Asymmetry Inside The Symmetry: Mechanisms and Disorders Of Laterality

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Asymmetry of the left-right (L-R) axis is a feature of vertebrates that is apparent in the viscera and vasculature. An understanding of L-R patterning in human development is aided by familiarity with key events and important pathways in mammalian embryogenesis. The induction of L-R asymmetry follows the establishment of the anteroposterior (A-P or rostrocaudal) and dorsoventral (D-V) axes and requires the pre-existing midline symmetry to be broken before sidedness is induced via a series of complex, coordinated molecular signaling events. In this review, our aim is to describe the molecular background of this embryological process, and give brief knowledge concerning its clinical consequences.
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Many living creatures in nature, from Ascidians to man, exhibit symmetry which can be described as correspondence in size, shape and relative position of congruent components about a plane or axis, as a feature of their external body plan. Most animals possess symmetrical body plans such as; spherical, radial, chiral, bilateral and pseudo-bilateral. In harmony with this, possession of a symmetrical body plan is one of the conspicuous morphological characteristics of vertebrates. Despite this symmetry in morphology, vertebrates also exhibit strikingly invariant left-right asymmetry of visceral organs. In the thorax, the cardiac apex points to the left and the aorta arches to the left. In the abdomen, while the liver and gallbladder are on the right, the stomach and spleen are situated on the left. Moreover, the blood vessels also exhibit apparent asymmetric development. Besides the asymmetric development of these impaired organs, asymmetric development, morphology, placement and even physiology of paired organs such as the lungs (the right lung consists of three lobes while the left has only two), kidneys and brain hemispheres also contribute to asymmetry.

Asymmetry in Humans

In human beings, possessing an asymmetrical internal architecture is not the only evidence of existing asymmetry. There are also many less defined asymmetries that have been the focus of many studies to date. Human hand preference and the functional asymmetry of the brain hemispheres are still attractive research areas, and the basics of genetic mechanisms are still controversial.[1] Cell mediated immune hypersensitivity is stronger on the left side of the body[2] and the left side of the scrotum descends lower than the right in correlation with handedness.[3] In addition, it has already been pointed out that non-visceral asymmetries exhibit an interesting relationship with gender.[4,5] Data obtained from human hermaphrodites is likely to support this relationship.[6,7]

Interesting information in this area of research is provided by the fact that non-conjoined monozygotic twins exhibit opposite directionality of markers such as hand preference, hair whorl direction, tooth patterns, unilateral eye and ear defects, cleft lip, cleft palate, supernumerary teeth and even tumor locations and undescended testicles without exhibiting any visceral laterality defects that characterize conjoined twins.[8]

In summary, although human beings seem to be bilaterally symmetric, they possess a strikingly invariant asymmetry beneath this symmetry that can be called "pseudo-bilateral" symmetry. Asymmetry, here, in fact is not only anatomic or morphologic but also functional and even physiologic. Deviations from normal development of left right asymmetry can lead to numerous defects with significant clinical implications such as bilateral symmetry (lack of asymmetry in organ physiology), isomerism (duplication of left or right sided paired organs), heterotaxy or situs ambiguous (randomization of placement of organs like the heart), situs inversus (reversal of a particular organ's orientation), situs inversus totalis (total left right mirror image inversion of organs).[9]

It is important to understand all aspects of left right asymmetry generating mechanisms in order to explain causes of these defects at the molecular level and clarify normal development of embryos. Three orthogonal axes are major references in embryological development; dorsal-ventral (DV), anterior-posterior (AP) and left-right (LR) axes. DV

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and AP axes have been well defined, but our knowledge of LR asymmetry is still limited and this study area is full of unanswered questions.

Up to the first molecular studies used to clarify the mechanisms playing a role in assembling left right asymmetry during embryological development, drugs that produce defects in left right asymmetric manner or randomize asymmetry were used for the same aim.[10] The greatest contribution to this field of research was obtained by molecular studies on many species including both invertebrates and vertebrates. By now, with studies executed at the molecular level, many endogen molecules thought to be related to LR asymmetry have been found and, via these endogen molecules, possible mechanisms and models have been suggested for LR asymmetry establishment. Besides providing valuable information about LR asymmetry generating mechanisms, these studies also help us understand the evolutionary process of asymmetry and conservation of mechanisms between species.

