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ORIGINAL RESEARCH

ATP1A3-Encoded Sodium-Potassium ATPase Subunit Alpha 3 D801N Variant Is Associated With Shortened QT Interval and Predisposition to Ventricular Fibrillation Preceded by Bradycardia

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BACKGROUND: Pathogenic variation in the *ATP1A3*-encoded sodium-potassium ATPase, ATP1A3, is responsible for alternating hemiplegia of childhood (AHC). Although these patients experience a high rate of sudden unexpected death in epilepsy, the pathophysiologic basis for this risk remains unknown. The objective was to determine the role of *ATP1A3* genetic variants on cardiac outcomes as determined by QT and corrected QT (QTc) measurements.

METHODS AND RESULTS: We analyzed 12-lead ECG recordings from 62 patients (male subjects=31, female subjects=31) referred for AHC evaluation. Patients were grouped according to AHC presentation (typical versus atypical), *ATP1A3* variant status (positive versus negative), and *ATP1A3* variant (D801N versus other variants). Manual remeasurements of QT intervals and QTc calculations were performed by 2 pediatric electrophysiologists. QTc measurements were significantly shorter in patients with positive *ATP1A3* variant status ($P<0.001$) than in patients with genotype-negative status, and significantly shorter in patients with the ATP1A3-D801N variant than patients with other variants ($P<0.001$). The mean QTc for ATP1A3-D801N was 344.9 milliseconds, which varied little with age, and remained <370 milliseconds throughout adulthood. *ATP1A3* genotype status was significantly associated with shortened QTc by multivariant regression analysis. Two patients with the ATP1A3-D801N variant experienced ventricular fibrillation, resulting in death in 1 patient. Rare variants in *ATP1A3* were identified in a large cohort of genotype-negative patients referred for arrhythmia and sudden unexplained death.

CONCLUSIONS: Patients with AHC who carry the ATP1A3-D801N variant have significantly shorter QTc intervals and an increased likelihood of experiencing bradycardia associated with life-threatening arrhythmias. *ATP1A3* variants may represent an independent cause of sudden unexplained death. Patients with AHC should be evaluated to identify risk of sudden death.

Key Words: AHC ■ alternating hemiplegia of childhood ■ ATP1A3 ■ D801N ■ sudden unexpected death in epilepsy

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CLINICAL PERSPECTIVE

What Is New?

- Patients hosting the D801N variant in *ATP1A3*-encoded sodium-potassium ATPase alpha-3 subunit (ATP1A3) that causes alternating hemiplegia of childhood have an abnormally short QT interval and a predisposition to life-threatening ventricular arrhythmias.

What Are the Clinical Implications?

- The ATP1A3-D801N variant is a susceptibility allele for sudden death in patients with alternating hemiplegia of childhood, and the *ATP1A3* gene may be associated with both sudden unexplained death in epilepsy and sudden cardiac arrest susceptibility.

Nonstandard Abbreviations and Acronyms

AHC	alternating hemiplegia of childhood
ATP1A3	alpha-3 subunit of the sodium-potassium ATPase
ATP1A3-D801N	D801N variant in the alpha-3 subunit of the sodium-potassium ATPase
QTc	corrected QT
SCA	sudden cardiac arrest
SUDEP	sudden unexpected death in epilepsy

The alpha-3 subunit of the sodium-potassium ATPase (ATP1A3), encoded by the gene *ATP1A3,* is responsible for maintaining the resting plasma membrane electrochemical gradient in neurons.[1,2] Genetic variants in *ATP1A3* are associated with several neurological disorders, namely rapid-onset dystonia parkinsonism; alternating hemiplegia of childhood (AHC); and a rare syndrome of cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss.[1–6] Patients diagnosed with AHC experience a spectrum of clinical symptoms, with the disease-defining features being sudden onset of episodes consisting of dystonia, quadriparesis, seizures/seizure-like events, and oculomotor abnormalities before 18 months of age.[1,4–6] Additionally, patients with AHC have an increased risk of mortality of 3.2 deaths per 1000 people per year, which increases to 4.5% by 29 years of age.[7,8]

The increased mortality observed in patients with AHC is attributable to sudden death. Sudden unexpected death in epilepsy (SUDEP) has been observed in association with seizure activity in patients with AHC with concurrent epilepsy.[7–9] SUDEP has also been observed in mouse models of AHC hosting the D801N and E815K variants of the *ATP1A3* gene[10,11]; however, the mechanism remains unknown. A recent study focusing on *ATP1A3*-related syndromes identified cardiac repolarization abnormalities, but the genetic underpinning of this finding remains unknown.[12] One potential explanation for sudden unexplained death in patients with AHC are cardiac arrhythmias, which may be fatal and have been associated with SUDEP in overlapping phenotypes.[13] Although the neurologic impacts of *ATP1A3* variants are well described, the role of *ATP1A3* in the heart is not well described, and the cardiac manifestations of disease-associated variants are not well delineated.

A known risk factor for the development of cardiac arrhythmias and a predisposition to sudden death is abnormal cardiac repolarization. Variants in genes encoding ion channels, which govern both depolarization and repolarization, have been shown to cause sudden death.[14] The rate of repolarization can be measured using the QT interval, with a shortened QT interval reflecting accelerated repolarization. An abnormally short QT interval can predispose individuals to ventricular fibrillation, and therefore, is associated with sudden cardiac death.[14] In this study, we aimed to determine if there is a unifying AHC genotype and phenotype associated with short corrected QT (QTc), and therefore, potentially an increased risk of sudden death. We demonstrate that the ATP1A3-D801N variant is associated with markedly short QTc measurements, which suggests a possible predisposition to lethal ventricular arrhythmias preceded by bradycardia among patients with AHC. This finding may suggest a high-risk genotype among patients with AHC and underscores a critical role for ATP1A3 in maintaining the timing of cardiac repolarization.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Participants

This study was approved by both the Baylor College of Medicine and Duke University School of Medicine institutional review boards, and all participants provided informed consent in accordance with the Declaration of Helsinki. Based on the current consensus on AHC diagnosis,[9] we adopted minimal criteria to define our AHC cohort. Typical patients with AHC were defined as patients who fulfilled the Aicardi criteria, which consists of 4 clinical findings: episodes of

alternating hemiplegia/hemiparesis, dystonia, abnormal eye movements, and epileptic seizures with events of autonomic dysfunction, all of which occur before 18 months of age.[8,15] Intellectual disability is also common. Patients experience dystonic attacks with distinctive notable triggers that can be identified such as heat and stress. Notably, sleep restores functionality following an attack, although patients still demonstrate abnormal movements between attacks.[8] Atypical patients with AHC were defined as patients who did not fulfill the entire Aicardi criteria but possessed enough AHC features to be on the AHC spectrum.[9] These patients had most of the major AHC features and did not fit into one of the other *ATP1A3*-related syndromes.

Inclusion criteria for this study were (1) a diagnosis of typical or atypical AHC, (2) clinical records available at Duke University Hospitals or Texas Children's Hospital, (3) documented informed consent, and (4) availability of clinical genetic test results for the major genes associated with AHC, including *ATP1A3, ATP1A2, CACNA1A, SCN1A,* and *SCN2A*. Patients found to have a pathogenic or likely pathogenic variant in one of these major genes were defined as genotype-positive, whereas patients found to not have a pathogenic/likely pathogenic variant in one of these major genes were defined as genotype-negative. Some patients included in this study did not have each of these major genes fully genotyped. Patients were excluded from analysis if (1) their diagnosis consisted of rapid-onset dystonia parkinsonism or syndrome of cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss; (2) no ECG data were available, or (3) they were unwilling or unable to provide consent for the study.

Study Design

A retrospective chart review of patients meeting the inclusion criteria was conducted. Patient history and family history were obtained. Echocardiographic data, when available, and ECG data were collected. Patients were divided into subgroups consisting of (1) typical AHC versus atypical AHC, (2) genotype-positive versus genotype-negative, and (3) ATP1A3-D801N variant positive versus all other *ATP1A3* variants. For studies of genetic yield, only unrelated probands were used. The primary outcome measure was sudden death or documented ventricular fibrillation. Additional ECG characteristics examined were the QT interval, heart rate, PR duration, QRS duration, P-wave axis, QRS-wave axis, and T-wave axis values between the cohorts. Early repolarization, ST morphology and elevation/depression, as well as the presence of U waves, T-wave alternans, and J-point elevation were also examined. Race and ethnicity were self-reported. Medications at the time of ECG was noted for each patient, with a focus on medications known to modulate the QT interval.

