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REVIEW ARTICLE

An insight into genetics of congenital heart defects associated with Down syndrome

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ABSTRACT:

Down syndrome is the most frequent live born human aneuploidy. Down syndrome is characterized by a set of phenotypically identified dysmorphism and various congenital malformations of which congenital heart defects is seen in about 50% of all cases. Atrio-ventricular Septal Defect is the most common form of congenital heart defect observed in Down syndrome. However, the exact molecular cause underlying the incidence of congenital heart defects and its differential phenotypic expression in DS are still not perfectly understood. In this review we have brought together findings from different studies and discussed multiple perspectives of the genetics of congenital cardiac defects in trisomy 21 background, viz., the contributions of triplicated dosage of chromosome 21 genes along with the effect of mutations in non-chromosome 21 disomic genes. Further, the roles of copy number variation and microRNAs have been discussed. We have also tried to shed light on the role of folate metabolism gene polymorphisms on congenital cardiac anomalies observed in Down syndrome. Lastly, we summarized the role of genes from different signaling pathways involved in cardiogenesis and also the recent developments in understanding of Down syndrome associated congenital heart defect from the studies on mice models. This review provides an overview regarding current state-of-art of knowledge related to Down syndrome associated congenital heart defects based on what have been discovered so far in this domain and hints at what prospect of research lies in this field in the future.

Introduction

Down syndrome (DS), caused by three copies of human chromosome 21 (Hsa21), is the most common live born aneuploidy in human (birth rate approximately 1 in 700/800)[1]. Individuals with DS suffer from various clinical conditions like Alzheimer's disease (AD), congenital heart defects (CHD), cancer, speech, learning and memory defects, Hirschsprung's disease, leukemia etc. Considering its impact on life and variations in phenotypic manifestation among the individuals with DS, congenital heart defects draws attention of clinicians and researchers[2] for elucidating its etiology. About 40-60% infants with DS are born with some sort of CHD that include 45% atrioventricular septal defects (AVSD), 35% ventricular septal defects (VSD), 8% isolated secundum atrial septal defects (ASD), 7% isolated persistent patent ductus arteriosus (PDA), 4% tetralogy of Fallot (TOF) and 1% other varieties[3–6]. The incidence of CHD is not universal among all individuals with DS and it occurs among euploid individuals without any syndromic features.

Though DS presents a unique model for understanding the etiology of CHD, dosage alterations of Hsa21 genes due to its triplication may not provide all answers regarding its cause, as many individuals with DS do not exhibit CHD phenotype. Studies have explored the genome of individuals with DS having CHD but haven't fully been able to elucidate the underlying genetic basis of the same. We aim to provide a comprehensive summary of current state of understanding of etiology of congenital heart defects in DS and to shed light on future research prospect in this subject.

CHD phenotypes associated with DS are considered as one of the complicated congenital defects and can be attributed to dosage imbalance of Hsa 21 genes, owing to their triplicated condition and their cross talking with the disomic genes located on the other chromosomes. Additionally, allelic variations of Hsa21 specific and other disomic genes complicate the phenotype[7–10]. One of the pioneering studies, conducted by Korenberg (1992), suggests a 4-5 Mb region on Hsa21 might be responsible for congenital heart defect phenotype in DS. This segment of chromosome termed as DS-CHD Critical Region (DSCR) and was identified to be located at the 21q22.2 cytogenetic locus[11]. Further studies have narrowed down this candidate region to 0.96 Mb long stretch which is suggested to interact with highly restricted DSCR (HR-DSCR)[12,13].

Phenotypically, CHD manifestation in DS represents alterations or disruptions in the formation of an extracardiac tissue, the dorsal mesenchymal protrusion (DMP)[14,15]. The dorsal mesenchymal protrusion is a cluster of mesenchymal cells of extracardiac origin and they arise from the posterior portion of the second heart field in the splanchnic mesoderm. Together with the mesenchymal cap of primary atrial septum, the dorsal mesenchymal protrusion fuses with the major atrioventricular cushions in due course of embryonic development and thus, closes the primary atrial foramen and forms the atrioventricular mesenchymal complex[16–18].

