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„Loss of functions mutations in Kir2.1 channels“

verfasst von / submitted by

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1 Acknowledgements

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2 Abstract

Mutations of the inward-facing potassium channel Kir2.1 can lead to a loss or decrease in the potassium flow. This channel is expressed in various regions of the human body, especially in the heart, brain and blood system and has a very important role in the repolarization of the cell. If small disturbances occur in the complex system of excitability of a cell, this can have drastic consequences. In this case, Andersen-Tawil syndrome develop, due to loss of Kir2.1 channel function, mostly caused by single point mutations. This condition is extremely rare, as it is approximately one in a million in frequency. It is an inherited disease that is characterized by the symptoms of long QT syndrome, neurological problems, and physical problems, such as short stature, low-set ears and widely spaced eyes.

The pathological alteration in the Kir2.1 is very complex and can have various causes. The mutation of certain amino acids in the protein can influence the binding or modulation of the essential activatory ligand PIP_2, (phosphatidylinositol-4,5-bisphophate) and/or the correct folding of the channel. In addition, the flow of potassium ions through the channel, as well as the transport and integration of the protein into the membrane, is influenced in some cases. It is difficult to infer the effect on the channel due to the mutation. Therefore, this diploma thesis deals with general information about the Andersen Tawil syndrome (ATS), such as its development, symptoms and its symptomatic treatment. The focus of this diploma thesis is to provide an overview of ATS causing mutations, the functional and structural consequences for potassium channel Kir2.1 function, as well as for the physiology of the body.

3 Zusammenfassung

Die Mutation des einwärtsgerichteten Kaliumkanal Kir2.1 führt zu einem Verlust oder Erniedrigung des Kaliumausstromes aus der Zelle. Dieser Kanal wird im Körper, vor allem in dem Herzen, dem Gehirn sowie dem Blutsystem produziert und hat eine sehr wichtige Rolle in der Repolarisation der Zelle. Wenn kleine Störungen in dem komplexen System der Erregbarkeit einer Zelle auftreten, kann dies drastische Folgen haben. In diesem Fall kommt es zur Ausbildung des Andersen-Tawil-Syndroms. Diese Erkrankung ist extrem selten, da sie eine Häufigkeit von etwa eins zu einer Million aufweist. Es ist eine vererbte Krankheit, die durch die Symptome eines Langes-QT-Syndroms, neurologischen Problemen sowie physischen Problemen, wie zum Beispiel Kleinwuchs, tiefsitzende Ohren und weit auseinander liegenden Augen gekennzeichnet ist.

Die pathologische Veränderung des Kir2.1 ist sehr komplex und kann verschiedene Ursachen haben. Die Mutation bestimmter Aminosäuren in dem Protein kann Einfluss auf die Bindung des essentiellen, aktivierenden Liganden PIP_2, (Phosphatidylinositol-4,5-bisphophat) und/oder auf die korrekte Faltung des Kanals haben. Außerdem wird bei bestimmten Mutationen der Durchfluss der Kalium Ionen durch den Kanal, sowie der Transport und die Integration des Proteins in die Membran beeinflusst. Es ist schwierig aufgrund der Mutation auf die Auswirkung auf den Kanal rückzuschließen. Daher befasst sich diese Diplomarbeit mit allgemeinen Informationen zur Erkrankung Andersen Tawil Syndrom (ATS), wie der Entstehung, den Symptomen und dessen symptomatischer Behandlung. Der Fokus der Diplomarbeit liegt auf den Mutationen welche ATS auslösen und deren physiologischen und strukturellen Veränderungen für den Kaliumkanal Kir2.1, als auch für die Physiologie des Körpers.

4 Abbreviations

A	Ala	Alanine
R	Arg	Arginine
N	Asn	Asparagine
D	Asp	Aspartic acid
C	Cys	Cysteine
E	Glu	Glutamic acid
Q	Gln	Glutamine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
L	Leu	Leucine
K	Lys	Lysine
M	Met	Methionine
F	Phe	Phenylalanine
P	Pro	Proline
S	Ser	Serine
T	Thr	Threonine
W	Trp	Tryptophan
Y	Tyr	Tyrosine
V	Val	Valine

ATS	Andersern-Tawil syndrome
BLAST	basic local alignment search tool
ECG	electrocardiogram
E_K	equilibrium potential
GOF	gain of function
G_{rel}	relative conductance
K+	potassium

KCNJ2	potassium voltage-gated channel subfamily J member 2
Kir	inwardly rectifying potassium channel
LOF	loss of function
LOS	loss of selectivity
LQT	long QT syndrome
mRNA	messenger ribonucleic acid
pdb	protein data bank (file format)
PIP_2	phosphatidylinositol-4,5-bisphosphate
SNP	single nucleotide polymorphism
SQT	short QT syndrome
TM	transmembrane
WT	wild type

5 Introduction

5.1. Kir2.1

Kir2.1 is an inward rectifying potassium channel and the protein consists of four subunits. They can assemble to form homotetramers or with different subunits from the Kir2.x family or for example the Kir3.1 to form homotetramers. They are located in the cell membrane and are characterized to have a higher K^+ flux into the cell rather than out of it. That is the reason why they are called anomalous. Kir2.1 has a strong rectification in comparison with other inward-rectifier channels. This characteristic does not follow the Hodgkin-Huxley kinetics. It depends on the electrochemical gradient, owing to the asymmetric open

channel pore block by cations and other molecules. Examples of blockers of the channel are magnesium, barium or polyamines such as spermine. [1]

A negative membrane potential results in a higher flux of ions and a positive potential to a lower one. It has also been shown that this channel is voltage independent. These features explain why the Kir2.1 channel, expressed by the KCNJ2 gene, plays an important role stabilizing the resting membrane potential. [1]

The genetic information KCNJ2 is located on 17q24.3. This gene locus expresses the mRNA of the Kir2.1. At the rough endoplasmic reticulum the polypeptide is produced and the Golgi makes post-translational modifications. A specific amino acid sequence at the cytoplasmic COOH terminus, F-C-Y-E-N-E, is responsible for the transport and integration to the cell membrane. Mutations in this motif lead to retention of the protein in the cytosol. But also other mutations as the missing of amino acids 95-98, 314-315 and V302M affect the transport of the channel to the membrane. Thus we can imagine the transport signal is important for channel integration in the membrane as well as other factors. [1]

The activity of the channel is also determined by its internalization. This mechanism depends on GTPase Rho family proteins and dynamin, which are associated with endocytosis. [2]

KCNJ2 is expressed in the heart, brain, lung, placenta, skeletal muscle cells and the kidney.

The Kir2.1 is part of the classical Kir family. The channel consists out of 427 amino acids. Two transmembrane domains, TM1 and TM2 are linked by a pore forming region (H5). The TM1 has a cytoplasmic amino terminus and the TM2 a long carboxy terminus. The whole amino acid sequence is mapped in Figure 1 to give an overview of the construction of the protein.

```
        10         20         30         40         50
MGSVRTNRYS IVSSEEDGMK LATMAVANGF GNGKSKVHTR QQCRSRFVKK
        60         70         80         90        100
DGHCNVQFIN VGEKGQRYLA DIFTTCVDIR WRWMLVIFCL AFVLSWLFFG
       110        120        130        140        150
CVFWLIALLH GDLDASKEGK ACVSEVNSFT AAFLFSIETQ TTIGYGFRCV
       160        170        180        190        200
TDECPIAVFM VVFQSIVGCI IDAFIIGAVM AKMAKPKKRN ETLVFSHNAV
       210        220        230        240        250
IAMRDGKLCL MWRVGNLRKS HLVEAHVRAQ LLKSRITSEG EYIPLDQIDI
       260        270        280        290        300
NVGFDSGIDR IFLVSPITIV HEIDEDSPLY DLSKQDIDNA DFEIVVILEG
       310        320        330        340        350
MVEATAMTTQ CRSSYLANEI LWGHRYEPVL FEEKHYYKVD YSRFHKTYEV
       360        370        380        390        400
PNTPLCSARD LAEKKYILSN ANSFCYENEV ALTSKEEDDS ENGVPESTST
       410        420
DTPPDIDLHN QASVPLEPRP LRRESEI
```

Figure 1 shows the amino acid sequence of the Kir 2.1 from number 1 to 427. (https://www.uniprot.org/uniprot/P63252)

To give a good overview about the channel, I will explain the structure in detail. An overview of the channel is presented in Figure 2 The first amino acids from number 1 to 82 are at the NH_2 terminus in the cytoplasmic region. The next amino acids to 106 are located in the membrane. This region is called TM1 (transmembrane helix 1) and can be described as the outer helix of the channel. The extracellular area is at position 107 to 128 and 148 to 156. The pore forming region consists of 18 amino acids and is important for selective flux of potassium ions. This ion-selectivity motif of the Kir2.1 is TTIGYG (141-147). The rest is the helical pore forming region, named H5. The inner transmembrane helix, TM 2, spans residues 157 to 178. The rest of the polypeptide until residue 427 forms the intracellular carboxyl terminus.

Intracellular	2-81	
Transmembrane	82-106	Helical TM1
Extracellular	107-128	
Intramembrane	129-147	Pore-forming region
Extracellular	148-156	
Transmembrane	157-178	Helical TM2

Intracellular	179-427	

Table 1 gives detailed informations about the structural distribution of the protein.

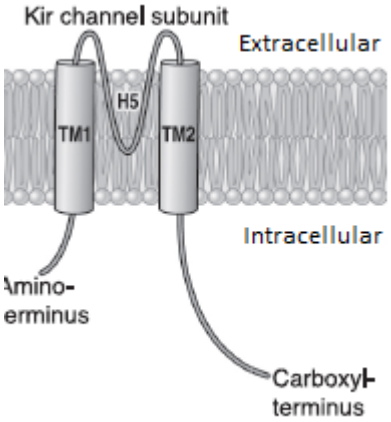


Figure 16

Figure 2: The different regions of one subunit of a Kir2.1. The aminoterminus and the carboxylterminus are in the intracellular regions. The TM1, TM5 and the H5, the pore forming domain are situated in the transmembrane domain. There are also some extracellular loops.

Figure 16Figure 16

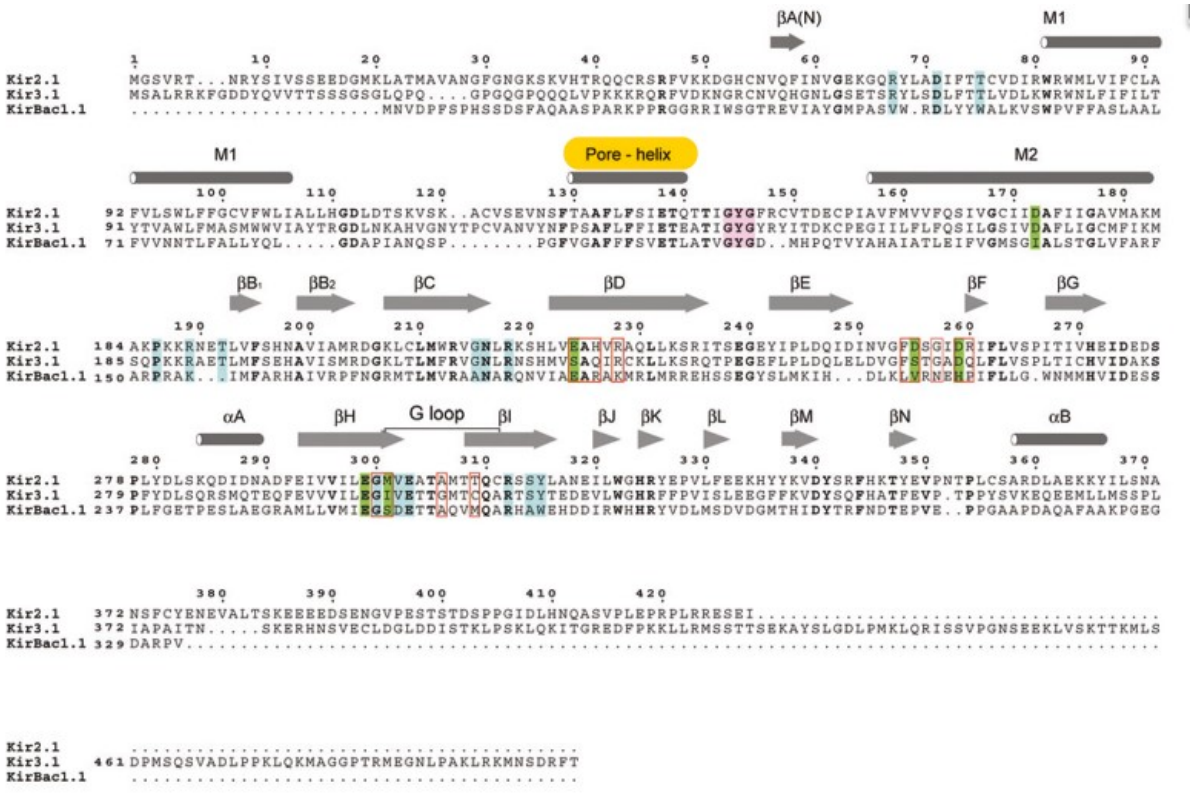


Figure 3. the different domains of the channel are combined with an alignment of the Kir2.1 with Kir3.1 and the KirBac1.1. The conservation of the important domains can be compared easily. https://www.uniprot.org/

[3]

The TM2 helices form a barrier to control ion permeation. The narrowest part of the cytoplasmic pore is a flexible G-Loop, which forms a ring and influences the flux of ions. PIP_2 could have an affect to flexibility and structure of the G-loop. [1][3]

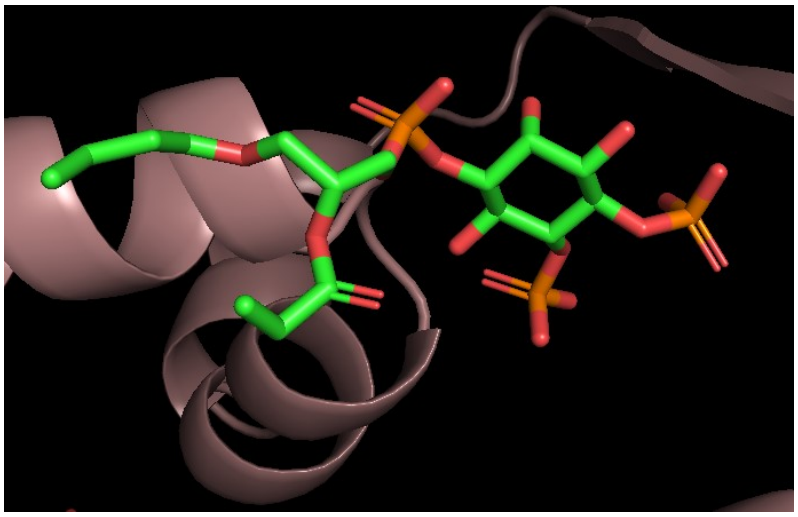
5.2. Gating regulation of Kir2 channels by Phosphoinositides

The N and C terminus form an enhancement of the pore. This region includes several binding sites for phosphatidylinositol 4,5-bisphosphate. The molecule PIP_2 binds with basic and polar amino acid regions, which are near to cell membrane. This interaction is important for the channels activity. Huan et al were able to prove this by removing PIP_2. By doing that the current of the channel got reduced. [4]

The authors of the paper “Direct and Specific Activation of Human Inward Rectifier K^+Channels by Membrane Phosphatidylinositol 4,5-Bisphosphate” used $^{86}Rb^+$ and giant patch-clamp liposomes to measure the concentration of PIP_2, which is needed to activate the channel. In an assay N. D’Avanzo et al could prove that the activation of the channel rises with 0.01% of PIP_2 and gets higher the more concentration was added. [3]

Interestingly the Kirbac1.1 gets inhibited by PIP_2. This phenomena could be explained because of the evolution of Kir channels. An important role in this theory could be ATP. The missing of 3 highly conserved regions of the KirBac1.1 could be a factor for this inhibition. [3] [5][6]

Phosphoinsotides are phospholipids having a phosphorylated inositol. This phosphorylation can be at position three, four or five.



