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THE DETERMINATION FACTORS OF LEFT-RIGHT ASYMMETRY DISORDERS- A SHORT REVIEW

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Abstract

Laterality defects in humans, situs inversus and heterotaxy, are rare disorders, with an incidence of 1:8000 to 1:10 000 in the general population, and a multifactorial etiology. It has been proved that 1.44/10 000 of all cardiac problems are associated with malformations of left-right asymmetry and heterotaxy accounts for 3% of all congenital heart defects. It is considered that defects of situs appear due to genetic and environmental factors. Also, there is evidence that the ciliopathies (defects of structure or function) are involved in development abnormalities. Over 100 genes have been reported to be involved in left-right patterning in model organisms, but only a few are likely to candidate for left-right asymmetry defects in humans. Left-right asymmetry disorders are genetically heterogeneous and have variable manifestations (from asymptomatic to serious clinical problems). The discovery of the right mechanism of left-right development will help explain the clinical complexity and may contribute to a therapy of these disorders.

Keywords: situs inversus, heterotaxy syndrome, left-right asymmetry determination factors, Nodal, Kartagener syndrome

Introduction

Laterality defects in humans are rare disorders with an incidence of 1:8000 to 1:10 000 in the general population. Left-right asymmetry (LRA) can cause randomization (situs ambiguus/heterotaxy) or complete organ position reversal (situs inversus totalis) [1]. Numerous case reports and research articles have been published, and yet the etiology of left-right asymmetry disorders remains unclear. Several molecular studies suggest that laterality malformations have a multifactorial etiology with heterogeneous phenotypes. Although the defects in left-right (LR) development may seem not that important for a human life (most of the patients, with situs inversus especially, are asymptomatic), it has been proved that 1.44/ 10 000 of all cardiac problems are associated with malformations of left- right asymmetry and heterotaxy accounts for approximately 3% of all congenital heart defects [2,3].

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Terminology

The term *situs* and its accompanying terms is used to summarize the left- right anatomy of the entire organism. Situs solitus for normal, and situs inversus (SI), situs ambiguus (HTX) for disorders of laterality in which the internal organs do not have their typical pattern of asymmetry [3].

While only 5-10% of patients with SI (complete, mirror-image reversal of all asymmetrical structures) may present congenital heart defects [4], those with HTX usually have multiple congenital malformations. Situs ambiguus (heterotaxy) is the abnormal arrangement of the thoracic and/or abdominal organs. Its major morbidity and mortality result from complex cardiovascular malformations. Also, patients with situs ambiguus may frequently present asplenia or polysplenia, liver misposition, dextrocardia or mesocardia, malrotation, microgastria, vertebral or rib abnormalities. It is more rare than situs inversus [3].

HTX often includes isomerism. Isomerism is characterized by asymmetric defects of paired organs that generally have obvious left and right shape, and it can be

separated into two classes: left isomerism (both lungs are bilobed with long hyparterial bronchi, both atria and left atrial appendages and polysplenia) and right isomerism (both lungs with trilobed with short eparterial bronchi, both atria with right atrial appendages, and asplenia) [3].

Pathogenesis

Situs inversus and situs ambiguus have a multifactorial etiology. It is considered that defects of situs appear due to genetic and environmental factors.

The role of the environmental factors in the LR development defects has not been determined precisely yet, although there are some candidate factors. For example, retinoic acid (RA), a vitamin A derivative, implicated in embryonic development, when exposed to in utero, causes laterality defects in several vertebrates. Also, maternal diabetes, maternal cocaine use, parental exposure to hair dye, smoking and laboratory chemicals can be associated with LRA disorders [5].

LR asymmetric defects have been identified in 59 syndromes [6]. Several genes have been demonstrated to be involved in syndromic situs inversus, some ciliopathies, including primary ciliary dyskinesia (PCD) and Kartagener syndrome (KS) [7]. Genetic studies in isolated situs inversus are very rare. Most of the genetic studies involved in situs inversus pay close attention to syndromic situs inversus, especially to PCD with situs inversus, which accounts for only 20-25% of individuals with situs inversus [8].

Cilia disorders (ciliopaathies)

Cilia are long hair-like cytoplasmic protuberances covered by plasma membrane, projecting from the cell body of certain eukaryotic cells, with particular motile and sensory functions [9].

Ciliary disorders (defects of structure or function) lead to several problems beginning with development abnormalities to serious clinical conditions. They are known as ciliopathies [10].

Being known that cilia are spread in various types of cells, ciliopathies can affect many organs or body parts such as: eye, kidney, lung and brain. Over 40 genes have been reported to be involved in ciliopathies up to now [11].

