The role of MAPRE2 and microtubules in Brugada syndrome pathogenesis

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Background: Brugada syndrome (BrS) is associated with loss-of-function variants in SCN5A (encoding NaV1.5), which are found in only ~20% of patients. Recent genome-wide association studies identified a novel locus within an intron of MAPRE2 (encoding microtubule end-binding protein 2, EB2), implicating microtubule (MT) involvement in BrS.

Purpose: To understand the role of MAPRE2 and MT in BrS pathogenesis.

Methods: A mapre2 knock-out (KO) zebrafish model was generated using CRISPR/Cas9 and validated by western blot. Larval hearts at 5 day-post-fertilization (dpf) were isolated for voltage mapping and immunohistochemistry with confocal imaging. Morpholino targeting mapre2 or ttl (encoding tubulin tyrosine ligase) were injected into zebrafish embryos at the one-cell stage. A transgenic zebrafish line with cdh2 (encoding N-cadherin) tandem fluorescent timer (tFT) was used to study adherens junction. MT plus-end tracking experiments were performed in iPSC-derived cardiomyocytes (CM) with MAPRE2 knockdown (KD) using siRNA. ECG was recorded in adult fish.

Results: Voltage mapping of larval hearts (14 WTs vs. 19 KOs) showed a significant decrease in ventricular conduction velocity (17.4±1.5 vs. 13.4±0.8 mm/s; p<0.05) and action potential upstroke velocity (Vmax; 86.1±3.6 vs. 73.9±3.2 1/s; p<0.05), suggesting loss of cardiac NaV function. Correspondingly, ECG of adult fish (11 WTs vs. 10 KOs) showed a significant increase in QRS duration (27.8±0.5 vs. 32.3±0.7 ms; p<0.0001). Confocal imaging of KO hearts showed disorganized adherens junction based on N-cadherin staining. Similarly, morpholino KD of mapre2 in the cdh2 tFT line showed ~40% mislocalization of mature N-cadherin relative to ZO-1 staining in larval ventricles (8 KDs vs 9 control hearts; p<0.05), suggesting disruption of adherens junction. In terms of MT, immunohistochemistry showed a 23% decrease of detyrosinated tubulin relative to total alpha tubulin in KO hearts (16 vs. 8 WT hearts; p<0.01). MAPRE2 KD in iPSC-CM (337 control vs. 285 KD MT in 4 sets of cells) showed an increase in MT velocity (7.9±0.2 vs. 8.8±0.2 µm/min; p<0.001), distance (5.8±0.2 vs. 7.1±0.2 µm; p<0.0001), and duration (46.2±1.4 vs. 52.2±1.6 s; p<0.001), suggesting changes to MT dynamics. Finally, morpholino KD of ttl in mapre2 KO embryos restored the fraction of detyrosinated tubulin (46% increase; 16 controls vs. 16 KDs; p<0.01) as well as Vmax (74.3±3.0 vs. 88.3±2.1 1/s; 13 controls vs. 9 KDs; p<0.01) to WT levels.

Conclusion: Genetic ablation of mapre2 led to a decrease in cardiac NaV function, a hallmark of BrS. This is associated with disruption of adherens junction, a decrease of detyrosinated tubulin as a marker of MT stability, and changes in MT dynamics. Restoration of detyrosinated tubulin fraction with ttl KD led to a rescue of NaV function in zebrafish larval hearts. Taken together, MAPRE2 loss-of-function may contribute to BrS pathogenesis by disruption of adherens junction via changes in MT dynamics.