Human heterotaxy syndrome is characterized by a wide variety of cardiac and extracardiac congenital malformations that are primarily induced by disorders of the left-right axis determination during early embryonic development. The cellular and molecular mechanisms of the left-right asymmetry have been extensively investigated in the past decade and the developmental mechanisms of the syndrome have been considerably elucidated. Medical and surgical management and treatment of heterotaxy syndrome have advanced as well. However, prognosis of the disease still remains unsatisfactory because the syndrome is often associated with a combination of complicated congenital heart diseases. Management of heterotaxy patients, particularly those who have undergone the Fontan procedure, is now one of the most important issues in pediatric and adult congenital heart disease clinics. In this review, we focus on the recent advances in knowledge of the genetic and molecular pathogenesis of heterotaxy syndrome, as well as its clinical features, management, and prognosis. (Circ J 2012; 76: 2066–2075)

**Key Words:** Congenital heart disease; Genetics; Molecular biology; Surgery

Congenital heart diseases (CHD) affect approximately 0.75–0.9% of newborns and are the leading cause of death in neonates and infants.1–2 The clinical issues of CHD were once limited to pediatric cardiologists and pediatric cardiac surgeons, because the survival rate of patients was, in general, low. Because medical and surgical treatments of CHD have become extremely advanced, over 90% of CHD patients survive until adulthood.3–4 Consequently, the total number of adult patients with CHD currently exceeds that of children and CHD is now becoming an essential part of healthcare not only for pediatric but also for adult cardiologists.

Among the various kinds of CHD, heterotaxy syndrome is one of the most serious. This syndrome occurs in approximately 1 to 5,000–7,000 of live births with CHD.5–7 Heterotaxy syndrome is primarily induced by disorders of left-right axis determination during early embryonic development. Recently, the molecular mechanisms of the left-right axis determination have been extensively investigated in animal models including genetic engineering of mice.8–11 Briefly, an initial break in symmetry occurs at the primitive node as a leftward “nodal flow”, which is created by unidirectional rotation of nodal cell cilia. This flow provokes asymmetry signals that are transmitted toward the left lateral plate mesoderm (LPM), where downstream left-side specific growth and transcription factors, Nodal, Lefty2, and Pitx2c, are activated. As a result, Pitx2c and other undetermined factors regulate genetic programs in the left side of the body and create asymmetric organ morphogenesis. Human genetics have also revealed several genes that are responsible for left-right laterality and heterotaxy syndrome, including ZIC3, NODAL, LEFTY2, NIP1, and SIM2.12–14

In the clinical field, precise diagnosis and surgical treatment of patients with heterotaxy syndrome have recently advanced as well. However, outcomes and the survival rate of patients are not satisfactory because this syndrome is often associated with combinations of serious CHDs.13–14 The mid- to long-term prognosis of heterotaxy patients who undergo the Fontan procedure for single ventricular physiology is now an important issue in pediatric and adult CHD clinics.15–18

**Morphological Characteristics of Heterotaxy Syndrome**

Patients with heterotaxy syndrome are subdivided into “bilateral right-sided” (right isomerism) or “bilateral left-sided” (left isomerism) according to the characteristic morphology of the atrial appendages of the heart.13–14 However, there is a wide spectrum of pathology, with considerable overlap of the anatomical features. The morphological characteristics of heterotaxy syndrome are summarized in Table.

**Molecular and Cellular Mechanisms of Left-Right Determination**

To elucidate the etiology of heterotaxy syndrome, it is neces-
Although the nodal flow is the initial break in body symmetry, the precise mechanism of how this flow is perceived by nodal and perinodal cells remains uncertain. One hypothesis (chemosensory model) is that the nodal flow produces a gradient of left determinant particles (node vesicular parcels, NVPs) containing hedgehog proteins and retinoic acid secreted by node pit cells (Figure 1D).27,33 The secretion of NVPs is regulated by fibroblast growth factor 8.35 These morphogens, in association with Nodal secreted in the node, activate downstream signaling of Nodal in the left-side perinodal cells. For instance, a transcription enhancer, ANE and Smad2/3, is activated by Nodal signaling and exhibits left dominant asymmetric expression in perinodal cells.20 Consequently, the left-side dominant active Nodal in the node is translated into asymmetry in the left LPM.

