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Cardiac phenotype in *ATP1A3*-related syndromes: A multicentre cohort study

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Abstract

Objective. To define the risks and consequences of cardiac abnormalities in *ATP1A3*-related syndromes.

Methods. Patients meeting clinical diagnostic criteria for Rapid-onset Dystonia-Parkinsonism (RDP), Alternating Hemiplegia of Childhood (AHC), and Cerebellar ataxia, Areflexia, Pes cavus, Optic atrophy, and Sensorineural hearing loss (CAPOS), with *ATP1A3* genetic analysis, and had at least one cardiac assessment, were included. We evaluated the cardiac phenotype in an *Atp1a3* knock-in mouse ($Mashl^{+/-}$) to determine the sequence of events in seizure-related cardiac death.

Results. 98 AHC, nine RDP, and three CAPOS patients (63 females, mean age 17 years) were included. Resting EKG abnormalities were found in 52/87 (60%) AHC, 2/3 (67%) CAPOS, and 6/9 (67%) RDP patients. Serial EKGs showed dynamic changes in 10/18 AHC patients. The first Holter EKG was abnormal in 24/65 (37%) AHC and RDP cases, with either repolarization or conduction abnormalities. Echocardiography was normal. Cardiac intervention was required in 3/98 (~3%) AHC patients. In the mouse model, resting EKGs showed intra-cardiac conduction delay; during induced seizures, heart block or complete sinus arrest led to death.

Conclusions. We found increased prevalence of EKG dynamic abnormalities in all *ATP1A3*-related syndromes, with a risk of life-threatening cardiac rhythm abnormalities equivalent to that in established cardiac channelopathies (~3%). Sudden cardiac death due to conduction abnormality emerged as a seizure-related outcome in murine *Atp1a3*-related disease. *ATP1A3*-related syndromes are cardiac diseases as well as neurological diseases. We provide

guidance to identify patients potentially at higher risk of sudden cardiac death who may benefit from insertion of a pacemaker or implantable cardioverter-defibrillator.

Introduction

The *ATP1A3* gene encodes the alpha-3 catalytic subunit of the neuronal ouabain-sensitive Na/K-ATPase complex. Na+/K+-ATPases are membrane-bound transporters regulating Na+ and K+ gradients through active ATP-dependent transport.[1]

The *ATP1A3*-related disorders are clinically heterogeneous, and include a spectrum of at least three distinct, although overlapping, phenotypes: Rapid-onset Dystonia-Parkinsonism (RDP);[2] Alternating Hemiplegia of Childhood (AHC);[1] and Cerebellar ataxia, Areflexia, *Pes cavus*, Optic atrophy, and Sensorineural hearing loss (CAPOS).[3] Although rare, these conditions are important as they are generally severe, including paroxysmal events and chronic severely disabling neurological deficits,[4] with increased rate of premature mortality. Epilepsy is often present, mostly in AHC; sudden death, including death during seizures or status epilepticus and apparent sudden unexpected death in epilepsy (SUDEP), is increasingly reported,[5,6] but in fact the cause of death is usually unexplained. Newer phenotypes are emerging,[7,8] suggesting that there may be additional unsuspected cases, perhaps also in sudden death cohorts.

In AHC, we previously demonstrated resting EKG abnormalities resembling those seen in inherited cardiac channelopathies, most commonly dynamic alteration of the repolarization phase.[9] Additional published data in mice and humans suggest cardiac dysfunction in early

death in AHC.[6,10-13] There are no previous studies investigating the cardiac phenotype of CAPOS or RDP.

We sought to determine whether the suggestions of cardiac involvement in *ATP1A3*-related disease had manifest clinical consequences beyond an EKG phenotype alone, noting that the single patient requiring intervention reported before[10] might have had a coincidental comorbidity. The findings provide the basis for recommendations for clinical cardiac investigations and interventions in *ATP1A3*-related disease.

Methods

Patients

Patients were recruited from 19 participating centres in 13 different countries: Duke University Medical Center, Durham, USA (n=21); Istituto Giannina Gaslini, University of Genoa, Italy (n=17); Hospital Sant Joan de De´u Barcelona, Spain (n=13); Wake Forest School of Medicine, Winston-Salem, North Carolina, USA (n=11); The National Hospital for Neurology and Neurosurgery UK (n=8); Department of Pediatric Neurology, Medical University of Silesia, Katowice, Poland (n=6); University Clinic for Child Neurology and Psychiatry, Belgrade, Serbia (n=5); Great Ormond Street Hospital for Children UK (n=5); Royal Children's Hospital Melbourne, Australia (n=4); C. Besta Neurological Institute Milan, Italy (n=4); Department of Clinical Epileptology, Sleep Disorders and Functional Neurology in Children, University Hospitals of Lyon, France (n=2); Neuropediatric Department, Hospital Maria Pia do Centro Hospitalar do Porto, Portugal (n=2); IRCCS E. Medea, Italy (n=2); Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland (n=2); Department of Neurology, Institute of Psychiatry and Neurology, Warsaw, Poland (n=2);

Department of Developmental Neurology, Medical University of Gdansk, Poland (n=2); Department of Neurology, University Hospital Antwerp, Belgium (n=2); Hospital "Agia Sofia", Athens, Greece (n=1); University Medical Center of the Johannes Gutenberg University Mainz, Germany (n=1). Patients meeting the clinical diagnostic criteria for typical AHC or other *ATP1A3*-associated syndromes and who underwent genetic analysis of *ATP1A3*, with or without identified mutations in *ATP1A3*, were included. At least one cardiac evaluation was required from: EKG, echocardiogram and prolonged EKG (≥24 hour), or prolonged EKG recording from EEG-videotelemetry. For patients who had already been included in the first study[9], at least one further cardiac investigation was required. Eleven patients were excluded due to genetic testing being unavailable or not performed (n=3), poor quality/uninterpretable cardiac data (n=6), or pathogenic mutation in genes not associated with AHC being identified (n=2) (Fig 1). Recruitment and data collection were from 1.9.2015 until 1.9.2018. Clinical and genetic data were collected through a standardised questionnaire. *ATP1A3* mutations were identified by Sanger, whole-exome or whole-genome sequencing. *De novo* mutation status was evaluated by Sanger sequencing where parental DNA was available; where unavailable, pathogenicity was declared if the same mutation was previously reported as de novo in another patient with an *ATP1A3*-related condition.

A total of 110 cases were recruited, including 98 AHC, nine RDP, and three CAPOS. All cases had identified mutations in *ATP1A3* except for 9 AHC cases with no mutation in *ATP1A3* detected by direct gene sequencing (these were included as they all met the clinical diagnostic criteria for AHC[14]) and no other causative mutations identified on exome sequencing or gene panels. Of the 98 AHC cases, 22 had been reported in the previous study[9]. None of the RDP or CAPOS cases were previously reported.

