ANDERSEN-TAWIL SYNDROME WITH HIGH RISK OF SUDDEN CARDIAC DEATH IN FOUR MEXICAN PATIENTS. CARDIAC AND EXTRA-CARDIAC PHENOTYPES

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ABSTRACT

Background: Andersen-Tawil syndrome (ATS) is a cardiac channelopathy that is inherited in an autosomal dominant way, and it is characterized by a triad of periodic paralysis, ventricular arrhythmias, and includes some dysmorphic features with incomplete penetrance and variable expression that result in a challenging diagnosis. Objective: The objective of the study was to describe the cardiac and extra-cardiac phenotype in a cohort of patients with ATS at risk of sudden cardiac death (SCD) to improve its early clinical identification. Methods: In an observational, transversal study, with a deviant case sampling, four female patients with ATS at high risk of SCD were included in the study. They carried the heterozygous pathogenic variants c.407C>T [p.Ser136Phe], c.652C>T [p.Arg218Trp] (n=2), and c.431G>C [p.Gly144Ala] in the KCNJ2 gene. Patients were evaluated by a cardiologist, a clinical geneticist, and a physiatrist. Results: One patient had the classical facial phenotype and the other three had subtle manifestations. The group of patients presented a diverse set of clinical data such as: triangular face, broad forehead, broadening of medial eyebrows, auricular pits, low-set ears, eyelid ptosis, thin lips, mandibular hypoplasia, and diverse types of dental alterations, single transverse palmar crease, camptodactyly, and syndactyly. Long-exercise test showed a decrement in the percentage amplitude up to 44%, classifying patients in IV or V types according to Fournier’s scale. Conclusions: Extra-cardiac manifestations were a common finding in this series of ATS type1 at high risk of SCD. Its recognition could help the clinician in the early identification of patients with ATS, especially for the cardiologist since they are commonly referred only for evaluation of ventricular arrhythmias. (REV INVEST CLIN. 2021;73(3):XX-XX)

Key words: Andersen-Tawil syndrome. Phenotypic variability. KCNJ2 pathogenic variants. Long-exercise electrophysiological test. Periodic paralysis.
INTRODUCTION

In approximately 60% of the cases of Andersen-Tawil syndrome (ATS) (MIM# 170390), heterozygous pathogenic variants in the gene KCNJ2 (locus 17q23.1-q24.2) affect the alpha subunit of the inward rectifier potassium channel 2 (Kir2.1), causing alterations in the resting action potential in skeletal muscle and the depolarization phase in cardiac muscle. This cardiac channelopathy affecting the KCNJ2 is inherited in an autosomal dominant way, and in one clinical case by biophysical analysis is inferred KCNJ5 as a cause of ATS.

The KCNJ2 covers more than 330 variants informed in the different database (www.ncbi.nlm.nih.gov/clinvar) and near to 94 variants are classified as pathogenic or likely pathogenic as a cause of Short QT syndrome 3, familial atrial fibrillation or ATS (www.hgmd.cf.ac.uk), and the participation of additional variants in nuclear and mitochondrial genome is under analysis. This disease is characterized by a clinical triad of subtle dysmorphisms (mainly facial), ventricular arrhythmias, and periodic paralysis (PP). An important burden in cardiac alterations includes premature ventricular contractions (PVC), prominent U waves, wide T-U junction, prolonged Q-U interval, and ventricular tachyarhythmias. It also includes polymorphic or bidirectional ventricular tachycardia, syncope, and in some cases, a family history of sudden cardiac death (SCD).

In tertiary health centers, a significant part of the clinical cases is identified through the analysis of a classical cardiac rhythm disorder plus a high suspicious index by the cardiologists.

However, when the cardiac phenotype is not predominant, the cases can be derivate to neurology or rehabilitation services for the analysis of muscular weakness or PP, and in few cases, these are referred to the medical geneticist by general practitioners or specialists with interest in clinical genetics, where mandibular hypoplasia is the main symptom of suspicion, to rule out the Perre-Robin sequence or as a differential diagnosis of 22q11del syndrome. In this context, the participation of the medical geneticist is scarce in the analysis of the phenotype in the several ATS cases reported and the development of the "facial gestalt" is hard to develop for these diseases because the low frequency in Mexico and worldwide.