From the molecular point of view the establishment of LR asymmetry can be divided into four steps: 1) Initial breaking of LR symmetry in or near the node (Hensen's node) with respect to the DV and AP axes. 2) Transfer of LR asymmetric signals from the node to the lateral plate mesoderm. 3) LR asymmetric expression of signaling molecules (asymmetric gene expression). 4) LR asymmetric morphogenesis of the visceral organs that are induced by these signaling molecules.[11-14]

The process resulting in asymmetric gene expression, step 1, is noteworthy as it is the initiation part of established asymmetry. The question: "What determines the sidedness of the first gene that is asymmetrically expressed?" is the center of attention. Syndecans, adhesion junctions, gap junctions, ion fluxes, intracellular motor proteins and cilia models are shown to be parts of mechanisms previous to asymmetric gene expression.[15] Another important part of the LR asymmetric development is step 4, in other words asymmetric establishment of the viscera. Transfer of asymmetric signals (step 2) and asymmetric gene expressions (step 3) such as transforming growth factor-β related factors, Nodal and Lefty-2 result in asymmetric morphogenesis. During this process Nodal acts as a left side determinant, whereas Lefty-2 is an antagonist that restricts the duration and the site of Nodal action.[13] At least three different patterns can be distinguished for step 4; 1) directional looping of a tube (e.g. heart, stomach, intestine), 2) differential lobation (e.g. liver, lung), 3) one sided regression of a structure (e.g. blood vessels).[14]

The reality of LR asymmetry raises many questions. Why is asymmetry needed? What is its role and importance for living creatures? What is the position of laterality in the evolutionary process? How were molecular mechanisms conserved? Is it the same in every animate? What is the first event that initiates LR asymmetry? How does the embryo distinguish its left from right in the beginning of this process? What are the molecular cascades that play a role in the development of left right asymmetry? These and more questions will find their answers when molecular, genetic and biochemical levels of embryo asymmetry development are understood.

The aim of this review is to summarize the presently defined molecular structures, signal pathways and models explaining ciliary and node function and the related clinical problems commonly seen.

Pathway to Asymmetry : the NODE

Embryonic axes start to be set invisibly very early. Firstly, the anteroposterior axis is defined by the second polar body (which refers to anterior) then the dorsoventral axis is defined by the inner cell mass of blastocyst (which refers to dorsal) and trophoblast (which refers to ventral) opposite to it. After these axes are set, right-left axis occurs spontaneously. The appearance of symmetry is seen during the formation of the primitive streak which is made up of the epiblast cells moving from caudal to cranial area at gastrulation (3rd week). Chordin, nodal, cripto and Vg1 are major signalling molecules for formation of the primitive streak and are active in the caudal area of the axis.[16] At the anterior end of the primitive streak, the primitive node develops where the cells delaminate to form the mesoderm. Detailed studies in mice models have demonstrated that the mouse node (or organizer) for asymmetry exposes at the time of gastrulation in a region at the distal tip of the embryo where dorsal ectodermal and ventral endodermal cells each with a simple cilia are in contact, separated only by a basement membrane and when a node is grafted to an ectopic side, the host is induced for a second body axis.[17,18,19] When the primitive streak regresses, the node moves towards the posterior end of the embryo, and ingresses after somitogenesis. Firstly the prechordal plate, then the notochord are formed after gastrulation by the cells invading throughout the node.[17,20] The notochord is considered to be the primitive axis of the very early embryo.19 After the formation of the notochord, the embryo shows bilateral symmetry for some time. At initiation of functioning of node cells, asymmetry inside the symmetry starts to be generated. (figure 1)

Each node cell carries a monocilium and all monocilia are very closely placed.[17] An important question arises about monocilia; whether node monocilia were functional or not. For instance; one third of left-right asymmetry mutations is about the genes having a role in ciliary biogenesis and function.[17] Kif3a and Kif3b, polaris (TG737), which are needed for intra-flagellar transport (IFT);[21] left-right dynein (lrd, for mouse) and DNAH5 (for human) defects affect cilia and result in paralyzed cilia;[22,23] a protein localized to mouse node cilia which includes ankyrin-repeats and has an unknown function called inversin[24] and; a transcription factor Foxh1[25] can be given as examples. The importance of the ciliary function will be explained later in detail.