ECG Evaluation

Patients with typical and atypical AHC received ECG and 24-hour Holter monitor evaluation as part of routine care. All ECGs consisted of 12 leads and were recorded using a paper speed of 25 mm/s with an amplitude of 10 mm/mV. For patients with multiple ECGs, the first ECG study was used for all analyses. The parameters evaluated from each ECG were heart rate, PR duration, QRS duration, QT interval, P-wave axis, QRS-wave axis, and T-wave axis. Additionally, the presence of early repolarization and presence of U waves and T-wave alternans was recorded for each patient. The criteria for ST elevation or depression were the presence of elevation or depression in at least 2 leads that measured at least 0.1 mV. The QTc interval was calculated with Bazett's formula ($QTc=QT/RR^{1/2}$)[16] using the manually measured QT interval.[17] The automated QT/QTc calculation was not reported or used in the analysis. The threshold set for shortened QTc interval was <370 milliseconds established by the Gollob criteria.[18] To ensure reproducibility of QTc measurements, 2 pediatric electrophysiologists independently remeasured QT/QTc intervals blinded to clinical status and genotype. Both electrophysiologists manually remeasured the QT/QTc intervals of each ECG used in the study. Twenty ECGs were randomly selected to compare reproducibility of the measurements. The method for QT determination was based on Lepeschkin and Surawicz.[19] Lead II was the preferential lead for remeasurement of QT/QTc, but if the tracing in lead II could not be used for measurement, lead V5 was used. The QT interval was defined as the beginning of the Q wave to the point where a tangent from the steepest part of the T-wave downslope crossed the isoelectric baseline. If present, U waves were excluded. This method is demonstrated in Figure S1.

Cohort of SUDEP and Sudden Cardiac Arrest Referrals

To explore the possibility that *ATP1A3* variants are an independent cause of SUDEP, or sudden cardiac arrest (SCA), or death, we established a cohort using deidentified variant information from the clinical genetic testing company Invitae (San Francisco, CA). A referral database of individuals referred for evaluation of cardiac arrhythmic disease were queried based on the following inclusion criteria: (1) referred to Invitae for clinical genetic testing and (2) referral diagnosis of syncope, seizures, abnormal ECG, abnormal QT interval, SCA, ventricular tachycardia, ventricular fibrillation, or channelopathy. The exclusion criteria were (1) variants determined to be likely benign and benign at the time of genetic testing, (2) individuals with a pathogenic/likely pathogenic variant in another gene

associated with cardiac channelopathies or SCA, (3) cardiomyopathy as the indication for genetic testing, (4) atrial ectopy (premature atrial complex) or ventricular ectopy (premature ventricular complex) as the indication for genetic testing, (5) QT prolongation as the indication for genetic testing, and (6) no clear indication for testing available. All resulting variants that fulfilled inclusion criteria and did not meet exclusion criteria were subjected to the 2015 American College of Medical Genetics and Genomics guidelines[20] used for classifying the pathogenicity of genetic variants.

Statistical Analysis

Descriptive statistics were used to report the characteristics for patients. Values were expressed as mean±standard deviation unless otherwise noted. An independent *t* test and Mann-Whitney test were used to compare parametric and nonparametric demographic data, respectively, between subgroups. An independent *t* test was used to compare mean heart rate, PR duration, QRS duration, QT interval, QTc interval, P-wave axis, QRS-wave axis, and T-wave axis within each subgroup. A multivariate regression analysis was completed using sex, AHC presentation, and *ATP1A3* genotype status as predictors of QTc measurement as the response. Interrater reliability was used to measure agreement between the manually remeasured QT intervals, with 90% agreement being deemed as satisfactory. Agreement was defined as a difference in measurement of ≤10 milliseconds, and disagreement was defined as a difference in measurement of >10 milliseconds. To assess changes in QTc measurements as a function of age, we compared QTc measurements <10 years of age and ≥10 years of age for all individuals and compared several groups including ATP1A3-D801N–positive individuals, *ATP1A3* genotype–positive individuals (excluding ATP1A3-D801N), *ATP1A3* genotype–negative, and atypical AHC.

RESULTS

Cohort Characteristics

A total of 62 individuals met inclusion criteria. Fifty percent were female subjects, the mean age at diagnosis was 2.2±1.4 years, and the mean age at the most recent follow-up was 11.7±9.4 years. One patient with AHC was evaluated at Baylor College of Medicine, and the remaining patients were evaluated at Duke University School of Medicine. Thirty-four individuals were White (55%); 7 were Black (11%); 7 were Asian, multiracial, or Native Hawaiian/Pacific Islander (11%); and 14 patients did not report their race (23%). From a general cardiac perspective, a small minority, 2 of 62 (3%), had structural abnormalities by echocardiogram (1 atrial septal defect and 1 ventricular septal defect). No individuals in the study cohort had atrial arrhythmia, sinus pauses, or atrioventricular block. Furthermore, no individuals in the study had implantation of cardiac pacemakers or implantable cardioverter-defibrillators. No individual underwent electrophysiology study or cardiac ablation, sympathetic denervation, placement of a ventricular assistive device, received extracorporeal membrane oxygenation, or cardiac surgery/transplant. Fifty-six individuals (90%) met the criteria for typical AHC, whereas 6 patients (10%) met the criteria for atypical AHC criteria. Thirty-seven patients (77.1%) were genotype-positive for variants in *ATP1A3*. Of these 37 patients, 9 (24%) carried the D801N variant. There were no statistically significant differences in demographics between any of these subgroups. There was no significantly higher burden of QT modulatory medications or discernable differences among anticonvulsant therapy regimens between these subgroups. The cohort was composed of unrelated probands except for 3 families that included 2 separate pairs of siblings and 1 family consisting of a mother with familial hemiplegic migraine and her 2 children with AHC. These findings are summarized in Table 1 and Figure 1. Representative 12-lead ECGs from patients in the ATP1A3-D801N,

Table 1. Characteristics of the Study Cohorts

	Total cohort	Typical AHC	Genotype-positive	ATP1A3-positive	ATP1A3-D801N
No.	62	56	48	37	9
Sex, % female	50.0%	48.2%	43.8%	46.0%	44.4%
Race, %, White, Black, other*, not reported	W: 54.8%	W: 53.6%	W: 54.2%	W: 54.1%	W: 88.9%
	B:11.3%	B: 12.5%	B: 12.5%	B: 16.2%	B: 0%
	O: 11.3%	O: 12.5%	O: 10.4%	O: 8.1%	O: 0%
	NR: 22.6%	NR: 21.4%	NR: 23%	NR: 21.6%	NR: 11.1%
Mean age at diagnosis. y	2.2	2.3	2.1	2.1	2.2
Mean age at most recent follow-up, y	11.77 (1.2–46)	11.69 (1.2–46)	12.20 (1.2–46)	11.98 (1.2–46)	15.62 (5–46)
Experienced ventricular fibrillation, %†	3.2%	3.8%	4.2%	5.4%	22.2%
Alive at most recent follow-up, %	98.4%	98.2%	98%	97.3%	88.9%

ATP1A3 indicates the alpha-3 subunit of the sodium-potassium ATPase; ATP1A3-D801N, indicates the D801N variant in the alpha-3 subunit of the sodium-potassium ATPase; B indicates Black; NR, not reported; O, other; and W, White.

*Races in the other category include Asian, multiracial, and Native Hawaiian/Pacific Islander.

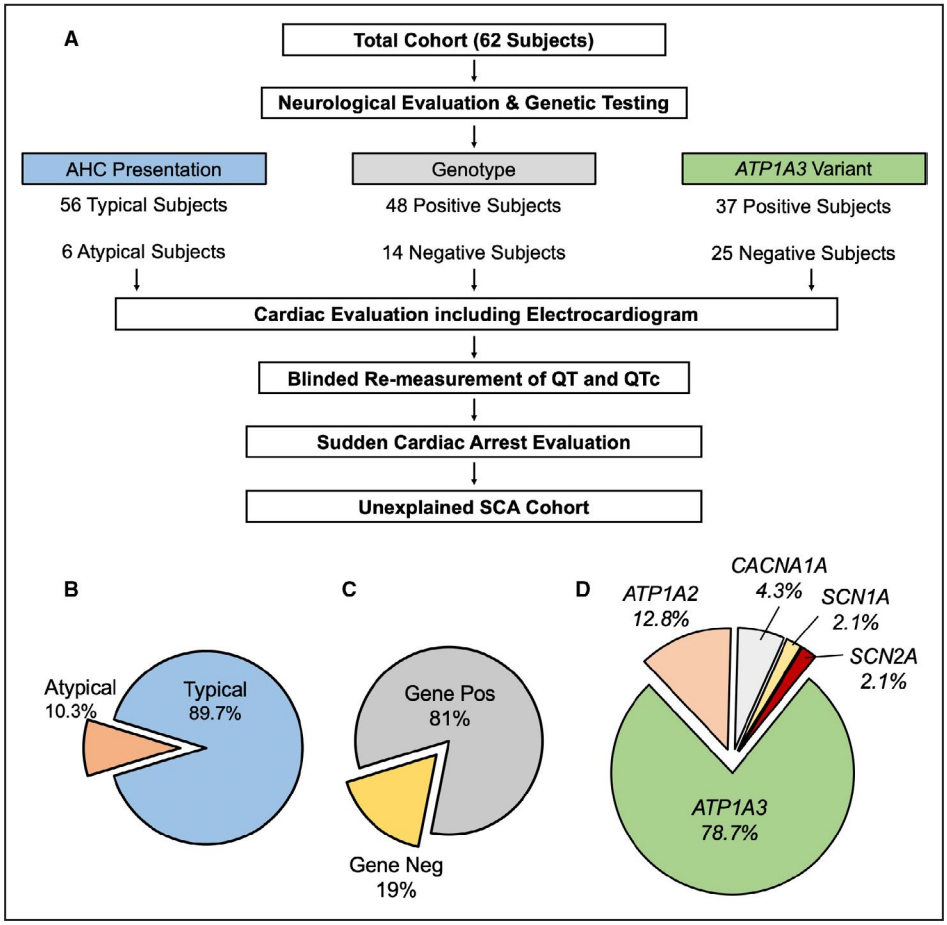

Figure 1. Study design and genetic distribution of subjects involved in the study.
A, Flowchart showing the design of this study and the distribution of subgroups used for analysis. Chart representing the study subgroups. **B**, Pie chart showing distribution of typical vs atypical alternating hemiplegia of childhood (AHC) in the study cohort. **C**, Genotype-positive vs genotype-negative individuals. **D**, Genes positive by panel genetic testing. Numbers represent the proportion of individuals present within each group among unrelated probands. Gene Neg indicates genotype-negative; Gene Pos, genotype-positive; QTc, corrected QT; and SCA, sudden cardiac arrest.

