# Novel KCNJ2 mutation associated with Andersen-Tawil syndrome

## Nova mutacija kanalčka KCNJ2 pri bolnici s sindromom Andersen-Tawil

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**Ključne besede:**
bidirekcijska prekatna tahikardija, genetska napaka, Andersen-Tawilov sindrom, mutacija KCNJ2, katetrska ablacija

## Abstract

Andersen-Tawil syndrome (ATS) is a rare inherited or sporadic disorder characterized by ventricular arrhythmias, characteristic QT-U wave patterns in electrocardiogram, periodic paralysis, and dysmorphic features. We describe a patient of Slovenian origin who exhibited mild dysmorphic features, repetitive bidirectional and monomorphic ventricular tachycardias, and characteristic QT-U wave patterns. Ventricular arrhythmias persisted despite several catheter ablation procedures and different anti-arrhythmic drug treatments, resulting only in pacemaker implantation and amiodarone toxicity. Cardioverter-defibrillator was implanted after a major syncopal attack. Finally, molecular genetic screening revealed a novel heterozygous mutation (c.424A>C/p.Thr142Pro) in *KCNJ2* gene consistent with the ATS.

## Izvleček

Andersen-Tawilov sindrom (ATS) je redka podedovana ali sporadična bolezen, ki se kaže s prekatnimi aritmijami, značilnimi spremembami vzorca QT-U v elektrokardiogramu, s periodično paralizo in dismorfnimi znaki. Predstavljamo slovensko bolnico z blagimi dismorfnimi znaki, ponavljajočo se bidirekcijsko in monomorfno prekatno tahikardijo in z značilnimi spremembami QT-U. Prekatnih aritmij nismo uspeli bistveno zmanjšati niti z antiaritmičnim zdravljenjem in tudi ne z ablacijskimi posegi. Nasprotno, vstaviti je bilo potrebno srčni spodbujevalnik. Zaradi toksičnosti smo morali ukiniti amiodaron. Zaradi sinkope smo ji vstavili kardioverter-defibrilator. Končno smo z genetskim testiranjem ugotovili novo heterozigotno mutacijo (c.424A>C/p.Thr142Pro) v genu za KCNJ2 in dokazali ATS.

**Key words:**
bidirectional ventricular tachycardia, genetic disorder, Andersen-Tawil syndrome, KCNJ2 mutation, catheter ablation

**Citirajte kot/Cite as:**
Zdrav Vestn 2013; 82: 55–60

Prispelo: 26. jan. 2012,
Sprejeto: 15. jun. 2012

## Introduction

Andersen-Tawil syndrome (ATS) is characterized by a triad of periodic flaccid muscle weakness, ventricular arrhythmias, characteristic QT-U wave patterns in standard electrocardiogram (ECG), and anomalies such as low-set ears, ocular hypertelorism, micrognatia, fifth-digit clinodactyly, and syndactyly.[1-5] Affected individuals present in the first or second decade with either cardiac symptoms (palpitations and/or syncope) or weakness that occurs spontaneously. ECG may reveal characteristic abnormalities including prominent U waves, prolonged QT-U interval, ventricular premature beats, polymorphic ventricular tachycardia (VT), and bidirectional VT.[5] Despite a large tachycardia burden, most ATS patients are remarkably asymptomatic and sudden cardiac death is rarely described.[1,4,6]

ATS is a unique channelopathy. The *KCNJ2* gene, encoding the inward rectifier potassium channel 2 protein (Kir2.1), is the only one known to be associated with ATS and more than 30 mutations have been described already.[4,7] The Kir2.1 channel normally sets the resting membrane potential and controls the duration of the action potential in many different types of excitable cells.[8] ATS is a rare condition with variable expression, caused by sporadic mutations, or inherited in an autosomal dominant manner. It has been diagnosed in only a few hundred families in the world. The phenotype is highly variable. Approximately 60 % of affected individuals manifest the complete triad of cardinal features and up to 80 % express two of them. [6,9] Approximately 60 % of individuals with ATS have a detectable mutation in *KCNJ2* gene. Non-penetrance is evident in 6–20 % of individuals with an identifiable mutation.[6,7,10] The presence of a pathogenic *KCNJ2* mutation confirms the diagnosis.[4,11]

We describe a case with novel *KCNJ2* mutation associated with ATS. Several catheter ablation procedures aiming to reduce the VT burden in this patient were unsuccessful or even harmful. The serum potassium and chloride ion imbalances may have a role in a pro-arrhythmic episode of our patient.