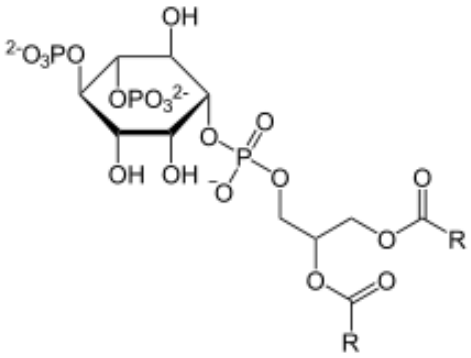


Figure 4 is the chemical structure of PIP_2 https://de.wikipedia.org/wiki/Phosphatidylinositol-4,5-bisphosphat

Figure 5 The figure shows phosphatidylinositol 4,5-bisphosphate visualized with Pymol.

The PIP_2 with the position four and five has the highest activation for Kir2.1. The specificity results from two negatively charged atoms in the lipid headgroup and because of the multiphosphorylation. The pocket of the channel for PIP_2 contains many basic amino acids, which influence the phosphates. So the molecule gets coordinated in the right position. The sensitivity is depending on other anionic lipids in the membrane. There is synergetic effect, which improves the activity. [6]

To identify the structure and location of PIP_2 many tests had been done. For example electrophysiology, computational docking, molecular dynamic simulations and x-ray crystallography helped to find information about the ligand and the channel. Many studies [7][8][9] assume, that a great number of positively charged residues determine the activation of Phosphatidylinositoldiphosphate. The amino acids include H53, R67, R82, K182, K185, K187, K188, R189, R218, K219, K228, and R312 have positively charged groups. Mutations at these positions for example to glutamine or alanine has a strong negative effect for the binding of Kir2.1channels. Further the binding of the ligand is affected by residues, which are not located in the binding pocket, but influence sensitivity allosterically. H53Q, R67Q, K228Q and R312Q have been shown to have a negative allosteric effect. [6]

PIP_2 causes a change of conformation of the channel. The flexible linker moves around six Å to the transmembrane domain. So the flexible linker cannot interact with the slide helices. [6]

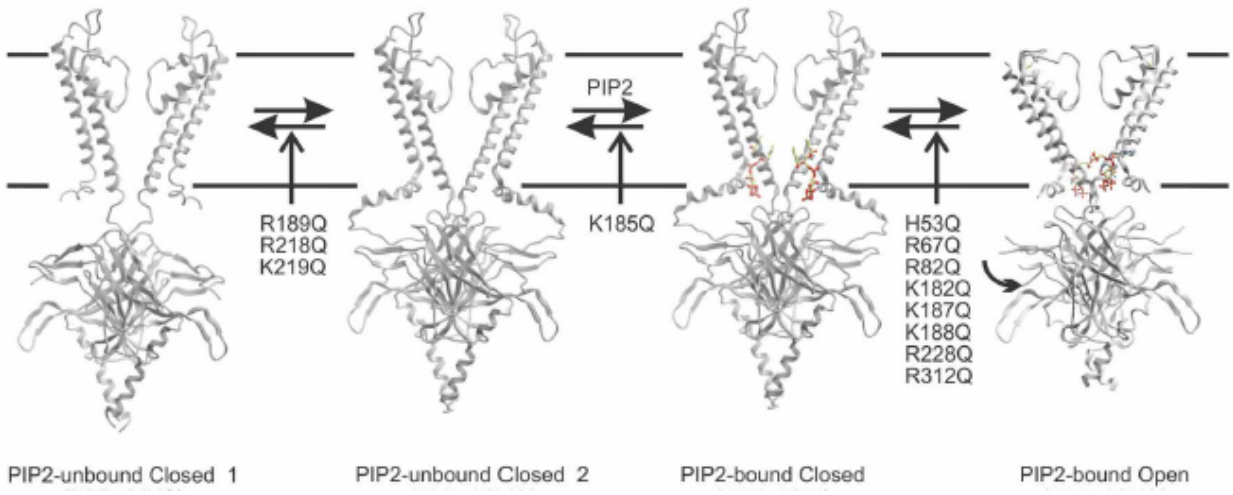


Figure 6 shows the conformation of the channel, if PIP_2 is bound. Bound PIP_2 may change the conformation and structural rotation. Many mutants affect the last step of opening the channel. So the change in the cytoplasmic domain is an important period in the channels activity. [6]

5.3. Physiological role of Kir2.1 channels

The Kir2.1 channel displays an important role for the resting membrane potential and to regulate the excitability of the cell. The Kir2.1 is a strong rectifying channel, with very little outward current occurring at voltages positive to the K^+ equilibrium potential. This arises at least in part due to electronegative regions, which influence polyamine block. These are Asp 172 in the TM2 domain and Glu 244/ 299 in the cytoplasmic region. Another important residue, influencing rectification is S165 in the transmembrane pore. [1]
The rectification of potassium ions results from magnesium and other polyamines, which bind to the cytoplasmic pore or to the inner vestibule of the channel. [3] Recent MD simulations suggest that polyamines can bind deep within the pore, preventing ion flux by blocking the selectivity filter. [10]

In the heart Kir 2.1 channels are important for stabilize the resting membrane as well as to the final action potential repolarization. There is a large inward current, when the E_m is more negative than E_k. A large outward current arises when E_m is slightly positive to E_k. This characteristics are important to stabilize the E_{res} of cardiac cells near the E_k. Inward rectification gets stronger when the membrane is more depolarized. So the flux of

potassium ions decreases and becomes practically zero when E_k is positive. The efflux of ions stops during the action potential plateau and results in the maintenance of the action potential. At the start of the repolarization the outward current get higher. [1]

To summarize the Kir2.1 channel is important for setting the resting potential, prolonging the action potential phase and inducing a fast repolarization. [1]

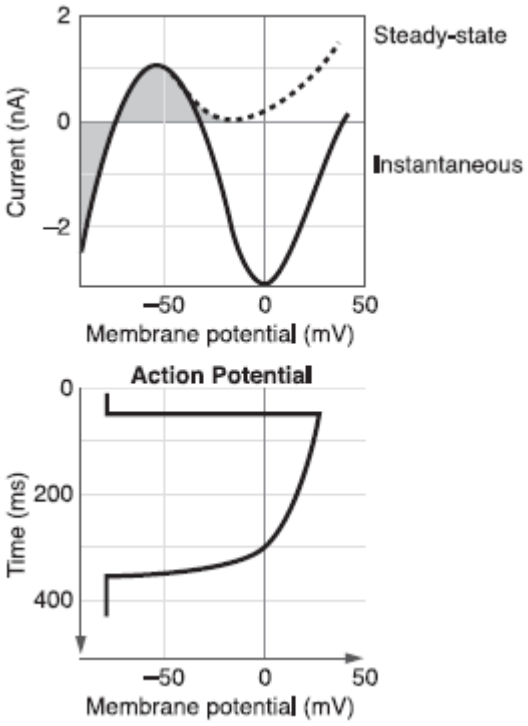


Figure 7 is a diagram, which shows the membrane potential versus the current and the time. [1]

In the blood system epithelial and smooth muscle cells express Kir2.1 channels. Kir2.1 sets the E_{res} to a negative potential in endothelial cells, which increases the Ca^{2+} flux into the cell. Ba^{2+} blocks the channel and due to the fact that there is more calcium in the cell the production of NO and phospholipase $_{A2}$ is higher. These factors cause vasodilatation and a lower blood pressure. In vascular smooth cells the mechanism is quite the same, but further research is needed to support this theory. [1]

In the brain Kir2.1 channels are considered involved in the maintenance of E_{res} and the excitability of neuronal cells. Apparently, there are different combinations of Kir 2.x tetramers important for this mechanism. The block with for example Ba^{2+} can cause depolarization and therefore an action potential. The classic Kir 2.x channels are expressed in all regions of the brain and have an unequal distribution. There are some theories about the exact mechanism of these channels in the brain, but they are hard to prove. [1] Many ATS patients suffer from periodic paralysis. They are often triggered by stress or sporting activity. [11]

5.4. Andersen-Tawil-Syndrome

Andersen Tawil syndrome is a rare genetic disorder, which manifests in variable symptoms. Heterozygous loss-of-function mutations in KIR2.1 (OMIM: 600681) is characterized by a triad of periodic skeletal muscle paralysis, developmental dysmorphic abnormalities, as well as biventricular tachycardia with or without the presence of long QT. A diagnose is based on the presence of at least two features of the triad. Even inside an affected family, the symptoms can be different. Long QT syndrome type 7 is another name for this disease, because the main symptom is a prolonged QT interval. This syndrome was mentioned first in 1963 by Klein et al. [12] An exact description of this disease was made by Ellen Damgaard Andersen. Rabi Tawil made clinical tests to understand the genetic and clinical background. This is the reason they named the disease Andersen-Tawil-syndrome (ATS). Another name for this disease is Andersen syndrome, which can be confusing, because that is the same name for a glycogen storage disease type IV. The prevalence of ATS is about one out of a million. [13][14]

The disease is divided in ATS 1 and ATS 2. Andersen Tawil syndrome 1 is associated with the loss of function of the Kir2.1, an inward rectifying potassium channel, due to a mutation in the gene KCNJ2. Patients with ATS type 2 have the same symptoms, but the cause of this disease is not yet explored. [13]

Andersen Tawil syndrome arises spontaneous or in an autosomal dominant way. The gene KCNJ2 is located on the 17^{th} chromosome. If a single chromosome mutates in this gene region, the channel can lose its function. The effect on the Kir 2.1. is different and very complex. [13] [14]

ATS associated KIR2.1 mutations fall into two categories: either affecting conduction properties, often due to mutations located in the putative PIP2 binding site of the channel, or impair channel trafficking to the plasma membrane. [15]

All ATS mutants have in common that they result in loss of function (LOF)

Cardiac symptoms:

The QT interval represents the time of the ventricular excitation. It is often prolonged in persons with ATS. The elongation is about 330ms to 500ms. **Fehler! Verweisquelle konnte nicht gefunden werden.**A shows a QT interval. The prolongation can lead to cardiac arrhythmias and in the worst case to sudden cardiac death. The existence of a higher U wave, seen in **Fehler! Verweisquelle konnte nicht gefunden werden.**B is an important sign, when the heart rate is increased. [13] [16]

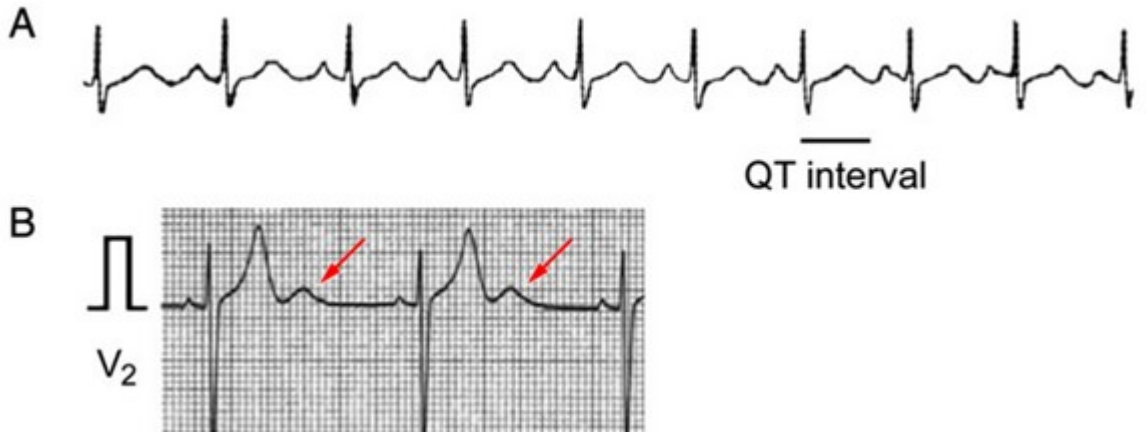


Figure 8: In this figure 4A there is ECG mentioned to show the QT interval. The QT interval takes a long period of one heartbeat and it is important for the normal rate. A higher U wave can be seen in figure 4B, which rises after an exercise. [13]

Obtained from Hoai-Linh Nguyen,Gerard H. Pieper,Ronald Wilders, "Andersen–Tawil syndrome: Clinical and molecular aspects," Int. J. Cardiol., vol. 170, no. 1, pp. 1–16, 2013 with permission from Elsevier.