An alternative hypothesis (mechanosensory model) is that the leftward nodal flow provokes an asymmetrical increase influx of Ca^{2+} ion in the sensory cilia cells through PKD2, a causative gene for human polycystic kidney disease.32,33 This Ca^{2+} influx is linked to the activation of Nodal in the left-side perinodal cells, which is consequently transferred to the left LPM (Figure 1D).

Experimental creation of the reverse nodal flow results in a reverse pattern of asymmetric gene expression.34 Mutations in the left-right dynein (Dnahc11)35 or kinesin (KIF13),36,37 which are the motor proteins of microtubules, give rise to randomization of the left-right asymmetry. Mutations in dynein assembly factor, DNAAF3, induce primary cilia dyskinesia and disturbance of left-right asymmetry in mice.38 In embryos with mutations of Noto, a transcription factor regulating cilia formation, left-right asymmetry of internal organs and expression of laterality markers is randomized.39 The Notch ligand DLL1-
mediated Notch signaling pathway plays a primary role in the establishment of left-right asymmetry by directly regulating expression of Nodal around the node. A t-box transcription factor, Tbx6, is also involved in left-right determination through Notch-mediated Nodal signaling and formation of node cell monocilia. A member of the GLI superfamily zinc finger transcription factor, Zic3, is also involved in left-right patterning. The precise mechanism of the laterality defect remains unknown, but Zic3 deficiency is associated with decreased expression of cardiac-specific genes, including Nkx2.5, Tbx5 and ANF. Epigenetic regulation with a chromatin modifier, Baf60c, has also been shown to control left-right asymmetry via Notch-mediated induction of Nodal. In addition to activation of Nodal in the left side of the node, an endogenous nodal antagonist, Cerl2, is expressed around the right side of the node and is responsible for downregulation of Nodal in the right side of the embryo.

An experimental perturbation of the left-right determination process induced by maternal administration of retinoic acid at E6.5 to E7.0 is shown in Figure 2. Figures 2A–D demonstrate dissected late fetal mice with right and left atrial isomerism, respectively. Nodal mRNA expression is limited in the left-side in a control mouse embryo (Figure 2J), and Nodal expression is randomly distributed in a RA-treated one (Figure 2K).

Asymmetry Signaling Transmits to the Left LPM and Upregulates Left Determinants Such as Nodal, Lefty2, and Pitx2

Transmission of Nodal to the left LPM followed by Pitx2 activation and the consequent heart morphogenesis in normal subjects is summarized in Figure 3A. Possible mechanisms of right/left isomerism and situs inversus from the viewpoint of nodal flow and the distribution of left determinants are shown in Figures 3B,C.

Actived Nodal in the left side of the primary node is transmitted toward the left LPM and midline. A member of the transforming growth factor (TGF)-β superfamily growth/differentiation factor 1 (GDF1) plays a role in the transportation of Nodal. Nodal activates other members of the TGF-β superfamily: lefty1 in the left LPM and lefty2 in the midline. Lefty1 and lefty2 compete and antagonize nodal activity to restrict the extent and duration of nodal signaling. Bone morphogenic protein (BMP) also plays a role in negatively regulating Nodal expression. Chordin and noggin, which are BMP signaling antagonists, promote Nodal signaling around the node, while BMP signaling represses Nodal expression in the left LPM.

Consequently, Nodal in the left LPM upregulates a transcription factor, Pitx2. Pitx2 is a major laterality gene and plays a direct and pivotal role in asymmetric organogenesis of the heart and other visceral organs. Pitx2c, a left-right asymmetric isoform, is expressed in the left LPM, outflow tract myocard...
Human Heterotaxy Syndrome

**Maternal RA administration at E6.5**
Right atrial isomerism, dextrocardia, common AV valve, double outlet right ventricle, doubly committed VSD, right Ao arch

**Maternal RA administration at E7.0**
Left atrial isomerism, dextrocardia, common AV valve, double outlet right ventricle, subaortic VSD, pulmonary stenosis, IVC interruption, left Ao arch

**Figure 2.** Experimental perturbation of the left-right determination process induced by maternal administration of retinoic acid at E6.5 to E7.0. A late fetal mouse treated with retinoic acid at E6.5 shows right atrial isomerism (A–D). A late fetal mouse treated with retinoic acid at E7.0 shows left atrial isomerism with multiple spleen (E–I). Nodal mRNA expression is limited in the left side of a control mouse embryo (J), while Nodal is randomly distributed in a RA-treated one (K).