Patients with ATS had a clinical variable expression and incomplete penetrance (~80%) hindering the diagnosis to the varied group of specialists attending these patients. These specialists belong to areas such as internal medicine, rehabilitation, medical genetics, cardiology, neurology, physical medicine, and rehabilitation. From a cardiologist perspective, the disease is related to heart rhythm disorders, specifically ventricular arrhythmias, in the presence of a long Q-U interval. The extra-cardiac phenotype produces facial dysmorphism. The significant features are low-set ears, a broad forehead, short palpebral fissures, hypertelorism, full nasal bridge with a bulbous tip, and maxillary and mandibular hypoplasia. In addition, the skeletal phenotypic alterations may also present dysmorphic features as clinodactyly, syndactyly, short stature, and scoliosis.

As PP is one of the main features of the disease, the screening of suspected cases has been proposed through post-exercise electrophysiological tests. These can be helpful to distinguish among different muscle disorders because different patterns of the compound muscle action potentials (CMAP) changes over time and correlate with specific pathogenic variants. Throughout a long-exercise test protocol, it is possible to differentiate patients with dystonic myopathy (a pattern I, II, or III in the Fournier scale) and PP (pattern IV and V). Specifically, in pattern IV, it is observed an initial increase in the CMAP amplitude posterior to the exercise and a posterior late decrease. Concerning the pattern V, it is noticing a late decrease in the CMAP amplitude.

The objective of this work was to describe the cardiac and extra-cardiac phenotype in a cohort of patients with ATS and the high risk for SCD, to increase the probability of finding patients without a classic phenotype, who are treated in neuromuscular and rehabilitation clinics. In addition, it is intended to increase the phenotypic heritage for a future genotype-phenotype correlation in a wider sample.

METHODS

Four female patients attending a cardiological center in Mexico City, with a confirmed diagnosis of ATS by
A genetic testing confirmed four different heterozygous \textit{KCNJ2} missense pathogenic variants, including c.652C>T [p.Arg218Trp]\textsuperscript{12} (two patients), c.407C>T [p.Ser136Phe]\textsuperscript{13}, and c.431G>C [p.Gly144Ala]\textsuperscript{13} (Supplementary Table 1), altering highly conserved sites in the protein (Supplementary Tables 1 and 2). From the patients considered at high risk of SCD, three of them already had an implantable cardioverter-defibrillator (ICD) for secondary prevention. They have been monitored for at least four years by a medical geneticist and a cardiologist specialized in cardiac channelopathies through a standard resting 12-lead electrocardiogram (ECG). Furthermore, the patients had a 24 h Holter monitoring at each 6-months visit at the Channelopathies Clinic of the National Institute of Cardiology.

A physiatrist in the Department of Muscular Dystrophy and Electrography at the Instituto Nacional de Rehabilitación Luis Guillermo Ibarra Ibarra analyzed the electrophysiological characteristics of skeletal muscle. Through various neurophysiological tests, the motor and sensory nerve conduction were analyzed through the study of the median, ulnar, peroneal, and sural nerves using surface electrodes and conventional techniques to analyze muscle weakness. To further study the muscle characteristics, electromyography (EMG) needle was inserted in proximal and distal limb segments to test muscular activity at rest and during exercise. Finally, it was carried out a set of special studies such as short and long-exercise tests according to Fournier’s method\textsuperscript{9} in an attempt to analyze a possible correlation and identification of cases with ATS and their pathogenic variants found in the genetic test.

All the procedures were made according to the declaration of Helsinki, and the patients signed informed consent. The protocol was approved by the Institutional Research and Bioethics Committees of the Instituto Nacional de Cardiología Ignacio Chávez (Project number 17-1003).

**RESULTS**

**Arrhythmic phenotype**

After explaining the benefits, risks, and limitations of the placement of an ICD, one of the patients decided that the device should not be placed. Therefore, three patients underwent an ICD implantation. In all subjects, the cardiac symptoms started before 25 years (Table 2). Cases 1 and 2 belonged to the same family. They had a family history of SCD in one sister and the mother. Table 3 shows the main ECG parameters. The mean QTc interval was 460 ms considering that only two patients had prolonged a QTc interval (441 and 544 ms). All patients showed prominent U waves with “U on P” sign and showed a prolonged Q-Uc interval up to 725 ms with a mean of 663 ms.
The results of 24-h Holter monitoring are described in Table 4. We documented in all patients PVC, with a 24-h burden, from 5% to 34%. The presentation included ventricular bigeminy, couplets, triplets, and episodes of ventricular tachycardia (VT): 50% with monomorphic VT and 50% had polymorphic VT. One subject had sustained VT with polymorphic VT. Bidirectional VT was present in one patient.