Effect of chromosome 21 gene dosage alteration on congenital heart defects in Down syndrome

Multiple triplicated genes on Hsa21 have been identified as potent troublemakers for increased risk of CHD in DS, and they are DSCAM, KCNJ6, RCAN1, COL6A1 and COL6A2[19–21] (Table 1). These genes are involved in embryonic cardio-genesis. Pathogenic mutations in these genes coupled with the 'gene dosage effect' might contribute significantly to the CHD pathogenesis in the trisomy 21 genetic backdrop. Polymorphisms in COL6A1[22] and COL6A2 gene cluster have been identified to be associated with CHD in DS[23]. DSCAM, a gene within the DSCR belongs to the immunoglobulin superfamily of cell adhesion molecules and takes part in the neural development[24]. It is reported to express in the human fetal heart tissues at 12 weeks of development and its overexpression possibly contribute to the abnormal atrioventricular septum development in trisomy 21 backdrop[25]. The genes DSCAM and COL6A1 have been suggested to have a co-operative effect on congenital cardiac defects[20]. Another Hsa21 gene DSCR1 or RCAN1 has been predicted to significantly contribute in the incidence of congenital heart defect in DS[26]. Overexpression of DSCR1 leads to calcineurin mediated dephosphorylation of NFAT and NFATc1 nuclear translocation in cardiac cells, affecting cardiac valve and septum development in trisomy 21 background, causing congenital cardiac defects[27–30]. Another gene on Hsa21 called KCNJ6 codes for the Kir3.2/GIRK2 subunits of G protein regulated K^+ (K_G) channels and in DS background overexpression of Kir3.2 protein alters cardiac arrhythmogenicity and overall regulation[31]. The gene RUNX1 codes for a transcription factor and is expressed in the developing cardiac mesenchymal tissue. Triplication of this gene and its over-expression affect functioning of extra cellular matrix related genes in trisomic cells and reduces the migrating activity of the trisomic fibroblasts[32,33].

Effect of non-chromosome 21 gene mutations on congenital heart defects in Down syndrome

A chromosome 1 gene, ALK2, codes for type I BMP receptor and plays a critical nonredundant role in the early development of the atrio-ventricular (AV) septum. Mice model based studies have reported that *Alk2* mutant mice carry defects in AV septum and atrioventricular valves[34]. Further study has reported p.His286Asp variant in ALK2 gene to be contributory to CHD in DS, and suggests the DS associated congenital heart defect to be moderated through ALK2-mediated reduction of BMP signaling[35]. Rare mutations of CRELD family genes that are not located on Hsa21 (Table 1), namely CRELD1 and CRELD2 have been implicated in the incidence of CHD in DS. CRELD1 codes a cell surface protein that is essential for endocardial cushion development[36]. Variations in CRELD1 were associated with congenital heart defects in

Down syndrome and euploid patients[36–39]. The gene CRELD2 codes for a protein that shows structural and functional similarity to CRELD1 and also shows spatio-temporal overlap in expression pattern in embryonic development[39,40].

Genome wide association study conducted on individuals with DS having CHD has suggested elevated risk of CHD in DS infants is not caused by a few common variants with large effect. However, the same study reports four non Hsa21 autosomal regions (1p36.3; 5p15.31; 8q22.3; 17q22.2), that gave comparatively stronger signals besides the well-established Hsa21 candidates[41]. A next generation sequencing based study has reported pathogenic variants in GUSB, GATA3, FLNA, KCNH2 and ENG genes as underlying risk factors for CHD in DS[42] (Table 1).

