Abnormalities May be Related Defective Somitogenesis: Multiple Vertebral Segmentation Defects and Neural Tube Defects

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✓ Multiple vertebral segmentation defects (MVSD) (MIM#277300) including hemi vertebrae, fused or absent ribs, and “crab-like” thorax has been reported under various names such as spondylothoracic dysplasia, spondylotic dysostosis, Jarcho-Levin Syndrome and others. Many different genetic mutations reported in order to explain these defects. But the manifestation of MVSD are various and complex, so these mutation couldn’t be satisfactory. Then, how can it be explained this maldevelopment? As a consequence of their segmented arrangement and the diversity of their tissue derivatives, somites are key elements in the establishment of the metameric body plan in vertebrates; probably, disregulation of somite differentiation and proliferation may disclose the problem. We conclude that defective somitogenesis should be investigated carefully to explain related clinical abnormalities.

Key words: Embryonic induction, multiple vertebral segmentation defects, neural tube defects

✓ Defektif Somitogenesis ile İlişkili Anomaliler: Multipl Vertebral Segmetasyon ve Nöral Tüp Defektleri


Anahtar kelimeler: Embryonik induksiyon, multipl vertebral segmetasyon defekleri, nöral tıp defekleri

INTRODUCTION

Many different genetic mutations reported in order to explain multiple vertebral segmentation defects (MVSD). But the manifestation of MVSD are various and complex[1], so these mutations couldn’t be satisfactory. Neural tube defects (NTD) are among the most common of congenital malformations, with an overall frequency of one to three per 1000 live births in the United
States[2]. Causes of improper development and the majority of cases, however, are idiopathic in nature[2]. Also, there have been some reports about the association of MVSD and NTD[2-5]. As a consequence of their segmented arrangement and the diversity of their tissue derivatives, somites are key elements in the establishment of the metameric body plan in vertebrates; probably, disorganization of somite differentiation and proliferation may disclose the problem. Embryonic induction is fundamental to the development of tissue diversity[3] and consists of an interaction between inducing and responding tissues that brings about alterations in the developmental pathway of the responding tissue[4-8]. Experimental analyses of inductive interactions have defined the inductive history of numerous organs[4,5]. Thus, we should know how vertebral morphogenesis and neurulation develop.

**Normal Early Vertebral Morphogenesis**

Gastrulation is the process by which bilaminar embryonic disc is converted into a trilaminar embryonic disc[9], by interposition of a mesoblastic layer between the epiblast and the hypoblast[3]. The first sign of gastrulation is the appearance of primitive streak at the caudal end of the embryo at the beginning of third week, usually embryonic day of 15[3,9,10]. The primitive streak results from the proliferation and migration of cells of the epiblast to the median plane of the embryonic disc. As the primitive streak elongates by addition of cells to its caudal ends, its rostral end proliferates to form a primitive node or Hensen’s node[3,9]. After the primitive streak appears, cells leave its deep surface and form a loose network of embryonic connective tissue called mesenchyme or mesoblast (gastrulation), thus creating the earliest stage of the trilaminar embryo[3,4,9]. Caudal to the primitive streak there is a circular area known as the cloacal membrane, which indicates the future site of the anus and urogenital structures[3,9].

Some mesenchymal cells migrate cranially from the Hensen’s node, forming a median cellular cord, the notochordal process during embryonic days of 16 and 17[3,9]. The notochordal process grows cranially between the ectoderm and endoderm until it reaches the prechordal plate. When fully developed, the notochordal process extends from Hensen’s node to the prechordal plate. Openings develop in the floor of the notochordal canal and soon coalesce, leaving notochordal plate (3,9,10). The notochordal plate infolds to form the notochord, the primordial axis of the embryo around which the axial skeleton forms[9].

The neural tube forms by a process called neurulation between embryonic days of 18 and 27[3]. As the notochord develops, the embryonic ectoderm over it thickens to form an elongated, slipper-like plate of thickened epithelial cells, the neural plate. The neural plate is induced to form by the developing notochord[9]. A longitudinal neural groove develops in the neural plate, which has neural folds on each side. The neural folds have begun to move together and fuse on the embryonic day of 22, converting the neural plate into a neural tube[3,9,10] (Fig.). As neurulation continues, the rostral and caudal neuropores gradually diminish in the size[10]. The rostral neuropore closes on the embryonic day of 24[10,11], and the caudal neuropore closes on the embryonic day of 26. Secondary neurulation begins during stage 12 (embryonic day of 26)[10,12,13] (Fig.).

