

Down Syndrome – Genetics and Cardiogenetics

Vasilica PLAIASU

Alessandrescu-Rusescu INSMC, Regional Center of Medical Genetics,
Bucharest, Romania

ABSTRACT

*During the last years, Down syndrome has been the focus of special attention. Down syndrome is a genetic disorder characterized by distinct physical features and some degree of cognitive disability. Patients with Down syndrome also present many other congenital anomalies. The mapping for phenotypes to specific regions of chromosome 21 permits to identify which genes (or small regions) contribute to the phenotypic features of Down syndrome and thus, to understand its pathogenesis. Mainly there are three cytogenetic forms of Down syndrome: **free trisomy 21, mosaic trisomy 21 and robertsonian translocation trisomy 21**. Prenatal and postnatal testing has become commonly used to diagnose different cases presenting the same pathology. Early clinical diagnosis is extremely important for patient prognosis.*

Lately, advances in Down syndrome research have been registered, but little is known about cardiovascular phenotype in Down syndrome. About half of patients with Down syndrome have congenital heart disease, and atrioventricular septal defects are the most common defects found.

Basic research on Down syndrome is now rapidly accelerating, using new genomic technologies. There were many studies performed to identify a correlation between genotype and phenotype in Down syndrome.

Keywords: Down syndrome, cardiogenetics, atrioventricular septal defects

BACKGROUND

Down syndrome (DS) is a clinical entity recognized for about 150 years (1), correlated 100 years later with trisomy 21 (2), represents the most common human autosomal aneuploidy and also the most common cause of intellectual disability (3).

The high degree of variability of the phenotype is the hallmark of DS clinical picture, not every patient having the same problems or associated conditions.

Down syndrome incidence is ranging from 1 in 650 to 1 in 1000 live births dependent on the population (4).

Genetic progress

The genetic basis for DS is trisomy 21: the presence in the genome of three chromosomes 21 instead of two, how it is normal. Chromosome 21 is the smallest human chromosome and contains 200 to 300 genes. Analysis of the chromosome revealed 127 known genes, 98 predicted genes and 59 pseudogenes (5).

Address for correspondence:

Plaiasu Vasilica, MD, PhD

Alessandrescu-Rusescu INSMC, Regional Center of Medical Genetics, Bucharest, Romania

E-mail: vasilica.plaiasu@gmail.com

Article received on the 19th of September 2017 and accepted for publication on the 28th of September 2017.

Down syndrome patients have an increased dosage or copy number of genes on chromosome 21. The genes that are involved are normal and their gene products are also normal. The genetic abnormality involves the production of increased amounts of products of the genes on chromosome 21 which have been overexpressed in cells and tissues of DS patients, showing phenotypic abnormalities (6). Because half of all patients with DS have a normal heart, this aspect suggests that genetic modifiers interact with dosage sensitive genes on chromosome 21 to result in congenital heart disease (CHD) (7).

Trisomy for functional non-protein-coding DNA elements could be involved in some of the abnormal phenotypes.

Diagnostic possibilities

Prenatal testing

For pregnancies, the high risk of DS is evaluated by fetal sample’s analyzing after invasive chorionic villus sampling (CVS) and amniocentesis, and by applying laboratory techniques such as conventional cytogenetic analysis (karyotype), Fluorescence *in Situ* Hybridization (FISH), Quantitative Fluorescence-Polymerase Chain Reaction (QF-PCR), Multiplex Ligation Probe Assay (MLPA) and array Comparative Genomic Hybridization (CGH), which are common techniques used for prenatal diagnosis of DS and each of them presenting with advantages and disadvantages. There is also a noninvasive technique for detection of trisomy 21 by Next Generation Sequencing (NGS) technology, known as Non Invasive Prenatal Diagnosis (NIPD). The process is based on analysis of extracted cell-free fetal DNA screening from maternal plasma samples.

Postnatal testing

Constantly conventional karyotype from peripheral blood is performed to confirm diagnosis for all patients suspected by Down syndrome.