Arrhythmias are common in ATS patients. In a cohort of 96 patients, 39 suffered from cardiac arrhythmias (Zhang et al). Only 2 out of all patients had problems with palpitations. Syncope and cardiac arrest were found in 10 diseased persons. [17]

Characteristically, a prolonged T wave downslope, a wide T–U junction, biphasic U waves, and enlarged U waves, were identified in 87 patients. These features can give a good prediction for Andersen Tawil syndrome. The risk of torsade de pointes and sudden cardiac death are low, because only a few incidents are acquainted. For these patients, it is important to reduce sport and stress to not overexert. **Fehler! Verweisquelle konnte nicht gefunden werden.** A gives an example of a patient suffering from arrhythmias and Fig. 5B shows a serious worsening, which is called torsade-de-pointes. [13], [17] [16]

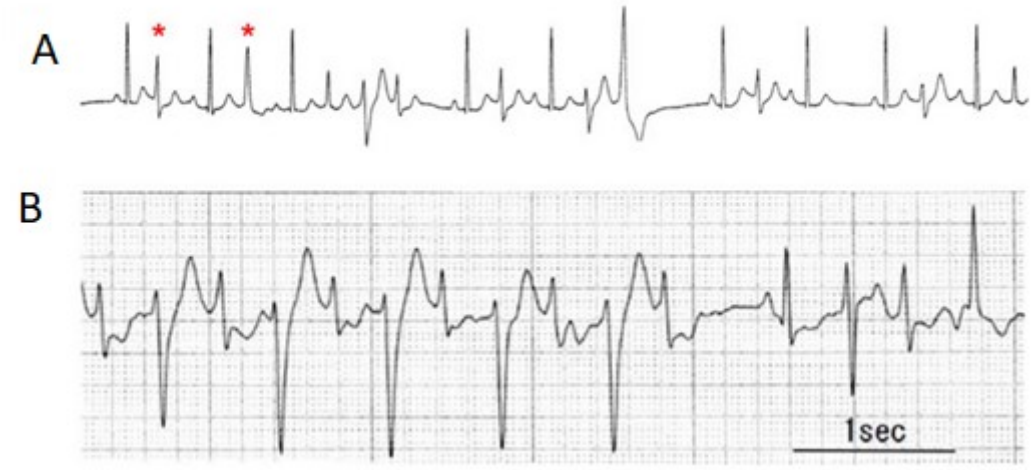


Figure 9 displays two different arrhythmias. The normal phases of depolarization and repolarization are totally shifted. [13] *Obtained from Hoai-Linh Nguyen,Gerard H. Pieper,Ronald Wilders, "Andersen–Tawil syndrome: Clinical and molecular aspects,"* Int. J. Cardiol.*, vol. 170, no. 1, pp. 1–16, 2013 with permission from Elsevier.*

Interestingly, it has been reported that pregnancy can improve the cardiac symptoms in ATS patients. Sabbia et al reported a patient, who got pregnant. During the gravidity, the cardiac disease was significant better, but the episodes of muscle weakness and paralysis got more frequent. A similar case was reported by Kamiya et al. A woman had the same characteristics in both of her pregnancies. [13]

Periodic paralysis and muscle weakness:

Muscle weakness and periodic paralysis have an extreme impact on the quality of life. In contrast to long QT syndrome and arrhythmias, these symptoms occur at the age of under a year and are better noticeable. Another interesting fact is that the periodic paralysis is not dependent of the potassium concentration. Patients with low, medium or high levels of potassium are affected equally. Further, the trigger for these attacks are different. Exercise, rest post exercise, stress, cold, heat and menstruation are examples of the main triggers. Muscle weakness affects the lower and upper extremities and sometimes the shoulders and the pelvic. Tests with potassium challenges are difficult to perform, because of the high risk of fatal ventricular arrhythmias. [13] [16]

Dysmorphology:

Patients with ATS suffer from many different dysmorphologies due to skeletal deformities. They have a shorter stature and a lower body weight than an average person. A long and narrow shaped head, a wider distance between the eyes, a broad nose, thin hair and an underdeveloped mandible are characteristics. Scoliosis, clinodactyly and syndactyly develop at an older age. Scoliosis characterises in a sideway curve of the spine. If a finger, commonly the fifth one, is slightly bend or makes a curve, this is called clinodactyly. Syndactyly is a malformation of the toes and fingers, where two are joint with soft tissue or fused. [13] [16]

Neurological and neurocognitive symptoms:

The research of neurological and neurocognitive symptoms is not far advanced. There are not many studies about this topic. Due to the fact that the channel is expressed in the brain, the cognitive ability could get worse. Some children with the mutation G300D have minor difficulties in school, but this was reported in one family. [13]

Some patients mentioned problems with concentration and memory. Other problems are mood swings and depressions. A few people also show major depressions and borderline symptoms. Further studies are needed before a conclusive evidence can presented. [13]

Diagnosis:

First, it is important to mention the high variety of the symptoms. Some ATS 1 patients are unaffected by their genetic mutation. Others show nearly all the characteristic symptoms. This circumstance makes it difficult to diagnose Andersen Tawil syndrome. The disease is typically diagnosed when patients are between three and thirty years. This age-range arises due to the fact that the symptoms are often inconspicuous. [13]

Ngyuen et al elaborated criteria to simplify the diagnosis. These criteria are presented in Figure 10. [13]

Diagnostic criteria for Andersen–Tawil syndrome.[a]

An individual is diagnosed with Andersen–Tawil syndrome if this individual meets at least one of the sets of criteria A and B
Set of criteria A
Two of the following three criteria:
1 Periodic paralysis
2 Ventricular arrhythmias (frequent premature ventricular contractions, bigeminy, ventricular tachycardia), prolongation of the rate-corrected QT or QU interval, and/or a prominent U wave
3 At least two of the following dysmorphic features:
a Low-set ears
b Wide-set eyes
c Small mandible
d Fifth-digit clinodactyly
e Syndactyly
Set of criteria B
1 One of the above three criteria
2 At least one family member who meets two of the above three criteria

[a] Modified from Venance et al. [49].

Figure 10 shows the criteria to diagnose Andersen Tawil syndrome. Due to the fact, that this disease is difficult to identify, Venance et al came up with a set of criteria to simplify the diagnosis.

Obtained from Hoai-Linh Nguyen,Gerard H. Pieper,Ronald Wilders, "Andersen–Tawil syndrome: Clinical and molecular aspects," vol. 170, no. 1, pp. 1–16, 2013 with permission from Elsevier.

The image displays the three characteristic symptoms as periodic paralysis, ventricular arrhythmias and dysmorphic features. If only two of the three features are perceptible, it is

acceptable to be diagnosed as Andersen Tawil syndrome. An exception is the appearance of the disease within the family, when only one criterion has to be met to have a high chance for ATS. [13]

To confirm this clinical diagnosis, a genetic test is unavoidable. The mutated gene KCNJ2 is located on the 17th chromosome. This section will be examined for mutations, which are related to ATS. For the future medication, it is quite important to know the background of the symptoms. Nguyen et al takes the position that a few patients with ATS 2 have a mutated Kir2.1, because of a failed gene test.[13]

Treatment:

There is no treatment available directed at the cause of this disease.

In general, the treatment of ATS patients is focused on reducing the severity of arrhythmias and periodic paralysis. Cardiac arrhythmias are primarily treated with beta-adrenergic-blockers. The life prolonging effect of beta blockers are proved in many studies. For example, Propranolol and Metoprolol are the most important substances and can reduce the amount of arrhythmias successfully. Other antiarrhythmics are also available for the therapy. Many ATS patients get a combination of flecainide and beta blockers. Flecainide is an unselective inhibitor of sodium potassium channels in cardiac cells. These channels are important for the flux of sodium into the cell during the upstroke of the action potential. All substances are comparable in their benefits. [13][18] However, treatment does not consistently reduce arrhythmia burden and is not specifically targeted towards Kir2.1. [19]

Carbonic anhydrase inhibitors are the best therapy for muscle weakness and periodic paralysis. They are sulphonamides, acetazolamides and dichlorphenamide. The problem is that many people are allergic to these medicaments. [13]

Oral potassium supplementation supports the medication, because a low concentration of potassium in the blood worsens the symptoms. Potassium wasting drugs such as diuretics should be avoided. [13] [20]

Patients with a high risk for recurrent arrhythmias and heart attack get an implantable cardioverter defibrillator. This device detects dangerous arrhythmias and can give small electric shocks to stabilise the heart rate. [18]

5.5. Electrocardiogram

The ECG, electrocardiogram, is produced by an electrocardiography. The electrical activity of the heart is mapped on a graph voltage versus time. The speed is 25 or 50 mm per second. The calibration peak shows how the writing speed is. There are many electrodes, which can discern small electrical changes, which result from cardiac muscle depolarization and repolarization. The electrocardiography is a non-invasive and painless intervention. It is unlimited repeatable and can be done at any time or place.

The ECG is used to check the electrical function of the heart. In this way the frequency and rhythm of the heart can be examined, but also the electrical activity in the atrium and ventricles. The structural characteristics are important, because you can detect a hypertrophy of the myocardium as well as inflammation of the pericardium and the myocardium.

In a normal ECG ten electrodes (Figure 11) are placed on the patient. The change of voltage in the heart can be measured on the surface of the body. Twelve angles can be evaluated, because of the ten electrodes, which are placed on predetermined regions as mentioned in the figure below.

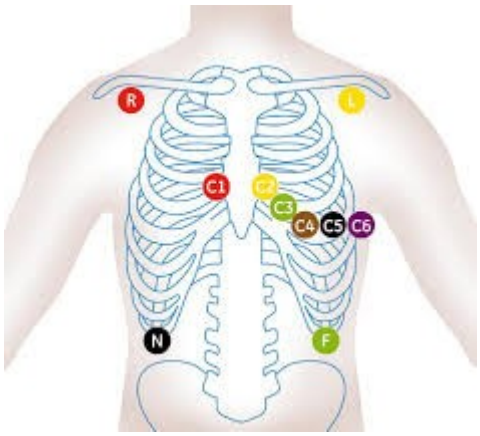


Figure 11 The electrodes in an ECG have to be positioned on exact places to get an accurate result. The electrodes are measuring the electrical differences during a heartbeat. If the angle changes, the electrocardiogram shows wrong outcomes and has to be repeated.
https://www.anandic.com/bausteine.net/f/11639/EKGElektrodenPositionierung.pdf?fd=2

The magnitude and direction of the heart´s electrical potential is captured in the cardiac cycle. It contains out of 3 important components, which are the P wave, the QRS complex and the T wave. The P wave rises, because of the depolarization of the atrium. The depolarization of the heart chambers is called QRS complex and its repolarization the T wave. Abnormalities in magnitude and length are associated with diseases like arrhythmias, cardiectasis, cardiac fibrillation or heart attacks.

A normal heartbeat starts with the pacemaker cells in the sinoatrial node. This depolarization moves to the atrium. Then the atrioventricular node and further the bundle of His and Purkinje fibers get depolarized. It ends in the left ventricle. This pattern repeats all the time to get normal heart beats. The direction of the depolarization and repolarization get detected by the electrodes. Either it spreads towards or away from the electrode, the signal is positive or negative. The charge of the heart muscle cells influences the surface of the body and therefore the electrodes.

The resting ECG is performed in a lying position. It needs only a few seconds for the test, so it can be done also in an emergency. The performance is extremely high and only temporary arrhythmias cannot be detected. This problem can be solved with a long time ECG. The patient has to wear the portable detector for one, two or three days.

Another important type is the stress ECG. The patient rides the bicycle or runs on a treadmill to get a higher blood pressure and pulse. Thereby small arrhythmias are more present. Another usage is to see how the heart frequency and blood pressure change, if the body is under pressure.

5.6. Arrhythmias

Cardiac arrhythmia is a disease in which the heartbeat is irregular. Arrhythmias arise from a disruption in the electrical system of the heart. The right excitability off the heart cells is complex and small problems, for example a mutation in an ion channel, can result in a different heartbeat. This rate should be between 60 and 80 per minute, but it depends on the age of the person. When the heart beats too slow, that is called bradycardia. The opposite is the tachycardia. There are four types of arrhythmias extra beats, ventricular arrhythmias, supraventricular arrhythmias and bradyarrhythmias.

The symptoms are quite different. Some people have no symptoms and often have no knowledge about their arrhythmia. The most common symptoms are palpitations and the feeling of a pause between the heartbeats. Other more disturbing ones are light-headedness, chest pain, passing out and shortness of breath. A heart stroke or failure are the most serious problem, if you suffer from arrhythmias.

This disease is treated with antiarrhythmics. They are divided into four different classes. Class number one are sodium channel blockers like procainamide, lidocaine, propafenone and flecainide. They block the voltage dependant channels to stabilize the membrane potential and weaken the excitation speed. Betablockers belong to the class two antiarrhythmics and block the beta receptors in the myocardium. They result in a negative ionotrope, chronotrope and dromotrope effect. Two types are the b_1 selective and b unselective blockers. The b_1 selective drugs, like Metoprolol, Atenolol and Bisoprolol have a huge advantage, because they are cardio selective and have less side effects. Class three antiarrhythmics are potassium channel blockers, amiodaron and class four are calcium channel blocker, which are diltiazem and verapamil. Besides all antiarrhythmics can have a proarrhythmic side effect. So the heart frequency and an ECG has to be screened quite often.

6 Methods

6.1 Literature review

This diploma thesis consists mainly of literature research. I started my research at the website of Uniprot (https://www.uniprot.org/). This website lists all mutations associated with Andersen Tawil syndrome with a link to the main publications. In addition, the review article from Hibino et al, published in 2010, provided a lot of information about the Kir2.1 channel in general and mutations leading to Andersen Tawil syndrome. [1] Further, I have searched for further studies using google scholar.

6.2 Sequence alignment

The pairwise sequence alignment between Kir2.2 (KCNJ2, several crystal structures available) and the closely related Kir2.1 channel (KCNJ12) shown in Figure 9 was made with the alignment tool in UniProt (https://www.uniprot.org/uniprot/P63252). Alignments are important to compare the sequence of a protein to others of the same or different family. It is simple to use and

understand. You get an understanding about conserved regions and thuus, the putative impact of a mutation.

6.3 Homology modelling

I constructed a homology. It was built using the swiss model webserver (https://swissmodel.expasy.org/). To build a homology model there four important steps. First you have to identify the structural template and make an alignment. This step is done by a BLAST (basic local search tool), which compares amino acid sequences with databases. Then a model is constructed by averaging position of the backbone atoms of the template. The similarity with sequences of other proteins is important to modulate the

structure. At least the quality of the model has to be evaluated. After all these steps I checked the model manually with Swiss-Pdb-Viewer. Many parameters like distances, angles and hydrogen bonds are easy to measure and verify. Figure 12 shows the Ramachandran plot of the homology model. As can be seen there are no critical outliers observed.