## Case presentation

A 20-year-old female was referred in 2002 for management of incessant repetitive VT occurring predominately during daily activities (Figure 1A). She was complaining of occasional chest discomfort and palpitations, but no muscular fatigue, dyspnea, dizziness or syncope were reported. Family history was uneventful. Physical examination was normal except for the mild hypertelorism and micrognatia (Figure 2). ECG revealed a prolonged QT-U interval (≥ 600 ms), prolonged terminal T wave downslope, wide T-U junction, biphasic and enlarged U waves, and normal QT interval length (Figure 1B). The R-on-U phenomenon of ventricular premature beats (VPBs) was noted (Figure 1A, B). Transthoracic echocardiography confirmed a structurally and functionally normal heart.

Several anti-arrhythmic drug regimens including propafenone and/or beta-blocking drug, diltiazem or verapamil, and sotalol were attempted during follow-up with only a slight effect on arrhythmia suppression. In addition, several electrophysiologic studies and radiofrequency catheter ablation procedures (RFAs) were attempted in our and other institutions from 2003 to 2009. The RFAs were directed towards the VPBs from the left anterior and posterior fascicles of the His-Purkinje system (H-P). However, these attempts were unsuccessful requiring permanent pacemaker implantation in 2004. Interestingly, a manifest bidirectional VT is rarely demonstrated after these left inter-ventricular septal RFAs (see Figures 1A and 1C). In 2005, we consulted genetic experts from two institutions about the possibility of Andersen's syndrome in our patient. Additional genetic testing was not recommended due to low probability of the disease. A cardioverter-defibrillator (ICD) was implanted in 2009 after a major syncopal episode with seizures and incontinence attributed to ventricular tachyarrhythmia. The sustained VT was documented at emergency outpatients department (Figure 1C). The electrolyte imbalances due to dehydration might have played a role in this pro-arrhythmic event since she had been working overnight before. The