Cytogenetics

Down syndrome is caused by trisomy of chromosome 21. Mainly there are three cytogenetic forms of DS:

1. *Free Trisomy 21* consists of a supplementary chromosome 21 in all cells (8).
2. *Mosaic Trisomy 21* means that there are two cell lineages, one with the normal number of

Form of Down syndrome	Standard trisomy 21	Robertsonian trisomy 21	Mosaic trisomy 21	Other
Frequency	95%	4%	3-5%	1%
Mechanism	Maternal meiosis 85-90%	Familial 25%	Mitotic	
	- Meiosis I 75%	<i>De novo</i> 75%		
	- Meiosis II 25%			
	Paternal meiosis 3-5%			
	- Meiosis I 25%			
	- Meiosis II 75%			

TABLE Origins of Trisomy 21 (Gardner RJ, Sutherland G, Shaffer L, 2012)

chromosomes and another one with an extra number of chromosome 21 (9). The mechanism of occurrence consists of an error or misdivision after fertilization during cell division.

3. *Robertsonian Translocation Trisomy 21* occurs only in 2-4% of the cases (10). The long arm of chromosome 21 is attached to another chromosome, generally an acrosome, mainly chromosome 14 (11).

Around 90% of *free trisomy 21* is due to a maternal meiotic error (13, 14) and only a small fraction is due to paternal errors (15).

Mosaic trisomy 21 occurs postzygotically due to a malsegregation of homologs or an anaphase lag (16).

Robertsonian translocation trisomy 21. There are two forms of Robertsonian translocation DS: familial and *de novo*. In case of the familial form, a parent is carrier of a translocation and this can transmit that translocation in an unbalanced form to the child, while for the *de novo* cases, parents have a normal karyotype and the abnormal chromosome occurs as a spontaneous event in maternal meiosis I from a chromatid translocation (17).

4. *Other forms of trisomy 21*

a) A terminal rearrangement of chromosome 21 around the telomeric region (18), the final chromosome having two centromeres and satellites on both ends.

b) As a component of a double aneuploidy (for example, 48,XY,+21 or 46,X,+21) (19, 20).

Molecular aspects

To understand DS it is crucial to know the genomic content of chromosome 21 and understand how the expression of these genes is altered by the supplementary chromosome 21. Many stu-

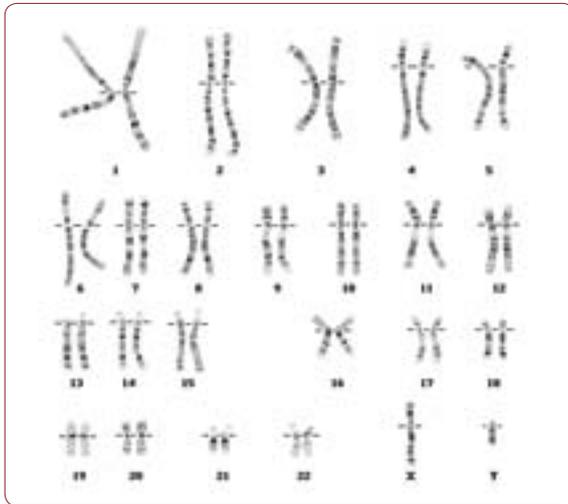


FIGURE 1. Normal karyotype: 46,XY (image by courtesy of genetic laboratory of Alessandrescu-Rusescu INSCM)

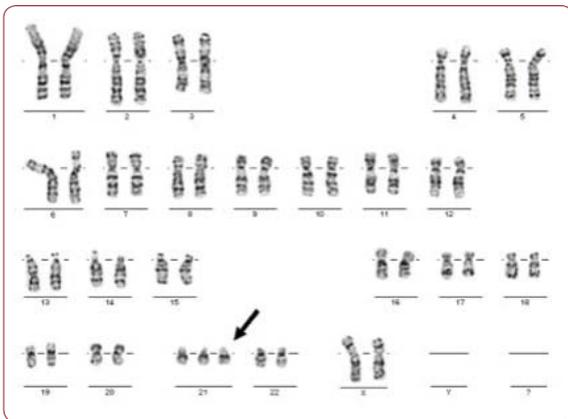


FIGURE 2. A female newborn with Down syndrome with a karyotype showing a free trisomy 21: 47,XX,+21 (image by courtesy of genetic laboratory of Alessandrescu-Rusescu INSCM)

