Perspective

Congenital heart defects in Down's Syndrome: *Identification of a unique molecular signature*

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Abstract: Congenital heart defects (CHD) are seen frequently in about 50% of patients with Down's syndrome (DS) and are the leading cause of death of DS in the early years of life. Recently, Lana-Elola et al. [1], in their seminal research article, showed that heart tissue from human fetuses with DS has characteristic transcriptional changes, as that of the embryonic hearts from the Dp1Tyb and Dp3Tyb mice models of DS that show CHD. They demonstrated that one of the causative genes for CHD in DS is *Dyrk1a*. They showed that an increased dosage of *Dyrk1a* results in impaired cell proliferation and mitochondrial respiration of cardiomyocytes and is necessary to cause CHD in DS. They reversed the CHD phenotype in Dp1Tyb mouse by reducing the copy number of *Dyrk1a* to two or inhibiting this gene expression.

Keywords: Congenital heart defects, Down's syndrome, mouse model, human heart, *Dyrk1a* gene, Notch signaling

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1. Introduction

In 1866, John Langdon Down described a slightly flattened facial profile, an upward slant to the eyes, low muscle tone, mental retardation and a single deep crease across the palm as the characteristic features of Down's syndrome (DS) [2]. DS is a common genetic condition and the most frequent cause of genetic mental retardation occurring in one out of every 700 newborns across all racial and economic groups [2]. Congenital heart defects (CHD) are seen frequently in about 50% of patients with DS and are the leading cause of death of DS in the early years of life [3-4]. Even 65 years after the discovery of Trisomy-21 in DS, chronic health issues and medical care of DS patients remain limited, resulting in premature deaths or mortality. With the advent of genomics technology, a wealth of new individualized information and diagnostics are possible, facilitating personalized treatments and predicting future risk factors for each individual. A seminal study by Lana-Elola et al., [1] identified the over-expression of the Dyrk1a gene in mice models of DS, which is responsible for the manifestation of CHD in DS. Here, their investigations and implications of their findings for possible treatments for DS are outlined.

1.1. Discovery of Trisomy-21 and risk factors for DS

After elucidating human chromosome number 2n=46 [5], Lejeune et al., [6] discovered the presence of an extra chromosome 21 in DS subjects through karyotypic analysis. Karyotypic analysis became the routine prenatal diagnostic test to confirm trisomy-21 in suspected DS fetuses. Subsequent studies found that chromosomes 21q21 to 21q22.3 form the critical DS region that causes most DS phenotypes [7]. The additional copy of chromosome 21 was seen in the abnormal eggs of mothers over 35 years of age [8], although the mother's lifestyle, environment, and occupational exposures may surpass maternal age as risk factors for chromosome nondisjunction leading to trisomy-21 [9].

Further karyotypic and fluorescence *in situ* hybridization analyses revealed that DS can be seen chromosomally as free trisomy (92-95%), translocation trisomy-21 (3-4%) and mosaic trisomy-21 (2-4%) [10]. A well-established precursor for trisomy-21 is meiotic nondisjunction, leading to abnormal segregation of chromosomes during gametogenesis in older mothers [11]. However, we have reported a contrasting trend where more DS children are born to younger mothers aged 18-29 years in India than to older mothers [12]. We found that younger mothers born to their mothers at the age of above 30 years produced more DS children. Therefore, besides the known risk factors, the maternal grandmother's age at the time of birth of the mother is a risk factor for DS [12].

1.2. Genes on chromosome 21

The Human Genome Project launched in October 1990 revealed that the average human genome is 3.2 billion bases per haploid set, encoding approximately 25,000 protein-coding genes. Further, chromosome 21 has about 329 genes with a size of 48 Mb [2]. This investigation opened the door for more significant advances in DS research. The over-expression of the genes in trisomy-21 is a challenge in understanding gene functions and interpreting biological characteristics. Gardiner et al., [13] reported that about 170 genes from chromosome 21 encode open reading frames conserved in orthologous regions of mouse chromosomes 16, 17 and 10. This has led to the development of mouse models to unravel the genotype-phenotype correlations in DS effectively.

2. Genes identified for DS-associated CHD

The development of the human heart is a complex process; any deviation can lead to CHD. In India and worldwide, the incidence of CHD exceeds 2% of all live-births globally [14-16]. CHD affects nearly 50% in babies with DS born with a partial or complete extra copy of chromosome 21 [4]. Over the years, Lana-Elola and colleagues have attempted to map the genetic determinants of CHD on chromosome 21 using human samples and mice models. They genetically engineered a mouse model, Dp1Tyb, with extra copies of 145 coding genes on mouse chromosome 16, corresponding to about 60% of the human chromosome 21, which exhibits a broad range of DS-like phenotypes. Lana-Elola et al., [1] further demonstrated that in embryonic DS mice models, three instead of the normal two copies of the dual-specificity tyrosine phosphorylation–regulated kinase 1A (Dyrk1a) gene caused the cardiac pathology as outlined below.