We calculated a severity index for the AHC patients based on clinical features previously associated with a gradient of clinical severity in a relatively large AHC cohort of AHC

patients.[15] A score of 1 was assigned for each of the following clinical variables: early onset of paroxysmal episodes (≤2 months), tonic/dystonic attacks, plegic attacks, seizures/epilepsy, status epilepticus, episodes of autonomic dysfunction, gait unsteadiness or ataxia, dystonia, language disorders including dysarthria, intellectual disability. We evaluated the correlation of this cumulative severity index obtained for each AHC patient with presence of EKG abnormalities and dynamic abnormalities.

Cardiac Investigations

Original cardiac investigations were anonymised, scanned, collected and reviewed centrally by two independent cardiologists with expertise in genetic cardiac disease and sudden cardiac death (R.A.G-R, J.P.K.). Abnormal repolarization was defined by the presence of abnormal T wave morphology (flattened or biphasic T waves; bifid or notched T waves) or T wave inversion in two or more of the following leads: I, aVL and V4–V6 (lateral repolarization abnormalities); II, III and aVF (inferior repolarization abnormalities); and V1–V3 in patients aged ≥14 years (anterior repolarization abnormalities); repolarization abnormalities of this type are seen in 2% of healthy adults.[16] Widespread repolarisation abnormalities were defined by abnormalities present in more than one group of leads. The corrected QT interval was calculated from lead II using Bazett's formula;[17] its normal range is 360–460 ms.[18] The Brugada pattern is characterized by a coved type ST-segment elevation ≥2 mm followed by a negative T-wave in ≥1 of the right precordial leads V1 to V2. Early repolarization (ER) was defined as a deflection in the R-wave descent (slurred pattern) or a positive deflection with a secondary r″ wave (notching pattern) in the terminal part of the QRS complex in at least two of the following leads: I, avL, v4-v6 (lateral ER); II, III, avF (inferior ER).[19] Lateral ER is seen in 3.5% of healthy individuals and inferior ER appears in 2.4%.[20] The U wave is defined as a small upward deflection following the T wave. It is discernible in about 25% of the

healthy population when the heart rate is within 80 to 95 beats/min and is not detectable when the heart rate is over 95 beats/min.[21] Right bundle branch block, complete (RBBB) and incomplete (IRBBB), and intraventricular conduction delays (IVCDs) were defined according to established criteria.[22] Isolated IVCD was considered normal in the absence of additional EKG abnormalities, as it is seen in up to 5% of the normal population.[23-24] Isolated RBBB is seen in 2–4% of healthy individuals.[24]

Pathology

The ATP1A3 subunit is expressed in the human heart and in neurons of the cerebral cortex and other brain structures,[25] with the highest expression detected in the frontal cortex.[26] Immunolabelling for ATP1A3 was performed in adult human heart, from a 75-year-old male who died from bronchopneumonia, after post mortem examination.

Mouse Model

Generation of the mouse model was performed as described before[12]. The aims of the *ATP1A3* D801N knock-in mouse (Mashl$^{+/-}$) model study were: 1) to establish the presence of baseline EKG abnormalities in this model as compared to wild type mice; 2) to determine the sequence of events in cardiac death resulting from seizure activity.

Experiment I

The interictal EEG of two groups of mice were recorded and compared. Group I consisted of adult wild type (WT) mice and Group II consisted of age-matched D801N (Mashl$^{+/-}$) mice of C57BL background. Electrocardiograms were obtained as described previously.[27-29]

Experiment II

EKGs were recorded in Mashl$^{+/-}$ mice during seizures induced by intra-amygdalar injection of kainic acid, ending in death, to determine the type of arrhythmias that may be associated with seizure-associated cardiac arrest and death. Mice underwent surgical implantations to record intracranial EEG and EKG to be recorded after kainic acid injection.

EKG analysis. All EKG data was saved in 2-second frame shots and analyzed with ImageJ software. The PR interval was calculated by horizontal measurement between the peak of the P wave to the peak of the R wave, QRS interval from the Q peak to the S peak, RR from two consecutive R waves, and the QT from the Q peak to the end of the T wave. The end of the T wave was defined using a tangent method in which a line was drawn representing the isoelectric line, which is defined by the base of the P wave to the base of the T wave, and a tangent drawn along the steepest slope of the T-wave repolarization. The end of the T wave is defined as the cross between the tangent and the isoelectric line. The QT interval was corrected using Mitchell's equation modified for mouse physiology.[28]

Statistical Analysis

Data were tested for normal distribution. The significance of differences in clinical and genetic factors potentially associated with EKG changes was estimated by Pearson χ^2 or two-sided Fisher's exact test, as appropriate, for categorical variables, and by Wilcoxon rank-sum test for continuous variables. Missing data were less than 5% of the total number of cases for all analysed variables; they were omitted from analysis. P value was considered significant at 0.05 (adjusted due to multiple testing using the Bonferroni method). Data were analyzed using Stata/IC 11.1(StataCorp, College Station, TX).

Standard Protocol Approvals, Registrations and Patient Consents

This research was approved by local review boards or ethics committees. For all cases written informed consent for research use of clinical and genetic data was obtained from patients or their parents, or legal guardians in the case of minors or those with intellectual disability. All animal procedures were approved by the Duke University Institutional Animal Care and Use Committee, and were conducted in accordance with the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals.

Data Availability

The authors confirm that the data supporting the findings of this study are available from the corresponding authors, upon reasonable request.

Results

Demographic and Clinical Features

110 patients (63 females, 47 males) were included in the analysis. Mean age at inclusion was 17 years (SD ±13; range 1 to 64), with 64 patients under the age of 17 years. A history of seizures or epilepsy was reported in 58 patients (53%), with status epilepticus in 21. Episodes of autonomic dysfunction including breathing difficulties were reported in 60 patients (55%). The only reported symptom considered to be related to cardiac function was syncope, reported in 3 patients (3%).

Cardiac Investigations

Investigations included: 12-lead EKGs in 99 patients (≥2 in 18 patients), Holter EKG in 65 patients (serial in 2 patients), echocardiogram in 80 patients, and an ajmaline provocation test in one patient.