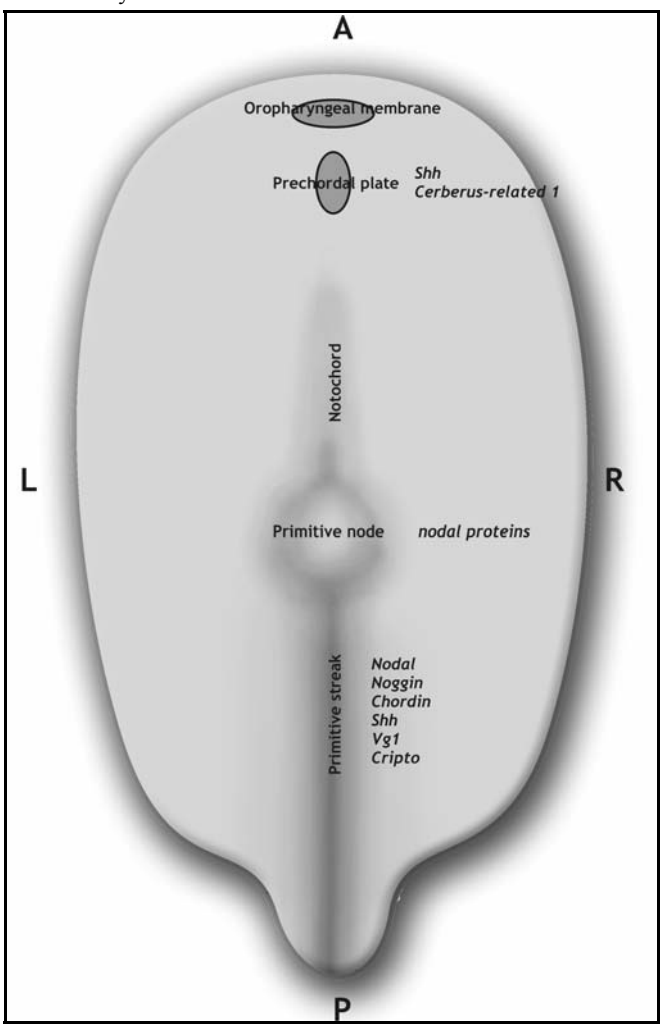

Figure 1. The establishment of symmetry during gastrulation. Nodal, chordin, Vg1 and cripto are major molecules those take place in the formation of primitive streak and they activate the caudal area. Firstly precordal plate then the notochord are formed after gastrulation by the cells invading the node. Notochord shows bilateral symmetry for some time. In the node nodal proteins are synthesized which regulate the symmetry and asymmetry. In the prechordal plate there are SHH and cerberus related 1 proteins.

The molecular structures, signal pathways, and three models explaining monocilia and node function in the development of asymmetry

Major Molecules:

H^+/K^+-ATPase

In xenopus and chick, the earliest component of LR asymmetry determination is H^+/K^+-ATPase. Drug sensitivity causes organ reversals by inhibition or misexpression of H^+/K^+-ATPase. Also asymmetric expression of SHH, fgf8 and Wnt8c loses the side. H^+/K^+-ATPase was firstly seen in chick embryos nearby the primitive streak andit was observed that its expression was symmetric. However, imaging studies suggested that H^+/K^+-ATPase functioned on the right, instead of the left side. Subsequently it was concluded that H^+/K^+-ATPase supplies the negative potential of cell membrane on the right.[15,19] The relationship between the potential and signal proteins will be mentioned in signal pathways.

Connexins

In early chick or Xenopus embryos, genetic or pharmacological perturbation of endogenous gap-junction communication interrupts the unilaterally expressed genes and changes organ situs.[11,26] Gap-junctions are composed of connexin proteins. Endogenous spreading of gap-junction communication is important, while experiments where "negative dominant connexin" is injected into dorsal blastomers of Xenopus embryos or "wild-type connexin" into ventral blastomers cause changes in laterality. This effect in blastomers demonstrates a signal that spreads out the dorsal blastomers and impedes at the ventral midline. One of these connexins; Cx43, seems to be important for the asymmetry of chicks, since it is present in cells which surround the node and when it is depleted by antisense oligonucleotides the sidedness of SHH and nodal expression is unbiased.[26]

Syndecan-2

In the right half of Xenopus embryo during gastrulation (at stage 11) the cytoplasmic domain of the heparin-sulphate proteoglycan, syndecan-2, is phosphorylated in animal cap cells (presumptive ectoderm).[13] During gastrulation, this phosphorylation is thought to affect the ability of the mesoderm contacting with ectoderm to decide LR pattern. Syndecan-2 might serve as a signal using the signal complex that includes 'growth factor like protein' at cell surfaces.[27]

Nodal

Nodals are a group of proteins within the TGF superfamily. It is assumed that nodals induce the formation of mesoendoderm and have a role in the left-right axis formation. Studies have suggested an asymmetrical distribution of the nodal genes. It is assumed that Nodals also have an important role in left-right asymmetry development.[28,29,30]

Furthermore, in the specification of ventral and posterior mesoderm Nodals heterodimerize with BMP4 and BMP7.[28]

Like the other signaling pathways, Nodal signaling pathways also have inhibitor proteins to regulate the signaling. The best studied inhibitor proteins are Lefty and Cerberus proteins.

