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Abstract Topic:- Molecular effects of genetic variation

Abstract Title:- Functional analysis of novel BMPs variants in isolated congenital heart disease

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Aims:-Congenital heart disease (CHD) is an umbrella term includes a wide range of heart defects with prevalence of ~ 6-8 per 1000 live births. Advance techniques brought up identification of >100 genes responsible for CHD. Several transcription factors (IRX4, NKX2.5), signaling molecules (BMPs, TGFβ) & contractile proteins (MYH6) are critical regulators of cardiogenesis. During cardiogenesis, BMP signaling is known to have a critical role. During valve formation, BMP2, 4, 5, 7 & 10 express in atrioventricular cushion and outflow tract. Previous studies report genetic variations in BMP2, BMP4 & BMP7 associated with CHD in humans.

Methods:- 285 isolated CHD cases including 174 males & 111 females & 400 age-matched controls were screened. Protein coding exons of human BMP2, BMP4, BMP7 genes were PCR amplified & Sanger sequenced. Varcard tool was used to predicts disease-causing potential of the mutants. In silico modelling of 2D & 3D structures of muteins were performed to visualize conformational changes. Transactivation assays for downstream promoters and immunostaining & immunoblotting were performed. Colocalization of BMP7 with ER-tracker & SERCA2 and qPCR was performed with downstream target genes.

Results:- Five non-synonymous variants (S37A, K241X, H321L, E328K, S351C) were identified in BMP2. Similarly, 4 missense variants (R113G, E151V, T197I, R226Y) in BMP4 & 5 variations in BMP7 (D85V, R175W, A283T, M315I, N321S) were identified. All BMP2, BMP4 & BMP7 variants were predicted to be damaging and pathogenic. Considerable changes were observed in 2D & 3D structures of all muteins of the three genes. Transactivation assay of Tlx2, SBE4, Id1 & Id3 promoters with BMP2 muteins namely S37A & K241X, H321L, E328K & S351C showed significant reduction in response to S37A while K241X, H321L, E328K & S351C caused enhanced expression of these promoters. Similarly, BMP7 variants D85V & R175W (propeptide domain) caused reduced expression contrary to A283T, M315I, N321S led to significant upregulation aforestated promoters. The localization of BMP2 was observed both in cytoplasm & nucleus while BMP7 was completely cytoplasmic, more specifically in endoplasmic reticulum which was confirmed by colocalization of BMP7 with ER tracker and SERCA2. Further, BMP2 & BMP7 variants also caused overexpression of pSMAD1/5 in immunoblotting. Real time PCR showed increase in expression of Id1, Id3, Smad1, Smad4, Smad5, Nkx2.5, Gata4 & Irx4 genes due to BMP2 & BMP7 variants

Conclusions:- This study supports the important role played by BMP2, BMP4 & BMP7 in cardiogenesis. In silco & in vitro functional analysis of different identified variants strength the primary role of these variants in inducing CHD.

Keywords:- Congenital Heart Disease, BMP, variant, missense, muteins