As the notochord and neural tube form, the intraembryonic mesoderm on each side of them proliferates to form a thick longitudinal
column of paraxial mesoderm. Toward the end of the 3rd embryonic week, the paraxial mesoderm differentiates and begins to divide into paired cuboidal bodies\cite{6,7,14} (Fig), under the inductive influences of notochord, neural tube, homeobox genes and cell adhesion proteins\cite{6,7,14,15}. These blocks of mesoderm are called somites and the original arrangement of the somites governs the subsequent metamericism of spinal ganglia, spinal nerves, intervertebral discs, and segmental vessels in the embryo\cite{6,7,14}. After forming, each somite separates into subdivisions that give rise to specific mesodermal components during the 4th embryonic week. The first of these subdivisions to appear are the sclerotomes, which will develop into the vertebrae and together with the notochord, give rise to the vertebral column\cite{6,7,14,15,16,17}.

During the precartilaginous or mesenchymal stage, mesenchymal cells from sclerotomes are found in the body wall, around the notochord and surrounding the neural tube\cite{6,7,14}. The sclerotomes appear as paired condensations of mesenchymal cells around the notochord during the 4th embryonic week. Each sclerotome consists of

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**Fig.** The lateral edges of the neural folds first begin to fuse in the occipitocervical region on the embryonic day of 22, leaving the cranial and caudal neuropores open at each end. The neural tube increases in length it zippers up both cranially and caudally, and the neuropores become progressively smaller. The cranial neuropore closes on the embryonic day of 24 and the caudal neuropore closes on the embryonic day of 26 (adapted from Larsen\cite{18}).
loosely arranged cells cranially and densely packed cells caudally. Some densely packed cells fuse with the loosely arranged cells of the immediately caudal sclerotome to form the mesenchymal centrum, the primordium of the body of a vertebra[5,6,14]. Thus, each centrum develops from two adjacent sclerotomes and becomes an intersegmental structure[5,14]. The notochord degenerates and disappears where the developing vertebral bodies surround it during the 4th embryonic week[10-20]. The mesenchymal cells, surrounding the neural tube, form the vertebral (neural) arch and the mesenchymal cells in the body wall form the costal processes that form ribs in the thoracic region during the 4th and 5th embryonic weeks[18-20].

Chondrification centers appear in each mesenchymal vertebra during the 6th embryonic week[10,20]. The two centers in each centrum fuse at the end of embryonic period to form a cartilaginous centrum. Concomitantly the centers in the vertebral arches fuse with each other and the centrum. The spinous and transverse processes develop from extensions of chondrification centers in the vertebral arch. Chondrification spreads until cartilaginous vertebral column is formed[8,14,17]. The ribs develop from the mesenchymal costal processes of the thoracic vertebrae[10,17]. Costal processes develop in association with the vertebral arches[8,10].

The ribs begin to form and lengthen on the embryonic day of 35 and develop as cartilaginous precursors that later ossify[10]. Primary ossification centers appear near the angle of each rib during the 6th embryonic week, and further ossification occurs in the tubercles and the heads of ribs during adolescence[9,10]. The original site of union of the costal processes with the vertebra is replaced with costovertebral joints[8,17]. The costovertebral joints form and separate the ribs from the vertebrae late the 6th embryonic week[10].

**Embryonic Induction**

Embryonic induction is fundamental to the development of tissue diversity[3] and consists of an interaction between inducing and responding tissues that brings about alterations in the developmental pathway of the responding tissue[3,5-8]. Experimental analyses of inductive interactions have defined the inductive history of numerous organs[4,5]. A number of genes or gene products have been identified whose activity in a given tissue is dependent upon a specific inductive interaction; the products of some of these genes serve as molecular markers of inductive interaction[4,5,8].

Experimental works on embryonic induction refer to the role of inductive interactions in the specification of cell fate[5,6,21]. The term 'embryonic induction' should refer only to tissue interactions that alter developmental pathways[22,23]. According to this definition, inductive interactions establish regions of developmental potency or morphogenetic fields[23]. Cell fates may be established by interactions within the morphogenetic fields over the course of organogenesis[4].

Through marking experiments, one can locate in an early embryo the group of cells, referred to as the anlage that will later form an organ. By microsurgical interventions, one can separate the organ anlage from its normal tissue environment to evaluate the state of developmental commitment of the organ anlage[4,21-29]. These microsurgical interventions include explanting the tissue and culturing it in isolation, transplanting the tissue to a heterotopic site, removing putative inductor tissues from the vicinity of
the anlage and removing the area fated to give rise to an organ to observe whether the surrounding tissue will regulate to form the organ\cite{14,21,24}.

