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Dose dependent Pitx2 loss of function impairs Zfhx3, Wnt8a and calcium handling; novel links to atrial arrhythmogenesis

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Atrial fibrillation (AF) is the most common cause of arrhythmogenesis in humans yet the genetic cause of AF remains elusive. Recent genome-wide association studies (GWAS) have reported risk variants in four distinct genetic loci (4q25, 1q21, 16q22 and 16q13) which have been associated with AF. Among them, the most significant are 4q25 risk variants, which are located in the vicinity of the PITX2 gene. Given the key developmental role of Pitx2 during cardiogenesis and particularly its role in pulmonary vein deployment, it has been postulated that Pitx2 dysfunction might be the molecular link to AF. Experimental evidences in distinct laboratories, including ours, have demonstrated that Pitx2 loss of function predisposes to atrial arrhythmogenesis. However, the molecular mechanisms driven by Pitx2 in this context remain somehow elusive, proposing either embryonic or mature gene expression defects. In order to get further insights into the molecular mechanisms driven by Pitx2 and their putative relation with novel AF GWAS associated genes, we have generated a new Pitx2 conditional mouse line, by intercrossing Sox2Cre and Pitx2floxed mice. Epiblast deletion of Pitx2 leads to the generation of heterozygous and systemic null Pitx2 null mutants, respectively. As expected, embryonic mortality and cardiac defects were similarly observed in Sox2CrePitx2 null mice as those previously reported for Pitx2 knock-out mice. Molecular analyses of the left atrial appendage in heterozygous Sox2CrePitx2 mice (20-30% reduction in Pitx2 expression) and atrial-specific NppaCrePitx2 null mice (60-70% reduction in Pitx2 expression) demonstrate that AF GWAS associated genes such as Zfhx3, Kcnq1 and Wnt8a are severely impaired while other such as Cav1, Synpo2l or Prxn1 are not. Surprisingly, beta-adrenergic signaling is not altered in these models whereas multiple calcium handling genes such as Serca2a, calsequestrin, phospholamban are severely impaired in atrial-specific NppaCrePitx2 null mice but not in heterozygous Sox2Cre-Pitx2 mice. Functional assessment of calcium handling further underscores these findings. Importantly, neither Zfhx3 nor Wnt8a gain-of-function or loss-of-function experiments impair Pitx2 expression, suggesting that Pitx2 is upstream of these genes. Furthermore, these data suggest a dose-dependent relation between Pitx2 expression and the susceptibility to display basal or only inducible electrophysiological defects. We are currently studying the hierarchical between Pitx2, AF GWAS associated genes and calcium handling, as well as to putative involvement of post-transcriptional modulators such as microRNAs.