ATP1A3-E815K, and *ATP1A3* genotype–negative cohorts are found in Figures S2 through S4. Although the ATP1A3-D801N variant was the most common, several other variants were identified, including G89D (4/37), E815K (5/37), and G947R (1/37), among others (Table S1).

ATP1A3-D801N Genotype Is Associated With a Shorter QTc Interval

Given the known incidence of SUDEP in patients with AHC, we investigated cardiac repolarization in our patient cohort. The interrater reliability between the 2 electrophysiologists for manually remeasuring the QT intervals was confirmed to be 90%. Comparison of QTc measurements among patients with typical AHC versus atypical AHC revealed that those with typical AHC had a shorter mean QTc; however, this difference did not meet statistical significance (387.4±4.6 milliseconds; 95% CI, 378.2–396.5 versus 400.8±15.3 milliseconds; 95% CI, 361.6–440.1, respectively; P=0.36). *ATP1A3* genotype–positive individuals had a significantly shorter QTc measurement when compared with genotype-negative individuals (375.4±5.6 milliseconds; 95% CI, 363.9–386.8 versus 408.4±4.8 milliseconds; 95% CI, 398.4–418.4; P<0.001). This reduction was driven by significantly shorter mean QTc measurements among subjects hosting the ATP1A3-D801N variant when compared with subjects with all other *ATP1A3* variants (344.9±7.0 milliseconds; 95% CI, 328.7–361.1 versus 385.1±6.0 milliseconds; 95% CI, 372.8–397.5; P<0.001). Furthermore, D801N variant–positive individuals had a shorter QT compared with *ATP1A3* genotype–negative individuals (408.4±4.8 milliseconds; P<0.001).

The QTc of male subjects versus female subjects in the cohorts was analyzed. Within the ATP1A3-D801N cohort, there was a statistically significant difference in QTc, with male subjects demonstrating a higher mean QTc of 357.6±5.4 (95% CI, 342.7–372.5) compared with female subjects , with a mean QTc of 329.0±9.9 (95% CI, 297.6–360.5; *P*=0.025). Conversely, there were no sex-based difference in the *ATP1A3* genotype–positive cohort or the *ATP1A3* genotype–negative cohort.

We next compared the ATP1A3-D801N QTc with the QTc of individuals hosting other *ATP1A3* variants. The ATP1A3-E815K variant is associated with more severe neurologic involvement,[21] yet in our cohort, this group had a significantly longer QTc compared with D801N QTc (398.8±7.9 milliseconds; 95% CI, 376.8–420.8; *P*<0.001). There was no significant difference in the QTc between patients with the E815K variant and all other genotype-positive patients who did not have the D801N variant (398.8±7.9 milliseconds versus 391.9±34.6 milliseconds; 95% CI, 379.8–404; *P*=0.25). Furthermore, the ATP1A3-G89D variant QTc was similar to E815K and was longer than D801N (409±5.9 milliseconds; 95% CI, 383.8–434.2; *P*=0.47). None of these differences were because of demographic differences such as age, sex, and race between each of the comparison groups. These results are summarized in Figure 2. This association was similar when comparing QTc intervals between subgroups (Figure S5). We next completed a multivariate regression analysis to determine the relationship between sex, AHC presentation, and *ATP1A3* genotype (predictors) to QTc measurement (response). *ATP1A3* genotype status was significantly associated with QTc measurement (*P*<0.001). Patients with a positive *ATP1A3* genotype have a 31.9-millisecond decrease in QTc as compared with those with *ATP1A3* genotype–negative status when accounting for sex (slope: 0.7 milliseconds, *P*=0.931) and AHC presentation (slope: 4.3, *P*=0.76). Overall, these findings suggest the ATP1A3-D801N variant is associated with an abnormally short QT interval (QTc ~345 milliseconds) when compared with other *ATP1A3* variants.

ATP1A3 Genotype Is Not Associated With Other Significant ECG Parameter Changes

To determine whether repolarization was the only electrocardiographic parameter impacted by the *ATP1A3* genotype, we compared other ECG measurements among subgroups. The QRS duration did not significantly differ between any of the groups. Individuals with typical AHC had a slightly left-shifted QRS axis compared with those with atypical AHC (63.1±3.6 versus 80.7±7.3; *P*=0.03). There were no other differences in ECG parameters. Additionally, when comparing the ECG parameters of patients who were genotype-positive with those who were genotype-negative, there were no significant differences in any of the examined ECG parameters. Finally, when the *ATP1A3* genotype–positive individuals were

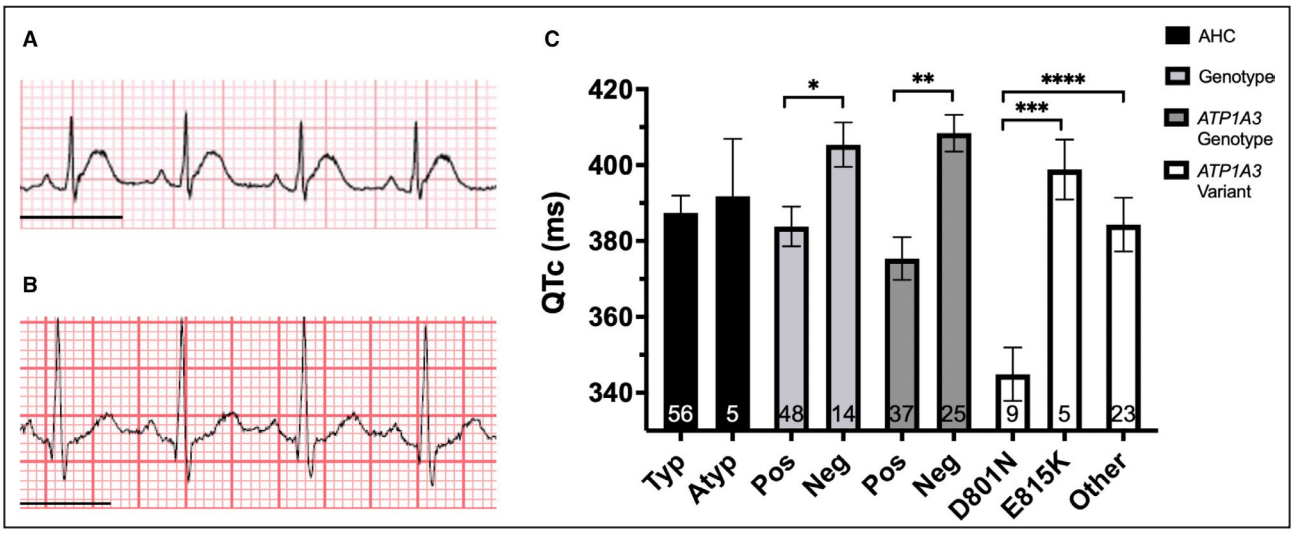

Figure 2. Comparison of corrected QT (QTc) measurements between study subgroups separated by genotype.
A, ECG of an 8-month-old girl who carries the ATP1A3-D801N variant; micron bar represents 0.4 seconds. **B**, ECG of a 16-year-old boy who is *ATP1A3* genotype-negative, typical alternating hemiplegia of childhood (AHC); micron bar represents 0.4 seconds. **C**, Chart with QTc (mean±SEM) for each study subgroup, including AHC (typical vs atypical, black fill), genotype (positive vs negative, dark gray fill), *ATP1A3* genotype (positive vs negative, light gray fill), and ATP1A3 variant (D801N variant vs E815K and other variants, white fill). Examples from lead II of ECGs for an ATP1A3-D801N female patient and an *ATP1A3* genotype-negative female patient are provided for comparison of QT/QTc measurements. Numbers on the bar graphs represent the total number of QTc measurements from each patient within the cohort. **P*=0.034. ***P*<0.001. ****P*<0.001. *****P*<0.001. ATP1A3 indicates alpha-3 subunit of the sodium-potassium ATPase; ATP1A3-D801N, D801N variant in the alpha-3 subunit of the sodium-potassium ATPase; Atyp, atypical AHC presentation; Neg, negative genotype; Pos, positive genotype; and Typ, typical AHC presentation.

compared with *ATP1A3* genotype–negative individuals, there were no significant differences in any ECG parameters except a modest change in P-wave axis (54.6±2.3 versus 43.3±4.3; *P*=0.01).