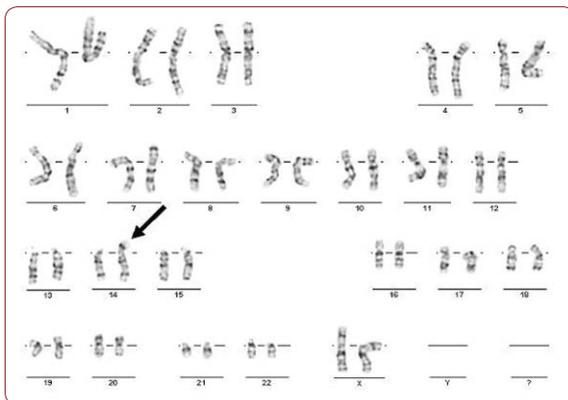


FIGURE 3. A girl with Down syndrome with a karyotype showing a Robertsonian translocation 14;21: 46,XX,t(14;21)(q10;q10),+21 (image by courtesy of genetic laboratory of Alessandrescu-Rusescu INSCM)

dies were performed to identify a correlation between genotype and phenotype in DS.

The duplication of a specific region of chromosome 21 could be responsible for the main features of DS. A critical region was suggested (21), namely Down Syndrome Critical Region (DSCR), which was defined with a proximal boundary between markers D21S17 (35 892 kb) and D21S55 (38 012 kb) and a distal boundary at *MX1* (41 720 kb) (22). Molecular studies of rare individuals with CHD and partial duplications of chromosome 21 established candidate gene *DSCAM*, which was expressed in the heart during cardiac development (23). Using the array comparative genomic hybridization technique to analyze patients with anomalies of chromosome 21, partial trisomy 21 and partial monosomy 21, the results suggested that there were more regions responsible for all aspects of the Down syndrome phenotype (24). The mapping phenotypes to specific regions of chromosome 21 permit to identify which genes (or small regions) contribute to DS phenotypic features, and thus to understand DS pathogenesis (24).

Basic research on DS is now rapidly accelerating, using new genomic technologies. More additional studies are needed to reduce the candidate regions for certain phenotypes.

Genetic counselling in Down syndrome

Cytogenetic investigation of all individuals suspected with DS is very important to establish a precise diagnosis and is mandatory in determining the recurrence risk of the syndrome in future generations.

Free trisomy 21 typically occurs as a sporadic event and recurrences are rare. When recurrence exists, the hypotheses are: gonadal mosaicism, a parental predisposition to nondisjunction, the effect of endogenous factors and environmental exposures and also chance (8).

Mosaic trisomy 21. Two different mechanisms were described for the formation of mosaicism: one is a mitotic error in a normal, euploid zygote resulting in a mosaic embryo having 46/47,+21 karyotype, the 45,-21 cell line being nonviable, and the other one is a nondisjunction in parental gametogenesis followed by an early postzygotic malsegregation of chromosome 21 (“trisomy rescue”). A significant proportion of the mosaic parents had been conceived as trisomics (25, 26).

Robertsonian translocation trisomy 21

Always a karyotype analysis of both parents is recommended if a case with DS is due to a translocation. Robertsonian translocations carry reproductive risks that are dependant on the chromosomes involved and the sex of the carrier from the family. If neither parent carries a Robertsonian translocation, the DS recurrence risk is low, similar to that of free trisomy 21. Advanced maternal age has been established to be a risk factor associated with DS (27).

First trimester screening in pregnancy by a combination of fetal echography (nuchal translucency) and biochemical serum maternal prenatal screening (free- β -human chorionic gonadotrophin and pregnancy-associated plasma protein-A) can identify about 90% of fetuses with trisomy 21 and other major aneuploidies for a false-positive rate of 5% (28).

Assessment of fetal nuchal translucency (NT) combined with screening of congenital heart diseases may predict many major cardiac defects in the first trimester (29).

Congenital heart diseases and Down syndrome

General information

The first report about an association between DS and heart malformation was in 1894 (30) and first correlation between atrioventricular septal defects (AVSD) and DS has been suggested almost 25 years later (31).

About half of patients with DS have CHD (32, 33), one of the major causes of morbidity and mortality (34), and the spectrum of CHD pattern varies widely, encompassing any structural abnormality in the heart and great vessels. Atrioventricular septal defects are the most common defects found. About half of the AVSDs occur in patients with DS (35). Although trisomy 21 is a risk factor for CHD, it is not a sufficient requirement (about 40–60% of people with trisomy 21 do not have CHD), so it is important to identify susceptibility genes.