The first 12-lead EKG available was performed at an average age of 18 years (SD ±15) and showed abnormalities in 60/99 patients (61%). The most common changes were T wave abnormalities anteriorly (32%), laterally (23%), and inferiorly (42%). Thirteen patients (13%) had widespread repolarization abnormalities. Lateral ER was seen in 5/99 patients and inferior ER in 8. In 3/99 patients there was ≥2 mm anterior J-point elevation, but no Brugada pattern was observed on the 12-lead EKG. The average QTc was 382msec (median 375 msec). Thirty-one (31%) patients had a corrected QT shorter than or equal to 360 msec. Only one patient had a prolonged corrected QT interval (500 msec), and was not on any drug treatment at the time of the EKG. U waves were present in 16 patients (16%), and in two of them the heart rate was >95 bpm. Other abnormalities included: left axis deviation (6%), right axis deviation (8%); IVCD (21%) and IRBBB (23%). The EKG was repeated in 18 patients once or more times (average interval time 2 years, SD ±2), and showed dynamic changes in 10/18 (56%). Details of the 12-lead EKGs are provided in Table 1.

The first Holter EKG was performed at an average age of 17 years (SD ±13) and showed abnormalities in 24 patients (24/65, 37%), mainly T wave abnormalities (n=20) including dynamic T wave inversion (n=2) or T wave notching (n=9), and dynamic J point elevation (n=9). We documented significant QT prolongation in two patients (505 msec and 480 msec, respectively), not exposed to QT-prolonging drugs, and QT shortening in one (330 msec). Isolated supraventricular ectopics were present in 34 patients (34/65, 52%) and ventricular ectopics in 23 (23/65, 35%); four patients had frequent ventricular ectopics, including couplets and bigeminy. Three patients (3/65, 5%) had evidence of conduction abnormalities on ambulatory Holter monitoring, in the form of pathological sinus pauses in all three, with additional atrioventricular block in one patient. Two patients had repeated Holter EKGs. One

patient had two Holter EKGs both showing no arrhythmias despite widespread repolarization abnormalities and IRBBB on the resting 12-lead EKG. The second patient was a 28 year-old lady with recurrent repolarization abnormalities on 12-lead EKGs; she had five repeat Holter EKGs. These showed sinus pauses, atrioventricular block, atrial and ventricular ectopics (Fig 2); she subsequently underwent insertion of an implantable cardioverter-defibrillator (ICD).

Overall, there were resting or ambulatory EKG abnormalities in 71 patients (71/110, 65%). A sensitivity analysis was conducted by excluding the 22 patients included in the previous study:[9] EKG (either 12-lead or Holter) abnormalities were present in 55 patients (55/88, 63%) and dynamic changes in serial 12-lead EKGs were present in 6 patients (6/13, 46%).

Echocardiography was performed at an average age of 16 years (SD 12) and did not show evidence of structural heart disease in any case. Only one AHC patient carrying the *ATP1A3* mutation c.2839G>C - G947R, aged 46 years, had left ventricular hypertrophy. This was likely associated with previously undiagnosed essential hypertension detected 17 months after the echocardiogram and for which he is currently on treatment with lisinopril.

One AHC patient who had previously been found to have features suggestive of Brugada syndrome (mild prolongation of QRS and J point elevation) on a single-lead EKG (modified V1) during EEG-videotelemetry recording[9] underwent ajmaline provocation testing which revealed an RSR' pattern to the QRS complex with subtle J-point elevation in lead V2 placed in the second intercostal space (high parasternal position) but did not meet the diagnostic criteria for Brugada syndrome.[30] Previous and subsequent 12-lead EKGs were unremarkable, confirming the dynamic nature of the abnormalities.

Patients Requiring Intervention

Three patients underwent implantation of a loop recorder and, based on the findings recorded, had subsequent implantation of a permanent pacemaker or ICD. None of them were on pharmacological treatment that could adversely affect the cardiac conduction system.

A female patient with AHC (c.2401G>A; p.D801N - mutation in *ATP1A3*) had syncopal episodes, in addition to hemiplegic attacks and epileptic seizures. She was included in the study at age 26 years. Her 12-lead EKGs showed dynamic changes, with repolarization abnormalities including lateral ER, inverted T waves in the anterior leads, and U waves. She also had consistently short QTc intervals (320-345msec). She had five repeat Holter EKGs and a loop recorder implanted that showed multiple asymptomatic pauses (up to 4.4 sec), paroxysmal complete atrioventricular block, atrial ectopics and polymorphic ventricular ectopics in couplets and bigeminy (Fig 2). Her echocardiogram showed a structurally and functionally normal heart. Aged 27, an ICD was implanted due to the combination of syncope, atrioventricular block, and ventricular ectopy. Only one episode of 'collapse' has occurred since (19 months follow-up), but no rhythm disturbances were seen on ICD interrogation during the episode.

A female AHC patient (c.410C>T; p.S137F - mutation in *ATP1A3*) started experiencing episodes of loss of consciousness with respiratory arrest at the age of 21 years.[10] Her routine 12-lead EKG was normal. She underwent implantation of a cardiac loop recorder aged 23, which documented three episodes of asystole longer than 3 seconds over a period of 4 months: a cardiac pacemaker was implanted. She had had EEG-videotelemetry prior to pacemaker implantation. She did not experience any episodes of loss of consciousness with respiratory arrest during the recording. The single-lead EKG that was part of the telemetry

showed no abnormalities. She has remained free of episodes of loss of consciousness with respiratory arrest following the pacemaker insertion, and there has been no evidence of ventricular arrhythmia on pacemaker interrogation over seven years of follow-up.

A female AHC patient (negative for *ATP1A3* mutation) had the onset of hemiplegic attacks at the age of 6 months, and the onset of syncopal episodes around the age of one year. Bradycardia was also noted during the fetal period. Her 12-lead EKGs showed dynamic changes with intermittent junctional rhythm, low QRS voltages, delayed RS progression, supraventricular ectopics, and widespread repolarization abnormalities. Holter EKG was abnormal with supraventricular and ventricular ectopy. Echocardiogram showed left ventricular hyper-trabeculation not fulfilling diagnostic criteria for left ventricular non-compaction, with preserved systolic function and was otherwise unremarkable. Due to the ongoing syncopal episodes, she had a loop recorder inserted aged 2 years which revealed sinus pauses longer than 4 seconds, and subsequently a pacemaker was inserted with no recurrence of the syncopal episodes over six years' follow-up.

Analysis of Potential Clinical and Genetic Risk Factors

The prevalence of repolarization abnormalities on 12-lead EKGs was greater in patients aged ≥16 years (36/48, 75%) than in those <16 years (24/51, 47%) (P=0.01). In contrast, the prevalence of repolarization and conduction abnormalities on Holter EKGs was greater in the younger patients (16/30, 53%) than in the older (8/35, 23%) (P=0.01). There was no difference in the prevalence of EKG abnormalities by sex, or in the prevalence of dynamic changes by age or sex.