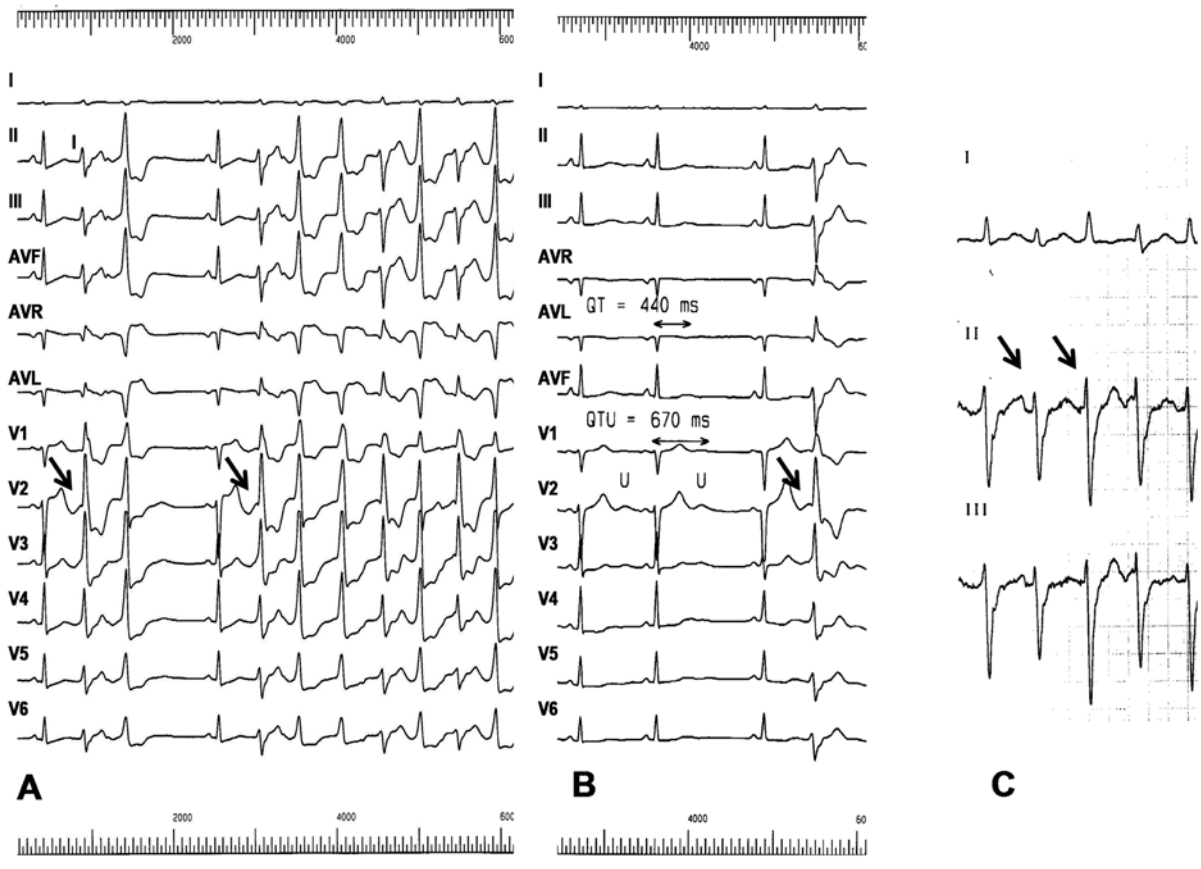


**Figure 1:** (A) Twelve lead ECG (25 mm/s) of our patient with repetitive sustained bidirectional ventricular tachycardia (VT) (130 beats/min). Right bundle branch block QRS morphology and alternating left- and right-axis deviations are seen during the VT. A ventricular premature beats with R-on-U phenomenon are marked with arrows (see A and B). (B) Characteristic T-U patterns: prolonged QT-U interval, prolonged terminal T down slope, wide T-U junction, prominent U waves in right pre-cordial leads, and normal QT interval. (C) Only mild alternating frontal plane axis-deviations are seen after extensive left septal radiofrequency catheter ablations–arrows in lead II.

serum potassium levels were at the lower border of the normal range (3.9 mmol/L) and the chloride levels were high (106–113 mmol/L). Both spontaneously normalized during hospitalization. Serum magnesium, sodium, and calcium levels were normal. After ICD implantation and combined amiodarone and bisoprolol treatment, she was doing well. However, amiodarone treatment had to be discontinued in 2010 due to thyrotoxicosis. She was treated with antithyroid drugs and is currently on levothyroxine replacement, bisoprolol and potassium supplements. She still has repetitive non-sustained VTs.

## Molecular genetic analysis

Molecular genetic analysis was performed after the informed consent of the patient had been obtained. Genomic DNA was extracted from peripheral blood of patient using Flexigene DNA kit (Qiagene, Hilden, Germany). Mutation screening was performed by sequence analysis of the coding region of the *KCNJ2* gene. Exon 2 was amplified by polymerase chain reaction (PCR) as four overlapping fragments using primers designed with Primer3 software (http://fokker.wi.mit.edu/primer3/input.htm), primer sequences are available upon request.

PCR was performed in total volume of 25 mL containing 75ng of genomic DNA, 0.4 pmol of each primer, 200 mmol of each dNTP, 1.75 mmol of $MgCl_2$, 0.8 U of *Taq* polymerase and 1X supplied buffer (Ampli-*Taq* Gold DNA polymerase, Applied Biosystems, Carlsbad, CA), using GeneAmp 9700 PCR system (Applied Biosystems, Foster City, CA). The PCR products of patient were purified with EXOSAP-IT kit (USB, Cleveland, Ohio, USA) and directly sequenced using Big Dye terminator cycle sequencing kit (Applied Biosystems) and an automatic sequencer (ABI Prism 310, Applied Biosystems, Foster City, CA). The cDNA sequence numbering was based upon GenBank reference sequence NM000891.2 (the first ade-