Embryology

Atrioventricular septal defects represent a spectrum of cardiac malformations including three subtypes: incomplete AVSD, transitional AVSD and complete AVSD (36, 37). Incomplete AVSDs are characterized by the presence of distinct mitral and tricuspid annuli, or left and right

valvar orifices. In transitional AVSDs, fusion of the anterior and posterior bridging leaflets results in a single valvar annulus. Complete AVSDs are characterized by the presence of a single, common AV valve orifice (36, 37). Complete AVSDs may also be classified according to the Rastelli classification system, which is based on the morphology, degree of bridging and chordal attachments of the superior leaflet (38).

These defects arise from abnormal development of the endocardial cushions, giving rise to partial, intermediate or complete AVSD. Septal formation begins at the end of the fourth week of fetal life, when the atrioventricular endocardial cushions appear at the superior and inferior borders of the atrioventricular canal. In addition, the two lateral atrioventricular cushions appear on the right and left borders of the canal. It is a defect in the fusion of the superior and inferior cushions which results in a persistent atrioventricular canal and thus an AVSD (39).

Understanding of the genes responsible for distinct steps of cardiac morphogenesis is necessary could help to define better all these aspects of embryologica framework.

Epidemiology

There are important differences among geographical regions. In Western European countries and the USA, endocardial cushion defect (43%), which results in AVSD/AV canal defect, was the main cardiac abnormality, followed by ventricular septal defect (VSD) (32%), secundum atrial septal defect (10%), tetralogy of Fallot (6%) and isolated patent ductus arteriosus (PDA) (4%) (32, 40). In Asia, isolated VSD has been reported to be the most common cardiac defect (40%) (41, 42). One study from Korea showed that atrial septal defect was the most common defect accounting for 30.5% of DS, followed by ventricular septal defect (19.3%), patent duct arteriosus (17.5%) and atrioventricular septal defect (9.4%) (43). The secundum type of ASD was the most common cardiac lesion from Latin America (44, 45). In Lybia, the most common isolated cardiac lesion was the atrial septal defect (ASD), found in 23% patients (46).

Genetics

The occurrence of CHD or the type of defect has little correlation with the chromosome 21 abnormality itself. Three copies of chromosome 21 increase the risk for CHD, but triso-

my 21 itself is not sufficient to cause CHD. Additional genetic variation and/or environmental factors could contribute to the CHD risk (47). Candidate non-chromosome 21 genes have also been identified for susceptibility to several CHDs and AVSD in particular (not related to DS) (48). Atrioventricular septal defects and *CRELD1* gene have been associated in the context of DS, mutations in this gene contributing to the pathogenesis of AVSD (49). Atrioventricular septal defects (AVSDs) occur as clinical defects of several different syndromes, autosomal dominant defects and sporadically occurring malformations (50). Also, *GATA4* gene mutations have been found in families with cardiac malformations that included AVSD (51).

Another study among individuals with DS and complete AVSD identified potentially damaging variants in six genes: *COL6A1*, *COL6A2*, *CRELD1* (already known), *FBLN*, *FRZB*, *GATA5* involved in VGFA pathway (52).

The next developmental studies and the new technologies will identify the exact mode of action by establishing the link between the genome variability and the phenotypic variability. □

Acknowledgments: The author would like to thank the Genetics Department team from Alessandrescu-Rusescu INSMC Bucharest for their ongoing support as cytogenetics laboratory assistance and for provision of karyogram images.

Conflicts of interest: none declared.