Abnormalities on the 12-lead EKG were found in all three *ATP1A3*-related disease categories with no difference in prevalence (P>0.99): 52/87 (60%) AHC patients, 2/3 (67%) CAPOS patients, and 6/9 (67%) RDP patients. Serial EKGs were available in 18 AHC patients, with ten (56%) showing dynamic changes, whilst in two CAPOS patients there were no dynamic changes (one had consistently normal EKG, one repeatedly abnormal).

Abnormalities on the Holter EKG were also found in all the categories except CAPOS, where Holter EKGs were performed in two patients and were unremarkable. Otherwise, Holter EKGs were abnormal with evidence of conduction disease in AHC patients only (4/61, 7%), and repolarization abnormalities in 19 AHC patients (19/61, 31%) and one RDP patient (1/2, 50%).

In 9 of the 98 AHC patients, no mutation in the *ATP1A3* gene was identified; three of them had serial EKGs and 2/3 presented dynamic changes. Abnormalities on the 12-lead EKG were present in 6/9 (67%), and abnormalities on Holter monitoring were seen in 5/9 (56%) of the *ATP1A3*-negative patients. These were the same type of abnormalities observed in the *ATP1A3* mutation carriers. There was no difference in the prevalence of EKG abnormalities in AHC patients with or without *ATP1A3* mutation (P>0.99).

The position of the mutation across the functional domains was not associated with different prevalence of EKG abnormalities or dynamic changes. Details of the different syndromes, mutations, and abnormalities are presented in Fig 3.

77/110 patients (70%) were exposed to treatment with a potential effect on cardiac conduction at the time of the investigation, the most frequent being flunarizine[31],

topiramate[32], and benzodiazepines[33]. There was no difference in the prevalence of EKG abnormalities (P>0.99) or dynamic changes (P>0.99) between patients exposed vs non-exposed to treatment with potential effect on cardiac conduction or repolarisation.

The average severity clinical severity index in the AHC cohort was 7 (range 1-10). No significant correlation emerged between phenotypic severity and prevalence of EKG abnormalities or dynamic abnormalities.

Pathology

Immunolabelling for ATP1A3 was performed in an adult human heart, from a 75-year-old male who died from bronchopneumonia. As shown in Fig 4, this confirmed strong expression of ATP1A3 in human cardiomyocytes.

Mouse Model

Experiment I:

At baseline, Mashl$^{+/-}$ mice had increased heart rate (532±3.5 vs 418±6.8 Hertz, P<0.001), prolonged QRS (0.0213±0.002 vs 0.009±0.00009 seconds, P<0.001), prolonged PR interval (0.099±0.0028 vs 0.05±0.002 seconds, P <0.001) and longer QTc interval (0.042±0.003 vs 0.0322±0.0014, P<0.01) compared to wild-type (WT) littermates (WT n=15; Mashl$^{+/-}$ n=3) (Fig 5).

Experiment II:

Following injection of kainic acid, all mice showed elevated JT intervals in response to ictal activity. In addition, one of the three Mashl$^{+/-}$ mice also showed a period with JT segment depression (Fig 6a). Two Mashl$^{+/-}$ mice died of atrioventricular block (Fig 6b).

Discussion

We show that *ATP1A3*-related diseases can cause heart abnormalities as well as neurological manifestations, with a requirement for life-saving cardiac intervention equivalent to that for the better-known genetic cardiac channelopathies.[18] Nearly 3% (3/98) of AHC patients showed asymptomatic and symptomatic asystole (and ventricular arrhythmias), and required the insertion of a pacemaker or ICD. Our previous observation in one *ATP1A3*-related condition of a significantly increased prevalence of abnormalities of the resting EKG, including abnormalities of repolarisation reminiscent of genetic cardiac channelopathies, when compared with healthy controls and disease controls with epilepsy[9], was replicated and extended here. We found EKG abnormalities in 65% of patients, with dynamic changes in the 56% of patients who had serial EKGs, across all *ATP1A3*-related syndromes and some AHC patients with no identified mutation in *ATP1A3*. The location of the mutation and the exposure to treatment with potential effects on cardiac conduction were not associated with different prevalence of EKG abnormalities or dynamic changes.

There are some limitations that need to be acknowledged: the study is unpowered to interpret the results in the non-AHC patients due the small sample size of non-AHC cohorts; data collection was partly retrospective; 22 AHC cases had already been included in the previous study[9] therefore introducing a potential selection bias. To minimize the latter, we have

conducted a sensitivity analysis which confirmed the high prevalence of resting and/or ambulatory EKG abnormalities and dynamic changes.

Nine patients with an AHC phenotype did not have a mutation in *ATP1A3* on direct gene sequencing but the majority (6/9, 67%) had the same dynamic cardiac electrical abnormalities observed in the *ATP1A3* mutation carriers, with one even requiring the insertion of a pacemaker due to symptomatic conduction disease. This suggests that either mutations might have been missed, a recognized phenomenon, or there may be mosaicism, or mutations in a different gene might be causative (suggesting cardiac dysfunction is related to shared pathophysiology rather than to *ATP1A3* mutations only).

The underlying basis of the EKG abnormalities observed is not yet explained, but, as suggested in our previous study, may be related to dynamic abnormality of cardiac repolarization reserve. In the present study, we confirmed the expression of ATP1A3 in the structurally and histologically normal heart of an adult male who died from bronchopneumonia and had post-mortem examination. The presence of dynamic EKG changes, the frequency of necessary preventative intervention (i.e., pacemaker or ICD), and the apparent age-related penetrance underpins similarities to the genetic cardiac channelopathies. Interestingly, although there was a lower prevalence of 12-lead EKG abnormalities in patients <16 years, Holter monitoring was more frequently abnormal in younger patients, suggesting less manifest and more dynamic cardiac electrical abnormalities at younger ages. This is in keeping with findings in cardiac sodium channel loss of function mutations, where presentation with more severe conduction disease and atrial arrhythmia is commoner in childhood and adolescence, whereas a Brugada phenotype is commoner in adulthood,[34] suggesting an age-related penetrance to the disease. Although the cardiac

abnormalities demonstrated in this study are not due to structural abnormalities detectable on echocardiography, it is theoretically possible that there may be structural abnormalities too subtle to be observed on echocardiography; additional investigations such as cardiac MRI with gadolinium contrast or histology from biopsies or post mortem heart tissue may provide further information.