Contribution of copy number variation (CNVs) on congenital heart defects in Down syndrome

Copy number variation (CNV) refers to variability in number of the copies of a particular gene or its segment (nucleotides) present in the genome of an individual. CNV includes insertion, deletions or duplications of part or whole of a gene. It accounts for a significant proportion of genetic variations between individuals. Studies have revealed a 1.8-kb copy number variation within ZBTB21 gene and another 4.9-kb upstream region of the RIPK4 gene on Hsa21 along with a couple of interacting *cis*-eQTLs from NRGN on chromosome 11 and CNOT11 on chromosome 2 to be risk loci for CHD in DS[43]. Another study has reported strong evidence that large, rare deletions increase the risk of DS-associated AVSD[44]. Further, next generation sequencing based study reported 17 *de novo* copy number variations in seven candidate genes in non-syndromic fetuses with atrioventricular septal defects and absent in healthy fetuses. These genes include NR2F2, SMC1A, NOTCH2, EHMT1, SHANK3, TBX1 and NIPBL[45]. It has been hypothesized that gene dosage effects might contribute through copy number variations on Hsa21[43]. Studies suggest that the differential phenotypic manifestation of CHD in DS from different ethnicities might stem from the population specific CNV or single nucleotide polymorphisms (SNP)[46,47] of genes related to embryonic cardiogenesis.

Effect of Pathway participants on congenital heart defects in Down syndrome

Various signaling pathways are involved in embryonic cardiogenesis and sub-optimal expression of candidate genes from these pathways increase the risk of CHD, with more vulnerability for trisomy 21 genetic background of the fetus. The mitogen VEGF-A is a well-recognized contributor in the morphogenesis of atrioventricular septum and atrioventricular valves. Rare missense variants in a total of six genes involved in VEGF-A pathway namely, COL6A1, COL6A2, CRELD1, FBLN2, FRZB and GATA5 were associated with congenital heart defects in Down syndrome[9] (Table 1). Whole genome sequencing studies investigated rare variants in Notch signaling pathway and ciliome genes, suggesting a link between these two pathways and CHD in DS[48]. Further a significant role of cilia genes and their

mutations in the pathogenesis of CHD in non-DS genetic backdrop has been elucidated in a number of studies[49–53]. The evolutionarily conserved cilia-dependent Sonic hedgehog (Shh) signaling plays an important role in dorsal mesenchymal protrusion development. Altered Shh signaling in mice, affects proliferation of dorsal mesenchymal protrusion in mice and leads to atrioventricular septal defects[17,49,54,55]. However, the contribution of Shh-signaling to CHD in DS remained elusive. The gene expression profile and pathway data of ten DS and five control samples analysed by the individualized pathway aberrance score (iPAS) method which predicted the cross-presentation of particulate exogenous antigens (phagosomes) and the methionine salvage pathway as good indicators of CHD in DS[56]. Further, CRELD1 is also reported to be associated with regulation of calcineurin mediated NFATc1 signalling crucial for cardiac development[56]. The precise functional mechanism is still not clearly understood.

Effect of miRNAs on congenital heart defects in Down syndrome

MicroRNAs or miRNAs are about 20 nucleotide long and are mostly associated with downregulation of gene expressions. It does so by binding to the 3' UTRs of target mRNAs, therefore inducing mRNA degradation or translation inhibition. Five DS-associated miRNAs (miR-99a, miR-125b-2, miR-155, miR-802 and let-7c), located on Hsa21 have been reported to be overexpressed in cardiac tissues of DS individuals. The overexpressing miR-99a/let-7c cluster might contribute to CHD in DS. Further studies speculate AUTS2 and KIAA2022 to contribute to CHD in DS through their interactions with miR-518a, miR-518e, miR-518f, miR-528a and miR-96[57–59].

The Folate saga: Effect of folate levels and folate metabolism genes on congenital heart defects in Down syndrome

Folate or vitamin B6 is obtained from green leafy vegetables and/or dietary supplements consumed. The folate metabolism pathway genes and their genetic variants have been well explored for their possible role in CHD in DS. Folate is extremely essential for DNA methylation. It acts as a methyl donor in purine and pyrimidine biosynthesis, enabling the conversion of homocysteine to methionine. Optimum intake of folate at a dietary level and uptake of the same at a cellular level are essential for these processes. Inadequate folate uptake alters the one-carbon metabolism leading to hyperhomocystenemia or DNA hypomethylation, increasing the risk of various developmental disorders[60]. A case-control study conducted on Hispanic and non-Hispanic populations reported significant association of the lack of maternal folic acid supplementation with cardiac septal defects among newborns with DS. This was also associated with altered methylation levels of DNA extracted from blood and cardiac tissues from DS fetuses[61]. Folate uptake in small intestine is mediated through a transmembrane transporter called reduced folate carrier (RFC1 or SLC19A1). Multiple studies have reported the SNP A80G (rs1051266) to be responsible for reduced folate uptake and to be associated with the