Other repolarization characteristics were assessed and were rarely abnormal without a clear association with genotype. Marked J-point elevation was not seen. Mild ST elevation, which was consistent with early repolarization, was occasionally observed, most often with 1-mV elevation in the anterolateral leads. Specifically, 2 out of the 9 patients (22%) with the ATP1A3-D801N variant were found to have early repolarization in the anterolateral leads. When analyzing the inferior leads, 1 out of 9 patients (11%) with the ATP1A3-D801N variant were found to have early repolarization. None of these patients had early repolarization in the septal leads. No septal lead J-point elevation was observed in general. Abnormal T-wave morphology was rare and found in 3 patients (4.8%) who demonstrated isoelectric T waves in inferior and anterolateral leads. U waves were not a common feature and were observed in 2 patients (3.2%). A summary of the ECG parameters can be found in Table 2. Overall, these findings suggest that repolarization timing (QT/QTc) is the predominant ECG difference when comparing AHC and *ATP1A3* status.

ATP1A3-D801N–Associated QTc Shortening Persists Into Adulthood

To determine whether QTc increases with age in patients with AHC, in particular those hosting the D801N variant, we compared QTc versus age at time of ECG study. The mean QTc for ATP1A3-D801N individuals was 344.9 milliseconds (95% CI, 328.7–361.1) and did not increase significantly based on age. The QTc measurements remained <370 milliseconds throughout childhood and adulthood (age range, 0.87–43 years) with no significant difference between mean QTc <10 and ≥10 years of age. In contrast, 7 out of 28 patients with *ATP1A3* genotype–positive not hosting the D801N variant demonstrated a shortened QTc <10 years of age, which increased with age (mean, 385.1 milliseconds; 95% CI, 372.8–397.5). Conversely, the vast majority of patients who were genotype-negative and had atypical AHC did not have short QTc measurements during their lifespan (mean, 408.4 milliseconds;

Table 2. Summary of ECG Parameters by Cohort and Genotype

	Total cohort	Typical AHC	Genotype-positive	ATP1A3-positive	ATP1A3-D801N
No.	62	56	48	37	9
Average heart rate, bpm	101.0±2.4	101.2±2.6	101.2±2.9	100.7±3.4	98.8±8.4
QT interval, ms	303.1±4.5	303.1±4.8	300.9±5.5	294.7±6.3	275.8±13.1
QTc measurement, ms	388.7±4.4	387.4±4.6	383.8±5.2	375.4±5.6	344.9±7.0
Average PR, ms	128.0±2.4	128.5±2.5	128.1±2.7	128.6±3.1	128.0±7.7
Average QRS, ms	74.5±2.0	74.8±2.2	74.8±2.5	74.4±3.1	82.4±10.5
Average P-wave axis	50.1±2.3	51.9±2.1	51.0±2.2	54.6±2.3	56.0±4.0
Average QRS-wave axis	64.7±3.3	63.1±3.6	66.0±4.0	63.9±4.8	73.4±5.5
Average T-wave axis	38.3±3.1	37.8±3.4	37.6±3.7	36.4±4.4	39.7±5.5
ST elevation, %	I: 8%, 0.14 mV	I: 9%, 0.14 mV	I: 10%, 0.14 mV	I: 11%, 0.15 mV	I: 11%, 0.2 mV
	A: 14.5%, 0.13 mV	A: 16%, 0.13 mV	A: 17%, 0.14 mV	A: 19%, 0.14 mV	A: 22%, 0.15 mV
	S: 3.2%, 0.1 mV	S: 3.6%, 0.15 mV	S: 4.2%, 0.15 mV	S: 5.4%, 0.15 mV	S: 0%
ST depression, %	I: 1.6%, 0.1 mV	I: 1.8%, 0.1 mV	I: 2%, 0.1 mV	I: 0%	I: 0%
	A: 1.6%, 0.1 mV	A: 1.8%, 0.1 mV	A: 2.1%, 0.1 mV	A: 0%	A: 0%
	S: 3.2%, 0.1 mV	S: 3.6%, 0.1 mV	S: 4.2%, 0.1 mV	S: 5.4%, 0.1 mV	S: 11%, 0.1 mV
Inverted ST segment, %	I: 3.2%	I: 1.8%	I: 2.1%	I: 2.7%	I: 0%
	A: 4.8%	A: 3.6%	A: 4.2%	A: 5.4%	A: 0%
	S: 1.6%	S: 1.8%	S: 2.1%	S: 2.7%	S: 0%
Isoelectric ST segment, %	I: 4.8%	I: 5.4%	I: 6.3%	I: 8.1%	I: 11%
	A: 4.8%	A: 5.4%	A: 6.3%	A: 8.1%	A: 11%
	S: 1.6%	S: 1.8%	S: 2.1%	S: 2.7%	S: 0%
Presence of U waves, %	3.2%	1.8%	4.2%	2.7%	0%
Presence of J points, %	1.6%	1.8%	2.1%	2.7%	11%
Presence of T-wave alternans	Not present	Not present	Not present	Not present	Not present

Voltage measurements are averages. Values are average±standard error of the mean unless indicated otherwise. A indicates anterolateral lead; AHC, alternating hemiplegia of childhood; ATP1A3, inidcates the alpha-3 subunit of the sodium-potassium ATPase; ATP1A3-D801N, indicates the D801N variant in the alpha-3 subunit of the sodium-potassium ATPase; I, inferior lead; and S, septal lead.

95% CI, 398.4–418.4; mean, 413.6 milliseconds; 95% CI, 392.9–434.2, respectively). These results are summarized in Figure 3. These findings suggest that patients with the ATP1A3-D801N variant have a lifelong shortened QTc. Furthermore, some patients who carry *ATP1A3* variants other than D801N may be predisposed to a shortened QTc in early childhood, specifically.

ATP1A3-D801N Predisposes to Ventricular Fibrillation With Sedation

Given the association between AHC and SUDEP, and our observation of a markedly reduced QTc in patients with the D801N variant, we next sought to determine whether QT shortening was associated with documented ventricular arrhythmic events. Two patients in our cohort with the ATP1A3-D801N variant and typical AHC experienced documented recalcitrant ventricular fibrillation, which resulted in fatal cardiac arrest for one of these patients.

The first patient was a 13.8-year-old boy who was in his usual state of health when awakening from general anesthesia, using inhaled sevoflurane, for brain magnetic resonance imaging, as is routine at our institution. He developed significant bradycardia that was treated with glycopyrrolate, as well as atropine, which increased his heart rate from 30 beats per minute (bpm) 130 bpm . Subsequently, he became restless and was given 2 incremental doses of dexmedetomidine. Seizure-like movements and irregular breathing were noted 3 to 4 minutes later. Because of concern for a seizure, midazolam was given, at which time the patient became pale and appeared cyanotic. Concordantly, he was noted to have no carotid or femoral pulse; therefore, cardiopulmonary resuscitation was initiated. Epinephrine was given, spontaneous circulation briefly returned, and he was endotracheally intubated. Cardiopulmonary resuscitation was initiated again because he was noted to have ventricular fibrillation on the monitor. Given that a secure airway had been established and he was adequately ventilated, it seemed plausible to rule out hypoxia as the cause for any bradycardia leading to subsequent arrhythmia. He was defibrillated 4 times before sinus rhythm was noted and maintained. During the cardiopulmonary resuscitation, he was given epinephrine 3 times, lidocaine, and sodium bicarbonate. Before the arrest, his ECG demonstrated a QTc measurement of 364 milliseconds. He had no prior episodes of unexplained syncope or documented arrhythmia. Following his arrest, the ECG demonstrated a QTc of 416 milliseconds which lengthened to 467 milliseconds in follow-up 10 days after the event before subsequently normalizing.

The second patient was a 4.5-year-old girl who was admitted to the hospital because of status epilepticus. She was intubated and sedated using dexmedetomidine, fentanyl, hydromorphone, midazolam, and chloral hydrate, and was in a nonepileptic state. Before the arrest, she experienced 2 episodes of bradycardia marked by a heart rate of approximately 40 bpm. Subsequently, she developed