Extra-cardiac phenotype

Table 5 shows the extra-cardiac phenotypic characteristics of the four cases. The most common facial features in the four patients were mandibular hypoplasia in all the cases (100%), plus triangular face, broadening of forehead, broadening of medial eyebrows, and eyelid ptosis in three cases (75%). Concerning the limbs, the most common finding was camptodactyly in hands (75%) and 2nd-3rd feet syndactyly (100%). All the patients had clinical history of a diverse set of dental alterations, though not assessable by physical examination in two patients because they had previous orthodontic treatment (Supplementary Fig. 1).

Electrophysiological testing

Table 6 shows the main results of the electrophysiological studies performed to identify alterations in
skeletal muscle due to the potassium channel dysfunction. In most cases, we found motor neuroconduction in normal ranges, but the patient number three had a decrement in the motor neuroconduction amplitude potential in median, ulnar, and peroneal nerves. Sensitive neuroconduction studies had normal parameters in all patients.

Needle EMG testing showed that 50% of membrane instability values mimic the typical pattern of myopathy in patients 2 and 3; the other patients had normal patterns. We did not find abnormalities short-exercise test, and the majority of the patients showed a minimum decrement in the amplitude voltage. The electrophysiological studies found a relevant result in the long-exercise test: a significant decrement in the amplitude percentage in the range of 44-64.5%, classifying patients in IV or V types according to the Fournier’s scale (Fig. 1). The decrement in the amplitude and area at the CMAP was up to 60% in all patients (Table 6).

Table 5. Phenotypic manifestations in patients with Andersen-Tawil syndrome

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low height</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Triangular face</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Broad forehead</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Broadening of medial eyebrow</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Ptosis palpebral</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Down-slanting palpebral fissures</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Myopia</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Flat nasal bridge</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Bulbous nose</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Malar hypoplasia</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Mandibular hypoplasia</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Dental alterations</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Thin lips</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Low-set ears</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Auricular pits</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Unilateral hypoacusia</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Scoliosis</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Camptodactyly in hands</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Clinodactyly</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Single transverse palmar crease</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Feet syndactyly</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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</tbody>
</table>

**DISCUSSION**

ATS is a disease with unknown frequency, due to the limited information contained in the literature that could be described as an ultra-rare disease (1:500,000-2,000,000)\(^{14}\). However, this number can be over-representative. A significant part of patients with ATS does not present the typical triad, which makes difficult the identification of the phenotype where there is not a significant cardiac affection.

The external phenotype identified in this group of Mexican patients with ATS agree with the clinical data previously informed in literature\(^{15,16}\), where the more common features were a triangular head, broad forehead, flared eyebrow, ptosis palpebral, mandibular hypoplasia, dental alterations, thin lips, camptodactyly, clinodactyly in feet, syndactyly, and muscle weakness. However, the case 2 had a typical appearance with the bulbous nose present and diverse
Table 6. Electrophysiology characteristics of the skeletal muscle in patients with Andersen-Tawil syndrome

<table>
<thead>
<tr>
<th>Case number</th>
<th>Symptoms</th>
<th>Motor neuro-conduction</th>
<th>Sensitive neuro-conduction</th>
<th>Needle EMG</th>
<th>Amplitude changes at CMAP in the SET (mv)</th>
<th>Amplitude decrement at CMAP in the LET</th>
<th>Area decrement at CMAP in the LET</th>
<th>Fournier scale in the LET</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Asymptomatic</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Increment from 7.6% to 18.7% and then decrement to basal level</td>
<td>50% (basal) 64.2% (maximum amplitude)</td>
<td>60.2% (basal) 71.2% (maximum valor)</td>
<td>Type IV</td>
</tr>
<tr>
<td>2</td>
<td>Weakness crisis</td>
<td>Normal</td>
<td>Normal</td>
<td>Repose: Membrane instability EMG patron look like myopathic</td>
<td>Decrement from 16.2% to 24.7%</td>
<td>Decrement from 5.5% to 10.5 after cooling</td>
<td>57.3% (basal) 77% (basal)</td>
<td>Type V</td>
</tr>
<tr>
<td>3</td>
<td>Weakness crisis</td>
<td>CMAP decreased in medianus, ulnar and peroneal nerves</td>
<td>Normal</td>
<td>Repose: Membrane instability EMG patron look like myopathic</td>
<td>Increment from 6.8 to 10% then decrement to basal</td>
<td>44% (basal)</td>
<td>74.5% (basal)</td>
<td>Type V</td>
</tr>
<tr>
<td>4</td>
<td>Little weakness</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Increment from 24.6% to 39%, then showed minimums decrement</td>
<td>51.7% (basal) 45.2% (basal)</td>
<td>64.5% (maximum amplitude) 62.7% (maximum valor)</td>
<td>Type IV</td>
</tr>
</tbody>
</table>