REFERENCES

1. **Down JLH.** Observations on an ethnic classification of idiots. *London Hosp Clin Lect Rep* 1866;3:259.
2. **Lejeune J, Gautier M, Turpin R.** Etude des chromosomes somatiques de neuf enfants mongoliens. *C R Acad Sci* 1959;248:1721-1722.
3. **Levenson D.** Talking about Down syndrome. *Am J Med Genet A* 2009;149A:vii-viii.
4. **Hook EG.** Epidemiology of Down syndrome. In: Pueschel SM, Rynders JE (eds), *Down Syndrome. Advances in Biomedicine and the Behavioral Sciences*, Cambridge: Ware Press (pub.) 1982; p. 11.
5. **Hattori M.** The DNA sequence of human chromosome 21. *Nature* 2000;405:311-319.
6. **Cheon MS, Shim KS, Kim SH, et al.** Protein levels of genes encoded on chromosome 21 in fetal Down syndrome brain: Challenging the gene dosage effect hypothesis (Part IV). *Amino Acids* 2003;25:41-47.
7. **Huiqing Li, Sheila Cherry, Donna Klinedinst, et al.** Genetic Modifiers Predisposing to Congenital Heart Disease in the Sensitized Down Syndrome Population. *Circ Cardiovasc Genet* 2012;5:301-308.
8. **Antonarakis SE.** Human chromosome 21: genome mapping and exploration circa 1993. *Trends Genet* 1993;9:142-148.
9. **Thuline HC, Poeschel SM.** Cytogenetics in Down syndrome. In: Poeschel SM, Rynders JE, *Down Syndrome. Advances in Biomedicine and the Behavioral Sciences*, Cambridge: Ware Press (pub.), 1982, p. 133.
10. **Asim A, Kumar A, Muthuswamy S, et al.** Down syndrome: an insight of the disease. *Journal of Biomedical Science* 2015;22:41.
11. **Mikkelsen M.** Down's syndrome cytogenetic epidemiology. *Hereditas* 1977;86:45-59.
12. **Gardner RJM, Sutherland RG, Shaffer GL.** Oxford Monographs on Medical Genetics, *Chromosome abnormalities and Genetic counseling*, fourth edition, Oxford University Press 2012, p. 281.
13. **Petersen MB, Mikkelsen M.** Nondisjunction in trisomy 21: origin and mechanisms, *Cytogenet Cell Genet* 2000;91:199-203.
14. **Yoon PW, Freeman SB, Sherman SL, et al.** Advanced maternal age and the risk of Down syndrome characterized by the meiotic stage of chromosomal error: a population-based study. *Am J Hum Genet* 1996;58:628-633.
15. **Savage AR, Petersen MB, Pettay D.** Elucidating the mechanisms of paternal non-disjunction of chromosome 21 in humans. *Hum Mol Genet* 1998;1221-1227.
16. **English C, Hammersley AL, Jackson A, et al.** Recurrent trisomy 21 resulting from gonadal mosaicism. *J Med Genet* 2001;38:S48.
17. **Petersen MB, Adelsberger PA, Schinzel AA, et al.** Down syndrome due to de novo Robertsonian translocation t(14q;21q): DNA polymorphism analysis suggest that the origin of the extra 21q is maternal. *Am J Hum Genet*, 1991;49:529-536.
18. **Pangalos C, Theophile D, Sinet PM.** No significant effect of monosomy for distal 21q22.3 on the Down syndrome phenotype in „mirror“ duplications of chromosome 21. *Am J Hum Genet* 1992;51:1240-1250.
19. **Zaki MS, Kamel AA, El-Ruby M.** Double aneuploidy in three Egyptian patients: Down-Turner and Down-Klinefelter syndromes. *Genet Couns* 2005;16:393-402.
20. **Hou JW, Wang TR.** Double aneuploidy with Down's-Klinefelter's syndrome. *J Formos Med Assoc* 1996;95:350-352.
21. **Rahmani Z, Blouin JI, Creau-Goldberg N, et al.** Critical role of the D21S55 region on chromosome 21 in the pathogenesis of Down syndrome. *Proc Natl Acad Sci USA* 1989;86:5958-5962.
22. **Korenberg JR, Chen XN, Schipper R, et al.** Down syndrome phenotypes: the consequences of chromosomal imbalance. *Proc Natl Acad Sci USA* 1994;91:4997-5001.
23. **Barlow GM, Chen XN, Shi ZY, et al.** Down syndrome congenital heart disease: a narrowed region and a candidate gene. *Genet Med* 2001;3:91-101.
24. **Lyle R, Bena F, Gagos S, et al.** Genotype-phenotype correlations in Down syndrome identified by array CGH in 30 cases of partial trisomy and partial monosomy chromosome 21. *European Journal of Human Genetics* 2009;17:454-466.
25. **Kovaleva NV.** Problems of chromosome 21 mosaicism. A review. *Tsitologiya* 2003;45:434-439.
26. **Parke JC Jr, Grass FS, Pixley R, et al.** Trisomy 21 mosaicism in two successive generations in a family. *J Med Genet* 1980;17:48-49.
27. **Hassold T, Chiu D.** Maternal age-specific rates of numerical chromosome abnormalities with special reference to trisomy. *Hum Genet* 1985;70:11-17.
28. **Nicolaidis KH.** Screening for fetal aneuploidies at 11 to 13 weeks. *Prenat Diagn* 2011;31:7-15.
29. **Mogra R, Alabbad N, Hyett J.** Increased nuchal translucency and congenital heart disease. *Early Hum Dev* 2012;88:261-267.
30. **Garrod AE.** On the association of cardiac malformations with other congenital defects. *St Bartholemew Hosp Rep (London)* 1894;30:53.