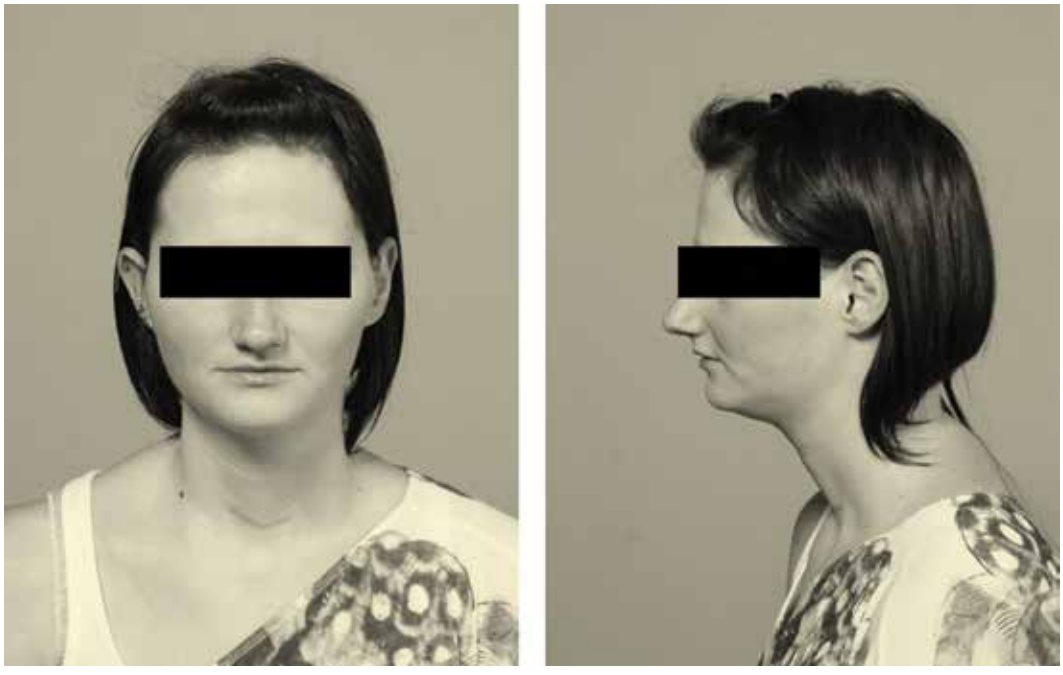

**Figure 2:** Our patient demonstrated mild dysmorphic features with hypertelorism and micrognatia.

nosine in initiator ATG was designed as +1). Novel mutation was verfied in 85 healthy unrelated control subjects by denaturing high-performance liquid chromatography method (dHPLC) using WAVE-3500 System (Transgenomic, Crewe, UK).

A novel heterozygous *KCNJ2* gene mutation, p.Thr142Pro (c.424A>C), was identified in our patient (Figure 3A). Absence of the mutation was confirmed in 85 unrelated and unaffected Slovene subjects (170 aleles). The novel mutation was named as recommended.[12] Unfortunately, proband's family members have not agreed to genetic testing. Multiple alignments of amino acid sequences between several members of Kir family have been made with multiple alignment tools (http://www.ncbi.nlm.nih.gov/tools/cobalt/) (Figure 3B).

## Discussion

We describe the first patient of Slovenian origin with proven ATS. The patient has clinically apparent phenotype with mild dysmorphic features including small mandible, characteristic QT-U wave patterns in ECG,[5] incessant ventricular arrhythmias, and rare syncopal episodes. In addition, the molecular genetic screening revealed a novel heterozygous *KCNJ2* gene mutation (c.424A>C/p.Thr142Pro), encoding the Kir2.1 potassium ion channel.

General topology of the Kir2.1 consists of two membrane spanning α-helical protein segments (M1, M2) separated by the extracellular pore-forming P-loop that confers potassium selectivity. The hydrophilic amino and carboxy termini are located intracellularly. Four Kir subunits oligomerize to form functional Kir2.1.[11,13] The Thr142Pro mutation was found in the P-loop replacing a highly conserved polar threonin on position 142 with non-polar imino acid prolin, and displays an increase in Kyte-Doolittle hydrophobicity level from -0.7 to -1.6. Substitution into proline may severely impair the secondary structure of the protein and its potassium selectivity. The functional effect of the Thr142Pro mutation is likely to affect the hydrophobic pore loop region near the extracellular surface of the membrane. Interestingly, the most frequently reported mutations in patients with ATS are located nearby at amino acid Gly144 and Gly146 locations.[9,11,14,15] We speculate that Thr142Pro mutation might have an even greater impact on the Kir2.1 pore structure and function due to its greater amino acid polarity alteration. The bioassay experiments or new reports on ATS patients with the same mutation would confirm the clinical role of Thr142Pro mutation. The novelty of this mu-