The loss-of-function of Pitx2c induces severe cardiac malformations including right isomeric heart. Recently, Pitx2 was identified as a suspect for human atrial electric and structural remodeling arrhythmias, because, for example, PITX2C is significantly decreased in human patients with sustained atrial fibrillation. Cited2, a transcriptional co-activator and is a negative regulator of HIF-1α, is also involved in left-right patterning. In association with AP2, Cited2 activates Nodal-mediated gene transcription such as Nodal, Lefty2, and Pitx2 in the LPM.

**Genes Associated With Human Heterotaxy Syndrome**
Recent human and animal model studies have provided insights into the genetic and developmental etiology of the heterotaxy syndrome. In humans, genes that are associated with heterotaxy syndrome are ZIC3, NODAL, CFC1, ACVR2B, LEFTY2, CITED2, and GDF1. Factors that deteriorate the prognosis of heterotaxy patients have been described as complications with pulmonary venous obstruction, pulmonary arterial distortion, regurgitation of the atrophicventricular valve, elevated pulmonary vascular resistance, and impaired ventricular function.

**Right Isomerism**
Neonates with right isomerism typically show a single atrium, single right ventricle, and a univentricular atrophicventricular connection often associated with atrophicventricular valve regurgitation. First stage palliation of such patients is control of pulmonary blood flow. If the pulmonary artery is atresic or severely stenotic, intravenous administration of prostaglandin E1 is necessary to open the left ductus, followed by the surgical operation of systemic pulmonary shunt (modified Blalock-Taussig shunt). If the pulmonary artery is not stenotic, pulmonary artery banding is necessary to protect the pulmonary vasculature until the Glenn and Fontan procedures can be performed. Both surgical interventions are generally performed approximately 2–4 weeks after birth, depending on the patient’s body weight and complications. Pulmonary venous obstruction because of total anomalous pulmonary venous drainage should be precisely diagnosed and immediately repaired by surgical operation. However, the complication of pulmonary venous obstruction in patients with right isomerism is prob-
lematic because recurrent obstruction often occurs despite surgical repair, including sutureless techniques. At 3–6 months after the first palliation, cardiac catheterization is performed to ensure that pulmonary arterial pressure and resistance are appropriate for the next procedure. In some patients with right isomerism, the normal 6th pharyngeal arch-derived pulmonary arteries are underdeveloped and pulmonary blood flow is supplied by major aortopulmonary collateral arteries (MAPCAs). In these cases, unifocalization of the MAPCAs in combination with a systemic pulmonary shunt is necessary in infancy if applicable.

Second-stage palliation is a bidirectional Glenn shunt, where-by the right and/or left superior vena cava is isolated and connected to the pulmonary artery. This operation is, in general, performed around 6 months after birth. If the atrioventricular valve regurgitation is hemodynamically significant, simultaneously repair or replacement of the common atrioventricular valve is necessary. At 4–6 months after bidirectional Glenn shunt, cardiac catheterization is again necessary to evaluate whether the hemodynamic conditions are satisfactory for the final palliation of a Fontan-type procedure, which nowadays means total cavopulmonary connection (TCPC). The criteria for successful Glenn shunt and TCPC include pulmonary arterial pressure <15 mmHg, pulmonary arterial resistance <2.5 U/m², atrioventricular valve regurgitation <mild, single ventricular ejection fraction >50%, and no significant distortion or stenosis of the pulmonary branch arteries. Significant stenotic lesions of the pulmonary arteries should be treated using percutaneous transluminal balloon angioplasty. In patients with severe hypoxia, aortopulmonary collateral arteries develop and supply blood to the pulmonary circulation. These collateral vessels seemingly improve cyanosis, but the retrograde blood flow interferes with normal antegrade pulmonary arterial flow and consequently raises the central venous pressure after TCPC completion. These collateral vessels should be occluded with catheter-based coil embolization before the Fontan procedure.