risk of CHD in DS and non-DS genetic backdrop from different populations [62–66]. The principal folate metabolism enzymes are methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR) and cystathionine β-synthase (CBS). Case-control studies conducted on some populations reported the SNPs C677T and A1298C of MTHFR gene contribute to the increased maternal risk of giving birth to DS children with CHD while other population based studies negated the association[66–72]. The promoter region of the MTHFR gene has been reported to be hypermethylated in DS individuals with CHD[73]. The MTRR C524T (rs1532268) and A66G (rs1801394) polymorphisms along with two novel polymorphisms T19775C and 19778_19778delG, have been reported to be associated with increased risk of CHD in DS from a population sample from south India[74].

Table 1: List of the genes studied in relation to the occurrence of congenital heart defects in Down syndrome

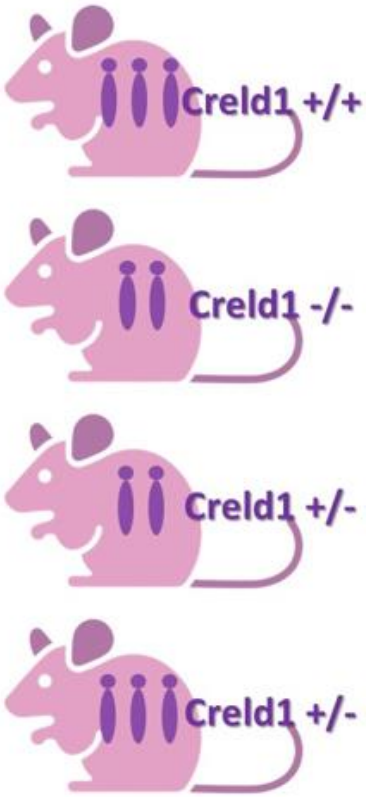
Gene	NCBI ID	Cytogenetic Location	Function of Gene	Reference
DSCAM	1826	21q22.2	Role in neural development	20,25
KCNJ6	3763	21q22.13	Codes for G protein regulated K+ channels	31
RCAN1	1827	21q22.12	Role in AV septum development through NFAT pathway	27–30
COL6A1	1291	21q22.3	Role in fetal heart development and AV septum development	9,22,23
COL6A2	1292	21q22.3		9,23
RUNX1	861	21q22.12	Encodes transcription factor expressed in cardiac development	32,33
SLC19A1	6573	21q22.3	Encodes for Folate transporter protein	62,63,66
ALK2	90	2q24.1	Codes for Type I BMP receptor having role in AV cushion development	34,35
CRELD1	78987	3p25.3	Overlapping roles in AV cushion development	9,36–39,75
CRELD2	79174	22q13.33		9,39,40,75

Gene	NCBI ID	Cytogenetic Location	Function of Gene	Reference
GUSB	2990	7q11.21	Encodes hydrolase enzyme that degrades glycosaminoglycans	42
GATA3	2625	10p14	Codes for a transcription factor involved in early development	
FLNA	2316	Xq28	Involved in cytoskeletal rearrangement in cell migration	
KCNH2	3757	7q36.1	Codes a component of voltage-gated K+ channels in cardiac development	
ENG	2022	9q34.11	Codes for a transmembrane glycoprotein	
FBLN2	2199	3p25.1	Involved in VEGF-A pathway and have role in AV septum and valve development	9
FRZB	2487	2q32.1		
GATA5	140628	20q13.33		
MTHFR	4524	1p36.22	Codes for an enzyme that converts folate into its active circulating form	63,69,70,73
MTRR	4552	5p15.31	Codes for an enzyme essential for methionine synthesis	66,71,74
MTR	4548	1q43	Codes for an enzyme essential for methionine synthesis	66

DS Mouse model in congenital heart defect study

One study showed the development of congenital heart defects when null allele of *Hey2* were introduced in Ts65Dn mice[75] while another study showed the same when null allele of *Creld1* introduced in Ts65Dn mice[76] (explained in Figure 1). Genetic analysis of congenital heart defects in Down syndrome in mouse mutants carrying *Dp(16Tiam1-Kcnj6)Yey* and *Df(16Tiam1-Kcnj6)Yey* have led to discovery of a narrowed down *Tiam1-Kcnj6* genomic region as a potent candidate region[77].