Lefty proteins are antagonists of the Nodals. Although it is not clear how they inhibit the function of Nodals, in the absence of Lefty molecules Nodal signaling is enhanced during mesoderm induction and left-right axis development.[17] The activity of Lefty is controlled at the level of transcription; consequently they act as classical feedback inhibitors.[28]

Dan Dan proteins are a group of proteins containing Xenopus Cerberus and Coco proteins, mouse Cerberus like protein, which bind and block Nodals. In some species they also inhibit Wnt and/or BMP signaling.[28] Unlike Leftys, Cerberus proteins that are also forebrain inducers are expressed in the anterior endoderm affecting the Wnt and BMP pathways. In contrast to Lefty proteins, they are independent of feedback inhibition.[28]

The Nodal signaling continues with the cytoplasmic factors such as Smads. Smad2 and Smad3 are phosphorylated in Nodal signaling[26,29,30] which in turn phoshorylate Smad4 and are translocated to the nucleus.[26,31,32] In the nucleus the P-Smad complex associates with transcription factors to regulate the expression of target genes.[26,31]

Signal Pathways :

A- NODAL/BMP

Transforming growth factor-β (TGF-β) superfamily members are important in determination of L-R asymmetry. Bone morphogenetic proteins (BMPs) are members of the TGF-β superfamily. In Xenopus, one of the BMP I receptors, ACVRI/ALK2, transduces right-sidedness in a manner that is antagonistic to VgI-dependent signaling.[33]

Smad5 can transduce BMP6 and BMP7 signals through ACVRI in vitro.[33] Ligands are important in the function of ACVRI in mouse embryos. BMP4 is expressed in the extra-embryonic region in pre-/early primitive streak stage embryos and in the most posterior part of the mesoderm. It seems not to play a role at the expression of nodal but BMP2, BMP4 and BMP7 can stimulate expression of nodal in the lateral plate mesoderm (LPM) in chick embryos.[34,35] All this information emphasizes the existing confusion and that more experiments are needed for identification.

ACVRI signaling is necessary for LR patterning and it is conserved from Xenopus to mouse. In 2004, Kishigami and colleagues carried out experiments on mice embryos to determine the molecular details of BMP signaling and defined the roles of distinct BMP receptors during LR axis determination. They used ACVRI null embryonic stem (ES) cells and injected ES cells to wild type blastocysts. Their findings show that ACVRI plays an important role in establishing LR asymmetry in the node and is required for expression of lefty1 in the midline to maintain LR asymmetry. ACVRI signaling in the midline is essential for induction of lefty1 and lefty2 in the prospective floor plate (PFP) and to form the midline barrier.[36]

Asymmetric expression of nodal in the perinodal region is temporally limited to the two to three-somite stage during embryogenesis in wild-type embryos. Kishigami and colleagues found that ACVRI-dependent signaling is required for asymmetric expression of nodal in the node. In the same way, ACVRI acts as an upstream way of smad5, which is required for inducing lefty1 at the left side of midline. SHH is normally expressed at the midline and ACVRI signaling is suggested to be parallel with SHH signaling at the midline. BMPs could induce the expression of nodal at LPM but different kinds of BMP receptors might play roles in this way.[36] (figure 2)

B- Ca++/NOTCH/NODAL

The Node is placed at the end of the primitive streak and it precedes posteriorly leaving midline tissues (notochord and the floor plate of the neural tube) behind it. Calcium ions (Ca+2) are an intermediate between an initial imbalance in electrical potential across the node and the definitive asymmetric activation of nodal expression to the left of the node by notch signaling.[37] This theory seems to be an explanation for the fluid flow model about the uncertain state of Ca+2. At the node asymmetric Ca+2 distribution is showed via fluorescent extracellular Ca+2 indicator. For understanding the importance of Ca+2 function, Ca+2 concentrations are changed in vivo and the activities of Notch pathway are researched. These studies show that there can be a transient electrochemical bias which interacts with the spatiotemporal distribution of gene expression along the anterior-posterior axis to generate a robust and heritable LR asymmetry in gene expression.[38] Every step of the Ca+2 pathway is not yet understood, and these studies represent new windows for new experiments, especially the relationships between intra and extracellular Ca+2 initiation of H^+/K^+-ATPase function, and the role of node cells. (figure. 2)

C-NODAL/LEFTY/ PITX2C

Nodal expression starts in the left of the node then this expression continues in the left LPM .