Hans Spemann and Hilda Mangold described one of the most dramatic examples of embryonic induction, which is a basic developmental process\cite{20}. They showed that the transplantation of the blastopore (a region homologous to the primitive node in humans) from one frog embryo to another stimulated the development of a second complete embryonic axis. The result was a pair of embryonic "Siamese twins," one resulting from gastrulation of the host’s own blastopore and the other induced by gastrulation of the implanted blastopore. These discoveries stimulated a massive search for the organizing substance or inducing molecule involved, and it has just been in recent years that we are beginning to obtain some insight into some of the mechanisms that underlie this process\cite{10,28}.

Inductive interactions are critical to elaboration of the body plan of all vertebrate embryos\cite{5,24}. Embryonic mesoderm is formed by an inductive interaction between endoderm and ectoderm\cite{3}. The growth factors are family of structurally related signal molecules that have been isolated from organisms throughout the animal kingdom, including humans\cite{5-8}. Experimental studies show that this induction is likely to be caused by endogenous peptide growth factors (PGFs), including members of the fibroblast growth factor (FGF) and transforming growth factor-β (TGF-β) families during early development\cite{20,23,24}. This newly induced mesoderm must attain regional differences that lead to axial patterning and the development of different tissues such as notochord, muscle and blood\cite{25}. Ectoderm is induced to differentiate into mesoderm by underlying endoderm in the frog embryo\cite{24}. TGF-β-like molecules and FGF play a role in mesoderm induction also comes from investigations of a homeobox gene\cite{24,25}. Homeobox genes are thought to be the immediate target of an inductive signal and to act as a trigger setting off the coordinated cascade of gene activations and biochemical changes that cause a cell to differentiate into a specific tissue type\cite{25}. Many inductive interactions seem to involve the activation of homeobox genes\cite{18}. It seems possible that the development of the cranio-caudal axis in Xenopus is controlled partly by differences in the level of Xho3 expression of homeobox genes\cite{21,24,25,27}.

The establishment of antero-posterior polarity requires a system of positional information that specifies different fates for mesodermal cells according to their position at the end of gastrulation\cite{25,27}. The system probably includes a diffusible graded signal to account for the observed graded axial deficiencies in embryos obtained after perturbation by ultraviolet irradiation, gastrulation arrest or Xho3 over-expression\cite{25,27}.

Data show that the frog ectoderm/endoderm system is the best-understood example of embryonic induction\cite{4,21,24,25,27}. The general model of induction may very well continue to be supported by new evidence and will serve as a guide in studies on inductive interactions in human. In several organism, proto-oncogenes (genes that can lead to cancer if their normal regulation is disturbed) and genes that contain homeobox or zinc-finger domain, appear to be involved in a number inductive cascade suggest that the researches on mechanisms of induction may lead to a better understanding of the roles of human genes in congenital disease\cite{10,25,27}.
Several recent studies have concentrated on the mechanisms that might underlie the mesenchymal-epithelial transitions characteristic of somite development. Using an assay for cell adhesion, Bellairs et al. showed that disaggregated somite cells are more adhesive than those of the segmental plate. Cell-cell adhesion increases at the anterior end of segmental plate, immediately before somite formation. These findings suggest that the epithelialization of the segmental plate is accompanied by an increase in cell-cell adhesion.

Several molecular mechanisms have been identified that could mediate cell-cell adhesion during somite formation in the chick embryo. For example, the calcium dependent adhesion molecule N-cadherin is expressed at low levels in posterior regions of the segmental plate, but increases in the more anterior regions. When segmentation occurs, the molecule becomes concentrated at the apical part of the newly epithelial cell surface. The subsequent disaggregation of the ventro-medial portion of the somite to form the mesenchymal sclerotome is preceded by a loss of N-cadherin immunoreactivity in precisely this region of the epithelium; and Fab fragments of monoclonal anti-N-cadherin antibody cause disaggregation of chick somites in vitro. N-cadherin appears to be closely related to adhesion molecule A-CAM (adherens junction-specific cell adhesion molecule) that localized to adherens junctions between the epithelial cells. Thus, it is reasonable to expect that the components of epithelial cell-cell associations, such as N-cadherin, will appear at some stage during somitogenesis.

