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A novel mechanism of sinus node dysfunction: intergenic deletion between PITX2 and ANK2 disrupts chromatin structure in pacemaker cell differentiation

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Background: Genetics of sinus node dysfunction (SND) remains poorly understood with few genes identified (HCN4, SCN5A, GJA5, KCNQ1, MYH6 and ANK2) and further genetic heterogeneity.
Purpose: We report two families with SND segregating with an intergenic deletion associated with long-range cis-regulatory elements (CREs) of PITX2.
Methods: We applied 30x whole genome sequencing (WGS) to two French families in which exome sequencing did not allow to detect the causing genes of SND and analyzed them bioinformatically.
Results: We first applied WGS to a 4-generation family presenting 16 patients with SND, atrial fibrillation and/or repolarization abnormalities and identified a 15-Kb deletion within a 16.5-cM linkage interval (Zmax = 5.9, θ = 0) on chromosome 4q25. We also applied WGS on a second family presenting 5 patients with SND and identified a 91-Kb deletion overlapping the initial deletion. This intergenic deletion, which was located between PITX2 and ANK2 and contained a binding motif of CCCTC-binding factor (CTCF), was segregating in all patients and was not found in 855 French control genomes. CTCF functions as a genome organizer mediating genomic interactions between genes and CREs. To clarify a possible regulatory function of the 1.5-Mb intergenic region containing the deletion, we interrogated 3 epigenetic databases. High-throughput chromosome conformation capture (Hi-C) (1) revealed that this intergenic region was placed in a topologically associating domain (TAD) containing PITX2. Chromatin interaction analysis by paired-end tag (ChIA-PET) of CTCF (2) suggested several possible functional chromatin loops in the TAD. Of them, a CTCF-mediated chromatin loop corresponding to a 300-Kb genomic region was clarified as a possible CRE using histone modification data from Roadmap. The region ranging between the intra-deletion CTCF motif and another distal motif was repressed in H3K27me3 profiles of fetal heart, while the region was activated in H3K27ac profiles of H1 ESC-derived mesendoderm.
Conclusion: This CRE may regulate expression of PITX2 and/or ANK2 in pacemaker cell differentiation. Ongoing experiments on patient's iPSC and transgenic mouse will further characterize the disease mechanism.