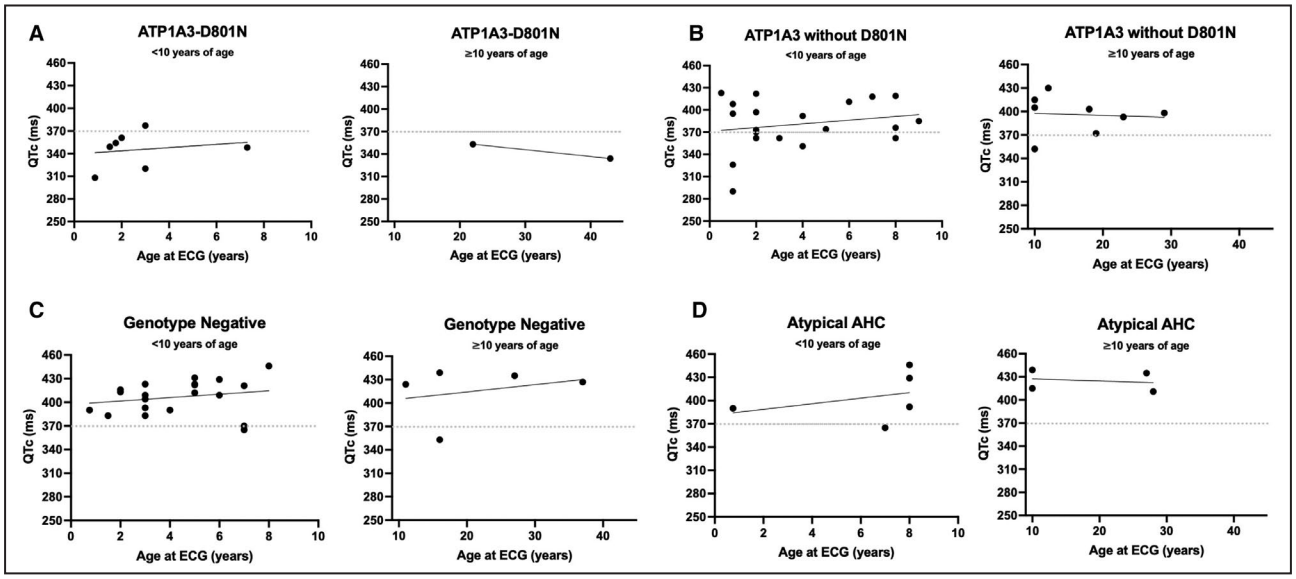

Figure 3. Scatter plots of corrected QT (QTc) (ms) as a function of age and linear regression model for (**A**) ATP1A3-D801N–positive individuals separated by <10 years of age and ≥10 years of age, (**B**) *ATP1A3*-positive individuals excluding those with the D801N variant separated by <10 years of age and ≥10 years of age, (**C**) genotype-negative individuals separated by <10 years of age and ≥10 years of age, and (**D**) individuals with atypical alternating hemiplegia of childhood (AHC) separated by <10 years of age and ≥10 years of age. Light gray line denotes 370-milliseconds threshold. ATP1A3 indicates alpha-3 subunit of the sodium-potassium ATPase; and ATP1A3-D801N, D801N variant in the alpha-3 subunit of the sodium-potassium ATPase.

ventricular fibrillation refractory to multiple defibrillations and administration of epinephrine, atropine, lidocaine, amiodarone, lidocaine, calcium, magnesium, and sodium bicarbonate. She ultimately died because of refractory ventricular fibrillation. Of note, her ECG at 8 months of age demonstrated a QTc value of 308 milliseconds. Immediately after arrest and before her death, her ECG demonstrated a QTc value of 514 milliseconds.

Bradycardia occurring just before arrest was a feature common in both of these patients. Both patients who arrested experienced QTc prolongation following the arrest. This finding is nonspecific to this patient population and is a well-documented occurrence following cardiac arrest.[22–24] No other subjects in our cohort experienced a documented ventricular arrhythmia, sudden death, or mortality. An ECG of the second patient before her arrest and cardiac telemetry during the arrest demonstrating ventricular tachycardia/ventricular fibrillation can be found in Figure S6. ECGs of the first patient at a time period before his arrest and following his arrest can be found in Figure S7. Interestingly, there was no prior history of syncope in the 2 individuals who experienced ventricular fibrillation, and only 1 individual out of 62 individuals in the entire cohort (2%) had a history of syncope or nonictal loss of consciousness. The individual with a history of syncope carried the ATP1A3-E815K variant and had typical AHC. Overall, these findings suggest that patients with the ATP1A3-D801N variant have an increased susceptibility to potentially fatal arrhythmias. Furthermore, because both patients who developed ventricular fibrillation lacked a history of syncope, the only indicator of a potential arrhythmia in the setting of sedation/general anesthesia may be bradycardia. This raises the possibility that risk of fatal arrhythmia is highest when sedation and bradycardia are present in individuals with shortened QTc at baseline (Figure 4).

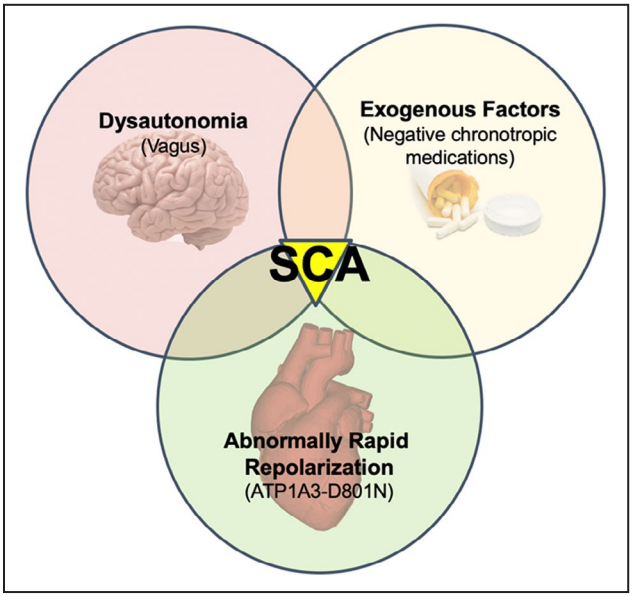

Figure 4. Venn diagram detailing the multifactorial contributions to sudden cardiac arrest (SCA) in patients carrying ATP1A3 variants.
The upper left circle defines the central nervous system factors contributing to SCA such as the vagus nerve contributing to bradycardia. The upper right circle defines exogenous factors contributing to SCA such as medications. The lower circle represents the cardiac factors that contribute to the development of SCA such as dysfunction of cardiac myocytes. ATP1A3 indicates alpha-3 subunit of the sodium-potassium ATPase; and ATP1A3-D801N, D801N variant in the alpha-3 subunit of the sodium-potassium ATPase.

ATP1A3 Variants Are Associated With Sudden Death and Sudden Cardiac Arrest

To examine if *ATP1A3* variants are independently associated with sudden death or SCA, we used a cohort consisting of 6437 individuals referred for cardiovascular disease or SUDEP genetic testing. Among these, 8 individuals (0.12%) carried rare variants found in *ATP1A3* that were otherwise negative for pathologic or likely pathologic variants in known sudden death or SCA genes (Table 3). All variants were classified as variants of uncertain significance at the time of genetic testing. Furthermore, when applying the 2015 American College of Medical Genetics and Genomics criteria to these variants, they were confirmed to be variants of uncertain significance. Within this group of 8 individuals, there were 3 broad categories of indications for genetic testing: an abnormal ECG/QT interval; possible channelopathy; or SCA, ventricular fibrillation, and ventricular tachycardia. A total of 5 out of 8 (62.5%) of these individuals had a referral indication of SCA or ventricular arrhythmia. Two of these 5 individuals had a reported positive family history of SCA. With this cohort of genotype-negative individuals who experienced sudden unexplained death, these findings suggest that *ATP1A3* variants may be associated with SCA and lethal ventricular arrhythmias with a less-pronounced neurological phenotype.

DISCUSSION

Assessing the underlying clinical phenotype of patients with AHC is critical, because this population is at increased risk for SUDEP; however, the underlying cause of the susceptibility to sudden death is not fully understood. This poses challenges in attempting to identify both individuals who are at risk of sudden death and exogenous risk factors that may place these individuals at even higher risk. Although our study contained one of the largest cohorts of patients with AHC and concurrent *ATP1A3* variants with ECG data available, AHC is a rare condition. The exact prevalence

Table 3. Characteristics of Genotype-Negative Sudden Unexplained Death Cohort

	Sex	Race/ethnicity	Age at time of genetic testing, y	Indication, abnormal ECG/QT, channelopathy, SCA/VT/VF	Family history
c.46G>A (Gly16Ser)	Male	Hispanic	46	Channelopathy (Brugada type 1)	None
c.1472A>G (Asn491Ser)	Male	White	42	SCA (VF followed by arrest at age 32 y)	None
c.2638T>C (Trp880Arg)	Female	White	37	SCA (irregular polymorphic VT)	None
c.1282G>A (Asp428Asn)	Female	White	24	SCA	None
c.2095-5_2095-4insTACCCTGCACCCT, intron variant	Male	White	69	SCA	SCD in father, PGF, MGM; brother bradycardia
c.1472A>G (Asn491Ser)	Male	Black	14	Channelopathy (CPVT)	None
c.2885C>A (Pro962His)	Male	White	18	SCA (VF followed by arrest)	SCD in MGM siblings/children
c.1312G>A (Ala438Thr)	Female	White	53	Channelopathy (CPVT)	None

CPVT indicates catecholaminergic polymorphic ventricular tachycardia; MGM, maternal grandmother; PGF, paternal grandfather; SCA, sudden cardiac arrest; SCD, sudden cardiac death; VF, ventricular fibrillation; and VT, ventricular tachycardia.

has been reported to be approximately 1:100 000 children,[25] therefore, we used a retrospective design in our study. We acknowledge that the necessary retrospective nature of this study means that the findings reported are associative.