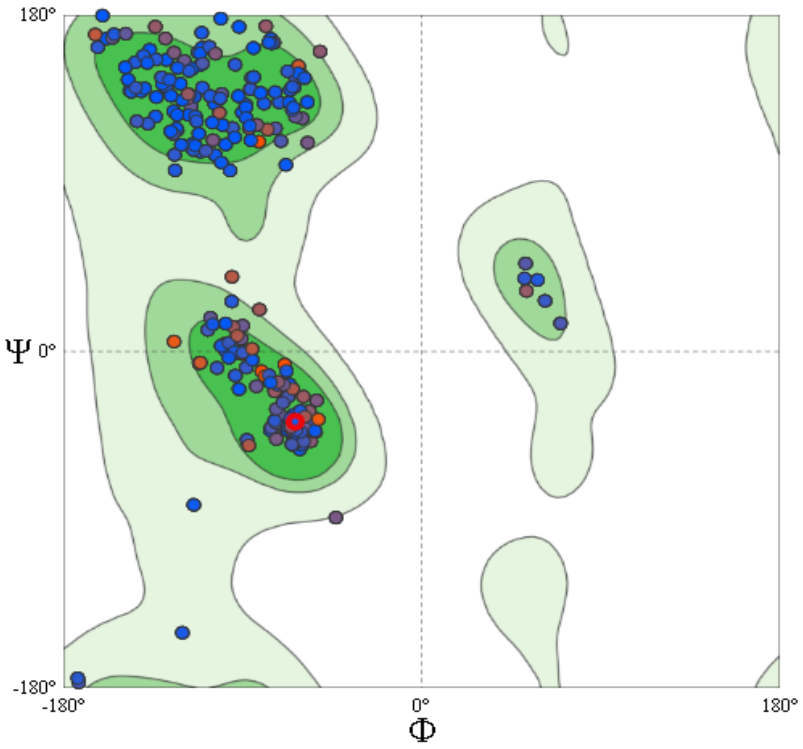


Figure 12 A ramachandran plot is an important tool to test the quality of modelled structures. The distribution of backbone torsion angles of the homology model of Kir2.1 are shown. No outliers (white space region) are observed.

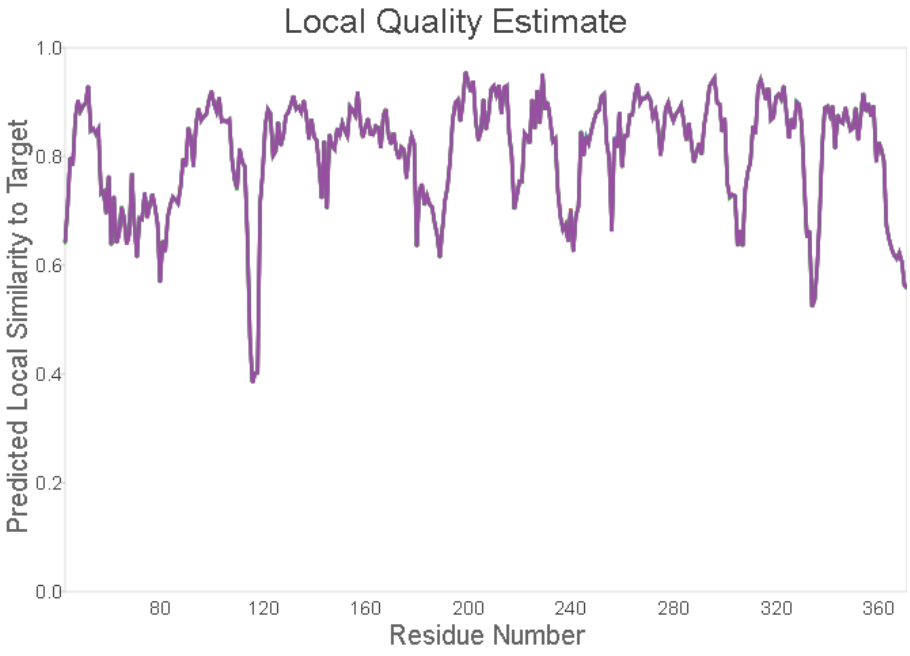


Figure 13 – Local quality estimate

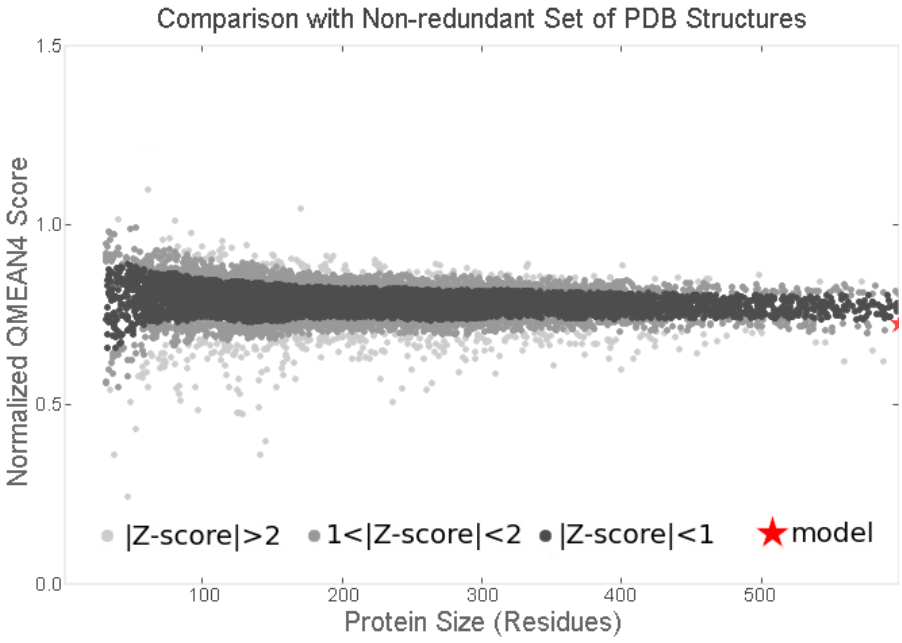


Figure 14 – overall quality estimate of the homology model

Figure 13 provides a local quality estimate of the homology model as provided by the swiss-model server (https://swissmodel.expasy.org/). The per-residue plot, shown on the x-axis reflects the expected similarity to the native structure, shown on the y-axis. As reported on the swiss-model homepage, values below 0.6 are expected to be of low quality. This is the case only in a small stretch around residue 120, which is in an external loop region and thus might not be critical. Figure 14 depicts the overall quality of the homology model (red star), in comparison to scores obtained from high-resolution crystal structures (grey dots). Since the Kir2.1 homology model is in the same range as high-resolution structures it can be expected that the model has comparable quality to the experimental structures.

In a next step I visualized this model with the program PyMOL (https://pymol.org/2/). PyMOL is a great tool to visualize proteins and label atoms, residues or total chains. **Fehler! Verweisquelle konnte nicht gefunden werden.** shows two subunits, which are green and orange, of the Kir2.1 homology model. The position of the potassium ions and the PIP2 binding sites were taken from the Kir2.2 crystal structure template and are shown as purple spheres and green sticks respectively. The C-alpha atoms of ATS and short QT

syndrome causing mutations are highlighted as coloured spheres. Mutations, which affect PIP_2 modulation of the channel are highlighted in pink. The white dots denote positions that influence trafficking to the membrane and the blue mutations lead to short QT syndrome.

Figure 15: A Pymol picture of two subunits of the Kir2.1 channel to show an overview about the location of mutations. The pink dots in the figure are single point mutations influencing the PIP_2 regulation of the channel. The mutations which affect channel trafficking are marked white. The dark red spheres are located in the selectivity filter, so perhaps less potassium can flow through the channel. The blue dots are associated with short QT syndrome.

7 Results 1- Clinical data

7.1.1 Overview of mutations causing Andersen-Tawil Syndrome (LQT7)

Gene	Channel	Mutation	Effect	Ref.	Preserv. time	Predicted effect
KCNJ2	Kir2.1.	C54F	Affects the binding of PIP_2	[21]	1036	probably damaging
		R67W	Affects the binding of PIP_2	[16]	1036	probably damaging
		D71V	Influences channel trafficking	[22]	1629	probably damaging
		T75R	Affects the binding of PIP_2	[23]	1237	probably damaging
		95-98 missing	Influences channel trafficking	[22]		
		S136F	Affects the binding of PIP_2	[22] [24]	1237	probably damaging
		G144S	Affects the binding of PIP_2	[22] [24]	1629	probably damaging
		P186L	Affects the binding of PIP_2	[9]	308	possibly damaging
		N216H	Influences channel trafficking	[9]	308	possibly damaging
		R218Q	Influences channel trafficking	[22] [25]	1237	probably damaging
		R218W	Influences channel trafficking	[22]	1237	probably damaging
		G300D	Affects the binding of PIP_2	[21]	1629	probably damaging

		G300V	Affects the binding of PIP_2	[21] [22]	1629	probably damaging
		V302M	Influences channel trafficking	[21] [22] [9]	1237	probably damaging
		E303K	Influences channel trafficking	[21] [22]	1237	probably damaging
		T305P	Affects the binding of PIP_2	[21]	1237	probably damaging
		T309I	No clear data	[21]	456	probably damaging
		314-315 missing	Influences channel trafficking	[22] [21]		

Table 2 A list of mutations causing Andersen-Tawil syndrome (LQT7) with data from Panther-tool.

7.1.2 Overview of mutations causing Short QT Syndrome

Gene	Channel	Mutation	Effect on the protein	Ref.	Preserv. time	Predicted effect
KCNJ2	Kir2.1	V93I	Affects the binding of PIP_2	[26]	308	possibly damaging
		D172N	Affects the binding of PIP_2	[26]	308	possibly damaging

Table 3 A list of mutations causing short QT syndrome with data from Panther-tool.

These tables provide an overview of the most important mutations in Kir2.1, which result in Andersen Tawil syndrome or short QT syndrome. The table information from the panther classification system tool. This software provides predictions on the pathogenicity of

mutations based on sequence conservation of homologous proteins. It estimates the probability of a nonsynonymous coding SNP to cause a defect on the protein. The calculation of how long an amino acid is preserved in the lineage gives an impact of how important this residue is. This method is called PANTHER-PSEP (position specific evolutionary preservation) and is based on evolutionary conservation. The number gives an impression of how many years an amino acid is conserved and that correlates with the pathogenicity of this region. [27]

7.2. Results 2 Functional consequences of ATS mutations- literature review

7.2.1 C54F and T305P

A mutation in the KCNJ2 gene at position G489T results in a change of the amino acids from cysteine to phenylalanine at the position 54, which is located in the well-conserved region of the N terminus of the protein. The other mutation T305P comes from the modification A1141C. Both mutants result in a loss of function of the channel. The expression in mammalian cells revealed a dominant-negative effect, when the mutant and the wildtype channel are co-expressed in one cell. It has been suggested that residues 54 and 305 may have an important role for PIP2 binding. Mutations might prevent the ligand from opening the channel and less potassium can flow out of the cell. [21]

S. Bendahhou et al performed an electromyographic test to measure the compound muscle action potential. The results were typical for the reduced muscle excitability of patients with KCNJ2 mutations (Figure 16).

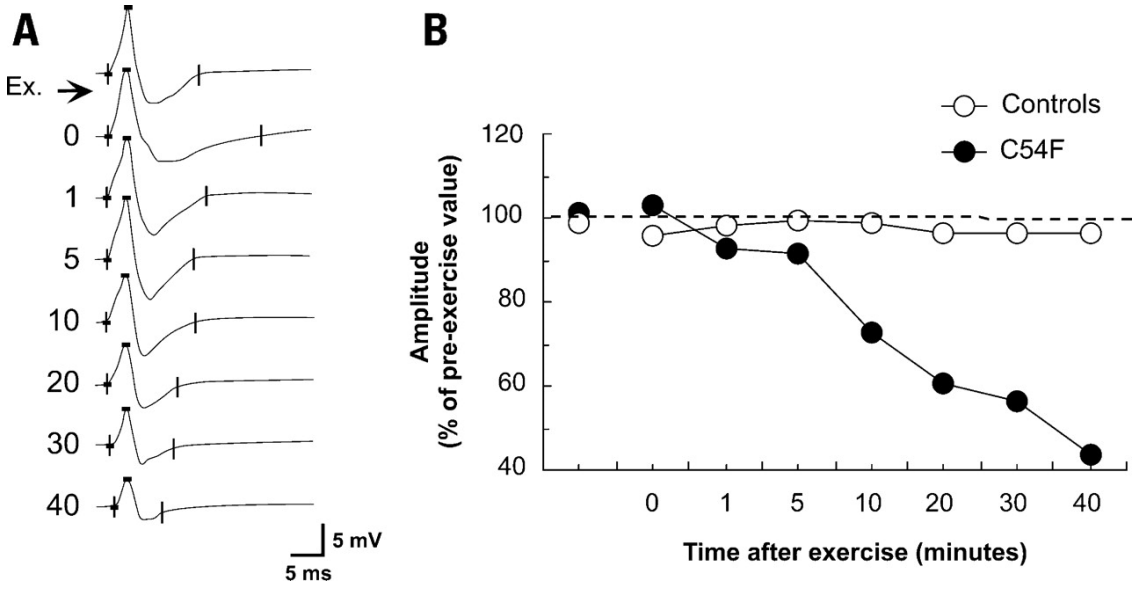


Figure 16 Long exercise test of the ATS patient with C54F mutation. The CMAPs were recorded with skin electrodes. The figure 1B shows the percentage of amplitude of pre-exercise value. [21]

Obtained from Bendahhou, Saïd; Fournier, Emmanuel, "Corticosteroid-exacerbated symptoms in an Andersen's syndrome kindred," Hum. Mol. Genet., vol. 16, no. 8, pp. 900–906, Apr. 2007. with permission from Human Molecular Genetics.