CMAP: compound muscle action potential; EMG: electromyography; LET: long-exercise test; SET: short exercise test.

dental anomalies, which in conjunction with the cardiac alterations previously informed, helped to identify in 2015 the second pathogenic variant for Mexicans, and a variant of unknown significance in the CACN1C (under functional genomic study) since the first cases by Canun et al. and Plaster et al. carrying the pathogenic variant c.212A>T [p.Asp71Val]. However, the cases 1, 3, and 4 had attenuated facial alterations and were identified because in a detailed exploration, subtle alterations were showed and, in conjunction with a history of weakness, makes mandatory to perform KCNJ2 Sanger sequencing. A personal observation was the presence of broadening of the medial eyebrow in three of four cases; the frequency of this data must be verified in patients with ATS and risk of SCD. With the development of new technologies such as 3D facial shape modeling, facial discrimination, including subtle features, a better diagnosis of subtle facial phenotypes can be made in individuals with ATS.

A relevant observation of this work is the dental alterations were a constant in the group studied; abnormalities identified included dental crowding, enamel hypoplasia, hypodontia, hyperdontia, microdontia, and macrodontia. The dental anomalies are considered underreported in ATS and the study of alterations in the development and growth of teeth could help to identify in a better way the participation of potassium channels in ATS.
Due to the leading group of patients described in the literature are young people, we identify additional alterations developed as part of the natural history of the disease, for example, subtle psychological changes in the behavior in two patients were detected. In this context, the participation of Kir2.1 in the neuronal potential of action and the effect in the neurophenotype is under scrutiny.

The information published related to electrophysiological studies analyzing the skeletal muscle response either because of screening identification for genetic testing or weakness as the main clinical sign is scarce. In this respect, Fournier et al. described that performing a long-exercise test protocol can be able to detect a decrement in up to 40% in the CMAP. The results obtained, which present an early decrease in the CMAP (nadir 34-40 min in all patients), agree with a recent report by Song et al., that an initial decrement in the CMAP amplitude during prolonged exercise test in ATS cases is concordant with the clinical history in our Case 1 (a case with two SCD in the family) but with a different morphology compared to Case 2 which has more alterations and also belongs to the same family. This pathogenic variant was included in a cohort of 11 patients with ATS, where 100% had modification >40% in CMAP. In addition, Tan et al. (2012) proposed muscle velocity recovery cycles combined with repetitive stimulation as an alternative to long test protocol were also patients with c.431G>C [p.Gly144Ala] pathogenic variant supports the finding obtained in one patient by Ballester et al.,. This case was a Hispanic patient positive in clinical history for muscle weakness, but the findings are opposed to the clinical data found by Rai et al., where did not find muscle affection when the LET

Twelve patients carrying the c.652C>T [p.Arg218Trp] pathogenic variant evaluated with the long test protocol in a recent Chinese cohort were studied. Authors were able to identify a substantial CMAP increase followed by a gradual reduction in amplitude which is concordant with the clinical history in our Case 1 (a case with two SCD in the family), but with a different morphology compared to Case 2 which has more alterations and also belongs to the same family. This pathogenic variant was included in a cohort of 11 patients with ATS, where 100% had modification >40% in CMAP. In addition, Tan et al. (2012) proposed muscle velocity recovery cycles combined with repetitive stimulation as an alternative to long test protocol were also patients with c.431G>C [p.Gly144Ala] pathogenic variant supports the finding obtained in one patient by Ballester et al.,. This case was a Hispanic patient positive in clinical history for muscle weakness, but the findings are opposed to the clinical data found by Rai et al., where did not find muscle affection when the LET