The asystolic episodes recorded in our three patients occurred independently to, and showed a different semiology from, other paroxysmal events (e.g. hemiplegia or seizures). This is very important to consider in the clinical assessment of *ATP1A3*-related disorders, where multiple types of paroxysmal episodes often co-exist and there is increasing evidence of the risk of sudden death or progressive disease course.[4,6] The episodes of sudden death reported in AHC patients[6] have usually been ascribed to SUDEP, but they may be directly caused by fatal cardiac arrhythmias. Cardiac causes of sudden death have different implications in terms of preventative strategies: achieving better seizure control can reduce the risk of SUDEP, whilst pacemaker and/or ICD insertion can be life-saving for life-threatening conduction disease or cardiac arrhythmias.[35]

We evaluated the cardiac phenotype in Mashl$^{+/-}$mice, carrying the heterozygous D801N mutation, present in about one third of AHC patients.[1] Resting EKGs in the mutation-carrying mice under general anaesthesia revealed evidence of intra-cardiac conduction delay, with a prolonged PR interval, indicative of likely conduction delay in the atria and atrioventricular node. Additionally, there was a prolonged QRS duration revealing probable delayed conduction in the His-Purkinje system and ventricle. When challenged by induced seizures, the mice were initially in sinus rhythm but with time abnormalities emerged, with heart block or complete sinus arrest leading to death. In wild type mice receiving kainic acid,

pre-ictal, but not ictal, tachycardia was observed and the squared coefficient of variation (SCV) of R-R intervals was significantly elevated before and during seizures compared to control conditions.[36] In wild type rats, kainic acid induced-seizures produced an immediate initial bradycardic response coinciding with initial low-level seizure activity followed by subsequent tachycardia with QTc prolongation and T wave elevation with increasing seizure activity.[37] Our observations of multiple cardiac rhythm abnormalities during kainic acid-induced seizures appear to be more remarkable than those described above in wild type rodents in the literature.[36-37] Our animal data showed similar resting EKG abnormalities to those observed in humans with AHC and other *ATP1A3*-related conditions. An important difference is that the murine conduction abnormalities deteriorate in the context of seizure activity, which so far has not been recorded in humans, but we note that the seizure paradigm in the experimental setting differs from most self-terminating seizures in humans. However, we cannot exclude that seizures represent a precipitating factor for cardiac arrhythmias in compromised tissue also in humans.

Atp1a3 mutations may indirectly affect the heart rhythm in mutant mice through the excessive excitability of the brain and its predisposition to spreading depression,[11] which has been shown to be a mechanism for autonomic dysfunction leading to SUDEP.[27] A direct effect of the mutation on the heart is another potential mechanism: although Atp1a3 has not been detected in the heart of >2 month old adult mice, it has been shown to be expressed in myocardium in E10.5 and E16.5 mouse embryos,[38] raising the possibility of a congenital effect of the mutated gene on cardiac electrophysiology. Further work is required and may help in the understanding of the cardiac pathophysiology in humans.

Conclusion

Our study offers an example of the 'precision medicine' paradigm for rare conditions, where the underlying genetic aetiology informs management and treatment strategies. Our findings provide further robust evidence that all patients with *ATP1A3*-related conditions should have longitudinal and systematic cardiac assessment by cardiologists with expertise in inherited cardiac disease. Our findings also provide evidence to recommend implantation of a loop recorder in all patients with *ATP1A3*-related disorders with atypical events (i.e. syncope or any other paroxysmal event with features atypical for seizures or hemiplegic attacks) to identify patients potentially at higher risk of sudden cardiac death who may benefit from insertion of pacemaker or ICD. Whether implantation of loop recorders in individuals with abnormal EKGs but no cardiac symptoms is warranted remains to be determined. We also recommend adoption of the current accepted practice for cardiac ion channel disease, which is also characterized by dynamic EKG changes.[18] Baseline EKG, cardiac ultrasound and Holter EKG should be undertaken in every patient with an *ATP1A3*-related condition, and then annual 12-lead EKG screening should be continued, with additional investigations (e.g. Holter monitoring) guided by symptoms or clinical status. Further prospective studies of cardiac disturbances in *ATP1A3*-related conditions to test the utility and cost-effectiveness of this approach, and to identify potential precipitating factors for life-threatening cardiac arrhythmias, are warranted.

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the participating Centres. The Data Management Service for this Study was provided free of charge by the IAHCRC Data Manager Rosaria Vavassori, appointed by the Scientific Coordinators of the Consortium, Prof. Alexis Arzimanoglou and Prof. Mohamad Mikati. We also thank the Italian family association AISEA.

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Table 1. Summary of the findings of the serial 12-lead EKGs performed in our cohort.

		EKG1 (n=99)	EKG2 (n=18)	EKG3 (n=9)	EKG4 (n=3)
Age in years, mean (SD)		18 (15)	19 (12)	21 (10)	25(6)
Abnormal, n(%)		60(61)	11(61)	5(56)	3(100)
Heart rate in bpm, mean (range)		89(51-169)	84(46-127)	70(52-93)	75(62-95)
QRS duration in msec, mean (range)		80(50-122)	84(70-102)	90(68-110)	88(84-90)
PR duration in msec, mean (range)		136(90-200)	133(110-170)	145(122-174)	152(140-160)
QTc duration in msec, mean (range)		382(330-500)	386(325-445)	365(320-410)	360(345-375)
T-wave abnormality	Anterior, n(%)	13(13)	1(6)	1(11)	1(33)
	Lateral, n(%)	2(2)	0(0)	0(0)	0(0)
	Inferior, n(%)	15(15)	4(22)	1(11)	0(0)
	Antero-lateral, n(%)	0(0)	0(0)	0(0)	0(0)
	Antero-inferior, n(%)	6(6)	0(0)	1(11)	0(0)
	Latero-inferior, n(%)	8(8)	3(17)	0(0)	0(0)
	Widespread, n(%)	13(13)	2(11)	0(0)	1(33)

IVCD	Anterior, n(%)	15(15)	4(22)	1(11)	2(67)
	Lateral, n(%)	0(0)	0(0)	0(0)	0(0)
	Inferior, n(%)	10(10)	2(11)	1(11)	0(0)
IRBBB, n(%)		22(22)	2(11)	2(22)	0(0)
J wave elevation, n(%)		3(3)	2(11)	1(11)	0(0)
U wave, n (%)		16 (16)	3 (17)	2(22)	1 (33)
Anterior J point elevation, n (%)		3(3)	2(11)	1(11)	0(0)
Lateral ER, n (%)		5 (5)	1 (6)	0 (0)	0 (0)
Inferior ER, n (%)		8 (8)	2 (11)	3 (33)	0 (0)

Abbreviations. bpm=beats per minute; IVCD=intraventricular conduction delay; IRBBB= incomplete right bundle branch block; ER=early repolarisation.

Figures

Figure 1. Study design. Diagram illustrating study design with included and excluded cases.