The probands were heterozygous for the two mutations. Both mutations are in high conserved regions and were inserted in a COS7-cell for a patch-clamp technique. The currents were measured and compared to the ones of the wild-type channel (Figure 17). The wild-type channels showed a normal inward rectifying current, but the current of the mutants was not detectable at all. A combination of the mutated channel and with wild-type subunits was measured, because humans with Andersen Tawil syndrome have both type of channels. These cells had only a small current. Both mutations result in a loss of function, when they were expressed alone or co-expressed with their wildtype channel. Bendahhou et al´s data suggests that if there is only one mutated subunit in the complex the whole channel could be silenced. A small current arises from channels, which consist out of pure wild-type subunits. [21]

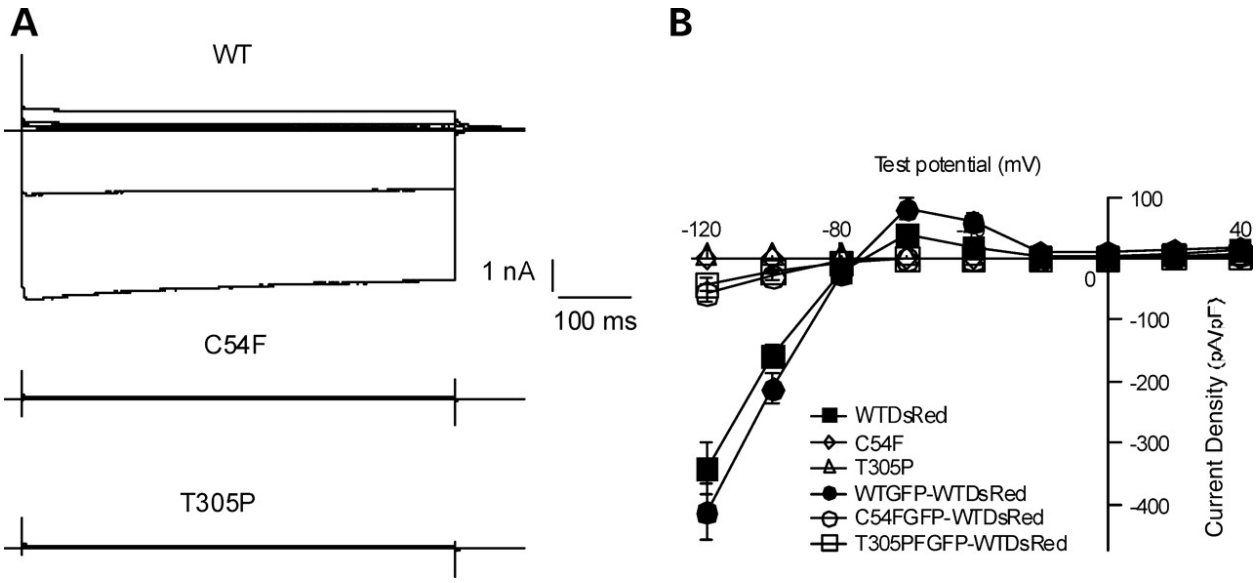


Figure 17 patch clamp technique of the mutant C54F, T305P, its wild type channels and the combination of the mutated and wild type channels. [21]

Obtained from Bendahhou, Saïd; Fournier, Emmanuel, "Corticosteroid-exacerbated symptoms in an Andersen's syndrome kindred," Hum. Mol. Genet., *vol. 16, no. 8, pp. 900–906, Apr. 2007. with permission from Human Molecular Genetics.*

Mutation T305P is located at the G-loop region, harbors intercellular and extracellular salt bridges. This region from residue 300-315 affects channel gating. There are eight other mutations located in this region, which are linked to Andersen-Tawil-syndrome. They are G300V/D, V302M, E303K, T305P, T309I, R312C and the missing of 314-315. [4]

In the study by S. Bendahhou et al they reported a patient who had exacerbations when treated with corticosteroids. His symptoms after the intake of corticosteroids were severe periodic paralysis, which disappeared after he stopped his treatment. Thus, the authors suggest that patients with ATS should be careful with this type of medication. [21]

7.2.2. R67C/W/Q

The study of "KCNJ2 Mutation Results in Andersen Syndrome with Sex-Specific Cardiac and Skeletal Muscle Phenotypes" connected the mutation R67W in the channel with Andersen-Tawil-syndrome. This mutation is produced by a transition from cysteine to threonine at position 199 in the KCNJ2 gene. G. Andelfinger et al examined large kindred of patients with Andersen-Tawil-syndrome to test, if they are associated with this kind of

disease too. The symptoms like ventricular arrhythmias and periodic paralysis were common in these kindred. The family tree in Figure 18 shows the connection between the gender of the patients and the sex-specific symptoms. [16]

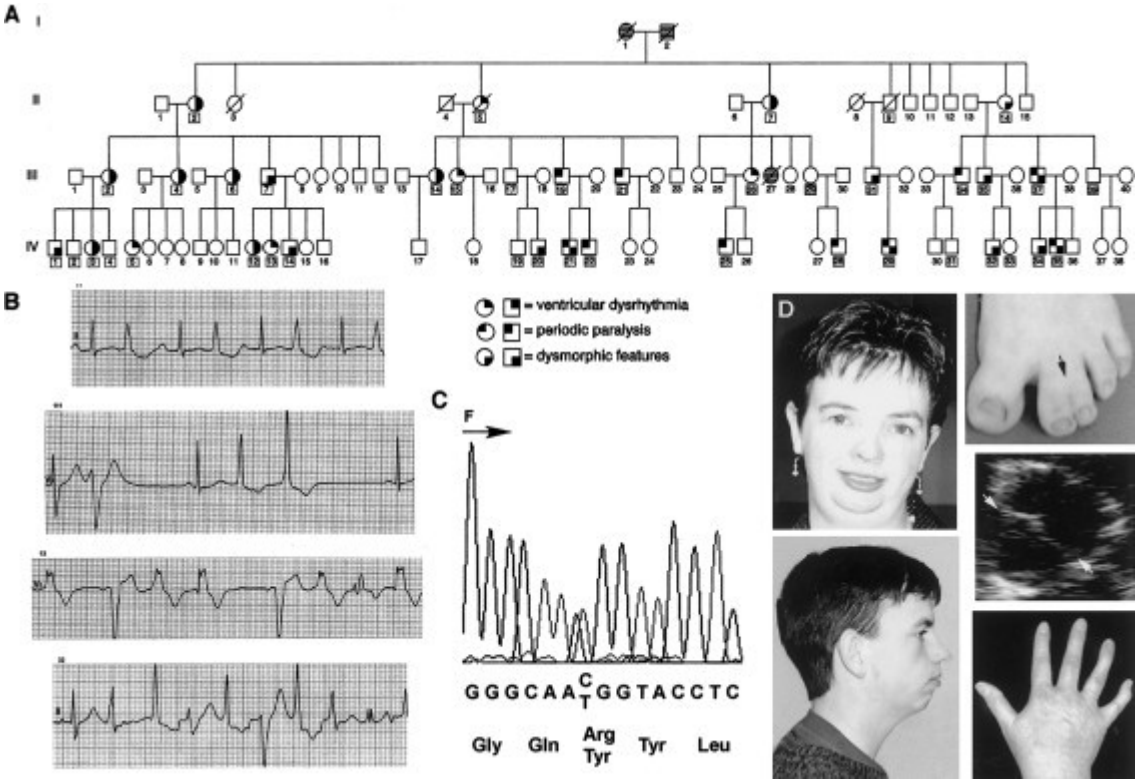


Figure 18A shows genetic test of a kindred to understand the inheritance of the R67W mutation. B demonstrate different ECGs of people affected by ATS. In figure 4D common physical symptoms are shown like low-set ears, wide ranged eyes and syndactyly. [16]

Obtained Gregor Andelfinger,Andrew R. Tapper,Richard C. Welch,Carlos G. Vanoye,Alfred L. George,D. Woodrow Benson, "KCNJ2 Mutation Results in Andersen Syndrome with Sex-Specific Cardiac and Skeletal Muscle Phenotypes," Am. J. Hum. Genet.*, vol. 71, no. 3, pp. 663–668, Sep. 2002., with permission from Elsevier.*

They identified 41 of 77 people with the mutation R67W and 16 women with this mutation had ventricular arrhythmias. Also 10 out of 25 genotype-positive men were diagnosed with periodic paralysis. Interestingly, not a single person with this mutation at position 67 had a prolonged QT-interval.

In this study, G. Andelfinger et al tested the electrophysical parameters in the human cell line tsA201 and in Xenopus oocytes. Cells with the wild type channel (see Figure 19 A and C) showed a typical characterisation of the current. However, the transfected cells with R67W had no visible currents. Owing to these results of the study they proved that mutation R67W influences the Kir2.1 channel to a loss of function and a dominant-

negative way. The correlation between the sex gender and specific diseases could not be proven. [16]

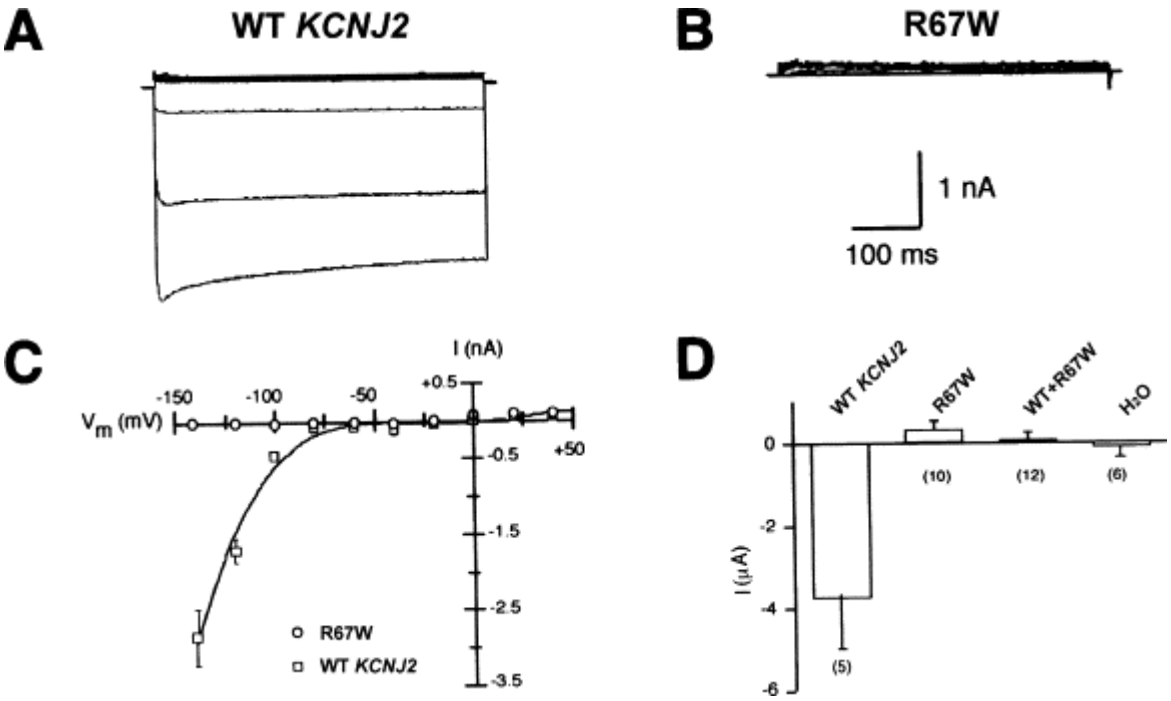


Figure 19 The picture A and C show the normal potassium flow in wild type cell, but in the picture B and D there was not any current measured in the R67W cells. [16]

Obtained Gregor Andelfinger,Andrew R. Tapper,Richard C. Welch,Carlos G. Vanoye,Alfred L. George,D. Woodrow Benson, "KCNJ2 Mutation Results in Andersen Syndrome with Sex-Specific Cardiac and Skeletal Muscle Phenotypes," Am. J. Hum. Genet., *vol. 71, no. 3, pp. 663–668, Sep. 2002., with permission from Elsevier.*

7.2.3. D71V

The study "Mutations in Kir 2.1 cause the Developmental and Episodic Electrical Phenotypes of Andersen´s Syndrome" focused on nine mutations, which were all in a high conserved regions in the channel. These were D71V, missing of 95-98, G144S, S136F, R218W/Q, G300V, E303K and missing of 314 to 315. The mutations were mapped on a

schematic representation of the channel, as shown in Figure 20. Some of them were found in the genes of the Andersen-Tawil-syndrome probands, but only D71V was pervasive and the mutation D71V was not found in any unaffected person. Therefore, N. M. Plaster et al suspect that this mutation is highly linked to Andersen-Tawil-syndrome. It is located on the N terminus of the alpha helix. [22]

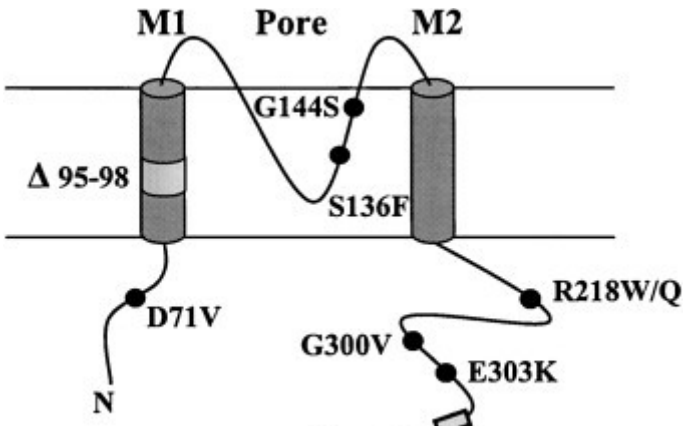
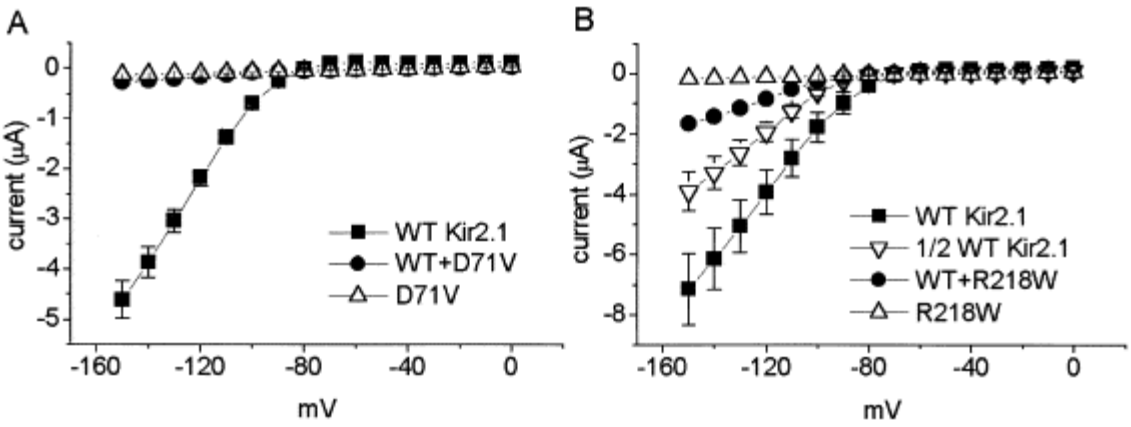


Figure 20 shows all mutations that were mentioned the study of Plaster et al for a better imagination , where the mutant amino acids are located.

