechanisms derived from animal studies. In Handbook of Teratology, eds. J.G. Wilson and C.F. Fraser, p. 47. New York, NY: Plenum Press
Wlodarczyk, B.J., Bennett, G.D., Calvin, J.A. et al. 1996. Arsenic-induced neural tube defects in mice: alterations in cell cycle gene expression. Reprod. Toxicol. 10:447–454.
Yelin, R., Kot, H., Yelin, D. et al. 2007. Early molecular effects of ethanol during vertebrate embryogenesis. Differentiation 75:393–403.
Yon, J.M., Baek, I.J., Lee, S.R. et al. 2008. The spatio-temporal expression pattern of cytoplasmic Cu/Zn superoxide dismutase (SOD1) mRNA during mouse embryogenesis. J. Mol. Histol. 39:95–103.
Zaken, V., Kohen, R., Ornoy, A. 2000. The development of antioxidant defense mechanism in young rat embryos in vivo and in vitro. Early Pregnancy 4:110–123.
Zakeri, Z.F. and Ahuja, H.S. 1997. Cell death/apoptosis: normal, chemically induced, and teratogenic effect. Mut. Res. 396:149–161.