Nodal/Lefty/Pitx2c pathway can be divided into three phases. In the first phase, a Ca+2 signal occurs. In the second phase, Nodal, a TGF-β like factor is produced on the left LPM. In the third phase, a transcription factor called pitx2c is expressed.[8,39] Pitx2c is a controller of stereotypic asymmetry anatomy of developing tissues, cell migration, shape, proliferation and survival.[18] Lefty1 protein affects Nodal at the dorsal midline so Nodal stays only on the left side of the embryo. Lefty2 protein affects nodal expression on the left LPM so Nodal does not function excessively. Like Nodal, lefty 2 is expressed in the left LPM as well as the midline, with a slightly larger expression domain than Nodal.[40] It is found that lefty proteins and nodal bind competitively and act antagonistically on the same receptor complex, suggesting that lefty1 and lefty2 facilitate a finely tuned control of nodal during LR patterning. (figure. 2)

MODELS FOR ASYMMETRY FUNCTION

A- Morphogen-Flow (Gradient) Model

This model suggests that consistent signaling molecules such as SHH, FGF, nodal and related proteins are swept from one side to the other to initiate downstream signal pathways. This hypothesis claims that symmetrically expressed signaling factors around the node are disturbed asymmetrically by nodal flow[41,42] Since asymmetric protein distribution has not been detected and most growth factors cannot diffuse freely, this hypothesis needs more discussion. Also the strong external flow used experimentally to renew normal LR development, is expected to move away or dilute signaling factors in mutant mice.[43]

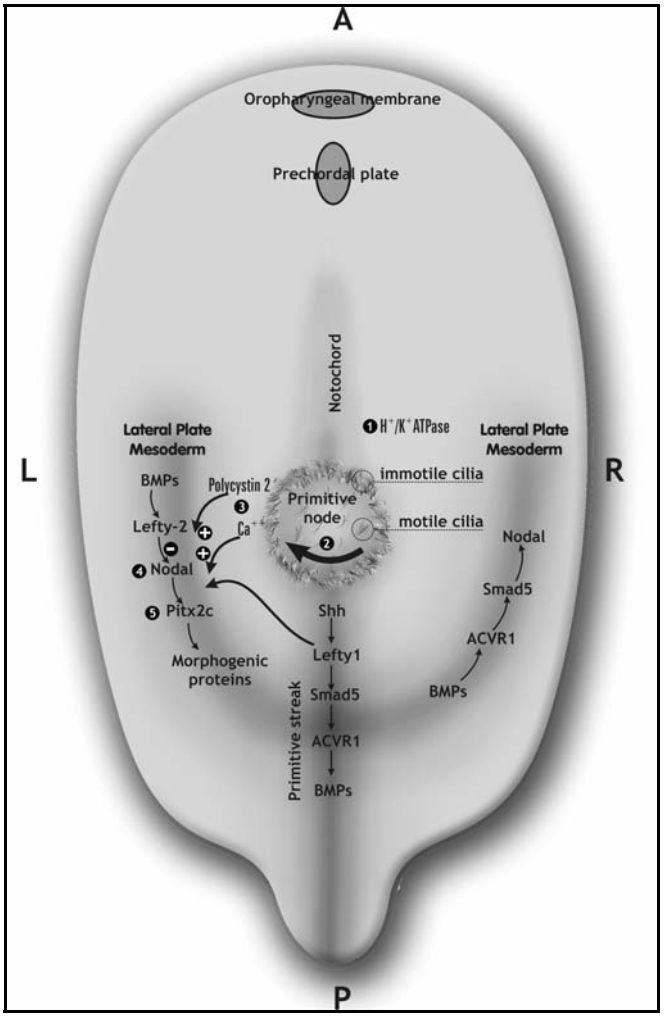

Figure 2. Chematic view of signalling pathways which regulate the left-right asymmetry. Firstly H^+/K^+ ATPase creates negative membrane potential on the right side of the node related with Ca++ signalling on the left side of the node.[1] The motile cilia in the center of the node causes a leftward nodal flow sensed by the immotile cilia in the left peripheral node cells.[2] These immotile cilia together with polycystin-2 and gap-junctions trigger intracellular Ca++ increase. The signalling wave spreads through the node cells, creating a Ca++ wave which causes nodal synthesis.[3] Nodal activity is regulated by the BMPs, SHH and lefty proteins. On the right side nodal activity is inhibited, on the contrary it is excitated.[4] On the left lateral plate mesoderm Pitx2c is activated, which then triggers the downstream morphogenic pathways.[5]

