

UC Office of the President

Research Grants Program Office (RGPO) Funded Publications

Title

Studying Long QT Syndrome Caused by NAA10 Genetic Variants Using Patient-Derived Induced Pluripotent Stem Cells

Permalink

<https://escholarship.org/uc/item/6dv41294>

Journal

Circulation, 148(20)

ISSN

0009-7322

Authors

Belbachir, Nadjat
Wu, Yiyang
Shen, Mengcheng
et al.

Publication Date

2023-11-14

DOI

10.1161/circulationaha.122.061864

Copyright Information

This work is made available under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives License, available at <https://creativecommons.org/licenses/by-nc-nd/4.0/>

Peer reviewed

RESEARCH LETTER



Studying Long QT Syndrome Caused by *NAA10* Genetic Variants Using Patient-Derived Induced Pluripotent Stem Cells

Nadjet Belbachir, PhD; Yiyang Wu, MD, PhD; Mengcheng Shen¹, PhD; Sophia L. Zhang², MS; Joe Z. Zhang, MD, PhD; Chun Liu³, PhD; Bjorn C. Knollmann⁴, MD, PhD; Gholson J. Lyon⁵, MD, PhD; Ning Ma⁶, PhD; Joseph C. Wu⁷, MD, PhD

Patients carrying rare genetic variants in the gene *N-α-acetyltransferase 10 (NAA10)* exhibit various symptoms including developmental delay, intellectual disability, and cardiac dysfunction.¹ A phenotypic similarity of Ogden syndrome (OS; *NAA10p.S37P*) and Timothy syndrome (a long QT syndrome [LQT]) was identified, and a potential shared molecular mechanism between the 2 involving calcium channels was hypothesized and tested using an OS patient-derived induced pluripotent stem cell–derived cardiomyocytes (iPSC-CMs) model.² Characterization of this model recapitulated OS relevant cardiac arrhythmia in a dish for the first time, including prolonged QT intervals and abnormal intracellular calcium transients.² In this study, we further investigated *NAA10*, the variant-mediated electrical phenotype, using patient-specific iPSC-CMs (Figure [A]). Two patients were recruited, including the one used in the above-mentioned OS iPSC-CMs model. One patient harboring the p.Y43S variant showed mild symptoms: mild intellectual disability, facial dysmorphism, and LQT.³ The patient with p.S37P exhibited severe symptoms—aged appearance, global developmental delays, and heart defects⁴—and died at 4.5 months of unknown cause. Patient-specific iPSC-CMs were generated with approval of institutional review committees and with subject informed consent. Results were compared with clustered regularly interspaced short palindromic repeats (CRISPR)-

corrected induced pluripotent stem cell lines named *NAA10p.Y43Scor* and *NAA10p.S37Pcor*, respectively.

To assess if iPSC-CMs carrying the *NAA10* variants recapitulated the LQT phenotype observed in some affected patients, single-cell action potential (AP) recordings by patch clamp in a current clamp mode were performed. AP durations (APDs) of *NAA10p.Y43S* and *NAA10p.S37P* iPSC-CMs at 30%, 50%, and 90% of repolarization were significantly increased compared with *NAA10p.Y43Scor* and *NAA10p.S37Pcor* iPSC-CMs: APD at 30% of repolarization: 526.7±142.7 and 659.1±493.9 ms versus 365.2±116.8 and 196.3±64.0 ms; APD at 50% of repolarization: 614.6±167.2 and 748.4±550.3 ms versus 419.8±118.8 and 207.8±73.6 ms; and APD at 90% of repolarization: 679.5±176.4 and 809.4±565.4 ms versus 480.5±113.1 and 281.2±86.2 ms (Figure [B and D]). Furthermore, arrhythmias such as early afterdepolarizations and delayed afterdepolarizations were observed in 50% of AP records from *NAA10p.S37P* iPSC-CMs (Figure [C]), consistent with a previous report.²

