Doctoral Dissertation Defense Announcement

Suma K. Thareja.

“Synthesizing Clinical, Genetic, Cellular, and Pathway Insights to Understand a Familial Case of Ebstein’s Anomaly and Left Ventricular Noncompaction”

Candidate for Doctor of Philosophy in Cell and Developmental Biology
Graduate School of Biomedical Sciences
Medical College of Wisconsin

Committee in Charge:
Aoy Tomita-Mitchell, PhD (Advisor)
Paul Burridge, PhD
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Gabrielle Geddes, MD
Michael Mitchell, MD
Curt Sigmund, PhD

Tuesday, April 19th, 2022 at 3:00 PM (CST)

Room: MEB M1061 Alumni Center
Live Public Viewing:
https://mcw-edu.zoom.us/j/94814638781?pwd=TDRJaWhXamprMVZQZDVblFxZUV4UT09
Zoom Meeting ID: 948 1463 8781
Passcode: Suma22!*
GRADUATE STUDIES

Advanced Cell Biology

Biostatistics I

Developmental and Stem Cell Biology

Developmental and Stem Cell Journal Club

Ethics & Integrity in Science

Reading and Research

Research Ethics Discussion Series

Team Science in Clinical Research

Doctoral Dissertation
ABSTRACT

Congenital heart defects (CHDs) are cardiogenic malformations present at or before birth and account for a high burden of neonatal morbidity and mortality. While traditional definitions of Ebstein’s anomaly (EA) and left ventricular noncompaction (LVNC), two rare CHDs, confine disease to either the right or left heart, respectively, patients with EA commonly manifest with LVNC cardiomyopathy. Combined EA and LVNC (EA/LVNC) poses a higher risk of poor clinical outcomes such as heart failure and sudden cardiac death than either disease alone. Apart from a few sarcomeric variant associations, the genetic etiology and pathogenesis of EA/LVNC remain largely unknown and disease treatment is non-specific, ranging from symptomatic management to invasive surgery. Investigating the mechanisms underlying EA/LVNC will permit understanding and may enable the development of novel and effective personalized therapeutics that can halt abnormal and irreversible cardiac development. Here, we synthesize the clinical, genetic, cellular, and pathway insights from a familial case of EA/LVNC.

In a familial case where 10 of 17 members, including four fetal deaths, inherited EA/LVNC in an autosomal dominant pattern, we discovered a novel and damaging missense variant in the gene KLHL26 that segregates with all genotyped EA/LVNC-affected subjects. The KLHL26 (p.R237C) variant comprises an altered electrostatic surface profile, likely decoupling the CUL3-interactome and altering ubiquitin-mediated protein turnover. While little is known about KLHL26 specifically, the KLHL family of proteins play crucial roles in striated muscle.

To study the cellular phenotypes of EA/LVNC, we differentiated a family trio (a heart-healthy daughter and EA/LVNC-affected mother and daughter who carry the KLHL26 (p.R237C) variant) of induced pluripotent stem cells into cardiomyocytes (iPSC-CMs) and characterized structure and function in a blinded fashion. Using flow cytometry, immunofluorescence, electron microscopy, and biomechanical, electrophysiological, and automated contraction methods, we investigated differentiation efficiency between days 10-20 (D10-20), contractile properties at D20-60, cell cycle regulation at D20, and sarcomere organization at D60. Between disease and wild-type lines at D20, we analyzed differential mRNA expression using RNA Sequencing (RNASeq) and RT-PCR and protein abundances via label-free quantification at D20.

While all lines differentiated into CMs, the affected daughter iPSC-CMs differentiated into more cTnT+ cells. In comparison to the unaffected, affected iPSC-CMs had longer cell lengths, fewer contractions per minute, and altered calcium transients. The affected daughter iPSC-CMs further had higher proliferation and a smaller cell surface area per cardiac nucleus. While there were no gross differences in sarcomere organization through α-actinin immunostaining, we discovered distended sarcoplasmic reticulum (SR) in the affected lines via electron microscopy. Enrichment analyses following RNASeq and label-free proteomics further implicated pathways in muscle contraction and regulation and proteolysis in EA/LVNC development. We also found altered expression of several cardiac genes, including decreased Sarcoplipin (SLN), a regulator of SR-Serca2-ATPase and increased Junctophilin 2 (JPH2), a junctional complex between the SR and membrane, relative to GAPDH via RT-PCR in affected iPSC-CMs.

Together, these results from cell and developmental studies suggest that this familial EA/LVNC associated with the KLHL26 (p.R237C) variant develops from decreased contractile ability, altered calcium transients, cell cycle dysregulation, and abnormal signaling of the SR and the cardiac sarcomere.
CURRICULUM VITAE

Suma K. Thareja
(Formerly Sai-Suma K. Samudrala)

EDUCATION:
2016 – Present **MD and PhD (Cell and Developmental Biology) Candidate**, Medical College of Wisconsin, Milwaukee, WI
2013 – 2016 **Bachelor of Science, Molecular Biology**, University of Wisconsin-Madison, Madison, WI