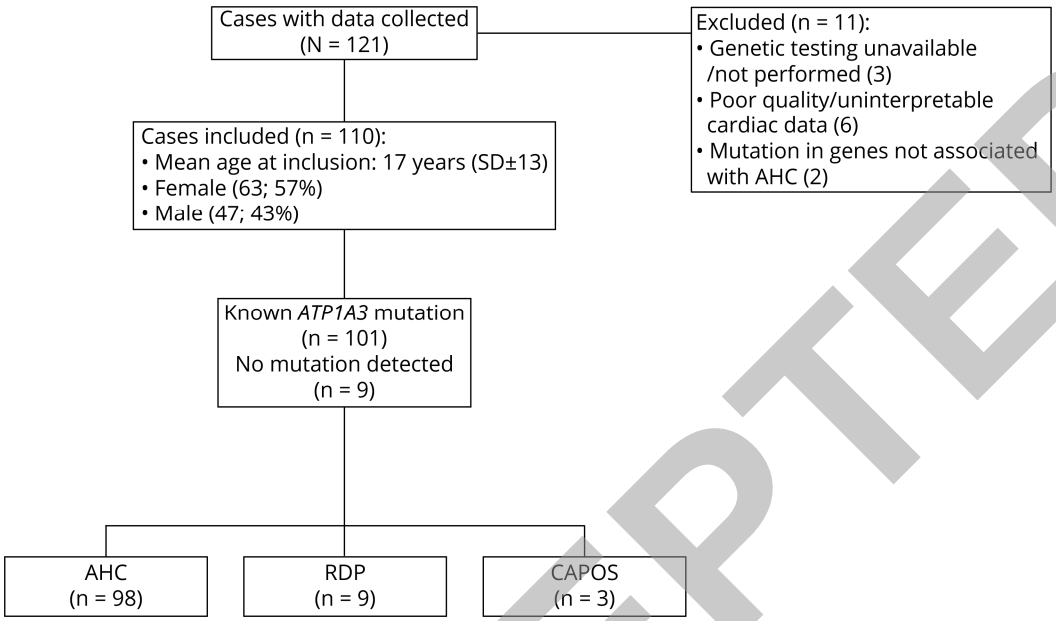
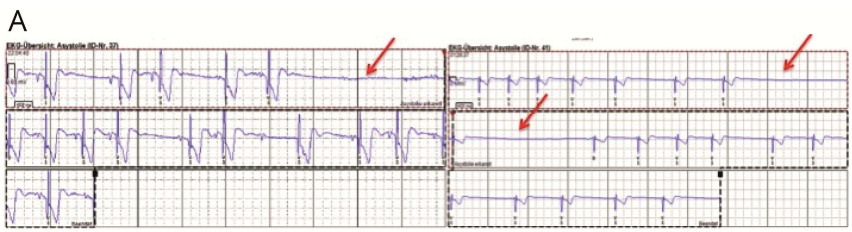


Figure 2. Holter abnormalities in a young AHC patient requiring the insertion of an implantable cardioverter-defibrillator. A) Abnormal Holter EKG showing asymptomatic sinus pauses of up to 4 seconds in duration (red arrows), and B) polymorphic ventricular ectopics in couplets and bigeminy, in a young patient who required the insertion of an implantable cardioverter-defibrillator (ICD) at the age of 27 years (patient with alternating hemiplegia of childhood (AHC), c.2401G>A – p.D801N mutation).

A



B

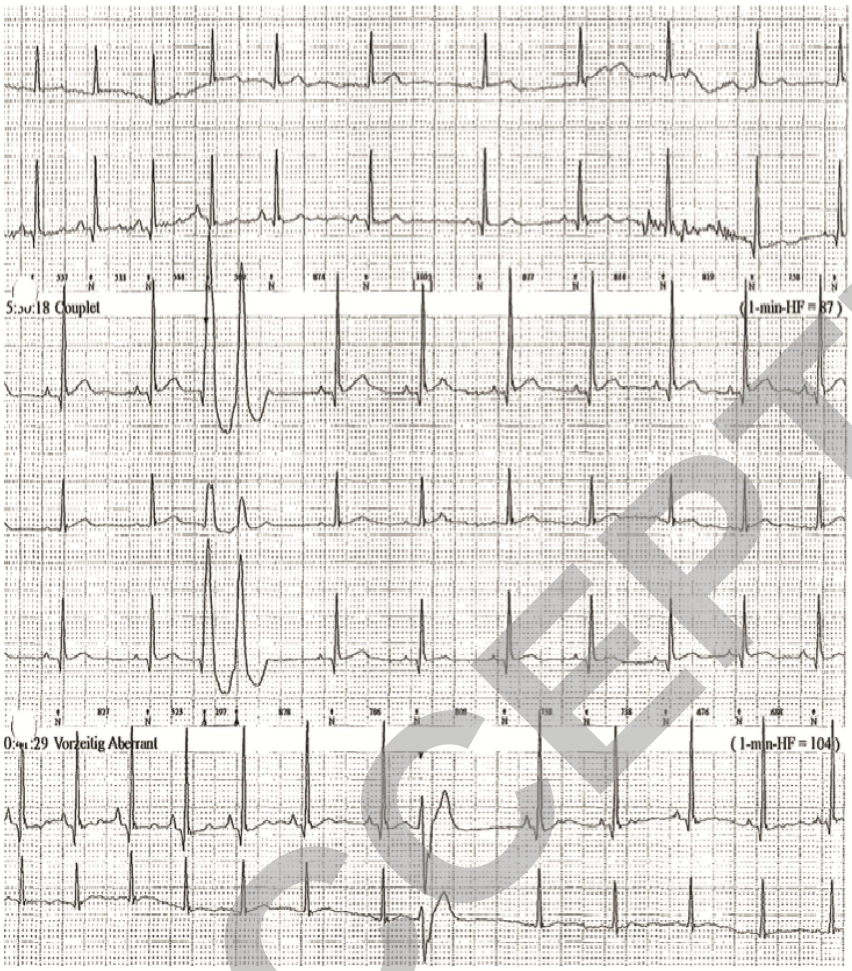


Figure 3. EKG abnormalities in *ATP1A3*-related syndromes. Graphical representation of the *ATP1A3*-related syndromes (AHC= rectangles, CAPOS= triangles, RDP= hexagons) (n=110), associated mutations and prevalence of EKG (12-lead and/or Holter) abnormalities. Each box includes the number of cases with a specific mutation and EKG abnormalities in the upper part, or without EKG abnormalities in the lower part. Reference sequences for the corresponding ATP1A3 transcript and protein were NM_152296.4 and Uniprot P13637, respectively. T1-T10= transmembrane domains. The position of the mutation across the functional domains was not associated with different prevalence of EKG abnormalities or dynamic changes. In 9 AHC patients, no mutation was identified in *ATP1A3*. D= dynamic changes (when serial tests were available).

