**PO-629-01**

LEVERAGING SIGNAL-TO-NOISE ANALYSIS TO EXPAND CLINICAL UTILITY OF PATHOGENICITY CRITERIA FOR INCIDENTAL VARIANTS IN HYPERTROPHIC CARDIOMYOPATHY-ASSOCIATED GENES

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**Background:** Hypertrophic cardiomyopathy (HCM) is the most common heritable cardiomyopathy and can predispose individuals to sudden death. Most HCM patients host a known pathogenic variant in a sarcomeric gene. With the increase in exome sequencing (ES) in clinical settings, incidental variants in HCM-associated genes are being identified more frequently. Diagnostic interpretation of incidental variants is crucial to enhance clinical patient management.

**Objective:** We sought to use amino acid-level signal-to-noise (S:N) analysis to establish pathogenic hotspots in sarcomeric HCM-associated genes as well as to refine the 2015 American College of Medical Genetics (ACMG) criteria to predict incidental variant pathogenicity.

**Methods:** Incidental variants in HCM genes (MYBPC3, MYH7, MYL2, MYL3, ACTC1, TPM1, TNNT2, TNNT3, TNNC1) were obtained from a clinical ES referral database (Baylor Genetics) and compared to rare population variants (gnomAD) and variants from HCM literature cohort studies. We compared the frequency of ES and HCM variants at specific amino acid locations in coding regions to rare variants (MAF < 0.0001) in gnomAD. S:N ratios were calculated at the gene- and amino acid-level to identify pathogenic hotspots. ES cohort variants were re-classified using ACMG criteria with S:N analysis as a correlate for PM1 criteria.

**Results:** We identified 7,066 unrelated probands in the ES cohort, with 509 individuals hosting a rare variant in an HCM gene. Variants in sarcomeric genes, especially radical variants, were enriched in the HCM cohort compared to the ES and gnomAD cohorts (p < 0.05). In the HCM cohort, five out of nine genes carried a S:N ratio greater than 6.0, suggesting a higher relative frequency of disease-associated variants in these genes. Among ES referrals, no gene had a global S:N greater than 3.0. Within each gene, localization of variants found in the ES cohort was more similar to those found in gnomAD than those found in the HCM cohort. In MYH7, most pathogenic hotspots were in the head domain.

**Conclusion:** Incidental variants in HCM-associated genes were common among clinical ES referrals, though the majority were not disease-associated. Leveraging amino acid-level S:N as a clinical tool may improve the diagnostic discriminatory ability of ACMG criteria by identifying pathogenic hotspots.

**PO-629-02**

NEW EVIDENCE TO CHALLENGE CLINGEN’S “DISPUTED EVIDENCE” DESIGNATION FOR AKAP9 AS A BONA FIDE SUSCEPTIBILITY GENE FOR CONGENITAL LONG QT SYNDROME

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**Background:** A-kinase-anchoring protein-9 (AKAP9) mediates the phosphorylation state of the KCNQ1-encoded Kv7.1 potassium channel. Loss-of-function (LOF) variants in KCNQ1 cause type 1 long QT syndrome (LQT1). In 2007, we published data suggesting AKAP9-S1570L as a novel LQTS-causative variant and AKAP9 as a novel LQTS-susceptibility gene. However, in a recent re-appraisal of LQTS genes, the Clinical Genome Resource (ClinGen) demoted AKAP9 to a “disputed evidence” gene due to apparent lack of sufficient evidence to support causation of LQTS.

**Objective:** To characterize the electrophysiology of a LQTS patient-derived pluripotent stem cell-cardiomyocyte (iPSC-CM) AKAP9-S1570L model and its corresponding CRISPR/Cas9 gene-editing/variant-corrected isogenic control (IC).

**Methods:** iPSC-CMs were generated from an AKAP9-S1570L positive female diagnosed with LQTS at 14 years of age with a QTc > 480 ms, history of syncope, a LQT1-suggestive treadmill stress test, and a positive family history of QT-prolongation. Standard whole-cell patch clamp technique was performed to measure the action potential duration (APD) of both the AKAP9-S1570L and IC iPSC-CMs. To determine if KCNQ1 contributes to the APD prolongation of the variant iPSC-CMs, the KCNQ1 channel specific activator, ML277 was used in additional APD measurements.

**Results:** APD50 (457 ± 21) and APD90 (549 ± 22 ms) were significantly prolonged in the AKAP9-S1570L iPSC-CMs compared to the IC iPSC-CMs (APD50, 335 ± 15 ms; APD90, 413 ± 16 ms; p < 0.05). Addition of ML277 resulted in a greater degree of APD shortening in AKAP9-S1570L iPSC-CMs (APD50 shortening, 349 ± 40 ms (p < 0.05); APD90 shortening, 397 ± 42 ms (p < 0.05)) than in IC iPSC-CMs (APD50 shortening, 219 ± 31 ms; APD90 shortening, 242 ± 33 ms). This indicates KCNQ1 LOF may contribute to the mechanism underlying APD prolongation in the patient-specific AKAP9-S1570L iPSC-CMs, consistent with our previously published data.

**Conclusion:** Using patient specific iPSC-CMs and isogenic controls, we provide new evidence bolstering AKAP9-S1570L as the pathogenic LQTS-causative variant responsible for this patient’s LQTS. Whether ClinGen deems this sufficient evidence to rescue AKAP9 from genetic purgatory and re-classify it as a sufficient evidence LQTS-susceptibility gene, remains to be seen.

**PO-629-03**

IN-DEPTH ANALYSIS OF THE SCN5A LOCUS HIGHLIGHTS DISTINCT GENETIC ARCHITECTURES FOR BRUGADA SYNDROME IN DIFFERENT ANCESTRIES AND IDENTIFIES A NOVEL RARE ENHANCER VARIANT ASSOCIATED WITH DISEASE IN SOUTHEAST ASIAN PATIENTS