The third-stage palliation for right isomerism patients is to connect the inferior vena cava and hepatic veins to the pulmonary artery. Recently, a modification using an extracardiac artificial conduit type TCPC is most often used, because the

Figure 3. Signal transmission of Nodal to the left lateral plate mesoderm followed by Pitx2 activation and consequent heart morphogenesis in normal embryos (A). Possible mechanisms of right/left isomerism and situs inversus from the viewpoint of nodal flow and distribution of left determinants (B, C). Right isomerism is a condition where production of left determinants is low and/or nodal flow is abnormal. As a result, the left signal is not activated in both sides. Left isomerism is a condition where production of left determinants is normal but nodal flow is abnormal. As a result, the left signal is activated in the both sides. Normal production of left determinants and inverse nodal flow results in situs inversus.
long-term prognosis of the conventional procedure (atriopulmonary connection, APC) has proved to be unsatisfactory, characterized by enlargement of the atrium, intractable atrial tachyarrhythmias, and thromboembolism.

After successful completion of the TCPC, cyanosis disappears and the general condition of the patient improves. However, the number of right isomerism patients who have undergone successful Fontan procedure is approximately 50%, because right isomerism is often accompanied by a combination of severe and complicated CHDs.77 Biventricular repair can be achieved in only a few patients with right isomerism.78 The surgical procedures for right isomerism are illustrated in Figure 4A.

**Left Isomerism**
In contrast to right isomerism, the combination of the cardiac malformations in left isomerism is not so complicated. Left isomerism is typically associated with atrioventricular septal defect (AVSD), persistent left superior vena cava, interrupted hepatic portion of the inferior vena cava, and atrioventricular conduction disturbance.13 Patients with the complete type of AVSD may undergo pulmonary artery banding in the neonatal period to protect the pulmonary vasculature. In left isomerism, the sinus node and atrioventricular node are usually hypoplastic and sinus bradycardia or complete atrioventricular block is a frequently accompaniment. In patients with severe bradycardia because of complete atrioventricular block from the fetal period, implantation of a pacemaker should be considered immediately after birth. Several months after pulmonary arterial banding, cardiac catheterization should be performed to ensure decreased pulmonary arterial pressure and resistance. Biventricular repair with a 2-patch method is usually performed for the complete type of AVSD with balanced ventricles.79

AVSD with unbalanced ventricles is sometimes seen in patients with left isomerism, where either of the ventricles and the inflow tract is significantly hypoplastic, for instance in less than 30%.80 In these cases, biventricular repair is not feasible. Bidirectional Glenn anastomosis followed by extracardiac shunt between the hepatic veins and pulmonary artery should be considered according to the criteria of the operation. If the patient’s systemic outflow tract exhibits signs of potential stenosis, the double-barrel Damus-Kaye-Stansel operation is also
Hepatic Dysfunction

Hepatic dysfunction, liver fibrosis, and cirrhosis are common complications of the Fontan operation. These complications are due to the shunting of blood from the systemic to the pulmonary circulation, which can lead to increased portal pressure and subsequent liver injury. The exact mechanism of liver dysfunction is not fully understood, but it is thought to be related to the increased portal pressure and the shunting of blood around the liver.

In patients with Fontan physiology, the liver is exposed to a high volume of systemic venous blood, which can lead to increased portal pressure and subsequent liver injury. This can result in liver dysfunction, which may progress to liver fibrosis and cirrhosis. The degree of liver dysfunction and the rate of progression are influenced by various factors, including the type of Fontan circulation, the presence of other cardiac anomalies, and the overall health status of the patient.

In summary, the Fontan procedure is a complex and challenging operation that requires careful monitoring and management of postoperative complications. Liver dysfunction is one of the most common long-term complications of the Fontan procedure, and it can be a significant contributor to patient morbidity and mortality. Treatment of hepatic dysfunction in these patients requires a multidisciplinary approach, including medical therapy, liver transplantation, and other interventions as necessary.
Future Directions

In the basic science field, embryonic development of left-right asymmetry has been uncovered by means of genetic engineering of mice. In addition, advanced human genetics has uncovered many of the genes responsible for heterotaxy syndrome. By means of innovative technologies, such as next-generation sequencer or patient-based human inducible pluripotent stem cells, novel genes will be clarified and analyzed. In the clinical field, anatomic and physiologic diagnosis from the fetal period, better clinical management after birth, tailor-made surgical operations, and systematic follow-up of the patients will improve their prognosis. Cell or tissue-based regeneration therapies could recover cardiac function of the failing Fontan patients. Multiple approaches including basic and clinical science are necessary to improve the prognosis and quality of life of heterotaxy patients.

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Disclosures

None.

References