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**Figure 1.** CMAP amplitude changes during long-exercise test protocol in four ATS cases with risk of SCD.
was performed in seven Indian patients from two related families. In the present study, the patient carrier of the same variant had little weakness evaluated by LET, and she coincided with the patient informed by Ballester et al. Until now, there is no published information of positive LET patients carrying this pathogenic variant to compare the results obtained. Further studies, including ethnic differences and hormonal changes, will improve the results obtained in this part of the phenotype. On the other hand, to our knowledge, there are no data for LET studies, including the variant c.407C>T [p.Ser136Phe] and this study includes the first patient with specific electrophysiological studies in the skeletal muscle, including this pathogenic variant.

In ATS, the phenotype is remarkably heterogeneous, and the diagnosis has been firmly successful in some cases due to arrhythmic manifestations, combined with clinical experience in rare disease and availability of genetic testing to identify heterozygotes pathogenic variants in KCNJ2 and some cases, pathogenic variants in KCNJ5 (<1%). However, considering the frequency of this rare disease, there must be >100 patients in the country. This makes it mandatory to review the clinical, demographic, and physiological factors that could help to improve the identification of ATS cases in Mexico, taking into account that a great part of the patients reaches adulthood.

Furthermore, in a review of the literature performed by our research group until December 2018 in PubMed (pubmed.ncbi.nlm.nih.gov/), it was found 28 patients carrying the pathogenic variant c.652 C>T [p.Arg218Arg] (14 female- 14 male), this pathogenic variant causes a change of the arginine-basic amino acid into a tryptophan-nonpolar residue or an uncharged polar residue(p.Arg218Gln)17, this site is located within the C-terminus domain of the alpha subunit of the strong inwardly-rectifying channel protein Kir2.1, causing a dominant-negative effect and diminished current of potassium, and also a decreased affinity for PIP2 has been reported for this domain26,27. The group of patients informed in literature had a range of 5-73 years old. This variant is considered as a hotspot according to literature1,7,8,12,16,17,28-33, male cases have less affectation in cardiac and skeletal muscle, and facial and skeletal studies are under-reported, the clinical variable expression is a constant, and sudden death in 11% of the patients informed in the literature7,16,34.

The pathogenic variants c.407C>T [p.Ser136Phe], and c.431G>C [p.Gly144Ala], are less informed in literature, both variants are located in the M1 pore region, causing alterations in the selectivity of the pore channel leading to nonfunctional Kir2.1 channel4,17. In a study performed by Zhang et al., analyzing a subset of patients with pathogenic variants in the M1 pore region (including the two variants of these two patients) was found to a less dysmorphic phenotype, similarly in two patients included in the report. However, cardiac alterations were found in many of the patients included in the study. In this study, the muscle was evaluated through the research of PP finding in a low proportion of this clinical data, but specific information concerning the facial phenotype is scarce4.

Concerning the differences between cases with the same pathogenic variant, epigenetic, hormonal, and additional genetic factors are under scrutiny. The main factor currently under analysis by the research group is the participation of some haplotypes of the mitochondrial genome on the function, biogenesis, and protein interaction in the cardiac phenotype of ATS. As a first result, a greater presence of variants in the mitochondrial DNA of patients with ATS has been identified, which could have an influence on the manifestation of the disease13.

A limitation of this work is the size of the sample, as only four subjects affecting from this rare disease, with a particular phenotype and risk of SCD were included and analyzed.

The benefits of the early identification of patients with ATS is determining an adequate treatment, management, stratification of the risk of SCD and avoiding the use of amiodarone to prevent fatal outcomes35,36, the monitoring of potassium levels avoids related hypokalemic alterations with weakness, and in some rare cases refer for plastic surgery intervention, to improve the quality of life of the patient. More studies are needed with a focus on the participation of genetics factors that lead to SCD and their interaction with environmental variables.

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SUPPLEMENTARY DATA

Supplementary data are available at Revista de Inves-tigación Clínica online (www.clinicalandtranslational-investigation.com). These data are provided by the corresponding author and published online for the benefit of the reader. The contents of supplementary data are the sole responsibility of the authors.

REFERENCES