To investigate the mechanism underlying the AP prolongation, we measured late Na current and L-type Ca currents (I_{CaL}). Late Na current density was not significantly different between patient and corrected isogenic lines (data not shown). On the other hand, I_{CaL} was significantly different between *NAA10p.Y43S*, *NAA10p.S37P* and *NAA10p.Y43Scor*, *NAA10p.S37Pcor* iPSC-CMs. Peak I_{CaL} density measured at 0 mV was

Key Words: Cav1.2 calcium channel ■ iPSC ■ long QT ■ *NAA10* ■ rare disease

Correspondence to: Joseph C. Wu, MD, PhD, 265 Campus Dr G1120B, Stanford, CA 94304, Email joewu@stanford.edu; Ning Ma, PhD, 188 Kaiyuan Ave, Science City, Huangpu District, Guangzhou, Guangdong 510530, China, Email ma_ning@gzlab.ac.cn; or Gholson J. Lyon, MD, PhD, 1050 Forest Hill Rd, Staten Island, NY 10314-6399, Email gholson.j.lyon@opwdd.ny.gov

For Sources of Funding and Disclosures, see page 1601.

© 2023 The Authors. *Circulation* is published on behalf of the American Heart Association, Inc., by Wolters Kluwer Health, Inc. This is an open access article under the terms of the [Creative Commons Attribution Non-Commercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use, distribution, and reproduction in any medium, provided that the original work is properly cited, the use is noncommercial, and no modifications or adaptations are made.