One of the most popular ciliopathies is immotile cilia syndrome, now known as primary ciliary dyskinesia (PCD). PCD is a rare autosomal- recessive disorder, with an incidence of 1:4 000 to 1:60 000, noticed more frequently in families with consanguineous marriages [12].

PCD is caused by congenital abnormalities of the function and structure of the cilia. Multiciliated epithelial cells move the mucus and debris outward to protect the upper and lower airways from chronic bacterial infections. So, in patients with PCD, because of the deficient mucociliary clearance, mucus and pathogens accumulate in the airways and recurrent respiratory infections occur very frequently [12,13]. Also, immotile cilia lead to organ laterality defects and fertility problems [14].

The clinical features of PCD are: variable age of presentation, neonatal respiratory distress, situs inversus or heterotaxy, chronic sinusitis, otitis media or bronchiectasia, persistent cough, nasal congestion or respiratory infection, male infertility, hearing loss [15].

Patients with PCD and situs inversus (50%) present Kartagener Syndrome (KS).The association with situs inversus is based on the hypothesis that during embryogenesis, the ciliary beat pattern determines the laterality of the organs. When they are immotile, the organ placement is random [16,17,18].

Kartagener syndrome, first described in 1933 [19], is characterized by the triad of: bronchiectasis, sinusitis and situs inversus [20]. These symptoms appear due to ultrastuctural anomalies of cilia (especially missing or abnormal dynein arms) of epithelial cells covering the upper and lower airways and spermatozoa flagellae [21].

A case of monozygotic female twins with PCD, one with situs inversus and the other with situs solitus has been reported, what suggests that the laterality of the organs is random in patients with PCD [22].

Molecular genetics of Human LRA Malformations

Several genes are expressed asymmetrically during gastrulation and lead to asymmetrical development of the organs primordia. Although no mechanism has been totally proved yet, there are two theories that try to explain the asymmetrical expression of the genes. One suggests that the clockwise rotation of monocilia of the node produces a leftward flow of the extraembryonic fluid, creating a gradient of growth and transcription factors in the left side of the node. The alternative theory says that asymmetric calcium signaling appears at the left margin of the node and then transferred to the right [23,24].

Errors of LR development during embryogenesis are characterized by several common human birth defects. Situs inversus, heterotaxy, dextrocardia or Kartagener syndrome are among common human birth defects [25].

Situs inversus displays autosomal recessive inheritance [26], while heterotaxy is a X-linked, autosomal dominant with reduced penetrance, or a autosomal recessive malformation [3].

Complex chromosomal rearrangements, small deletions or duplications, balanced translocations, insertions have a key role in determining LRA disorders [27].

Over 100 genes have been reported to be involved in left-right patterning defects in mice, but only a few are likely to candidate for LRA defects in humans [28] (Table 1). Up to now, the human genes identified have either mutations at low frequency or have not been tested in larger population [29].

NODAL

Nodal is one of the well-known conserved asymmetric gene and plays a key role in the left- right development in all vertebrates [30].

It is initially expressed symmetrically in the crown cells surrounding the node, followed by asymmetrical left-sided expression. The right mechanism by which *Nodal* is expressed asymmetrically at the node is yet unknown [31,32].

The asymmetrical expression of *Nodal* determines a cascade of left-sided gene expression in the left lateral plate mesoderm (LPM). The Nodal signaling pathway is mediated by an activin receptor complex: *ACVR1B* and *ACVR2B*. Nodal induces the expression of other members of TGF-*β* superfamily: *Lefty 1* (*LEFTYA* in humans), expressed in the midline, and *Lefty 2* (*LEFTYB* in humans), expressed in the LPM. These activated genes restrict the Nodal expression and inhibit the transfer of left-sided gene expression across the midline of the embryo. Also, *Nodal* activates a homeobox transcription factor, *Pitx*2 [33,34,35].

Mutation within the Nodal signaling pathway have been found in gene *NODAL*, as well as in ligand co- receptor (*CFC-1*), receptor(*ACVRIIB*), transcriptional co-activator (*FOXH1*) and midline inhibitor (*LEFTYA*) [36].

SHH

Shh is a member of the Hedgehog family. *Shh* expression is maintained on the left and repressed on the right by *Activin beta* and *Activin receptor IIa*. Its asymmetrical expression is essential in transfer of laterality information to organs progenitors. The role of Shh may differ from specie to specie, being demonstrated in chick, but not in mouse [37].

