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RESEARCH LETTER

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

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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; *NAA10*p.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 defects[4]—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 *NAA10*p.Y43Scor and *NAA10*p.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 *NAA10*p.Y43S and *NAA10*p.S37P iPSC-CMs at 30%, 50%, and 90% of repolarization were significantly increased compared with *NAA10*p.Y43Scors and *NAA10*p.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 *NAA10*p.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 *NAA10*p.Y43S, *NAA10*p.S37P and *NAA10*p.Y43Scor, *NAA10*p.S37Pcor iPSC-CMs. Peak I_{CaL} density measured at 0 mV was

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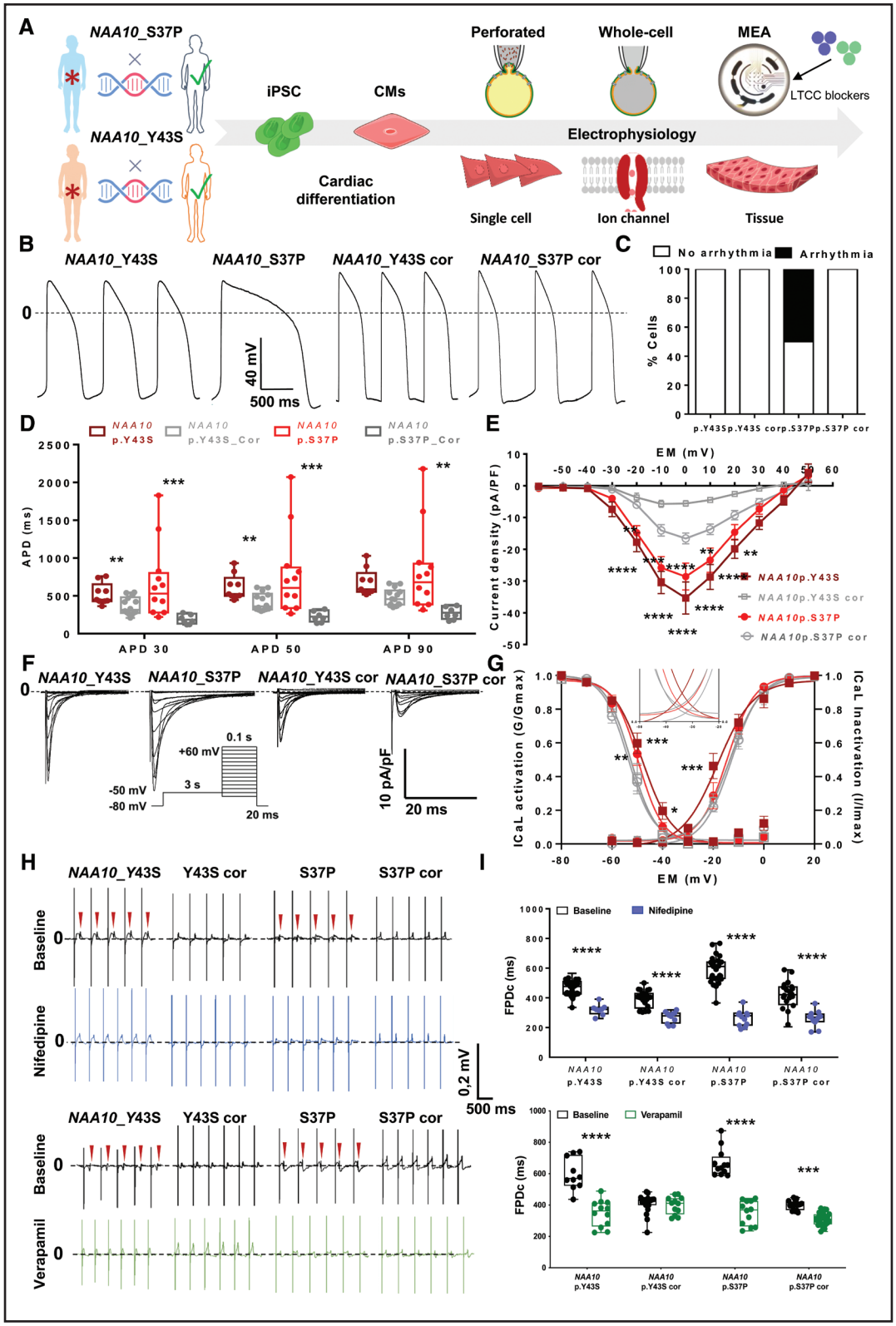

Figure. Studying N-α-acetyltransferase 10 variants using patient-derived induced pluripotent stem cells.
A, iPSCs were reprogrammed from 2 patients carrying mutations on the *NAA10* gene (p.S37P and p.Y43S),[3,4] and corresponding clustered regularly interspaced short palindromic repeats (CRISPR)-corrected 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 *NAA10*p.Y43S, *NAA10*p.Y43Scor, *NAA10*p.S37P, and *NAA10*p.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 *NAA10*p.Y43S and *NAA10*p.S37P iPSC-CMs, compared with −5.6±2.6 and −16.6±5.4 pA/pF in *NAA10*p.Y43Scor and *NAA10*p.S37Pcor iPSC-CMs, respectively (Figure [E and F]). Steady state activation of 50% channels was shifted toward negative potentials ($V_{1/2}$ = −16.9±6.4 and −14.5±5.4 mV versus −12.7±5.5 and −12.6±3.7 mV) in *NAA10*p.Y43S and *NAA10*p.S37P compared with corresponding CRISPR-corrected lines. Steady state inactivation was significantly shifted toward positive potentials only for the *NAA10*p.Y43S iPSC-CMs ($V_{1/2}$ = −48.1±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 *NAA10*p.Y43S and *NAA10*p.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 *NAA10*p.Y43S and 588.6±85.7 versus 266.8±52.4 ms for *NAA10*p.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 *NAA10*p.Y43Scor and 414.9±99.4 versus 260.9±52.2 ms for *NAA10*p.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 *NAA10*p.Y43S and 668±87 versus 351±77 ms for *NAA10*p.S37P). No changes (407±60 versus 397±55 ms for *NAA10*p.Y43Scor) or minor changes (399±28 versus 310±40 ms for *NAA10*p.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.

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Disclosures

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

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