RESEARCH EXPERIENCE:
2018 – Present **PhD Graduate Research**, Medical College of Wisconsin, Milwaukee, WI
  Advisor: Aoy Tomita-Mitchell, PhD
2014 – 2016 **Undergraduate Research**, Wisconsin Institute for Discovery, University of Wisconsin-Madison, Madison, WI
  Advisor: Peter Lewis, PhD
2013 – 2015 **Undergraduate Research**, Genetics and Biotechnology Center, University of Wisconsin-Madison, Madison, WI
  Advisor: Barry Ganetzky, PhD

MEMBERSHIPS IN PROFESSIONAL SOCIETIES:
2021 – Present American Physiological Society
2021 – Present Association for Clinical and Translational Science
2018 – Present American Medical Association
2018 – Present American Heart Association

GRANTS/FELLOWSHIPS:
2021 – 2022 **Other Significant Contributor**, *The Ubiquitin-Proteasome System in Ebstein’s Anomaly and Left Ventricular Noncompaction*, MCW Cardiovascular Center, Aoy Tomita-Mitchell (PI)

AWARDS & HONORS:
2022 – Cardiovascular Section Research Recognition Award for Experimental Biology
  2022, American Physiological Society
2021 – MCW/Marquette Medical Alumni Association Poster Award, 31st Annual MCW Graduate School Research Poster Session, Virtual
2021 – MCW President’s Community Engagement Award
2021 – Fundamental of Team STEPPS and Team Science in Clinical Research Certificate
2021 – Friends of MCW Poster Award, 30th Annual MCW Graduate School Research Poster Session, Virtual
2020 – Highlighted Poster, Midwest Pediatric Cardiology Society, Virtual
2018 – Best Medical Student Poster Award, MCW Cardiovascular Research Center Retreat, Harley Davidson Museum, Milwaukee, WI
2017 – Best Medical Student Oral Abstract Presentation, 41st Midwest Pediatric
Cardiology Society Meeting, St. Louis, MO
2017 – Dr. Michael J. Dunn Poster Contest Award, Medical Student Summer Research Program, Medical College of Wisconsin, Milwaukee, WI
2015 – 2016 Hilldale Undergraduate/Faculty research Fellowship, University of Wisconsin-Madison, Madison, WI
2015 – Ann M. Dura Scholarship, University of Wisconsin-Madison, Madison, WI
2014 – Welton Summer Sophomore Honors Apprenticeship, University of Wisconsin-Madison, Madison, WI
2013 – 2016 Academic Excellence Scholarship, University of Wisconsin-Madison, Madison, WI

ABSTRACTS:


2019 – 29th Annual Medical College of Wisconsin Graduate School Research Poster Session, Milwaukee, WI. (Poster)
2019 – Weinstein Cardiovascular Development Conference, Crowne Plaza, Indianapolis, IN. (Poster)
2019 – Harmony 4 Hope Rare Storyteller Series: Rare Disease Research, Medical College of Wisconsin, Milwaukee, WI. (Poster)
2019 – Medical College of Wisconsin Cardiovascular Center Research Retreat, Boerner Botanical Gardens, Hales Corners, WI. (Poster)
2018 – Medical College of Wisconsin Cardiovascular Center Research Retreat, Harley Davidson Museum, Milwaukee, WI. (Poster)
2017 – 41st Midwest Pediatric Cardiology Society, Hyatt Regency, St. Louis, MO. (Oral)
2017 – Medical Student Summer Research Training Program, Medical College of Wisconsin, Milwaukee, WI. (Poster)


2021 – 4th Annual Medical College of Wisconsin Graduate Student Association Symposium, Virtual. (Poster)
2021 – Developmental and Stem Cell Biology Seminar, Medical College of Wisconsin, Virtual. (Oral)
2021 – 30th Annual Medical College of Wisconsin Graduate School Research Poster Session, Virtual. (Poster)
2020 – 35th Annual MD/PhD student Virtual Conference, Virtual. (Poster)
2020 – Midwest Pediatric Cardiology Society, Virtual. (Poster)
2020 – Herma Heart Institute Quality, Outcomes, and Research Seminar, Virtual. (Oral)

2022 – 5th Annual Graduate Student Association Symposium, Medical College of Wisconsin, Virtual. Abstract Accepted* (Poster)
2022 – Experimental Biology 2022, Pennsylvania Convention Center, Philadelphia, PA. Abstract Accepted* (Poster)
2021 – Medical College of Wisconsin Cell and Developmental Biology, Neurology, and Anatomy Seminar Series, Virtual. (Oral)
2021 – Medical College of Wisconsin Cardiovascular Center Research Retreat, Wisconsin Center, Milwaukee, WI. (Poster)
2021 – 31st Annual Medical College of Wisconsin Graduate School Research Poster Session, Virtual. (Poster)

PUBLICATIONS:
  - Journal Cover Image: https://doi.org/10.1002/mgg3.1271

LEADERSHIP AND COMMUNITY ENGAGEMENT:
2020 – Present Student Council Class Representative, Medical Scientist Training Program, Medical College of Wisconsin, Milwaukee, WI
2020 – Present Chair/Founder, The Food as Medicine (FAM) Initiative, Saturday Clinic for the Uninsured, Milwaukee, WI
2019 – Present Chair/Founder, The My Life, My Story (MLMS) Initiative, Milwaukee VA Medical Center, Milwaukee, WI
2016 – Present Columnist, MCW Magazine, Medical College of Wisconsin, Milwaukee, WI