CONCLUSIONS

Previously reported in patients association between MVSD and NTD suggest that the deformation likely occurs due to before the neural tube has closed at the end of the 4th week, which produces the notochord, neural tube and adjacent somites. MVSD arises as a result of defective embryonic induction (DEI), probably first 6th embryonic weeks. Recent researches support this idea that the process of embryonic induction is fundamental to the development of tissue diversity. Three structures possess spatial relationship each others during gastrulation. DEI may be cause a failure of midline axial integration during gastrulation, the primitive streak is abnormally wide and the prospective notochordal cells in Hensen's node begin ingressing more laterally than normal.

Mesodermal structures formed during the 3rd and 4th embryonic weeks participate in the development of most of the structures involved in caudal displasia and associated malformations. For example, the sacral and coccygeal vertebrae form from structures called sclerotomes that develop from the sacral and caudal somites. The intermediate mesoderm differentiates into kidneys in response to induction by the in growing mesodermal ureteric buds. Imperforate anus, previously described in patients with MVSD, may result from the incomplete migration of mesodermal structures.

Transplantation experiments have established that sclerotome cells differentiate to form either a vertebral body or a vertebral arch in the response to specific inducing substances. Vertebral bodies are formed in response to substances produced by the notochord, whereas vertebral arches form in response to substances produced by the neural tube. Molecules such as chondroitin sulfate, which are secreted by
the notochord, appear to be especially potent inducers of cartilage formation in the sclerotome\textsuperscript{[14]}. A number of spinal defects are caused by abnormal induction of the sclerotomes. Defective induction of vertebral bodies such as defective chondroitin sulfate secretion on one side of the body may result in a severe lateral scoliosis\textsuperscript{[10]}

Ablation and grafting experiments have shown that normal development of the vertebral bodies depends on close proximity of the notochord, whereas the vertebral arches require an inductive signal from neural tube\textsuperscript{[5-8,14]}. If a small segment of notochord is removed from an experimental animal, only vertebral lamina and arches develop in that location. Similarly, ablation of a segment of the neural tube results in the development of vertebral bodies alone \textsuperscript{[10,14,26]}

NTD originate during the 3rd embryonic week\textsuperscript{[10,35,36]}. A failure of part of the neural tube to close (spinal dysraphism) disrupts both the differentiation of the central nervous system and the induction of the vertebral arches and can result in a number of developmental anomalies. These malformations generally involve part of the cranial or caudal neuropore, resulting in a defect of the cranial or lower lumbar and sacral regions of the central nervous system, respectively\textsuperscript{[10,35,36]}. Failure of the neural tube to close disrupts the induction of the overlying vertebral arches, so that the arches remain underdeveloped and fail to fuse along the dorsal midline to enclose the vertebral canal. The resulting open vertebral canal is a condition called spina bifida\textsuperscript{[10,17]}. The fact that spina bifida is quite common in the lower lumbar and upper sacral region suggests that neuropore closure or secondary may be involved in the etiology of this malformation\textsuperscript{[10,35,37]}. At the mildest extreme of spina bifida, the vertebral arches of a single vertebra fail to fuse while the underlying neural tube differentiates normally and does not protrude from the vertebral canal. This condition is known as spina bifida occulta\textsuperscript{[0,19]}. Spina bifida occulta appears to be a common finding in reported MVSD cases\textsuperscript{[19-21,24,37]}

The mild severe defects of neural tube closure called meningomyelocele reported in previous reports\textsuperscript{[3,36-38]}. Meningomyelocele results from defective closure of the rostral neuropore\textsuperscript{[17,19]}. The most severe defects of neural tube development are those in which the neural folds not only fail to fuse but also fail to differentiate invaginate and finally separate from the surface ectoderm \textsuperscript{[3,12,16,33,37]}

NTD in humans have no clear-cut genetic or teratogenic cause\textsuperscript{[39-41]}. Although some animal mutants expressing similar defects exhibit chromosomal anomalies\textsuperscript{[42-44]}, the karyotype usually appears normal\textsuperscript{[45,46]}. Teratogens that induce NTD have been identified in animals and humans, opening the possibility that a proportion of human NTD are caused by environmental toxins or nutritional factors\textsuperscript{[36,38,47-50]}. Experimental studies have identified many teratogens that can induce NTD when applied during the period of neural tube closure. These agents include retinoic acid, insulin, high plasma glucose levels and trypan blue\textsuperscript{[51]}. Factors implicated in the induction of NTD in humans include valproic acid, maternal diabetes and hyperthermia\textsuperscript{[53,52]}

In summary, these findings indicate that important inductive interactions are taking place. However, very little is known about the molecular basis of these interactions. Studies of other inductive systems should be investigated to provide insight into the molecular and nature of induction.
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REFERENCES


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