Figure 1: Creld1+/- on a trisomy 21 background (Ts65Dn) increases risk of CHD



% with CHD observed	Type of CHD observed
5%	100% membranous VSD observed
Embryonic lethal	Endocardial cushion is formed, but hypocellular; few mesenchymal cells observed
0%	N/A
33%	50% membranous VSD 50% secundum ASD observed

Figure legend: The mouse model for trisomy 21 (represented by the 3 chromosomes), Ts65Dn, shows a low level of heart defects. The model with only 2 sets of Hsa21 genes, but with the CRELD1 gene completely knocked out (-/-) is an embryonic lethal, showing the importante role of CRELD1. The model with only 2 sets of Hsa21 genes, and with CRELD1 present on only one gene, but not the other; +/-, shows no heart defects, suggesting that lower levels of CRELD1 are sufficient for normal heart development. However, for the trisomic mouse model with CRELD1 present on only one gene, but not the other; +/-, there is a significant increase in those with septal defects. This shows that CRELD1 is important in heart development and that lowering the levels of CRELD1 on a trisomic background exacerbates the severity of the abnormal heart development. VSD: Ventricular Septal Defects; ASD: Atrial Septal Defects.

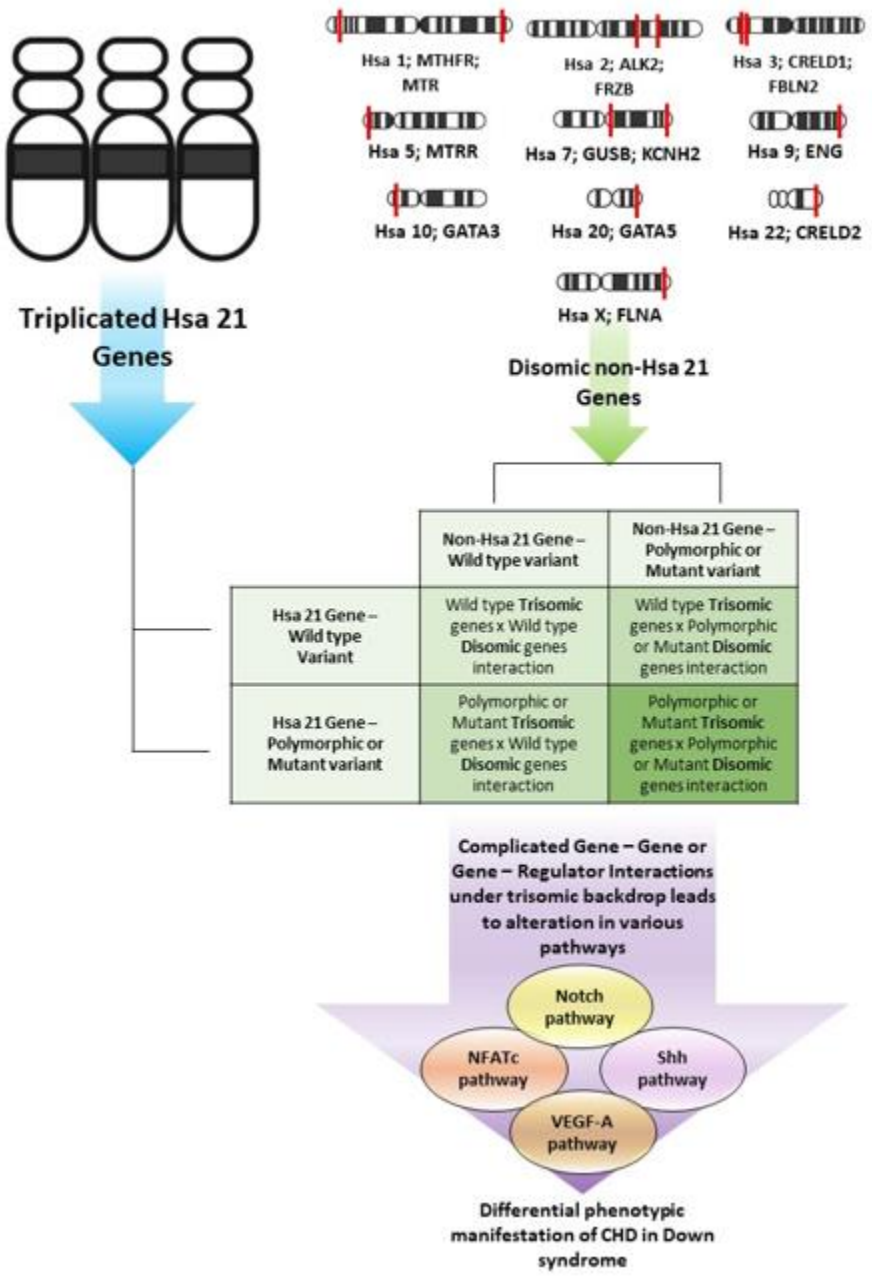
Conclusion

The cause of congenital heart defects is multifactorial and intriguing. The phenotypic manifestation of congenital heart defects among individuals with DS varies to great extent. The incidence of CHD or atrioventricular septal defects in DS is 2000-fold elevated than in euploid population. This review provides a brief description of the different genetic analyses conducted so far on

clinical samples as well as on preclinical DS mouse models to explore the molecular mechanisms associated with the frequent incidence of congenital heart defects in among the individuals with DS.

Among the individuals with DS, atrioventricular septal defects are the most frequent congenital heart defects observed. As far as published literatures are concerned several factors contribute into the etiology of CHD in DS. First, altered dose of Hsa21 specific genes is the primary cause. Second, these triplicated gene doses affect the expression of genes (or modifier loci) from other parts of genome in trans, i.e., on chromosomes other than Hsa21, through altered level of cross-talking. Third, both sets of genes, from Hsa21 and other chromosomes carry allelic variations that contribute in complication of genetics