B- Fluid-Flow Model

This model has three components; monocilia, gap-junction communication and polycystin-2. It suggests that monociliary action propels an extracellular determinant.[18] A nodal flow is driven by specialized cilia. The vortical movement of the cilia creates a leftward fluid flow across the node and this movement causes an increase of concentration of some diffusible determinant on the left side of the node.[41] The Fluid flow model suggests that the monociliary motion is an extracellular determinant, but another possibility is that monocilia initiate a signal such as a Ca+2 wave that spreads through the layer of cells surrounding the node. Monocilia, gap-junctions and polycystin-2 could function coordinately and trigger an intercellular Ca+2 wave. Around and in the chick node they can affect asymmetric early gene expression such as SHH, FGF8 and wnt8c.[44] Polycystic kidney disease (PKD) which is inherited as an autosomal dominant, is the source of this model, because PKD includes each of the proteins and structures of the model. In normal kidney tubules and ducts monocilia are located on the apical surface of epithelial cells and appear to sense lumen diameter via detecting changes in fluid dynamics. Motion of monocilia triggers Ca+2 influx through ciliary or nearby channels. Then Ca+2 is released from intracellular stores which are sensitive to inositol 1,4,5-triphosphate [Ins(1,4,5)-p3]. A second messenger follows the signal around the cells and diffuses to gap-junctions. However there are different opinions about the localization of polycystin-2 and it is thought to be a cation channel where Ca+2 enters from the plasma membrane and Ins (1,4,5)-p3 sensitive channel over ER.[45]

In this connection, asymmetry is inherited in monociliary structures, forming the embryonal asymmetry, thus leftward fluid flow might be the earliest known cause of asymmetry in mouse.[18] Mouse embryo forms asymmetry via inherited node properties whereas chick node receives LR pattern from surrounding tissues.

Overall, the simple result is that LR asymmetry determination pathways might be common or different according to species. Both genetic and epigenetic patterns cooperate with physical laws and affect the asymmetry process.

C- Mechanosensory Model

According to this model, there are two populations of cilia in the node; motile and non-motile. Motile cilia include lrd an axonemal motor protein localized in the center of the node. Non-motile cilia that lack lrd are distributed around the periphery of the node. Motile or immotile, all the cilia contain the cation channel polycystin-2. This model suggests that motile cilia generate a leftward nodal flow in the center and peripheral non-motile cilia detect the flow as mechanosensors.[46] After mechanosensory cilia detect the nodal flow, Ca+2 flux is activated on the left side of the embryo which results in the Ca+2 spike on the left side activating the downstream LR pathway of Nodal, Lefty and pitx2 expression on the left. This model is found at the earliest stage of LR development in mice.

In the mechanosensory model it seems likely that the symmetry-breaking process in left-right determination is initiated by the leftward nodal flow of the extra-embryonic fluid, although the mechanism of the signal transduction is not known

The timing of the initiation of LR asymmetry in various species is particularly controversial; however the mechanisms underlying different aspects of LR patterning in various species are beginning to be uncovered in significant detail.[15] Nevertheless, all these models are far from being competent to ex-

plain the initiation of LR patterning. The Mechanosensory model is possibly the closest approach among the ones described above. More experiments are needed to explain the initiation of LR development in different species, yet there are common ways such as nodal, lefty, pitx2c in progression.

Heterotaxy Syndromes

Body symmetry mainly corresponds to the external components of the mammalian phenotype. The cardiovascular, pulmonary and gastrointestinal systems are asymmetric. This particularly specific asymmetry is the consequence of a series of events led by genetic and molecular signals during the development of the midline structures during the embryonic period.

Synonyms: Heterotaxy=Visceral inversion=Laterality sequence=Situs anomaly=Situs inversus=Situs inversus viscerum=Visceral transposition=Visceral heterotaxia

- Situs inversus
- Thoracic isomerism
- Situs ambiguous
- Asplenia (Ivemark) syndrome
- Polysplenia syndrome
- Ciliary dyskinesia syndrome
- Kartagener syndrome

The normal arrangement of asymmetry is called situs solitus, a descriptive term derived from the Latin situs, which means position, and solitus, which means customary. Thus, situs solitus signifies the customary, or normal, asymmetric arrangement of the viscerovascular anatomy. Situs solitus is the evolutionarily conserved asymmetric arrangement of the thoraco-abdominal viscera and vasculature that is present in over 99% of the population.[47]

Situs inversus is a complete mirror image reversal of situs solitus. Although embryologically related to the other situs anomalies it should be evaluated separately from the others due to pathophysiological and clinical aspects.