Obtained Nikki M. Plaster et al, "Mutations in Kir2.1 Cause the Developmental and Episodic Electrical Phenotypes of Andersen's Syndrome," Cell, vol. 105, no. 4, pp. 511–519, May 2001., with permission from Elsevier.

Electrophysiological tests had been done to understand the effect of this mutation. Oocytes were injected with the wild-type and the mutant cRNA. The cells with the wild-type channels had a normal potassium inward flux. No current was measured with oocytes, which had the mutated subunits, so they argued that these channels could not form functional homomultimeric channels. When the cells with the genes of the wildtype and the mutant were measured, the current was extremely low, because only a few tetramers were composed out of 4 wild-type channels. In In Figure 21 the electrophysiological data is shown. In general, the mutation results in a strong dominant negative effect and induces a loss of function of Kir 2.1 channels. [22]



In Figure 21 the currents of potassium are mapped to see the contrast between the flow of ions through the wild type channels and channels with the mutation D71V or R218W. The D71V cells or D71V with wild type subunits have no potassium flow. However, the combination R218W subunits with wild type ones a show lower current. The pure cells with the mutated R218W channels could not show a potassium flux. [22]

Obtained Nikki M. Plaster et al, "Mutations in Kir2.1 Cause the Developmental and Episodic Electrical Phenotypes of Andersen's Syndrome," Cell, vol. 105, no. 4, pp. 511–519, May 2001., with permission from Elsevier.

7.2.4. T75R

The study identified a family, where three family members were affected with the T75R mutation in the Kir2.1. They tested the probands with direct DNA sequencing. These three patients suffered from typical symptoms like periodic paralysis and cardiac arrhythmias. The T75R mutation is situated in the highly conserved cytoplasmic region. At position 75 a threonine is replaced by arginine. This specific mutation was not found in any other family member or in 100 unaffected control individuals. [23]

The homotetramerisation and distribution of the mutated T75R subunits was tested. In Figure 22 there are the transfected mammalian cells with the mutation. The cellular distribution in the cells were similar, therefore C.-W. LU et al argued that the channel´s trafficking to the cell membrane and the homotetramerisation is not affected by this mutation. [23]

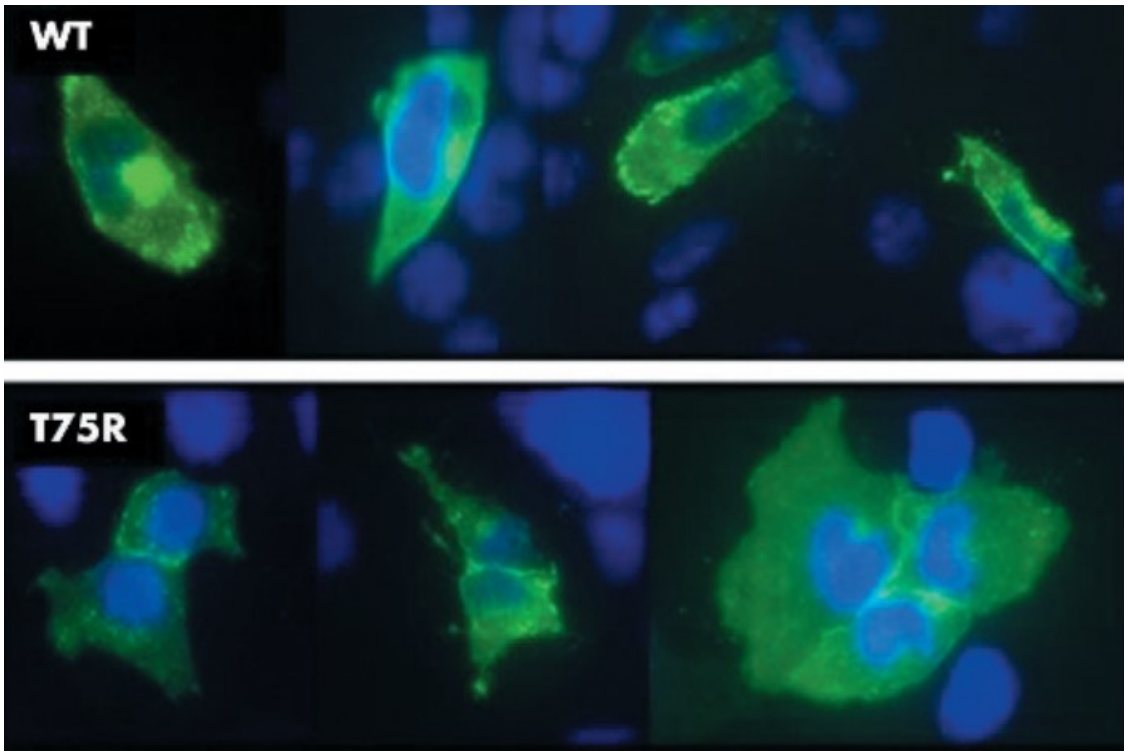


Figure 22: In a fluorescence detector the marked channels have a green colour. So, the right trafficking of the channel to the membrane can be checked. The wild type channel has nearly all his channels distributed to cell membrane. However, the Kir2.1 channels of the T75R mutated cells are are located anyplace. [23]

Obtained C-W Lu,J-H Lin,Y S Rajawat,H Jerng,T G Rami,X Sanchez,G DeFreitas,B Carabello,F DeMayo,D L Kearney,G Miller,H Li,P J Pfaffinger,N E Bowles,D S Khoury,J A Towbin, "Functional and clinical characterization of a mutation in KCNJ2 *associated with Andersen-Tawil syndrome,"* J. Med. Genet.*, vol. 43, no. 8, pp. 653–659, Aug. 2006., with permission from BMJ Publishing Group Ltd..*

The electrophysiological studies were performed in Xenopus oocytes. With the whole cell voltage clamp analysis, it was discovered that there is no current when the mutant is expressed alone. The expression with the same amount of wildtype subunits showed no current, because of a strong dominant negative effect. [23]

Mice were transfected with the mutated channel. These mice had a prolonged QT- Interval and 5 out 14 mice had ventricular arrhythmias. [23]

Overall, the mutation T75R in the Kir2.1 channel resulted in a complete loss of function and had a strong dominant negative effect. However, the tetramerization and distribution of the channel was not disturbed. [23]

7.2.5. V93I

This mutation is quite different to the others, because it results in a gain of function. The change from valine to isoleucine at the position 93 is in a highly conserved region along many species. A large Chinese kindred with atrial fibrillation and all members was tested and had this V93I mutation. 400 healthy people were checked and none of them had this specific mutation. In COS-7 cells functional tests were performed to determine the potassium flux and the outcome is shown in Figure 23. In the range from -140 mV to -40 mV the current of the mutated cells was higher in comparison to the wild-type cells. In HEK 293 cells the results were equal. The effect is a gain of function of the channel, because the inward and outward flux was higher compared to the wild type. M. Xia et al assumed that the higher potassium flux is due to higher conductance. [20]

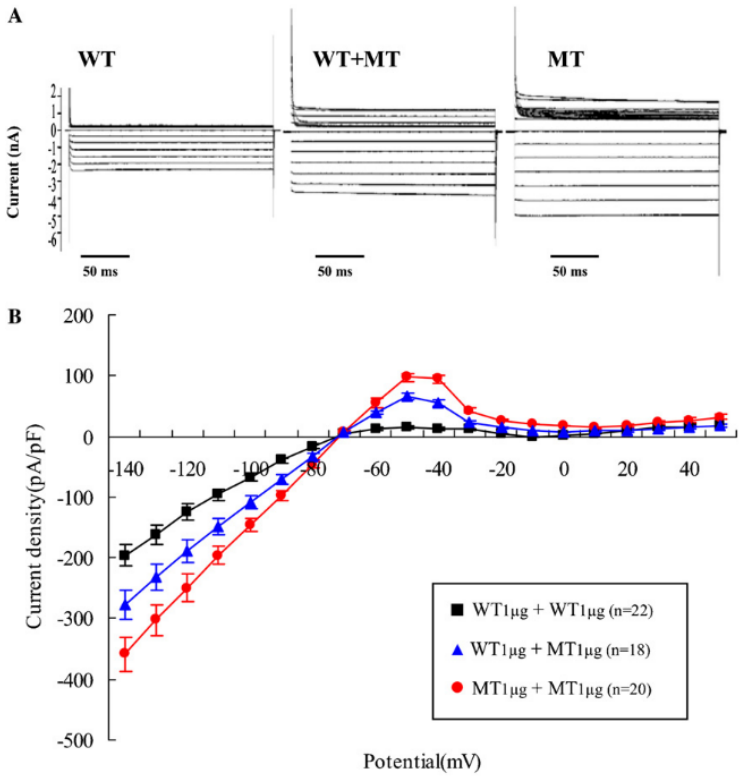


Figure 23 shows the different currents and current densities between the wild type and mutated cells and also in different concentrations.

Obtained Min Xia,Qingfeng Jin,Saïd Bendahhou,Yusong He,Marie-Madeleine Larroque,Yiping Chen,Qinshu Zhou,Yiqing Yang,Yi Liu,Ban Liu,Qian Zhu,Yanting Zhou,Jie Lin,Bo Liang,Li Li,Xiongjian Dong,Zhiwen Pan,Rongrong Wang,Haiying Wan,Weiqin Qiu,Wenyuan Xu et al., "A Kir2.1 gain-of-function mutation underlies familial atrial fibrillation," Biochem. Biophys. Res. Commun.*, vol. 332, no. 4, pp. 1012–1019, Jul. 2005., with permission from Elsevier.*

7.2.6. 95-98 missing

The amino acids 95 to 98 are situated in the TM1 transmembrane domain. N. Plaster et al suspect that the missing of these exact amino acids, the channel is prohibited to integrate into the membrane. The TM1 domain can not be inserted in the membrane structurally.[22]

7.2.7. S136F

When a serine is changed at position 136 to a phenylalanine the function of the channel decreased. It is a missense mutation. The position of that amino acid is in the pore region of the channel. The pore region is important for channel gating. The functional data showed that transfected oocytes with S136F could not show any flux through the channel.[1], [9], [28], [29]

7.2.8. G144S

This mutation results from the alteration in the gene KCNJ2 from G to A at the position 658. It is situated in the pore region. To be more precise it is located in the highly conserved region of the GYG sequence. This motif is important for the selectivity of potassium ions and mutations in this region affects ion selectivity. The fact that the subunits ca not assemble with others gets supported by an extremely decreased current in a patch clamp test with oocytes. This was tested with the genes from the mutant and the wildtype. [22]

7.2.9. D172N

This single point mutation at position 514 in the Kir-channel coding gene has a completely different effect on the channel function compared to the other mutants described so far. The mutation changes aspartic acid to asparagine at position 172. The acid group is replaced by a polar side chain, but it does not affect side chain size. This residue is

located in a conserved region in the Kir-family. Interestingly however, certain family members, including for example Kir6.2 have an asparagine at that position in the wilt type. Remarkably, the effect on the channel is a gain of function, which means the channel opens more often. This mutation influences the rectification and outward flux potassium ions and is located at the end of the second transmembrane region. This region is involved in the process of potassium rectification.

The functional data in Figure 24, which was performed in CHO cells, showed that the outward current at the potentials between -75 and -45 was much higher than the current of the wildtype or the combined wildtype with mutant cells. [26][24]

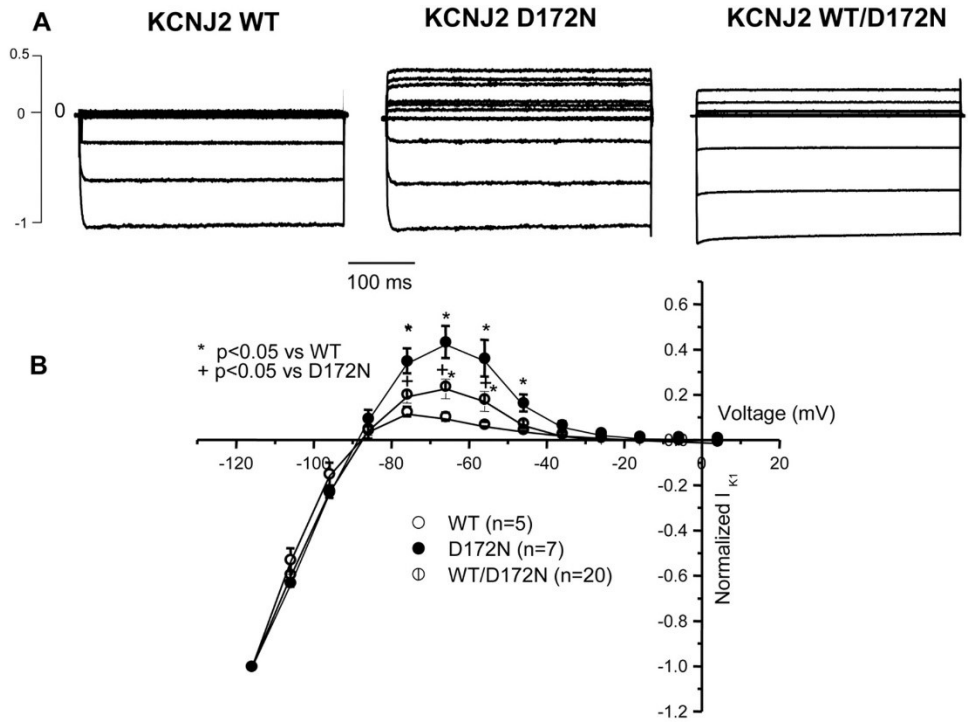


Figure 24: The test was performed in CHO cells to map the potassium flow through the Kir2.1. [26]

Two proband were diagnosed with short QT-syndrome and had the same change in the ECG. A genetic analysis of five different channels showed that they had the same mutation. [26]