of congenital heart defects in DS. Figure 2 summarizes the hypothesis that describes possible level and degree of interactions between sets of genes and their alleles from Hsa21 and non-Hsa21 that lead to spectrum of different CHD among the individual with DS. In addition, ethnic differences across the population divides contribute another degree of complexity as confounding factor for CHD manifestation in DS. This review summarizes the possible contributions of all these genes in DS associated CHD. Further, we collated the findings of genome wide association studies and outcome of CNVs analyses conducted on different ethnic populations. The role of different signaling pathways and implication of micro-RNA in cardiac development and congenital heart defects in trisomy 21 backdrop has also been summarized.

Figure 2: Hypothetical Interaction among genes involved in congenital heart defects in Down syndrome



Future possible research paths warrant a large-scale whole genome sequencing (WGS) study for discovering novel candidate genes and allelic alterations that might provide a better explanation of the genetic basis of congenital heart defects in DS individuals. Additionally, next generation sequencing approach is needed to characterize novel mutations and deep seated CNVs that may contribute into its complicated pathology.

Specific coding and non-coding regions of genome from chromosomes other than Hsa21 are needed to be explored in this regard. Animal model-based studies are also required to understand the probable mechanisms that bring together, at a translational level, the pathological conditions of CHD in trisomy 21 background. An approach through induced pluripotent stem cell (iPSC)-based studies might help in designing therapeutic strategies to combat DS associated congenital heart defects. Further, epigenetic status of regulatory regions of already identified loci through genome wide association studies is of scientific interest. More studies are warrant to characterize the epigenetic alteration of genome associated with congenital heart defects in DS. Characterization of genetic, epigenetic and epidemiological risk factors will bring us to a significant step closer towards understanding the exact etiology of CHD in DS in near future

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Conflicts of Interest statement
The authors have no conflicts of interest to declare.

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