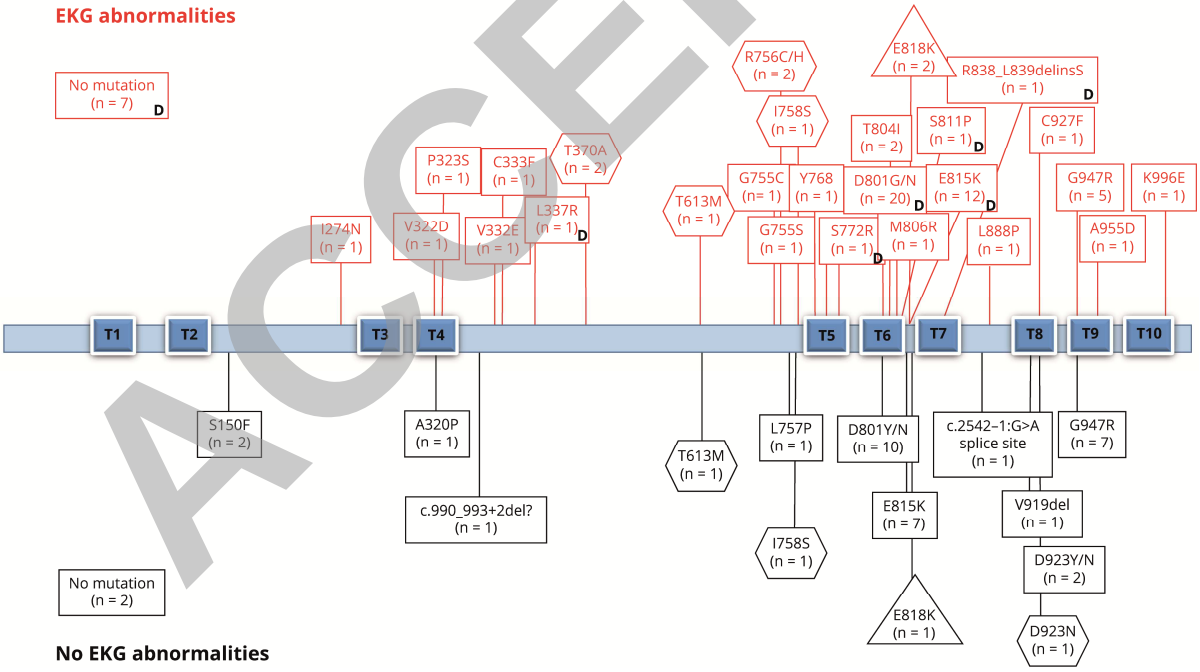


Figure 4. ATP1A3 expression in an adult normal heart. Dark brown stripes show strong immunolabelling for ATP1A3 corresponding to intercalated discs in adult myocardium from a 75 year-old male (cause of death at post mortem: bronchopneumonia). The tissue samples were formalin-fixed and paraffin-embedded. A standard immunohistochemistry method was applied to 5 micron thick sections with primary antibody Anti-ATP1A3 (Santa Cruz, Polyclonal, Goat, sc16052) at a dilution of 1/1000 overnight incubation at 4°C in diluent buffer (DAKO REAL, Ab diluent S2022). The immunostaining was qualitatively evaluated.

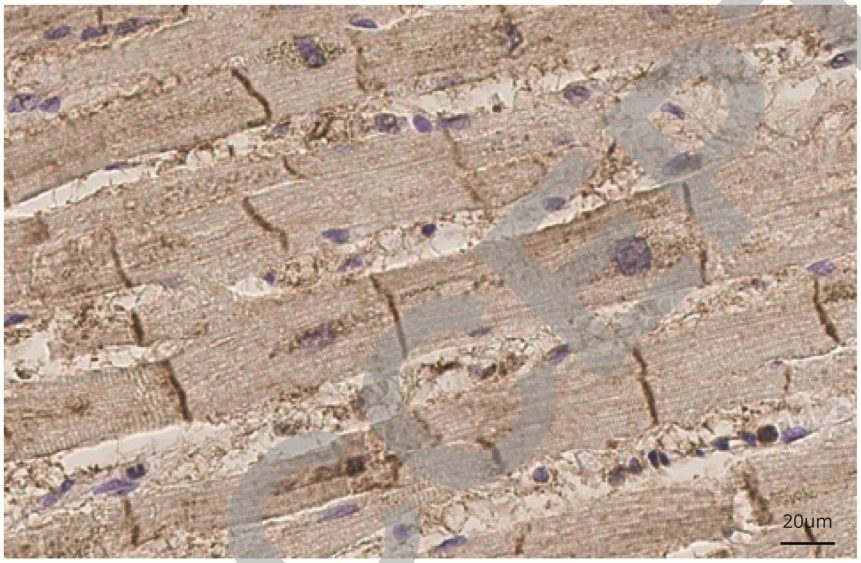
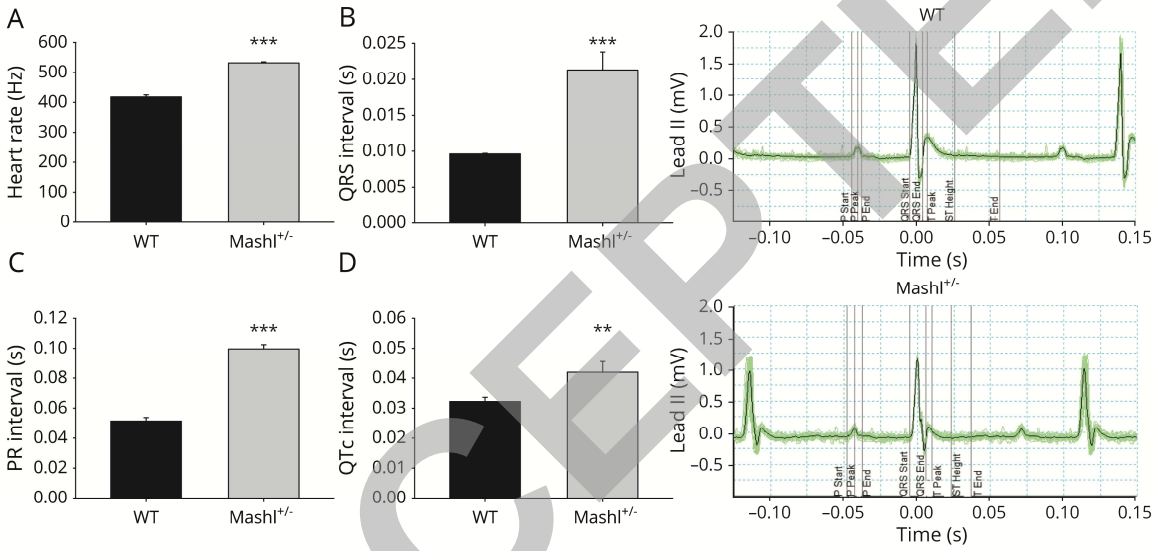