Cardiac situs refers to the position of the atria and viscera relative to the midline. The atrium whose appendage is broad-based, receives blood from the inferior vena cava is called the systemic or right atrium. In situs inversus, the morphologic right atrium is positioned left, while the left atrium is on the right side. The pulmonary anatomy is the opposite with the left lung with three, and right with two lobes. The liver and gall bladder are situated on the left while the spleen and stomach are on the right side. The remaining internal organs are mirror-images of the normal.

Situs inversus can be classified into two as situs inversus with dextrocardia or situs inversus with levocardia. Levo-or dextrocardia is determined by the position of the cardiac axis at birth. This does not indicate the positions of the cardiac chambers. Situs inversus is termed situs inversus totalis when there is accompanying dextrocardia because the position of the heart, the atrial chambers, and abdominal viscera are the mirror images of the normal.

Life expectancy of patients with situs inversus is normal. Dextrocardia is a common finding. The incidence of congenital heart defects is 3-5%.[48] Atrioventricular discordance and transposition are the most common abnormalities, with the aortic arch being right-sided in 80% of patients.[49] The exact incidence of situs inversus totalis is unknown, but it is estimated to be 1 in 8000 to 25000 live births. Almost 20-25% of this incidence is concurrent with immotile cillia or Kartegener's syndrome. If situs is incomplete, situs ambiguous or heterotaxia is present.

Situs Ambiguous

Situs ambiguous, or heterotaxy, refers to visceral malposition and dysmorphism associated with indeterminate atrial arrangement.[50] This abnormal arrangement of body organs is different from the orderly arrangement seen in situs solitus or situs inversus.[51] The incidence of situs ambiguous is believed to be 1.44/10000 live births.[47]

The complexity of this syndrome is reflected in the various terms used to subclassify it, including asplenia syndrome, double right-sidedness, right isomerism, or Ivemark syndrome and polysplenia syndrome, double left-sidedness, or left isomerism. Isomerism denotes the duplication of the left or the right side, which may be complete or partial.

The detailed description of situs ambiguous is determined with the dominance of the structures of the right or left side. Individuals with right-sided symmetry have asplenia, while left-sided symmetry involves a segmented spleen or multiple small spleens. Nomenclature is as follows: a) right isomerism or asplenia syndrome and b) left isomerism or polysplenia syndrome. Classical right isomerism demonstrates bilateral right sidedness with bilateral right atria, midline positioned liver, bilateral tri-lobed lung and absence of spleen. Classical left isomerism with bilateral left sidedness is related to polyspenia demonstrating bilateral left atria, and two lobed lungs on both sides. Inferior vena cava intersects with the azygous vein and continues with the hemiazygous.

The common misleading classification of these two conditions is asplenia and polysplenia respectively. In contrast, heterotaxy or situs ambiguous reflect the disruption of embryonic laterality leading to faulty development of asymmetric organs. Neonates with situs ambiguous should be evaluated for severe congenital heart anomalies. Knowledge of the vascular anatomy is essential before intervention.

Situs ambiguous or heterotaxia is associated with other clinical conditions, such as intestinal malrotation, biliary atresia, splenic abnormalities and consequent immunologic defects, abnormal gastric suspension mechanisms, displacement of abdominal viscera, and aberrant vascular structures and vascular connections. Each of these abnormalities is de-

rived from an embryologic inability to determine laterality and establish the complex solitus asymmetry, while symmetric structures remain unaffected.

Right-sidedness associated with asplenia can present with Howell-Jolly bodies in the peripheral blood. Defects in the immune system can lead to sepsis. In polysplenism, multiple splenic islands are recognized on both sides of the dorsal mesogastrium. Splenic structures may be present around the greater curvature of the stomach. Biliary atresia can be observed in individuals with polysplenia, while polysplenia is present in 10% of patients with biliary atresia. It is important to demonstrate the caval and portal venous systems before surgical intervention.[52]

A small subset of individuals with situs ambiguous also has a variety of midline defects, including central nervous system (CNS) anomalies (eg, holoprosencephaly, neural tube defects) and caudal regression syndrome. This association indicates the abnormal modeling of the midline developmental complex in the early genesis of situs derangements.