In the present study, we found that in patients with AHC who are *ATP1A3* genotype–positive, and in particular those with the D801N variant, the timing of cardiac repolarization is affected as evidenced by a shorter QT interval compared with others in the cohort. We also showed that in patients with the D801N variant, the QTc was pathologically short, which suggests that these patients may have a predisposition to bradycardia causing potentially fatal ventricular arrhythmias. Our results raise the possibility that the cause of SUDEP among patients with AHC is because of abnormal repolarization and arrhythmia. A minority of patients with the ATP1A3-D801N variant displayed early repolarization, especially in the anterolateral leads, and the clinical relevance of this remains unknown because it is a common feature in pediatric patients in the setting of normal repolarization times.[26–29] Furthermore, our analysis of QTc measurements as a function of age revealed that patients with the ATP1A3-D801N variant have shortened QTc measurements that persist through childhood and adulthood. Although patients with other *ATP1A3* variants displayed shortened QTc measurements in childhood, this propensity normalized with increasing age. The definition for short QTc adopted in this study comes from the proposed diagnostic criteria report by Gollob et al, which suggests that a QTc measurement <370 milliseconds can be pathologic.[18]

These data were further supported by patient survival, which suggested that individuals who experience ventricular fibrillation may have a series of risk factors present just before the arrest, including (1) a susceptible genotype, (2) a shortened QTc measurement, and (3) a sedation-induced bradycardia. Collectively, these findings suggest that patients with the ATP1A3-D801N variant may be predisposed to SUDEP because of shortened QTc measurements present throughout childhood and adulthood, particularly during times of anesthesia/sedation. Further investigation is needed to validate this finding and to determine the mechanism of disease, which can provide insight into actions and medications that should be included or omitted during necessary procedures for these patients.

One study utilizing an AHC registry documented 3 patients with AHC (D801N, P808L, and G947R variants) succumbing to sudden death because of cardiac dysfunction, and noted that their autopsy findings consisted of gross cardiology alterations.[2] One of the 3 patients in that study who experienced sudden death carried the ATP1A3-D801N variant. This supports the understanding that patients with the ATP1A3-D801N variant are more likely to experience sudden death,

possibly resulting from a shortened QT interval, and potentially also gross cardiac repolarization abnormalities that may increase the likelihood of a fatal cardiac arrhythmia. Additionally, one study analyzed 52 ECGs of an age-matched control group of patients with epilepsy to patients with AHC. They found that patients with AHC had significantly more repolarization abnormalities as well as a shorter mean QT interval,[30,31] specifically in patients with the ATP1A3-D801N variant.[31] This finding is supported by the results of our study showing that patients with the ATP1A3-D801N variant with typical AHC presentation are more likely to experience ECG abnormalities and have a shorter QTc. An additional alternative hypothesis not explored in this study is that individuals with *ATP1A3* variants may be predisposed to apnea rather than cardiac arrhythmias.[32] Sleep apnea has been documented as a risk factor for coronary events and cardiovascular death[33,34]; however, a link, if any, to cardiac disease remains unclear. It is critical to understand that SCA is multifactorial and can be attributable to many causes as outlined in Figure 4.

Abnormalities in cardiac electrophysiology have been scarcely reported in patients with AHC and are rare in patients with rapid-onset dystonia parkinsonism or syndrome of cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss. Previous work in principally neuronal models has revealed that differing protein variants in the alpha-3 subunit of the *ATP1A3* gene that result in the syndrome of cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss; rapid-onset dystonia parkinsonism; and AHC cause specific disruptions that cause reduced affinity at the cytoplasmic surface, widely distributed protein dysfunction, or dysfunction at the cytoplasmic surface in the transmembrane domain, respectively.[30,31,35–39] Although ATP1A3-D801N is associated with a mild–moderate form of AHC from a neurological perspective,[21,40] it has been implicated in more severe cardiac phenotypes.[2,31] Studies focusing on the D801N variant show through homology modeling that the D801N variant creates a positive dipole which consequently affects the flow of potassium through the pump via electrostatic repulsion[41] and reduced sodium-potassium ATPase activity and pump current in mammalian cells.[42–44] However, there remains a paucity of studies using cardiac myocytes and the mechanism of short-QT pathogenesis in the setting of ATP1A3-D801N, and the role of *ATP1A3* in cardiac repolarization more broadly, remain open questions.

Limitations

The present study has several identifiable limitations. The study is a single-center experience with a retrospective design that should be validated independently in other centers and prospectively, if possible. Furthermore, the link between ATP-D801N and short QT is associative and should be evaluated in experimental models to clarify the mechanism. Although there was no difference in the presence of QT modulatory medications between subgroups, polypharmacy is common in this cohort with multiple medical disabilities. Thus, we are not able to exclude drug interactions or the impact of potential drug effects on the cardiac phenotypes noted for different genetic substrates. Furthermore, the use of a genotype-negative sudden unexplained death cohort consisting of patients who experienced SCA and possess *ATP1A3* variants is limited by lack of detailed clinical information confirming short QT in ECGs. It is also limited by the constraint that *ATP1A3* as a gene is still under investigation for its role in cardiac disease. Therefore, applying the American College of Medical Genetics and Genomics criteria results in these variants satisfying the criteria for variant of unknown significance, because the ACMG criteria are based on the a priori association of a certain gene with a disease. Nonetheless, this genotype-negative sudden unexplained death cohort provides important information that contributes to the hypothesis that *ATP1A3* variants may be independently associated with SCA. Despite these limitations, our study provides important insights into the underlying pathophysiology associated with SUDEP in patients with AHC.

CONCLUSIONS

In this study, we report that patients who are *ATP1A3* genotype–positive, particularly patients with the ATP1A3-D801N variant, consistently experience short-QTc measurements regardless of age. Also reported is that these persistent repolarization abnormalities may be associated with an increased predisposition to bradycardia, which can cause life-threatening cardiac arrhythmias. It is crucial to monitor cardiac parameters in this patient population to reduce the likelihood of a fatal cardiac event.

ARTICLE INFORMATION

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Disclosures

M.A.M. has a provisional patent for treatment of AHC. J.G., R.N., R.T., T.E.C. received salary from and are stockholders of Invitae. The remaining authors have no disclosures to report.

Supplementary Material

Table S1
Figure S1–S7

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SUPPLEMENTAL MATERIAL

SUPPLEMENTAL TABLES

Table S1. Spectrum and prevalence of *ATP1A3* variants

ATP1A3 Variant Positive		*37/56 (66%)*
	D801N	9/37 (24%)
	E815K	5/37 (13.5%)
	G89D	4/37 (0.11%)
	G947R	1/37 (0.03%)
	E324G	1/37 (0.03%)
	P336S	1/37 (0.03%)
	Y768H	1/37 (0.03%)
	E818K	1/37 (0.03%)
	G371S	1/37 (0.03%)
	R463C	1/37 (0.03%)
	V589F	1/37 (0.03%)
	A681T	1/37 (0.03%)
	V129E	1/37 (0.03%)
	F857del	1/37 (0.03%)
	T360R	1/37 (0.03%)
	D801H	1/37 (0.03%)
	A333T	1/37 (0.03%)
	D923Y	1/37 (0.03%)
	L326R	1/37 (0.03%)
	L839P	1/37 (0.03%)
	P323S	1/37 (0.03%)
	G755C	1/37 (0.03%)

SUPPLEMENTAL FIGURES

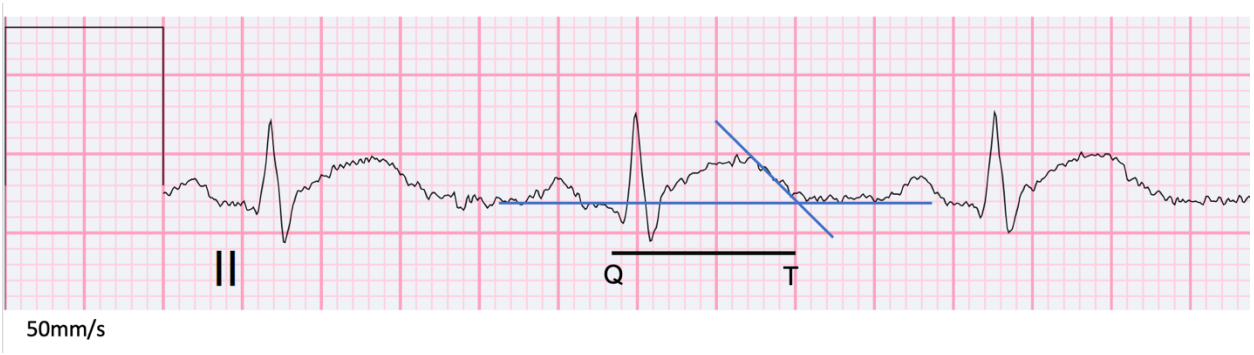

Figure S1: Demonstration of Lepeschkin's method to determine QT interval at paper speed of 50mm/second.