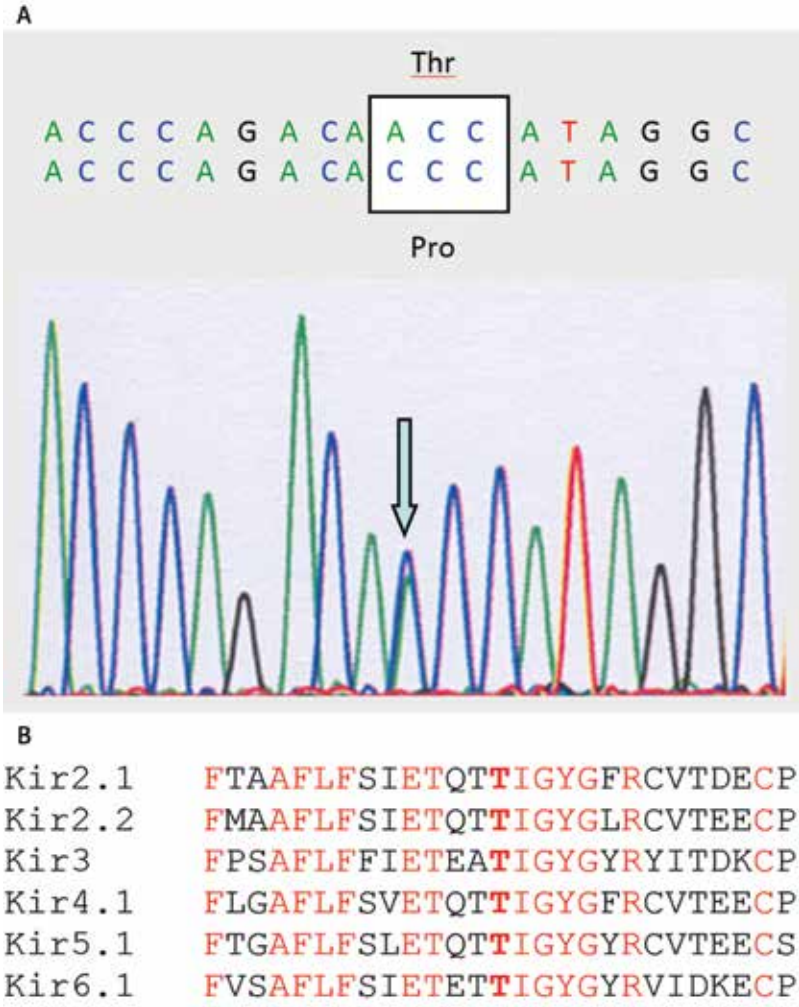


**Figure 3:** Identification of novel *KCNJ2* gene mutation p.Thr142Pro (c.424A>C) in our patient with Andersen-Tawil syndrome. (A) Sequence chromatogram of heterozygous mutation. (B) Amino acid conservation is seen at the p.Thr142Pro (T142P) mutation site in different members of the family of inward-rectifying potassium channel (Kir) proteins.

tation was checked in a large number of Slovene healthy unrelated controls. Unfortunately, proband's close family members refused to undergo the genetic testing.

Mechanosensitive ion channel balance may contribute to the genesis of the U wave in ECG, [16] and one might speculate that decreased function of delayed rectifier potassium channel could result in an augmentation of other mechanosensitive channels and thereby producing an enlarged U wave, as seen in our and other ATS patients.[5] This notion may explain, in part, the pro-arrhythmic episode of our patient in 2009 since elevated serum chloride and reduced serum potassium levels were documented that might acutely disrupt this mechanosensitive channel balance. This kind of electrolyte disturbances may increase the ventricular mechanoelectric feedback with a consequent increased susceptibility for delayed afterdepolarizations (DADs) and pro-arrhythmic events in ATS patients. The R-on-U phenomenon of ventricular premature beats demonstrated in our patient (Figure 1A, B) is in agreement with DAD-related triggered activity as the principal mechanism for ventricular arrhythmias. Higher ventricular arrhythmia burden during daily activities might also suggest the importance of autonomic modulating influences on mechanosensitive ion channel balance.

Both, bidirectional and monomorphic VTs of RBB morphology were documented in our patient. The electrophysiologic study revealed that monomorphic VT was a left fascicular VT (not shown). Interestingly, the bidirectional VT transformed to a VT with only slightly changing frontal plane axis deviations (Figure 1C), probably due to the left H-P system destruction after all previous ablation procedures. Therefore, the left H-P conduction system might be also the primary source of bidirectional VT in these patients. This observation is in agreement with the recently proposed concept of reciprocating bigeminy as a mechanism for bidirectional VT in which DAD-induced triggered activity develops at different heart rate thresholds in different regions of the H-P system.[17] In our patient, a pair of foci with reciprocating bigeminy might have been located in the left posterior and left anterior fascicles being modified or destroyed with RFA. According to our unsuccessful efforts in reducing the ventricular arrhythmia burden by catheter ablation procedures, we absolutely do not recommend this treatment approach in patients with ATS. This is in agreement with standard treatment recommendations.[4]

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