ZIC3

ZIC3 is a member of GLI superfamily and also involved in left-right pattering [38]. Mutations in *ZIC3* cause 1% of sporadic HTX cases, in males as well as in females, being responsible for the X-linked inheritance of HTX [32,389. Point mutations and chromosomal translocations have been found in affected females [40].

Except HTX, loss of function of *ZIC3* produces cardiovascular malformations such as: double outlet right ventricle, transposition of the great arteries and ventricular inversion. The *ZIC3* role in developmental function is not yet known, but there is evidence that ZIC3 acts on Nodal signaling at the node [41].

While all the male cases with *ZIC3* mutations reported are HTX, no female case with *ZIC3* mutations and HTX are identified to date. Female cases generally have SI and other clinical phenotypes [42].

PITX2

Pitx2 is a homeobox transcription factor and plays an important role in asymmetric development of the organ progenitors, especially the heart, gut and lung [43].

Pitx2 has an asymmetrical left-right expression initiated by Nodal, but persists long after Nodal expression ends [44]. Pitx2 is also involved in regulating the expression of adhesion molecules and participates in the development of the pituitary gland, the mutated gene being found in Rieger syndrome [37,45].

Loss of function of *Pitx2* can cause severe cardiac malformations, but information about cardiac specific targets is yet unclear [45,46,47].

LEFTY A, LEFTY B

Lefty A and *Lefty B* (*Lefty 1* and *Lefty 2* in mice) are members of TGF-β superfamily and serve a large variety of functions in growth and development. *Lefty A* and *Lefty B* are located on chromosome 1q42, separated by 50kb [48].

Both are expressed on the left side of the embryo; Lefty A in the midline and Lefty B in the LPM. Lefty A blocks the transfer of laterality information across the midline, while Lefty B induces Pitx2 in organ progenitors. Furthermore, Lefty A appears to regulate the expression of Lefty B, which shows that both genes are part of the same pathway, but have different functions [3,49].

One nonsense and one missense mutations have been found in these genes that lead to malformations in LR axis. Both mutations lied in exon 4, a region which encodes the cysteine knot [50].

Table I. Candidate genes for human LRA disorders.