Circulation is available at www.ahajournals.org/journal/circ

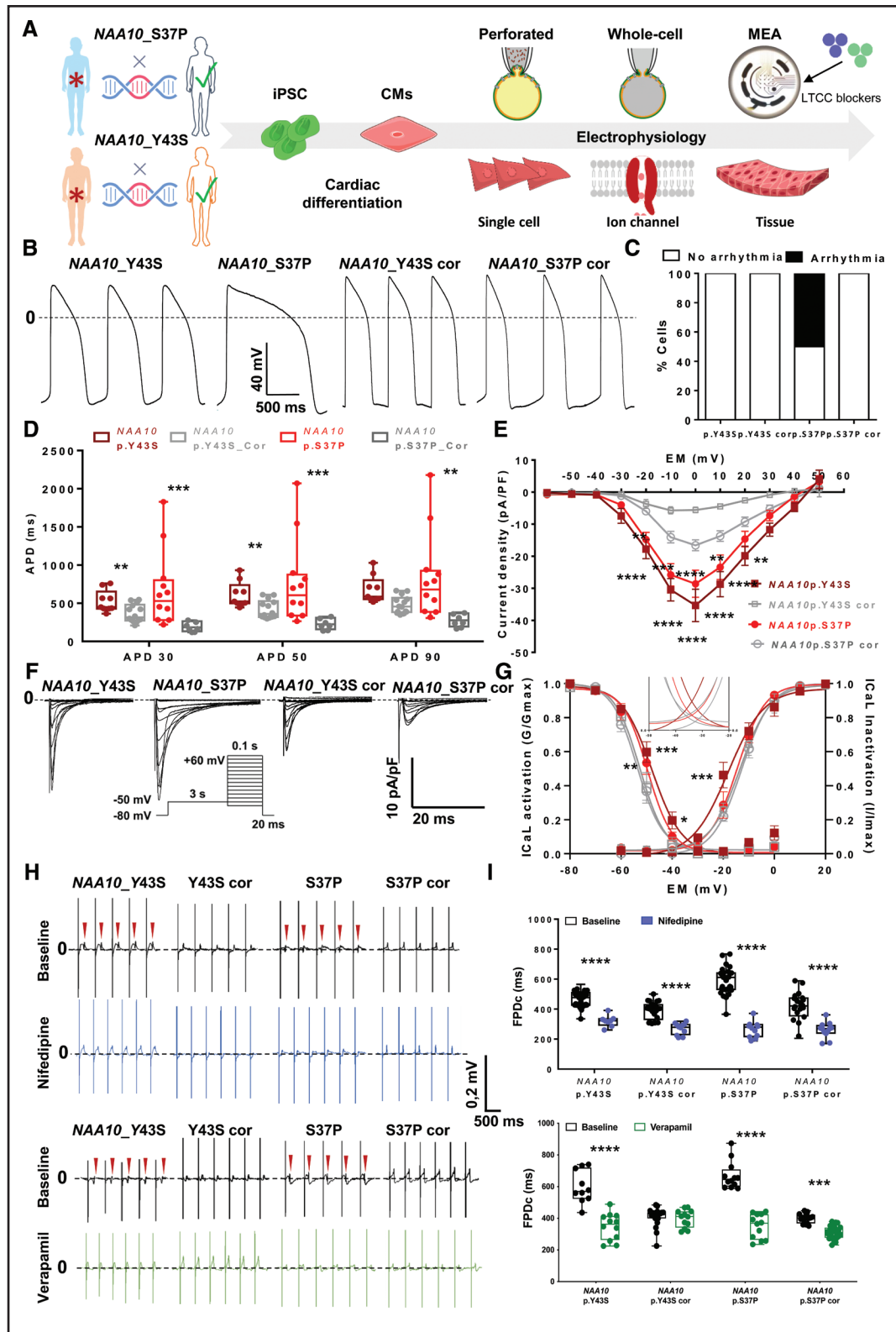


Figure. Studying N- α -acetyltransferase 10 variants using patient-derived induced pluripotent stem cells.

A, iPSCs were reprogrammed from 2 patients carrying mutations on the *NAA10* gene (p.S37P and p.Y43S),^{3,4} and corresponding clustered regularly interspaced short palindromic repeats (CRISPR)-corrected isogenic control iPSC lines were generated and then differentiated into iPSC-CMs. A full electrophysiology investigation was conducted, including patch clamp both in perforated and whole cell configurations and multielectrode array (MEA) measurements. **B**, Representative action potential recordings of iPSC-CMs from patient lines (*NAA10*p.Y43S and *NAA10*p.S37P) and isogenic CRISPR-corrected lines (*NAA10*p.Y43Scor, *NAA10*p.S37Pcor) by patch clamp. **C**, Early after depolarization observed in action potential recordings from mutated and corrected isogenic iPSC-CMs (*NAA10*_Y43S: n=9 vs Y43Scor: (Continued)

Figure Continued. n=14 and S37P: n=12 vs S37Pcor: n=10). **D**, Action potential durations (APDs) of patient lines and corrected isogenic lines. ** $P < 0.01$, *** $P < 0.001$ by Mann-Whitney test. **E**, L-type calcium current (I_{CaL}) current-voltage relationship of maximum current density (NAA10_Y43S: n=9 vs Y43Scor: n=9 and S37P: n=14 vs S37Pcor: n=11). ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ by 2-way ANOVA combined with multiple comparison test comparing mutated lines vs corrected lines at each voltage regardless of row and column. **F**, Representative I_{CaL} records of NAA10 variant lines (p.Y43S and p.S37P) and corresponding corrected isogenic lines using patch clamp. **Inset**, Voltage-clamp protocol. **G**, Overlap of I_{CaL} activation (G/Gmax) and inactivation (I/Imax) plots, both fitted using the Boltzmann equation. **Inset**, I_{CaL} window current (NAA10_Y43S: n=9 vs Y43Scor: n=9 and S37P: n=16 vs S37Pcor: n=9). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by 2-way ANOVA with multiple comparison between corrected and mutated lines at each voltage point. **H**, Representative MEA recordings of NAA10 variants (p.Y43S and p.S37P) and corresponding corrected isogenic lines before and after acute treatment with I_{CaL} inhibitors nifedipine (blue) and verapamil (green). Red arrows indicate arrhythmic events. **I**, Field-potential duration measurements of NAA10p.Y43S, NAA10p.Y43Scor, NAA10p.S37P, and NAA10p.S37Pcor iPSC-CMs before and after acute nifedipine and verapamil treatment. *** $P < 0.001$, **** $P < 0.0001$ (2-way ANOVA statistical analysis combined with multiple comparison test comparing baseline vs dose for each line). CM indicates cardiomyocyte; FPDC, corrected field potential duration; iPSC, induced pluripotent stem cell; iPSC-CM, induced pluripotent stem cell-derived cardiomyocyte; and NAA10, N- α -acetyltransferase 10.