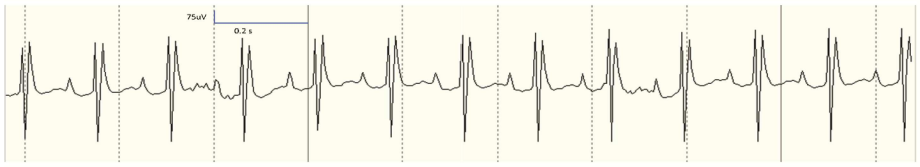
Figure 5. EKG data in the Mashl$^{+/-}$ compared to wild-type mice. Comparison of EKG data acquired from wild-type (WT, n=15) and Mashl$^{+/-}$ mice (n=3). (A) Heart rate, (B) QRS interval, (C) PR interval, (D) QTc interval. The traces are examples of EKG traces in WT and mutant mice. Heart rate, QRS and PR interval, were all higher in Mashl$^{+/-}$ mice. ***P=<0.001, **P<0.01 (Student *t*-test).



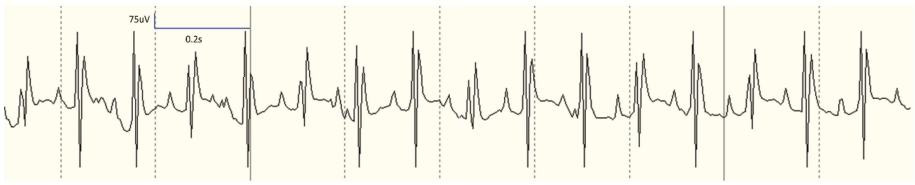
Mice	Heart rate (Hz)	QRS interval (s)	PR interval (s)	QTc interval (s)
WT (n = 15 mice)	418±6.8	0.009±0.00009	0.05±0.002	0.0322±0.0014
Mashl$^{+/-}$ (n = 3 mice)	532±3.5	0.0213±0.002	0.099±0.0028	0.042±0.003

Figure 6. EKG abnormalities in the Mashl$^{+/-}$ mice (n=3) after seizure induction. A) Mashl$^{+/-}$ #1 EKG traces: i. Baseline, with normal heart rate, noise present stems from skeletal muscle (breathing) activity; ii. Earlier changes started with onset of EEG seizures at 21 minutes after injection: Heart rate increase and JT segment elevation; iii. Later changes: Consecutive EKG traces shortly prior to sinus arrest showing heart rate fluctuation and atrioventricular block (red arrows), premature ventricular contractions (PVCs) are also visible (blue arrows); iv. Terminal change: Sinus bradycardia that was followed by sinus arrest. B) Mashl$^{+/-}$ #2 EKG traces: i. Baseline, with normal heart rate; ii. Earlier changes started with onset of EEG seizures after 5 min of injection and persisted to one minute prior to death (time of ii illustration): Increased heart rate with JT segment depression (red), and JT elevation (blue) widened QRS; iii. Later changes: Sequence of events <1 min before sinus arrest showing atrioventricular block (red arrows) and elevated JT segments (blue arrows); iv. Terminal change: Sinus bradycardia that was followed by sinus arrest. Mashl$^{+/-}$ #3 had similar EKG traces from baseline to terminal changes.

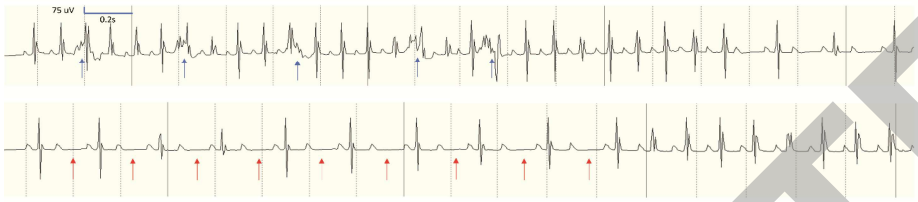
A.a Baseline



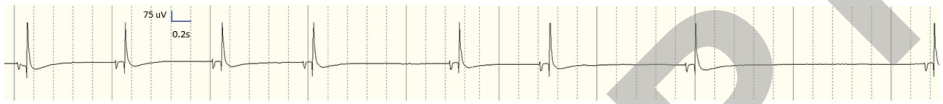
A.b Earlier changes



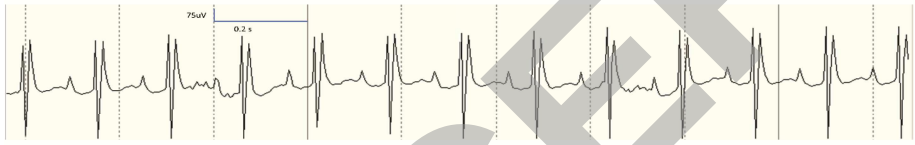
A.c Later changes



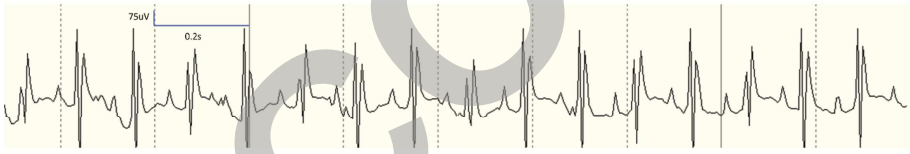
A.d Terminal bradycardia (was followed by sinus arrest)



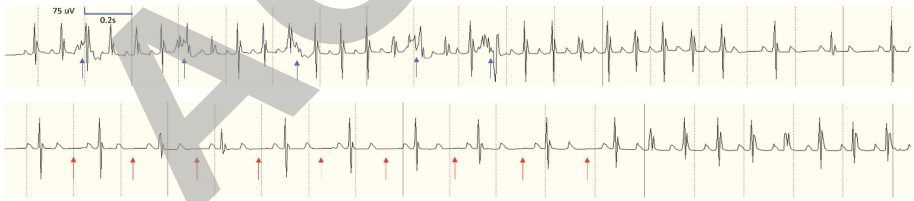
B.a Baseline



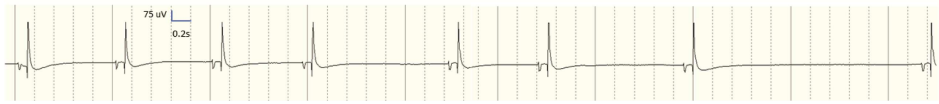
B.b Earlier changes



B.c Later changes



B.d Terminal bradycardia (was followed by sinus arrest)



Cardiac phenotype in *ATP1A3*-related syndromes: A multicentre cohort study

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