The diversity of findings in individuals with situs ambiguous mandates detailed evaluation of all organs and systems with imaging methods in order to demonstrate the position of abdominal organs, hepatic veins, superior vena cava, inferior vena cava, coronary sinus, pulmonary veins, cardiac atria, atrioventricular structures, the cusps as well as cardiac venricules, position of the cardiac apex, aortic arch and the great vessels.

Kartagener Syndrome

Kartagener syndrome (KS) affects 20% of patients with situs inversus. Kartagener syndrome is a subgroup of disorders of primary ciliary dyskinesia. In Kartagener syndrome the cilia have abnormal or no movement due to absence or irregularity of the dynein arms. The inheritance pattern of KS is autosomal recessive with no male or female predominance.

Kartagener syndrome is typified by bronchiectasis, sinusitis, and situs inversus, but only half the patients with Kartagener syndrome have situs inversus. The co-occurrence of situs inversus and chronic sinusitis was first reported by Siewert in 1904, but Kartegener was the first to describe it as an autosomal recessive syndrome.[53] In addition to this classic triad of abnormalities, patients also may have nasal polyps, an impaired sense of smell, recurrent otitis media, hearing loss, chronic respiratory infections, obstructive lung disease, reduced fertility (females), and/or infertility (males). Each of these conditions is related to impaired cilia or flagella. Today, the term "primary ciliary dyskinesia"(PCD) is used to indicate the underlying pathophysiology. In fact, KS is part of the PCD spectrum. Only half of the individuals with PCD have situs inversus, and therefore diagnosed as having KS. Abnormal sperm motility makes affected males completely infertile. Although ciliary dyskinesia of the fallopian tubes impairs female fertility, the tubes are not obstructed, and women with Kartagener syndrome can conceive.

Individuals with KS should undergo radiological evaluation including sinus and chest radiograms and high-resolution computerized tomography of the lungs. The saccharine test examines nasal mucosal clearance. Audiograms and respiratory function tests are helpful in management. Nasal endoscopy may be indicated for mucosal biopsies and nasal polyposis.

As discussed earlier the definite cause of anomalies associated with laterality is unknown. Situs is congenital and always present at birth. The variations in the clinical phenotype and the severity of the cardiac anomaly may delay accurate and early diagnosis

Familial situs anomalies in humans have been reported with both autosomal and X-linked inheritance patterns.[54] Situs inversus and situs ambiguous have been identified among members of the same family. ZIC3, is an X-linked gene and its defect has been reported in both sporadic and familial cases. Affected males tend to demonstrate situs ambiguous phenotype, while females have either situs solitus or inversus. This gene also plays a role in the development of the neural tube and alignment of the midline embryonic structures. Submicroscopic deletions are found in Xq26 and 18p in familial cases, while various balanced and unbalanced translocations have been reported in sporadic cases.[55,56] In addition to these cytogenetic abnormalities, retinoic acid and maternal diabetes can also lead to laterality defects.

Management

The concept of situs or laterality regarding viscerovascular anatomy is important in the evaluation and imaging of congenital and vascular malformations.

The involvement of the heart is the major determinant of morbidity and mortality among individuals with laterality defects. The failure of formation of the complex cardiac structure leads to ambiguity involving a single ventricle, or conotruncal abnormalities such as truncus arteriosus or transposition of great vessels. The highest mortality rate occurs with the total anomalous pulmonary venous return. Biliary atresia and absence of spleen are both life threatening visceral anomalies. Malrotation may be a clinical complication in the presence of a midgut volvulus.

The recognition of situs inversus is important for preventing surgical mishaps that result from the failure to recognize reversed anatomy or an atypical history. For example, in a patient with situs inversus, cholecystitis typically causes left upper quadrant pain, and appendicitis causes left lower quadrant pain. A trauma patient with evidence of external trauma over the ninth to eleventh ribs on the right side is at risk for splenic injury. If surgery is planned on the basis of radiographic findings in a patient with situs inversus, the surgeon should pay careful attention to image labeling to avoid errors such as a right thoracotomy for a left lung nodule. In conclusion, we would like to emphasize the fact that there are numerous endogenous molecules involved in

asymmetrical development and as many suggested mechanisms and hypotheses on this subject. Nevertheless, there are still many unanswered questions and controversial points which make the subject an open area for further researches. The "asymmetry inside the symmetry" will be in the focus of future researchers creating knowledge and interest in the field. In the meantime, clinicians of almost every field should be well aware of the clinical consequences in order to correctly manage affected individuals.

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