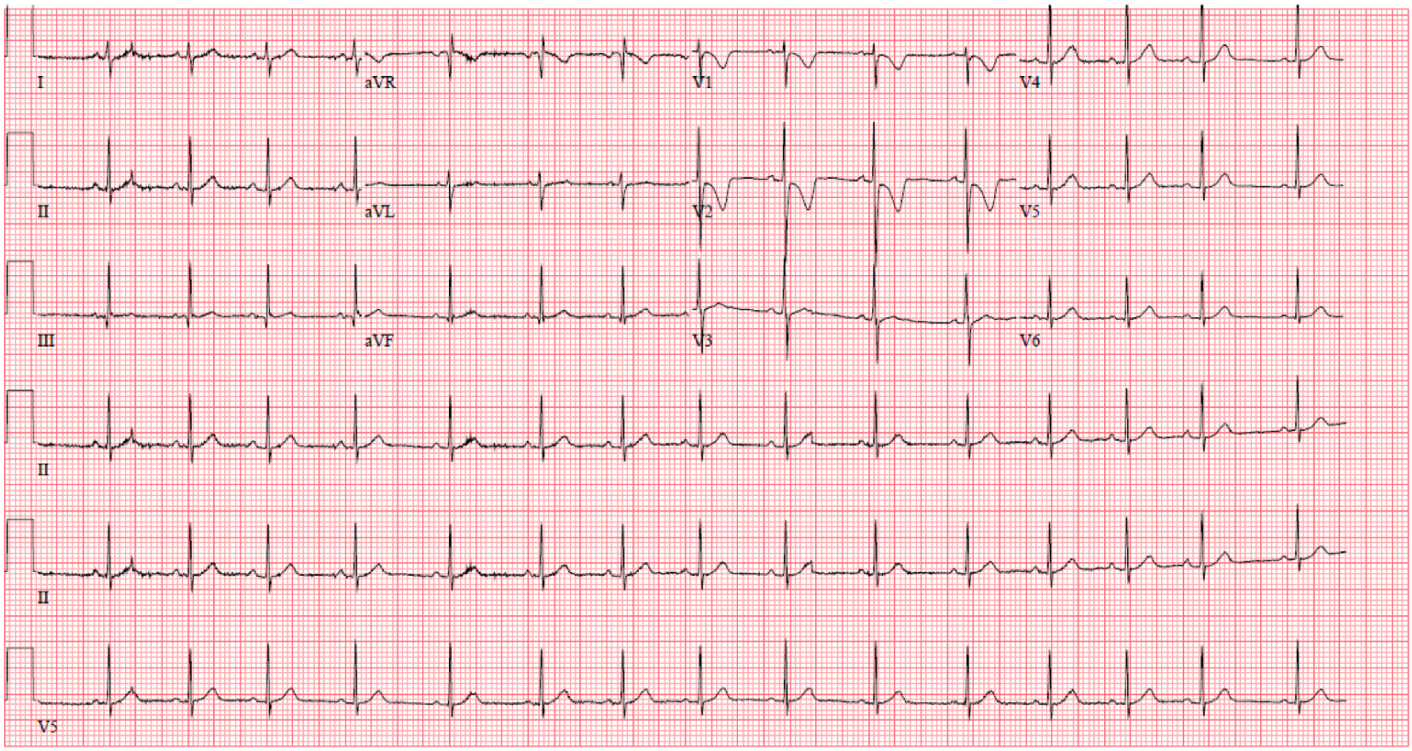

Figure S2: Representative 12 lead ECG from subject hosting the ATP1A3-D801N variant.

Paper speed of 25 mm/sec.

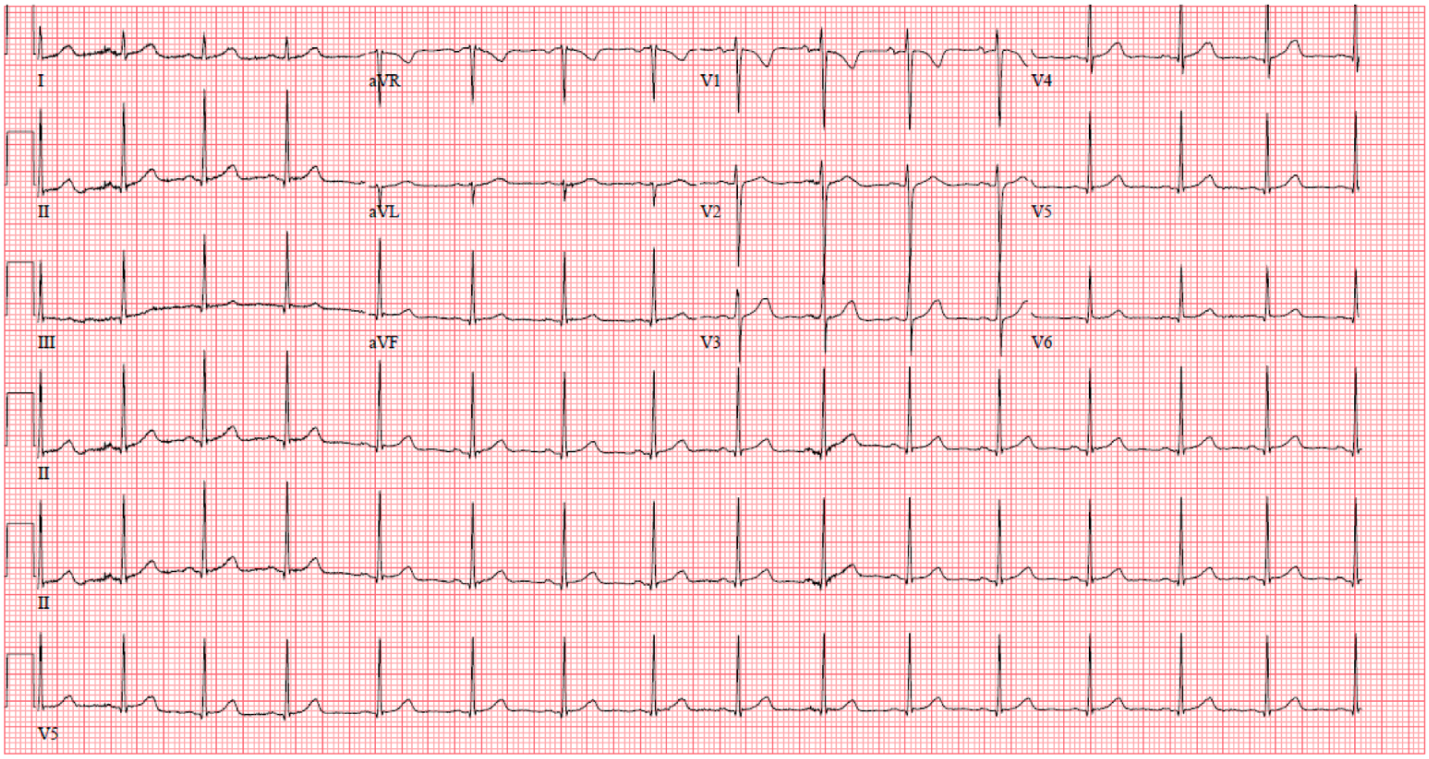

Figure S3: Representative 12 lead ECG from subject hosting the ATP1A3-E815K variant.

Paper speed of 25 mm/sec.

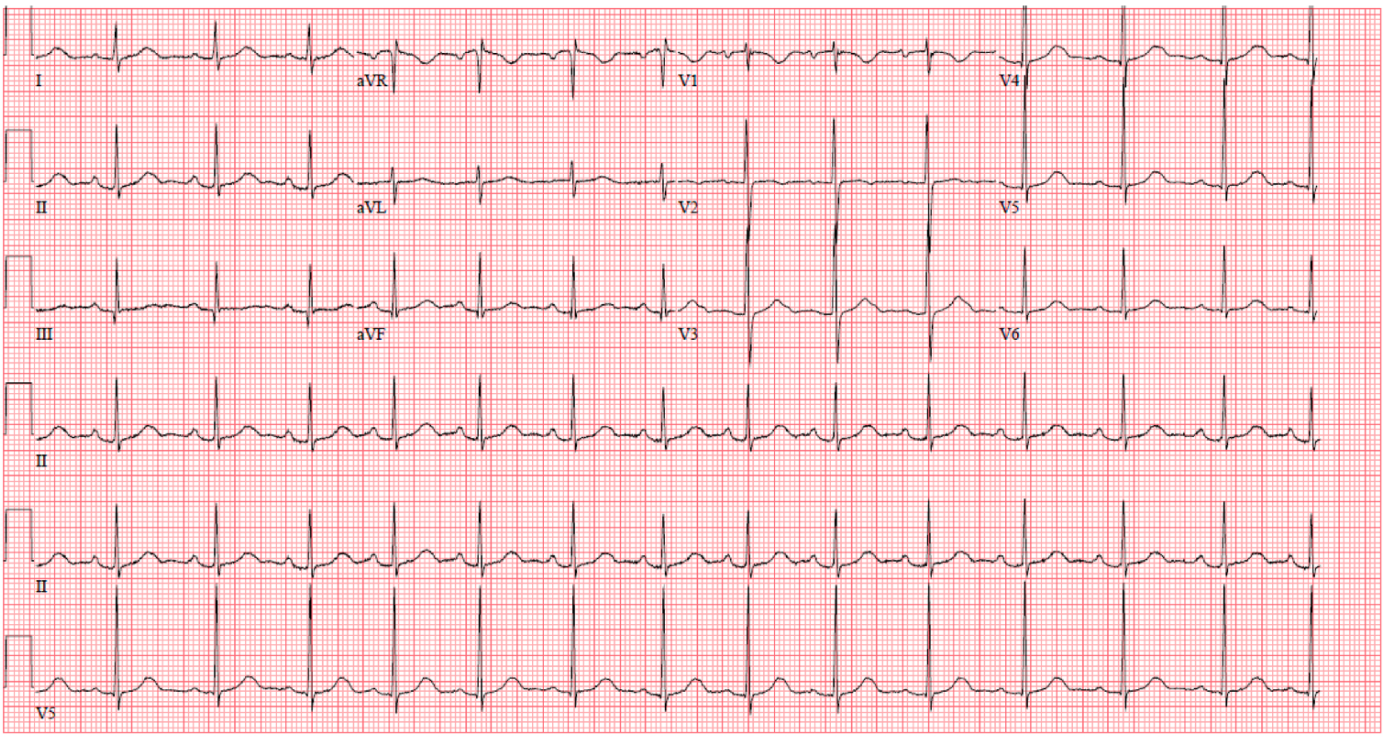

Figure S4: Representative 12 lead ECG from subject meeting criteria for AHC who is genotype-negative for variants in ATP1A3. Paper speed of 25 mm/sec.