Nonstandard Abbreviations and Acronyms

AP	action potential
APD	action potential duration
ICaL	L-type Ca current
iPSC-CM	induced pluripotent stem cell-derived cardiomyocyte
LQT	long QT syndrome
NAA10	N- α -acetyltransferase 10
OS	Ogden syndrome

-35.3±15.2 and -28.6±15.1 pA/pF in NAA10p.Y43S and NAA10p.S37P iPSC-CMs, compared with -5.6±2.6 and -16.6±5.4 pA/pF in NAA10p.Y43Scor and NAA10p.S37Pcor iPSC-CMs, respectively (Figure [E and F]). Steady state activation of 50% channels was shifted toward negative potentials ($V_{1/2} = -16.9 \pm 6.4$ and -14.5 ± 5.4 mV versus -12.7 ± 5.5 and -12.6 ± 3.7 mV) in NAA10p.Y43S and NAA10p.S37P compared with corresponding CRISPR-corrected lines. Steady state inactivation was significantly shifted toward positive potentials only for the NAA10p.Y43S iPSC-CMs ($V_{1/2} = -48.1 \pm 3.6$ versus -52.2 ± 5.4 mV) (Figure [G]). The combination of these gating kinetics abnormalities of I_{CaL} ultimately led to an increase in the window current of the NAA10 variant-carrying lines compared with CRISPR-corrected lines, which prolonged the AP plateau phase, delaying its repolarization and explaining the LQT phenotype of the affected patients (Figure [G, inset]). Taken together, our results demonstrated that NAA10p.Y43S and NAA10p.S37P variants caused electrical dysfunction that recapitulated NAA10 variant-mediated LQT without altering cell morphology and sarcomere organization (data not shown).

We next used the multielectrode array technique to test the effect of I_{CaL} blockers on iPSC-CMs harboring both variants. After acute treatment with nifedipine, the corrected field potential duration was significantly decreased to normal range values (476.3 ± 51.3 versus 320.6 ± 37.9 ms for NAA10p.Y43S and 588.6 ± 85.7 versus 266.8 ± 52.4 ms for NAA10p.S37P). Nifedipine application to the CRISPR-corrected iPSC-CMs

also decreased corrected field potential duration (391.5 ± 55.9 versus 269.8 ± 37.1 ms for NAA10p.Y43Scor and 414.9 ± 99.4 versus 260.9 ± 52.2 ms for NAA10p.S37Pcor), albeit to a lesser degree. We next tested verapamil as an additional I_{CaL} blocker. Similarly, corrected field potential duration was significantly reduced after acute administration toward normal ranges in both NAA10 variant lines (598 ± 102 versus 347 ± 84 ms for NAA10p.Y43S and 668 ± 87 versus 351 ± 77 ms for NAA10p.S37P). No changes (407 ± 60 versus 397 ± 55 ms for NAA10p.Y43Scor) or minor changes (399 ± 28 versus 310 ± 40 ms for NAA10p.S37Pcor) were observed in CRISPR-corrected lines. Arrhythmic events observed at baseline were suppressed by administration of either I_{CaL} blocker (Figure [H and I]).