In silico tests support that this alteration in the channel is responsible for the increased rate of repolarization in ventricular action potential and reduces its duration. [24]

7.2.10. P186L

An interesting mutation in the PKKR motif is P186L. The PKKR motif is from the amino acid number 186-189 and is involved in the binding of phosphoinositide-3,4-bisphosphate. The change from proline to leucine alters this interaction, but in the studies it is not mentioned how exactly this mutation influences the channel. [14][4][21][24]

In the study “Functional and clinical characterization of KCNJ2 mutations associated with LQT7” M. Tristani-Firouzi made electrophysiological tests in Xenopus oocytes. They compared the current from the wildtype channel with the mutated combined with the wildtype (Figure 25). The current was strongly decreased, because of the mutated binding site of phosphoinositide-3,4-bisphosphate. [14]

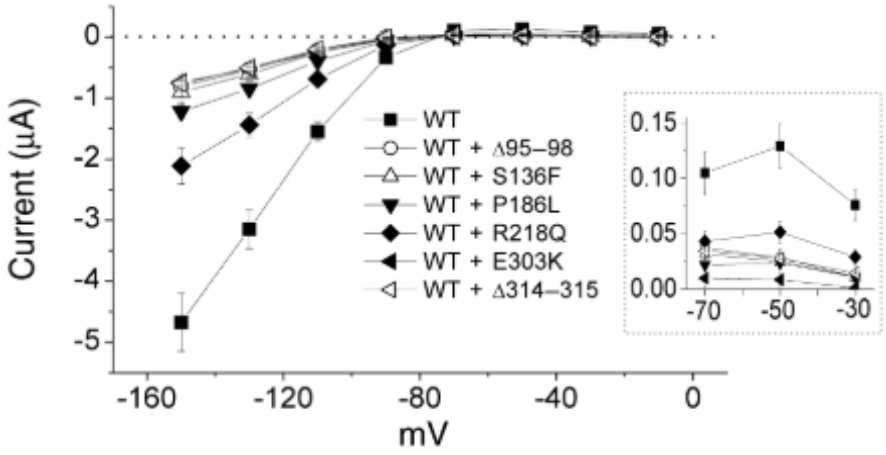


Figure 25 The figure shows the current produced with different mutations and wild-type subunits. [9]

7.2.11. N216H

The location of N216H is thought to be involved in the interaction phosphoinositide-3,4-bisphosphate. [14] The mutation in the gene KCNJ2 A874C changes the amino acid asparagine to histidine. It is a change from a neutral functional group to a positively charged one. Many studies [14], [21], [24] and [4] have shown that this mutation is involved in the Andersen Tawil syndrome.

When it is expressed alone it cannot produce any current. [21] The mutated subunit can assemble with subunits from the wild type. It embed in the membrane but loses its

function. The current of N216H combined with the wild-type subunits is reduced. For that reason, this mutation results in a loss of function. [14], [21]

7.2.12. R218W/Q

The study “Mutations in Kir 2.1 cause the Developmental and Episodic Electrical Phenotypes of Andersen´s Syndrome” includes also the mutation R218W. This abnormality evolves from a point mutation in the gene KCNJ2 at position 880, where an arginine is replaced by a tryptophan. In the channel it is located in the C-terminus interaction domain. [22]

Electrophysiological tests were made to understand the effect of this mutation. Oocytes were injected with the wild-type and the mutant cRNA. The cells with the wild-type channels had a normal potassium inward flux. The results can be seen in the In Figure 21 below.

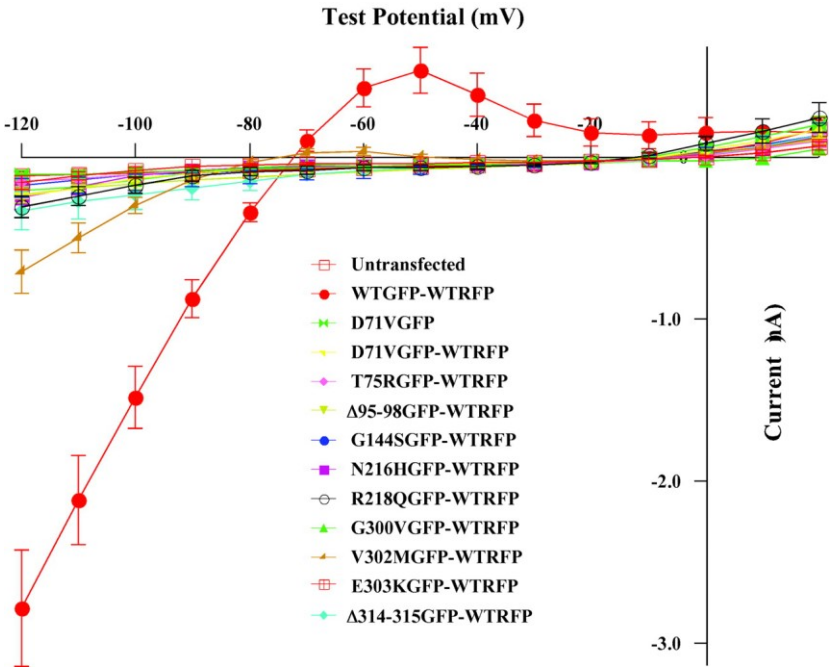
The inward current in mutant cells was not measurably. In comparison to D71V mutants, R218W can assemble better with the wild-type subunits. That is the reason why this combined oocytes have a much higher current than the mutants. In summary the change from arginine to tryptophan results in a weak dominant-negative effect and a loss of function of the channel [22] [9]

7.2.13. G300D

This mutant is situated in the C-terminus intracellular. The exact function of this region is not known, but it there is a connection with the binding of PIP_2. If this mutation is in combination, because homomeric channels are non-functional, with other subunits the affinity is reduced. [3] [29]

7.2.14. V302M

When the subunit of the KIR2.1 has a mutation at position 302, where a valine is changed to a methionine, it cannot assemble with other subunits from the wildtype. In the study [21] they marked mutated subunits with a colour to detect them in a fluorescent microscope (Figure 27). This technique showed that V302M interferes with normal channel trafficking and assimilation. The functional data in In Figure 26 reveals that there is potassium flow, however only through the WT constructs.[21]



In Figure 26 nearly all important single point mutation are mapped to compare the different currents. The interesting part of it is to see the results of various mutations.

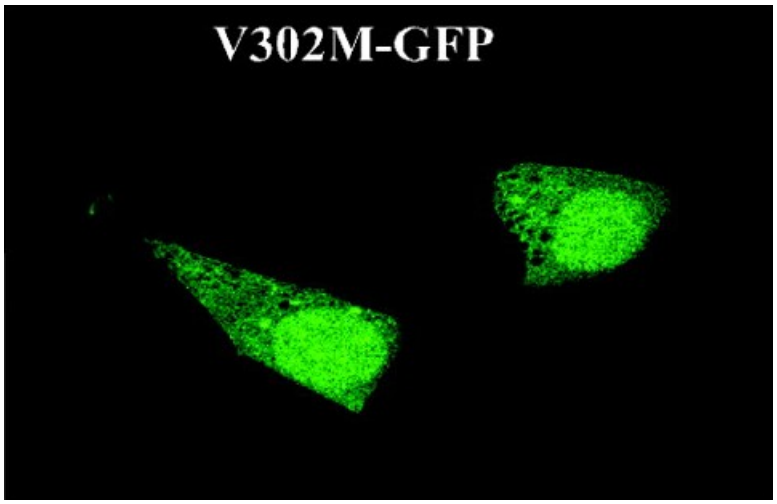


Figure 27 shows the fluorescent marked V302M channels in a cell. The study mentioned, that this diffuse location of the channels could be the result of a misfold of the protein. [30]

Possibly mutation V302M results in misfolding of the protein and is leading to incomplete post modifications. [21]

7.2.15 314-315 missing

A deletion of two amino acid at positions 314 and 315 leads to ATS. These amino acids are located in the G-loop of KIR2.1 and line the potassium ion conduction pathway. Due to this deletion the protein is unable to fold correctly and integrate into the cell membrane. Tests showed that the ability to assemble with wild type subunits is normal. The combined tetramers can integrate into the membrane, but cannot transport potassium ions. Therefore, this mutation results in a loss of function in a dominant negative way. The electrophysiological data support this theory because no current was measurable. [29]

8 Results 3: Modelling based interpretation of disease causing mutations

Figure 28 shows the pairwise sequence alignment of Kir2.1 (KCNJ2) with the template protein Kir2.2 (KCNJ12). Disease causing mutations have been highlighted. As can be seen in the Figure below, all positions are highly conserved, in agreement with their functional consequences.

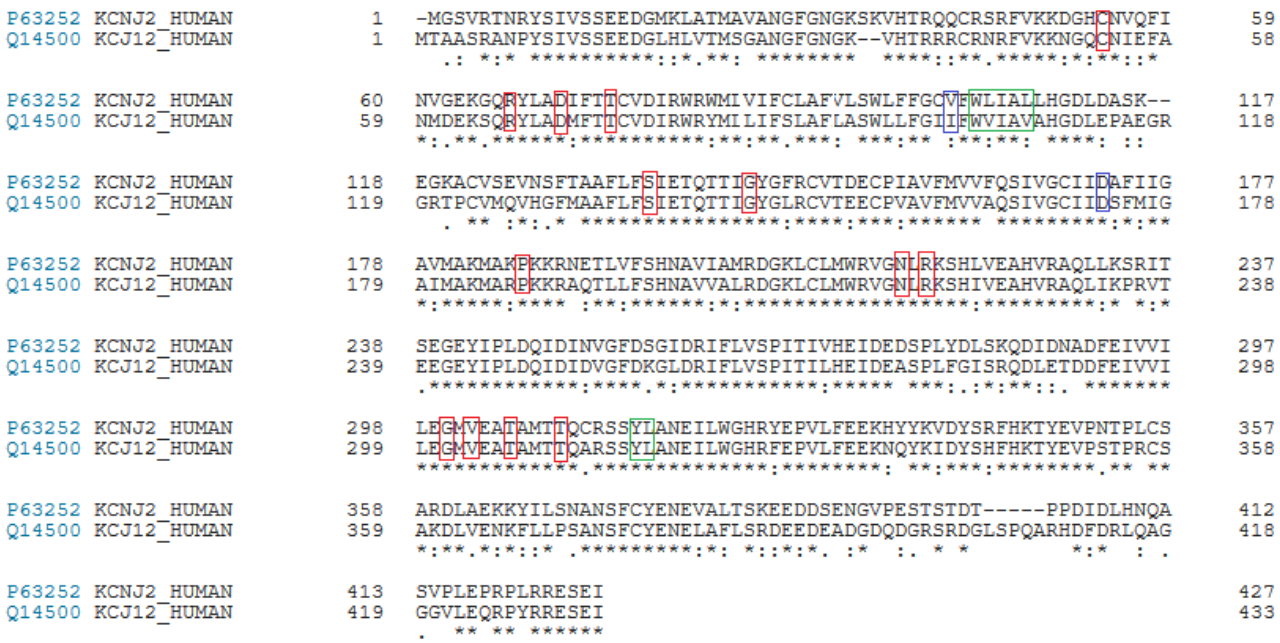


Figure 28 is an alignment of Kir2.1 and Kir2.2. with all mutations, which are mentioned in this thesis. The red marked mutations result in a loss of function of the channel. Deletions of more than one amino acid are highlighted in green. Blue marked are mutation with a gain of function of the channel.

R67W

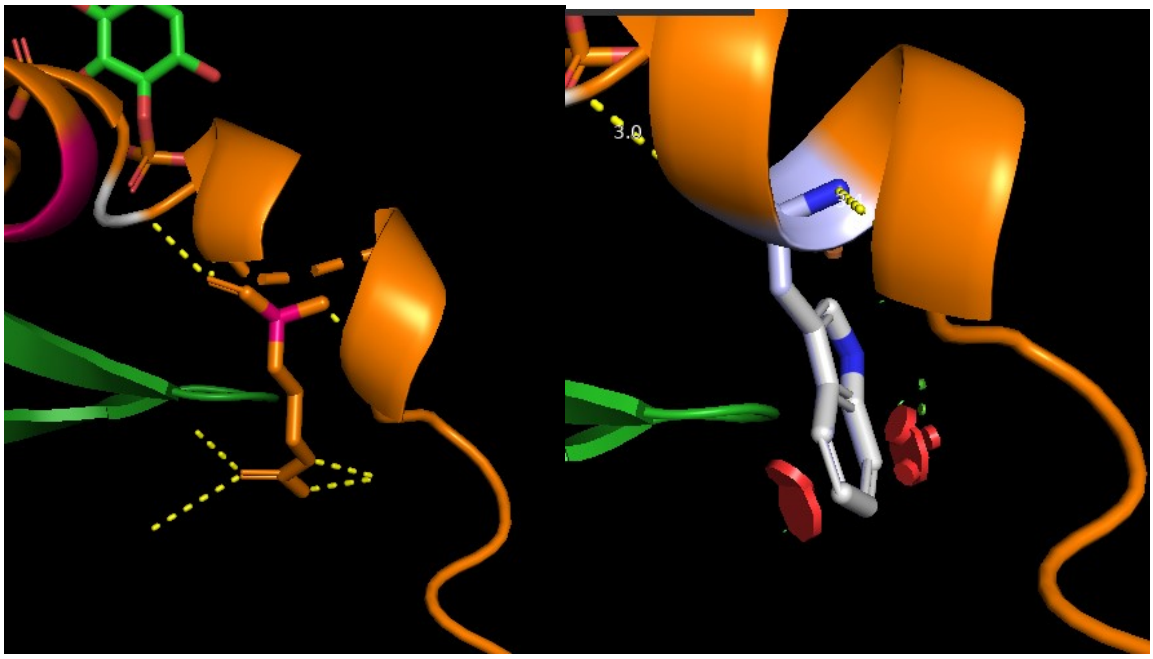


Figure 29 – close up view of amino acid R67 in WT (left side) and the in silico mutated W67 (right side).

This mutation is defined by a replacement of arginine by tryptophan. From the homology model it is not apparent, why the mutation leads to a loss of channel function.