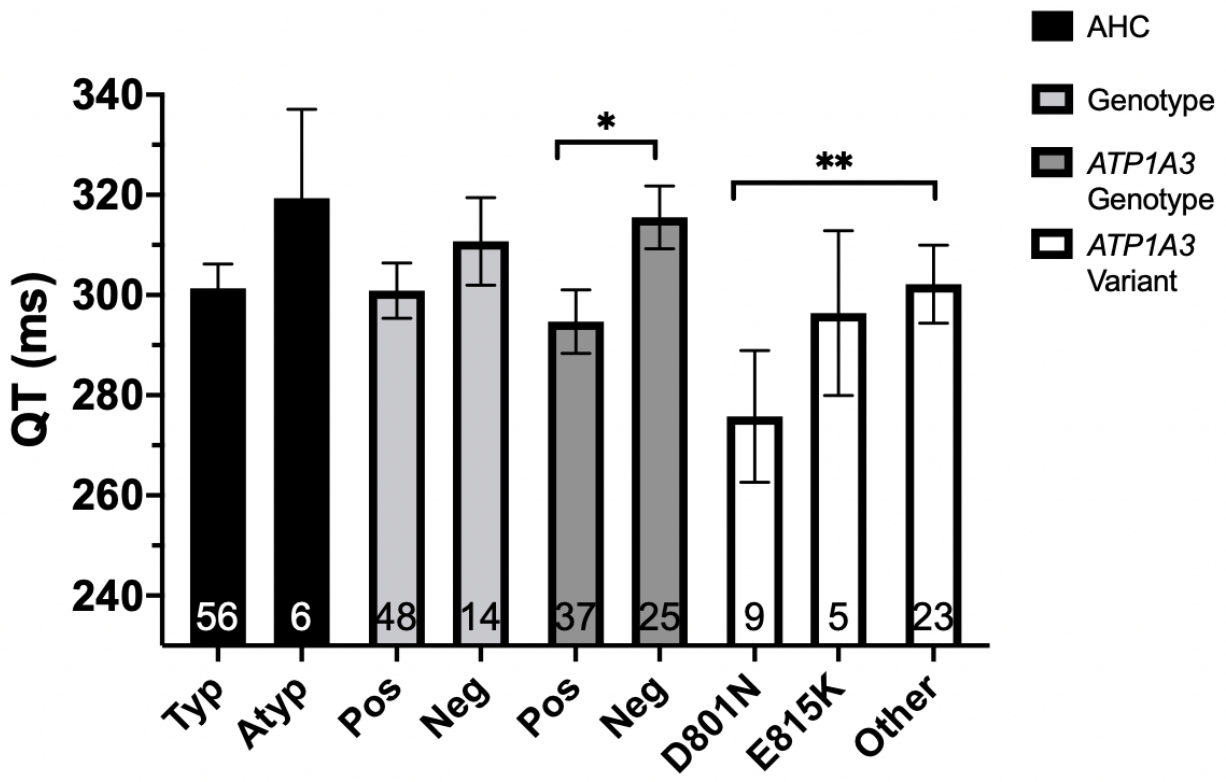

Figure S5. Bar graph summarizing QT (ms) means for study groups, including AHC (typical versus atypical, black fill), genotype (positive versus negative, dark grey fill), *ATP1A3* genotype (positive versus negative, light grey fill), and ATP1A3 variant (D801N variant versus E815K and other variants, white fill). Numbers on the bar graphs represent the total number of QTc measurements. **P*=0.028, ***P*=0.042. Ms: millisecond; Typ: Typical AHC presentation; Atyp: Atypical AHC presentation; Neg: Negative Genotype; Pos: Positive Genotype; Neg: Negative *ATP1A3* genotype; Pos: Positive *ATP1A3* Genotype.

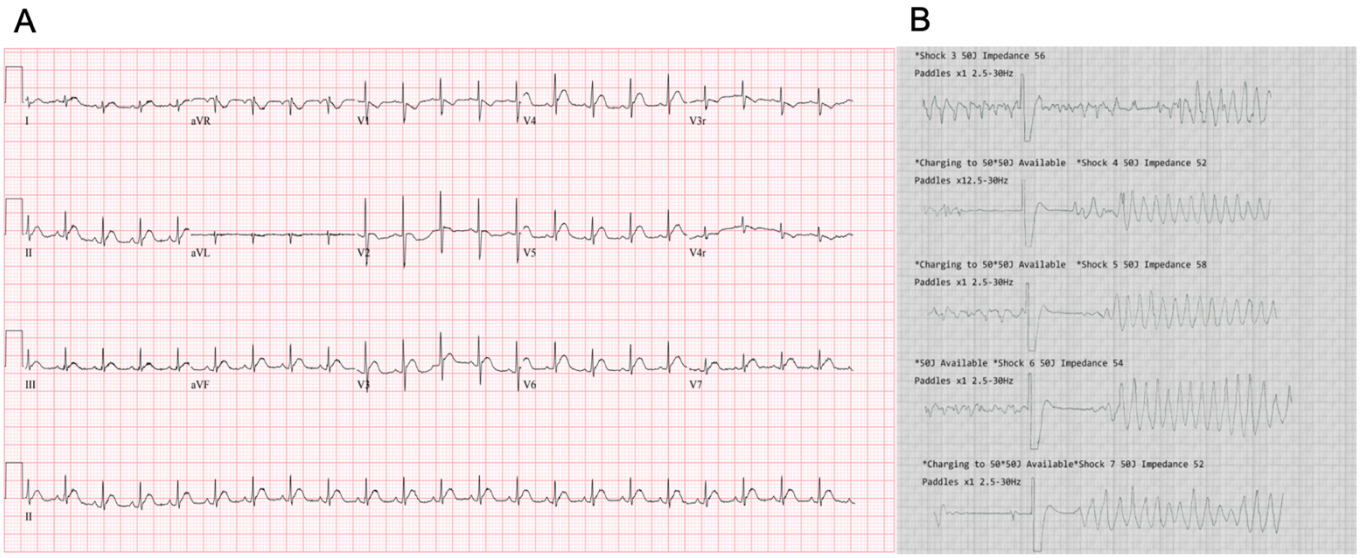

Figure S6: A, 12 lead ECG of 4.5-year-old female with the ATP1A3-D801N genotype prior to arrest. B, cardiac telemetry during the arrest demonstrates recurrent VT/VF refractory to defibrillation.

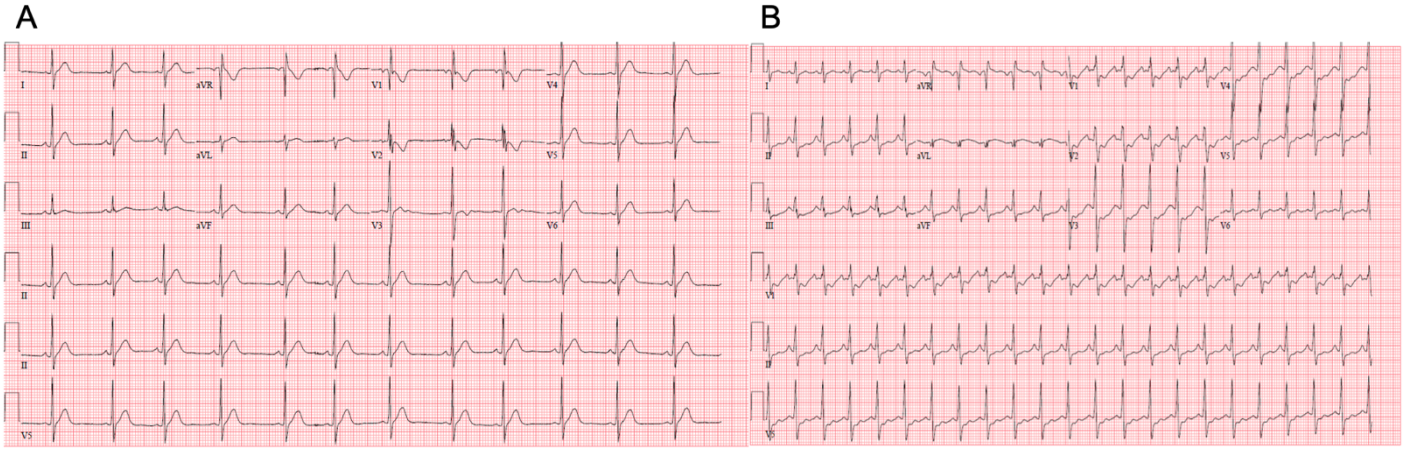

Figure S7: A, 12 lead ECG of 13-year-old male with the ATP1A3-D801N genotype at baseline.

B, 12 lead ECG of the proband approximately 1 hour following the arrest