31. **Abbott ME.** New accessions in cardiac anomalies: pulmonary atresia of inflammatory origin; persistent ostium primum with Mongolian idiocy. *Int A M Mus Bull* 1924;10:111.
32. **Freeman SB, Taft LF, Dooley KJ, et al.** Population-based study of congenital heart defects in Down syndrome. *Am J Med Genet* 1998;80:213-217.
33. **Urbano R.** *Health issues among person with Down syndrome.* Academic Press 2012:169.
34. **Marder LTR, Pascall E.** Cardiac problems in Down syndrome. *Pediatr Childhealth* 2015;25:23-29.
35. **Calkoen EE, Hazekamp MG, Blom NA, et al.** Atrioventricular septal defect: From embryonic development to long-term follow-up. *Int J Cardiol* 2016;202:784-795.
36. **Jacobs JP, Burke RP, Quintessenza JA, et al.** Congenital Heart Surgery Nomenclature and Database Project: atrioventricular canal defect. *Ann Thorac Surg* 2000;69:36-43.
37. **Briggs LE, Kakarla J, Wessels A.** The Pathogenesis of Atrial and Atrioventricular Septal Defects with Special Emphasis on the Role of the Dorsal Mesenchymal Protrusion. *Differentiation* 2012;84:117-130.
38. **Rastelli G, Kirklin JW, Titus JL.** Anatomic observations on complete form of persistent common atrioventricular canal with special reference to atrioventricular valves. *Mayo Clin Proc* 1966;41:296-308.
39. **Anderson RH, Ho SY, Falcao S, et al.** The diagnostic features of atrioventricular septal defect with common atrioventricular junction. *Cardiol Young* 1998;8:33-49.
40. **Vis JC, Duffels MG, Winter MM, et al.** Down syndrome: a cardiovascular perspective. *J Intellect Disabil Res* 2009;53:419-425.
41. **Jacobs EG, Leung MP, Karlberg J.** Distribution of symptomatic congenital heart disease in Hong Kong. *Pediatr Cardiol* 2000;21:148-157.
42. **Lo NS, Leung PM, Lau KC, et al.** Congenital cardiovascular malformations in Chinese children with Down's syndrome. *Chin Med J (Engl)* 1989;102:382-386.
43. **Min-A Kin, You Sun Lee, Nan Hee Yee, et al.** Prevalence of Congenital Heart Defects Associated with Down Syndrome in Korea. *J Korean Med Sci* 2014;29:1544-1549.
44. **De Rubens Figueroa J, del Pozzo Magana B, Pablos Hach JL, et al.** Heart malformations in children with Down syndrome. *Espanola Cardiol* 2003;56:894-899.
45. **Vida VL, Barnoya J, Larrazabal LA, et al.** Congenital cardiac disease in children with Down's syndrome in Guatemala. *Cardiol Young*, 2005;15:286-290.
46. **Z Elmagrpy, A Rayani, A Shah, et al.** Down syndrome and congenital heart disease: why the regional difference as observed in the Libyan experience? *Cardiovasc J Afr* 2011;22:306-309.
47. **Sailani MR, Makrythanasis P, Valsesia A, et al.** The complex SNP and CNV genetic architecture of the increased risk of congenital heart defects in Down syndrome. *Genome Res* 2013;23:1410-1421.
48. **Robinson SW, Morris CD, Goldmuntz E, et al.** Missense mutations in CRELD1 are associated with cardiac atrioventricular septal defects. *Am J Hum Genet* 2003;72:1047-1052.
49. **Maslen CL, Babcock D, Robinson SW, et al.** CRELD1 mutations contribute to the occurrence of cardiac atrioventricular septal defects in Down syndrome. *Am J Med Genet A* 2006;140:2501-2505.
50. **Guo Y, Shen J, Yuan L, et al.** Novel CRELD1 gene mutations in patients with atrioventricular septal defect. *World J Pediatr* 2010;6:348-352.
51. **Garg V, Kathiriya IS, Barnes R, et al.** GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5. *Nature* 2003;424:443-447.
52. **Ackerman C, Locke AE, Feingold E, et al.** An excess of deleterious variants in VEGF-A pathway genes in Down-syndrome-associated atrioventricular septal defects. *Am J Hum Genet* 2012;91:646-659.