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 ambiguous/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].

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 ambiguous (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 ambiguous (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 ambiguous 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
Pathogenesis

Situs inversus and situs ambiguous 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 (ciliopathies)

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 ultrastructural 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, *Pitx2* [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 | Family/Role                                   | Phenotypical effect                                      | Syndrome | Anomaly                  |
|------------|--------------|-----------------------------------------------|----------------------------------------------------------|----------|--------------------------|
| ACVR2B     | 3p22.2       | Transmembrane receptor                        | Complex heart malformations and other visceral anomalies  |          |                          |
|            |              |                                               | typical of situs ambiguous, right isomerism [51,52]      |          |                          |
| C10orf88   | 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 | PCD, SI, HTX, pronephric kidney cysts [54]                |          |                          |
|            |              | motor proteins                                |                                                          |          |                          |
| CCDC11     | 18q21.1      | Encodes structural outer dynein arm (ODA)     | LRA disorders, cardiac malformations [55]                 |          |                          |
|            |              | motor proteins                                |                                                          |          |                          |
| CCDC39     | 3q26.33      | Encodes structural inner dynein arm (IDA)     | PCD, SI, HTX [56]                                        |          |                          |
|            |              | motor proteins                                |                                                          |          |                          |
| CCDC40     | 17q25.3      | Encodes structural IDA motor proteins         | PCD, KS [57,58]                                          |          |                          |
| CFC1       | 2q21.1       | EGF-CFC/Co-receptor                           | Transposition of the great arteries, but without extra-   |          |                          |
|            |              |                                               | cardiac anomalies [59,60]                                 |          |                          |
| Chromosome 6p |           |                                               |                                                          |          |                          |
| CITED2     | 6q24.1       | Transcriptional co-activator                  | 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     | 1q21.3       | 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]                        |          |                          |
| DNAH3      | 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]                                             |          |                          |
| EB4AI5     | 6q24.3-21.2  | Encodes dynein heavy chain genes and a kinesin | LRA disorders, abnormal heart looping, right isomerism,   |          |                          |
|            |              | gene                                         | dextrocardia [63,64]                                      |          |                          |
| FOXI1      | 8q24.3       | Transcriptional co-activator within the Nodal  | LRA disorders [83,84]                                    |          |                          |
|            |              | signal transduction pathway                   |                                                          |          |                          |
| GALNT11    | 7q36.1       | Activates Nodal signaling, coordinates cilia   | LRA disorders, HTX [67,84]                                |          |                          |
|            |              | type                                         |                                                          |          |                          |
| GATA4      | 8p23.1       | Involved in heart morphogenesis, initiates     | Cardiac malformations (double-outlet right ventricle,     |          |                          |
|            |              | early NKX2-5 expression                       | defects in the semilunar valves), dextrocardia [85,86]    |          |                          |
| GDF1       | 19p13.11     | TGF-β/Growth/ Differentiation factor/ Transports| LRA disorders, right isomerism, cardiac defects [87,88]|          |                          |
|            |              | Nodal, regulates Nodal signaling              |                                                          |          |                          |
| Kif3b      | 6q24.1       | Kinesin family member/Encodes the kinesin     | Prenatal lethality, neural tube disorganization and        |          |                          |
|            |              | arms and has a role in the beating of the     | randomized LRA [89,90,91]                                 |          |                          |
| LEFTY A,B  | 1q42.12      | TGF-β/ / . Lefty A blocks the transfer of     | Left sided morphology of the lungs, cardiac malformations,|          |                          |
|            |              | laterality information across the midline and | abnormalities of the inferior vena cava and theazygous    |          |                          |
|            |              | regulates the expression of Lefty B. Lefty B | veins, left isomerism [3,49,50,92]                        |          |                          |
|            |              | induces Pitx2 in organ progenitors.           |                                                          |          |                          |
| 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     | LRA disorders, cardiac defects, glomerular kidney cysts    |          |                          |
|            |              | protein kinase-8                             | [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           | Malformation of the heart, gut and lung, Rieger syndrome  |          |                          |
|            |              | asymmetrically in the LPM and regulates the   | [43,44,45,57]                                            |          |                          |
|            |              | expression of the adhesion molecules         |                                                          |          |                          |
| PKD2       | 4q22.1       | Encodes polycystin protein products, implicated| LRA disorders, polycystic kidney disease [101]            |          |                          |
|            |              | in the transduction of the nodal flow         |                                                          |          |                          |
| SESN1      | 6q21         | Involved in Nodal signaling                   | LRA disorders [102]                                      |          |                          |
| SHH        | 7q36         | Hedgehog/Role in cell growth, cell signaling  | LRA disorders [37]                                       |          |                          |
|            |              | and the normal pattering of the organism      |                                                          |          |                          |
| 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        | LRA disorders, dextrocardia, unbalanced complete         |          |                          |
|            |              | expression in perinodal cells                 | 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/Transcript factor. Acts on    | Heterotaxy, cardiovascular malformations( abnormal heart   |          |                          |
|            |              | Nodal signaling at the node                   | 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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