These investigators employed embryonic DS mice models and human fetal heart tissue with DS, RNA sequencing, high-resolution episcopic microscopy imaging and 3D modelling to correlate the dose-sensitive expression of Dyrk1a with CHD phenotype, as well as changes in mitochondrial function and cell proliferation in the Dp1Tyb mouse. RNA sequencing analysis in Dp1Tyb mouse embryonic hearts, human DS hearts and also hearts of Dp3Tyb mouse strain having an extra copy of 39 protein-coding genes contained within the large duplication showed similar rates of CHD, indicating that these 39 genes were sufficient to cause CHD, associated with decreased expression of oxidative phosphorylation genes, which are correlated with CHD.

The Ts1Rhr mouse embryos have an extra copy of a slightly shorter region containing just 31 genes and they do not show CHD. The decreased expression of three proliferation gene sets was seen in Dp3Tyb mouse hearts, but only two of these were also decreased in Ts1Rhr mouse hearts. These findings suggest that impaired oxidative phosphorylation and potentially cellular proliferation may contribute to the etiology of CHD. Single-cell RNA sequence analysis revealed similar gene expression changes across Dp1Tyb mouse

embryonic heart cell types. The transcriptomics of human DS fetal hearts and mouse embryonic hearts from the DS mouse models showed that reduced expression of mitochondrial respiration genes and cell proliferation genes were correlated with CHD pathology. Flow cytometric analysis of Dp1Tyb mouse hearts showed an increased proportion of cardiomyocytes and endocardial cells in the G1 phase and fewer cells in the S phase of cell division, indicating that embryonic cardiomyocytes have defective mitochondria with reduced basal and maximal respiration rates consistent with impaired mitochondrial function.

Genetic crosses between the Dp1Tyb mouse model and mice strains deficient in each of the 39 candidate genes were conducted using a systematic genetic mapping approach. They observed that reduced copy number of dual-specificity tyrosine phosphorylation–regulated kinase 1A (Dyrk1a) gene from three to two completely rescued CHD indicating that three copies of Dyrk1a are necessary to cause CHD in the Dp1Tyb mouse [1-4]. An increased dosage of Dyrk1a causes key transcriptional changes in Dp1Tyb embryonic hearts. Dyrk1a is also broadly expressed in many cardiac cell types, indicating its role in causing the observed changes in cellular pathways leading to CHD. The hearts from human DS, Dp1Tyb, and Dp3Tyb mice also showed decreased expression of proliferation genes partially correlated with CHD.

Further, the reduction of phosphorylated retinoblastoma protein in Dp1Tyb mouse embryonic hearts was reversed by reducing the copy number of Dyrk1a from three to two and less expression of E2F-regulated genes that are required for the G1 to S phase transition, indicating that three copies of Dyrk1a can lead to mitochondrial dysfunction in embryonic cardiomyocytes. This suggests that an increased dosage of DYRK1A protein resulted in impairment of mitochondrial function and CHD pathology in the Dp1Tyb mouse. Pharmacological inhibition of DYRK1A also resulted in partially rescuing the DYRK1A-dependent CHD in Dp1Tyb embryos. Taken together, the findings demonstrate that the three copies of the Dyrk1a gene contribute to CHD in the Dp1Tyb mouse. This study adds to the growing literature on the genes and gene networks responsible for causing CHD in humans, similar to the abnormalities in embryonic Notch 1 signaling, explaining bicuspid aortic valve, or aortic stenosis, or hypoplastic left heart syndrome [17] or other genetic causes of obstructive left-heart lesions [18]..

3. Limitations and Future Directions

Although extensive investigations have been done to explain the three copies of Dyrk1a cause CHD, this study has several limitations. Firstly, it is unclear how defects in mitochondrial function and proliferation in most cardiac cells can cause localized defects in septation rather than broader cardiomyopathy. Further study is needed to understand the developmental defects in Dp1Tyb mouse hearts that may lead to ventricular and atrioventricular septal defects. Secondly, even though an increased dosage of Dyrk1a is necessary, it may be insufficient to cause mitochondrial defects and CHD; therefore, other genes associated with CHD phenotypes in DS need to be explored. Thirdly, DYRK1A phosphorylates many proteins that regulate transcription, splicing, and apoptosis. Further studies are needed to determine whether any of these DYRK1A targets are involved in DYRK1A-induced CHD, and why the inhibitor Leucettinib-21 does not have a more substantial effect on transcriptional changes in Dp1Tyb embryos? Further studies are needed to explore the cardiac pathology resulting from a complex genetic interplay between Dyrk1a and other unknown causative genes, the association of Dyrk1a with other known DS features such as neurodevelopment deficits [19].

4. Conclusions

Despite 65 years having passed since the discovery of the origin of Trisomy-21, DS patients continue to suffer from the consequences of this chromosomal anomaly. The extra free copy of chromosome 21 creates a remarkable change in the genome of the DS subjects to produce distinct phenotypes, including 50% of them suffering from CHD. However, it is noteworthy that advanced technology in this millennium is the most exciting time for investigating human genetic diseases, including DS. Although an extra copy of Dyrk1a was associated with heart defects in mouse, its contribution to human heart defects is yet to be fully explored. The

systematic genetic mapping approach for dosage-sensitive genes can be used to identify causative genes and mechanisms responsible for the many other phenotypes of DS. Further, identifying all the causative genes and their inhibitors would help study the pathological mechanisms for CHD that can be potential therapeutic targets for treating DS-associated heart defects, which is essential for most DS clinical conditions. Further research into the genetic and molecular basis of Trisomy-21 promises to transform the lives of DS individuals and their communities.

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