Altogether, we successfully recapitulated a NAA10 variant-mediated LQT phenotype using patient iPSC-CMs in a patient-specific manner. Electrophysiological investigation performed on the iPSC-CMs carrying the NAA10 variants demonstrated that both corrected field potential duration and APD prolongations were triggered by abnormal gating properties of the Cav1.2 channel, resulting in an increase of I_{CaL} current density and ultimately leading to a LQT phenotype. Furthermore, we explored potential therapeutic solutions with I_{CaL} blocker administration, which successfully rescued the field potential duration prolongation observed in patient iPSC-CMs. This study enhances our understanding of the link between NAA10 variants and cardiac arrhythmia, contributing to the study of NAA10 variant-related dysfunction. This study may facilitate development of novel therapeutic tools for the treatment of NAA10 variant-mediated LQT. The data that support the findings of this study are available from the corresponding authors upon request.

ARTICLE INFORMATION

Affiliations

Stanford Cardiovascular Institute, Stanford, CA (N.B., M.S., S.L.Z., J.Z.Z., C.L., N.M., J.C.W.). Division of Cardiology, Department of Medicine (N.B., M.S., S.L.Z., J.Z.Z., C.L., N.M., J.C.W.), Department of Radiology (J.C.W.), Stanford University School of Medicine, CA. Stanley Institute for Cognitive Genomics, Cold Spring Harbor Laboratory, Woodbury, NY (Y.W., G.J.L.). Vanderbilt Memory & Alzheimer's Center,

Vanderbilt University Medical Center, Nashville, TN (Y.W.). Greenstone Biosciences, Palo Alto, CA (C.L., J.C.W.). Vanderbilt Center for Arrhythmia Research and Therapeutics, Division of Clinical Pharmacology, Vanderbilt University School of Medicine, Nashville, TN (B.C.K.). Department of Human Genetics, New York State Institute for Basic Research in Developmental Disabilities, Staten Island (G.J.L.). Biology PhD Program, Graduate Center, City University of New York (G.J.L.). School of Basic Medical Sciences, Guangzhou Laboratory, Guangzhou Medical University, China (N.M.). The Sixth Affiliated Hospital of Guangzhou Medical University, Qingyuan People's Hospital, China (N.M.).

Acknowledgments

We would like to thank Dr Sally Lynch from University College of Dublin for providing the skin biopsies from NAA10_Y43S-carrying patients used to generate the patient-specific induced pluripotent stem cell line.

Sources of Funding

This study was funded by National Institutes of Health grants R01 HL113006, R01 HL141371, R01 HL150693, HL163680, P01 HL141084 (J.C.W.), and R35 GM133408 (G.J.L.); Leducq Foundation grant 18CVD05 (J.C.W. and B.C.K.); American Heart Association grants 20CDA35310038 (N.M.) and 22CDA940474 (N.B.); and Tobacco-Related Disease Research Program grant 30FT0852 (M.S.).

Disclosures

J.C.W. is a cofounder and scientific advisory board member of Greenstone Biosciences. The other authors report no conflicts.

REFERENCES

1. Wu Y, Lyon GJ. NAA10-related syndrome. *Exp Mol Med*. 2018;50:85. doi: 10.1038/s12276-018-0098-x
2. Wu, Y. *Toward Precision Medicine: From Clinical Genomics to iPSC Disease Modeling* (Publication No. 10281516) [Doctoral dissertation, Stony Brook University]. ProQuest Dissertations Publishing; 2017. <http://hdl.handle.net/11401/77611>
3. Casey JP, Stove SI, McGorrian C, Galvin J, Blenski M, Dunne A, Ennis S, Brett F, King MD, Arnesen T, et al. NAA10 mutation causing a novel intellectual disability syndrome with long QT due to N-terminal acetyltransferase impairment. *Sci Rep*. 2015;5:16022. doi: 10.1038/srep16022
4. Rope AF, Wang K, Evjenth R, Xing J, Johnston JJ, Swensen JJ, Johnson WE, Moore B, Huff CD, Bird LM, et al. Using VAAST to identify an X-linked disorder resulting in lethality in male infants due to N-terminal acetyltransferase deficiency. *Am J Hum Genet*. 2011;89:28–43. doi: 10.1016/j.ajhg.2011.05.017