**EXOME SEQUENCING AND FUNCTIONAL ANALYSIS FOR PATIENTS WITH EARLY ONSET CARDIAC CONDUCTION-SYSTEM DISEASES**

**K. Hayashi**1, A. Nomura1, Y. Asano2, M. Beerens3, Y. Kurata4, N. Fujino1, H. Tada1,

C. MacRae5, S. Takashima6, M. Yamagishi1

1Kanazawa University Graduate School of Medicine, Kanazawa, Japan

2Osaka University Graduate School of Medicine, Osaka, Japan

3Brigham and Women’s Hospital, Boston, MA, USA

4Kanazawa Medical University, Uchinada, Japan

5Brigham and Women’s Hospital, Boston, MA, USA

6Osaka University Graduate School of Medicine, Osaka, Japan

**Objective:** We here sought to identify pathogenic variants in cardiac conduction system disease (CCSD) patients using whole exome sequencing accompanied using targeted gene analysis and understand the primary molecular mechanisms that cause CCSD by cellular electrophysiological study and CRISPR/Cas9 mediated gene knock-out in zebrafish.

**Methods and Results**: We performed exome sequencing in 23 probands diagnosed with early-onset (<65 years old) CCSD, and analyzed 116 genes linked to arrhythmogenic diseases or cardiomyopathies. We used a standard variant quality control method and focused on rare variants (minor allele frequency < 0.5 %). Selected variants were subjected to in silico analysis combined with frequency data from public database. Nine probands had pathogenic variants in *EMD* (2 probands),*LMNA* (3 probands),*KCNA5*,*KCNH2*, *SCN5A*, and *RBM20* (2 probands). To evaluate the functional change of an *LMNA* c.339 dup T, we sought to generate and characterize a knockout zebrafish with CRISPR-mediated insertions or deletions (indels) of the human *LMNA* homolog, *lmna* in zebrafish. The mean heart rate and the mean conduction velocities of the CRISPR/ Cas9 injected embryos with lmna indels were significantly decreased compared to those of the CRISPR only injected embryos. Both*SCN5A* P1824A and *KCNH2* R269W showed loss-of-function by electrophysiological study and may contribute to CCSD by simulation study. Of remaining 14 probands, 6 probands had at least one likely pathogenic variant in 15 genes. Three probands harbored novel ‘likely pathogenic’ variants in *SCN10A* gene, which showed functional changes.

**Conclusion:**Nine in 23 probands with CCSD (39%) harbored pathogenic variants in known genes. CRISPR/Cas9 mediated gene knock-out in zebrafish might be useful for evaluating functional properties of protein truncating variants in patients with CCSD.