Gene	Localisation	Familiy/Role	Phenotypical effect Syndrome Anomaly
ACVR2B	3p22.2	Transmembrane receptor	Complex heart malformations and other visceral anomalies typical of situs ambiguus, right isomerism [51,52]
C1ORF88	1p13.2	Regulates primary cilia disassembly	LRA disorders, neonatal lethality, cystic kidneys, liver fibrosis [53]
CCDC103	17q21.31	Encodes structural outer and inner dynein arm motor proteins	PCD, SI,HTX, pronephric kidney cysts [54]
CCDC11	18q21.1	Encodes structural outer dynein arm(ODA) motor proteins	LRA disorders, cardiac malformations [55]
CCDC39	3q26.33	Encodes structural inner dynein arm(IDA) motor proteins	PCD, SI, HTX [56]
CCDC40	17q25.3	Encodes structural IDA motor proteins	PCD, KS [57,58]
CFC1	2q21.1	EGF-CFC/ Co-receptor/ Encodes an epidermal growth factor family protein	Transposition of the great arteries, but without extra- cardiac anomalies [59,60]
Chromosome 6p	6p24.3-21.2	Encodes dynein heavy chain genes and a kinesin gene	LRA disorders, cardiomyopathy, congenital heart malformations, left isomerism [61,62]
CITED2	6q24.1	Transcriptional co-activator/ Contributes to the initiation or maintenance of Nodal	LRA disorders, abnormal heart looping, right isomerism, dextrocardia [63,64]
CRELD1	3p25.3	EGF/Encodes a cell adhesion molecule	LRA disorders, partial atrioventricular septal defects [65,66,67]
DNAAF1	16q23.2-q24.1	Encodes structural ODA and IDA motor proteins	PCD, KS [68,69]
DNAAF2	14q21.3	Encodes structural ODA and IDA motor proteins	PCD, SI [70]
DNAAF3	19q13.42	Encodes structural ODA and IDA motor proteins	LRA disorders, PCD [21]
DNAH11	7p21	Encodes structural ODA motor proteins	SI, KS, cystic fibrosis [71,72,73]
DNAH5	5p15.2	Encodes structural ODA motor proteins	PCD, KS [74,75,76]
DNAI1	9p13.3	Encodes structural ODA motor proteins	PCD, KS [77,78]
DNAI2	17q25.1	Encodes structural ODA motor proteins	PCD, SI [79]
EPB41L5	2q14.2	Contributes to proper morphogenesis of the node and midline	LRA disorders, holoprosencephaly [80,81]
FOXH1	8q24.3	Transcriptional co-activator within the Nodal signal transduction pathway	LRA disorders [83,84]
GALNT11	7q36.1	Activates Nodal signaling, coordinates cilia type	LRA disorders, HTX [67,84]
GATA4	8p23.1	Involved in heart morphogenesis, initiates early NKX2-5 expression	Cardiac malformations(double-outlet right ventricle, defects in the semilunar valves), dextrocardia [85,86]
GDF1	19p13.11	TGF-β/ Growth/ Differentiation factor/ Transports Nodal, regulates Nodal signaling	LRA disorders, right isomerism, cardiac defects [87,88]
Kif3b		Kinesin family member/ Encodes the kinesin arms and has a role in the beating of the cilia	Prenatal lethality, neural tube disorganization and randomized LRA [89,90,91]
LEFTY A,B	1q42.12	TGF-β./ . Lefty A blocks the transfer of laterality information across the midline and regulates the expression of Lefty B. Lefty B induces Pitx2 in organ progenitors.	Left sided morphology of the lungs, cardiac malformations, abnormalities of the inferior vena cava and the azygous veins, left isomerism [3,49,50,92]
MED13L	12q24.21	Involved in early heart and brain development	LRA disorders, heart, brain defects, mental retardation [93]
NEK8	17q11.2	Encodes the NIMA- related serine/ threonine protein kinase-8	LRA disorders, cardiac defects, glomerular kidney cysts [94,95,96]
NKX2-5	5q35.1	Contributes to the normal heart morphogenesis	Embryonic lethality with abnormal cardiac development [97,98]
NODAL	10q22.1	TGF-β/ Expressed asymmetrically in LPM	LRA disorders, symmetrical hearts [7,34,99]
NPHP4	1p36.31	Regulates Nodal signaling	LRA disorders, cardiac malformations [100]
PITX2	4q25	Homeobox transcript factor/ Expressed asymmetrically in the LPM and regulates the expression of the adhesion molecules	Malformation of the heart, gut and lung, Rieger syndrome [43,44,45,57]
PKD2	4q22.1	Encodes polycystin protein products, implicated in the transduction of the nodal flow information	LRA disorders, polycystic kidney disease [101]
SESN1	6q21	Involved in Nodal signaling	LRA disorders [102]
SHH	7q36	Hedgehog/ Role in cell growth, cell signaling and the normal pattering of the organism	LRA disorders [37]
SHROOM3	4q21.1	Encodes an actin binding protein	SI, dextrocardia, pulmonic stenosis, bilateral keratoconus, sensorineural hearing loss [103]
SMAD2	18q21.1	TGF-β/ Exhibits left dominant asymmetric expression in perinodal cells	LRA disorders, dextrocardia, unbalanced complete atrioventricular canal, pulmonary stenosis [104,105]
TXNDC3	7p14.1	Encodes structural ODA motor proteins	PCD, HTX, SI [106]
UVRAG	11q13.5	Regulates Nodal	LRA disorders [107]
ZIC3	Xq26.3	GLI superfamily/Trascript factor. Acts on Nodal signaling at the node	Heterotaxy, cardiovascular malformations(abnormal heart looping, double outlet right ventricle, L-transposition of the great arteries, ventricular inversion), anal anomalies, anomalies of the ureter [32,41,42,108,109]

Discussion

LRA disorders are genetically heterogeneous and have variable manifestations (from asymptomatic to serious clinical problems) [2]. However, knowing the right position of the organs may be crucial in thoracic as well as in abdominal surgeries, imagine diagnosis or interpreting an EKG (the P wave appears negative for patients with dextrocardia) [1].

Up to now, previous studies have demonstrated the link between the function of the cilia and the lateralization determination [10,11,12,13,14]. Also, several studies reported that early cell signaling during embryogenesis is a well conserved mechanism in all organisms [30,31,32,33].

Being aware that most of the studies on LR development are made on model organisms, not humans, the question that arises is: to what extend will the genes involved in the development of model organisms have the same role in humans, knowing that even among model organisms the development mechanisms and genes are not the same? [2] Moreover, the number of the candidate genes identified as important in LRA in animal models exceeds 100, but only Nodal has been demonstrated to be involved in humans LR development [110,111].

In addition, to what extent do the environmental factors play a role in LR development?

More interesting questions remain unanswered and further studies on this subject are needed in the future.

Conclusion

In conclusion, the discovery of the right mechanism of LR development will help explain the clinical complexity and may contribute to a therapy of these disorders [7]. Furthermore, the knowledge of the right mechanism may lead to the discovery of a prevention method for LRA disorders and their clinical manifestations.

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