T75R

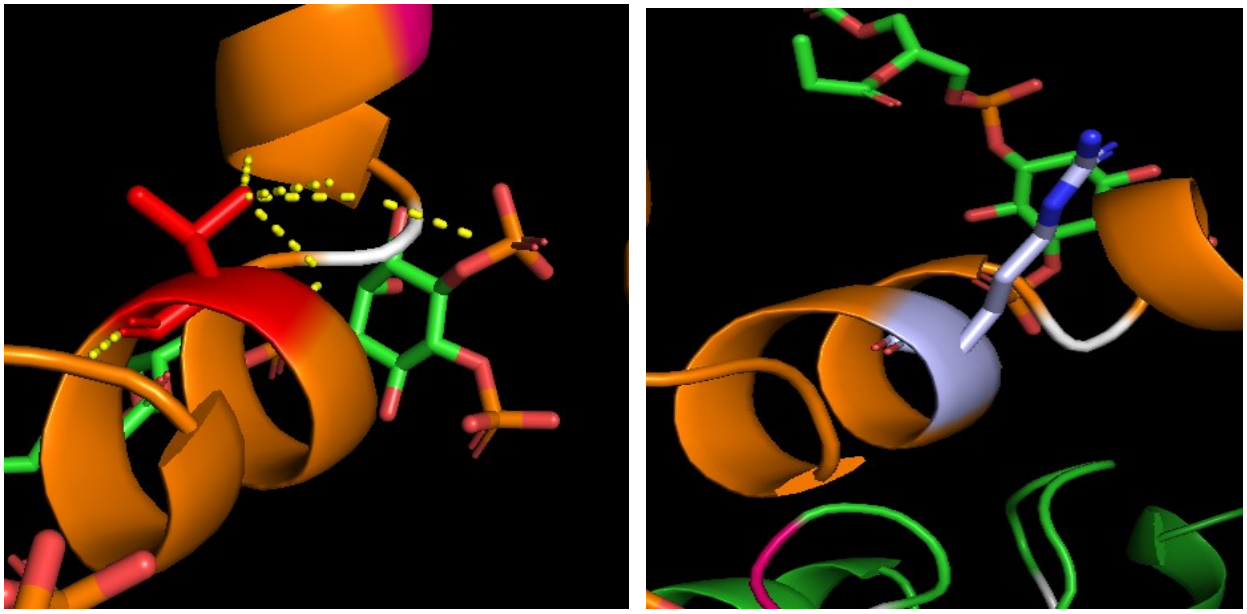


Figure 30 – location of WT (left side) and mutated channel (right side), the location of PIP2 is shown in sticks representation

The change from threonine to arginine at position 75 causes a large difference with respect to side chain properties. Instead of a small hydroxyl group, there is a long hydrophobic c-chain and a basic guanidino group. Based on in silico mutating this residue, only in one conformation the arginine side chain does not interfere with other residues in the vicinity. Interestingly, as can be seen in Figure 30 position 75 is in close proximity to the PIP2 head group, thus in most side chain orientations, arginine is predicted to interfere with proper PIP_2 binding.

S136F

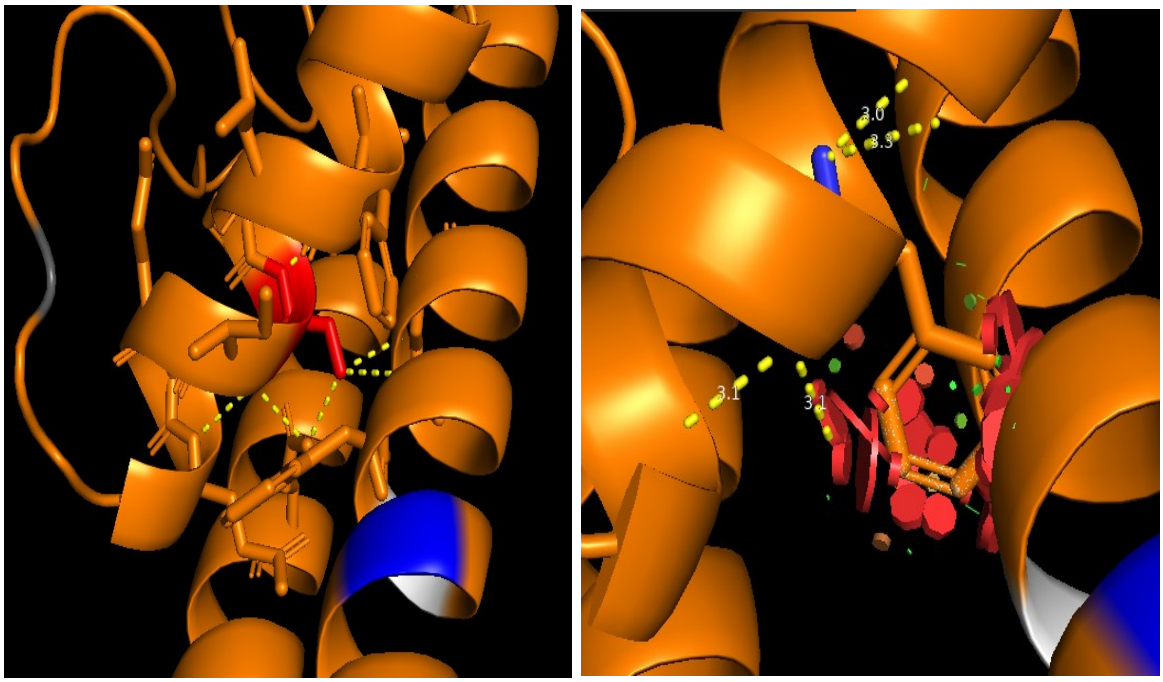


Figure 31 – WT representation (left side) with hydrogen bond network of S136 (yellow dots) and in silico mutated F136 (right side), with considerable steric clashes indicated as red shapes.

The mutation S136F (shown in red) is located in the pore helix. This helix is important for stabilizing the region behind the selectivity filter, which is essential for potassium flux. The serine forms important hydrogen bonds (yellow dotted lines in Figure 31) to TM1 and TM2 helices to stabilize the protein. The change from serine to phenylalanine results in a loss of function, because phenylalanine not only occupies too much space, as indicated by the steric clashes highlighted in red on the right side, but further this side chain cannot form any hydrogen bonds due to is aromatic/hydrophobic nature. In the figure, you can see that phenylalanine does not properly fit into the space, normally occupied by serine, thus it is

predicted that this mutation might seriously disrupt folding of the protein and or potassium flux.

G144S

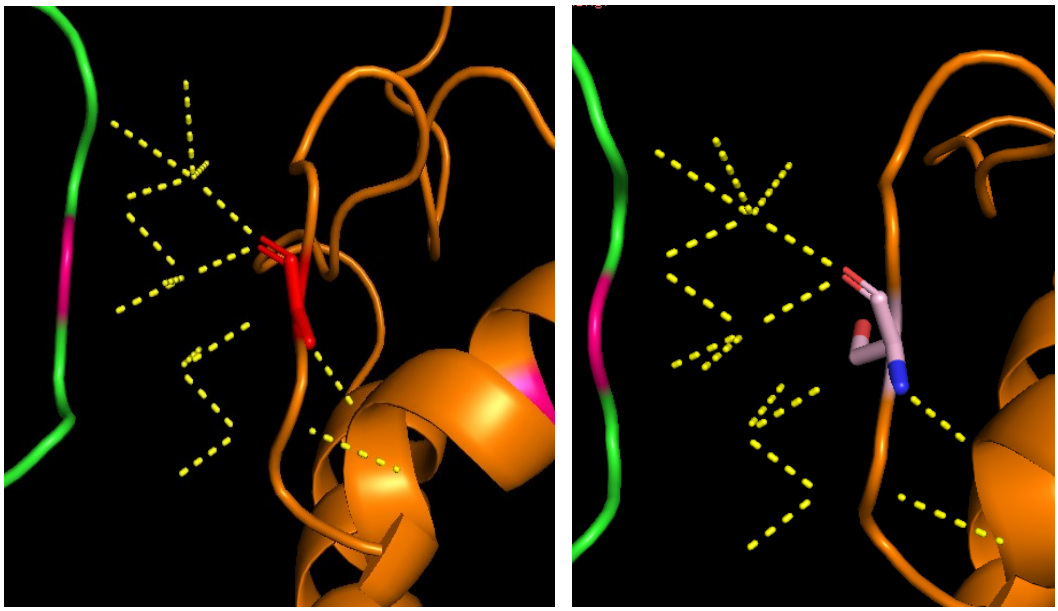


Figure 32 – WT G144 (left side), mutant S144 (right side).

The amino acid 144 is located in the selectivity filter. In comparison to glycine the additional hydroxyl group of serine might impact the selectivity of the channel. This polar group might prohibit the flux of potassium ions.

P186L

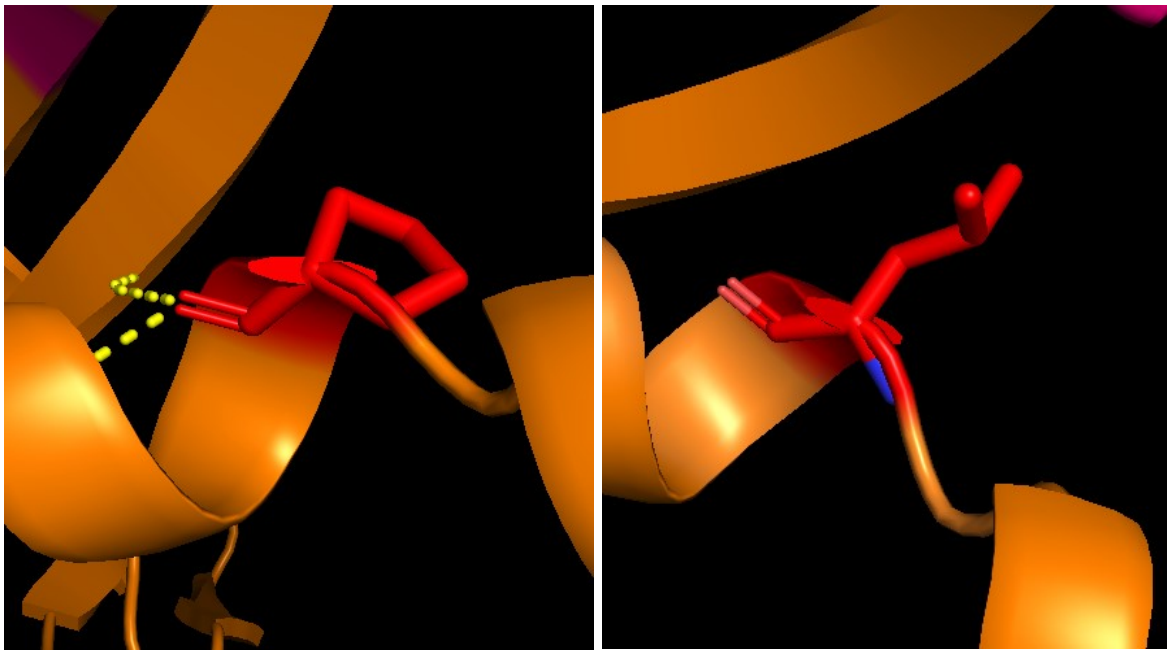


Figure 33 – left side: WT P186, right side: location of mutated leucine

The mutation P186L is located in the PKKR motif, which is involved in the binding of phosphoinositide-4,5-bisphosphate. A change from an aromatic ring to an alkyl group is predicted to influence the binding of PIP_2. Modelling predicts that the pocket for the ligand is different in the mutant and that PIP_2 cannot fit properly any more. Thus, one can speculate that potassium flux will decrease, perhaps due to reduced or hindered opening of the channel.

G300D

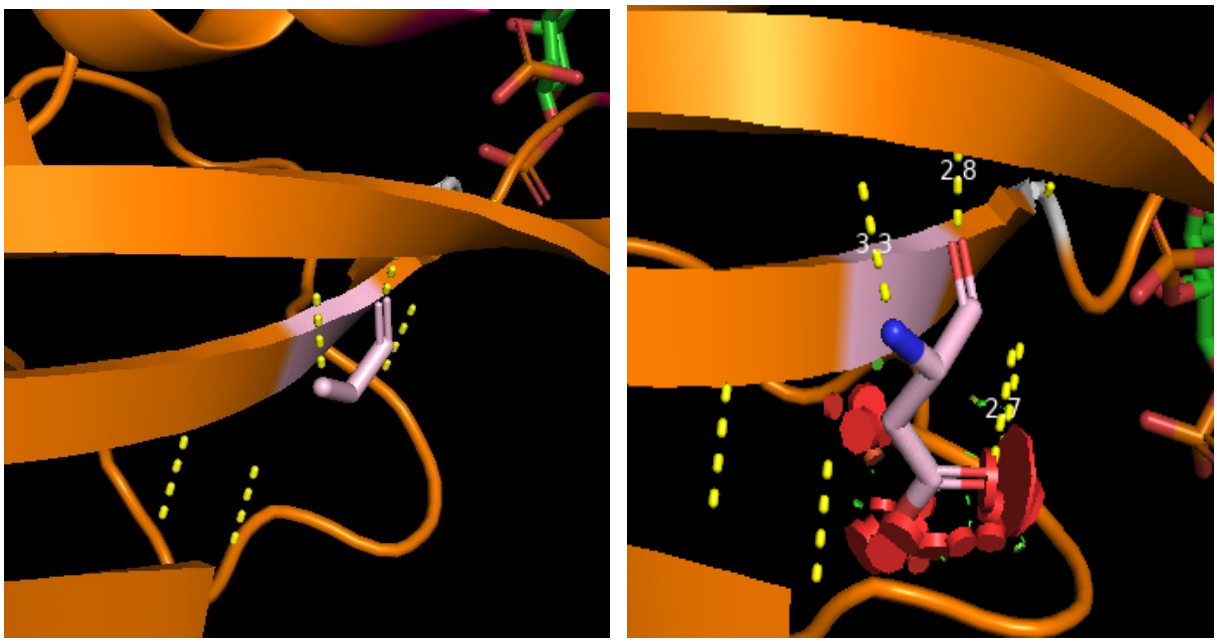


Figure 34 – location of the WT glycine is shown on the left side, right side: putative interactions of mutant aspartic acid with steric clashes indicated in red.

The bigger size of the side chain of aspartic acid might impede the normal folding of the cytoplasmic domain, as indicated by the red spheres, revealing steric clashes, when this side chain is placed in the wild-type model. Further, a negative allosteric effect might be the result of this mutation. Due to the fact that the protein cannot fold to his normal 3D structure, the binding of the ligand might be disturbed and the channel loses its function.

T305P

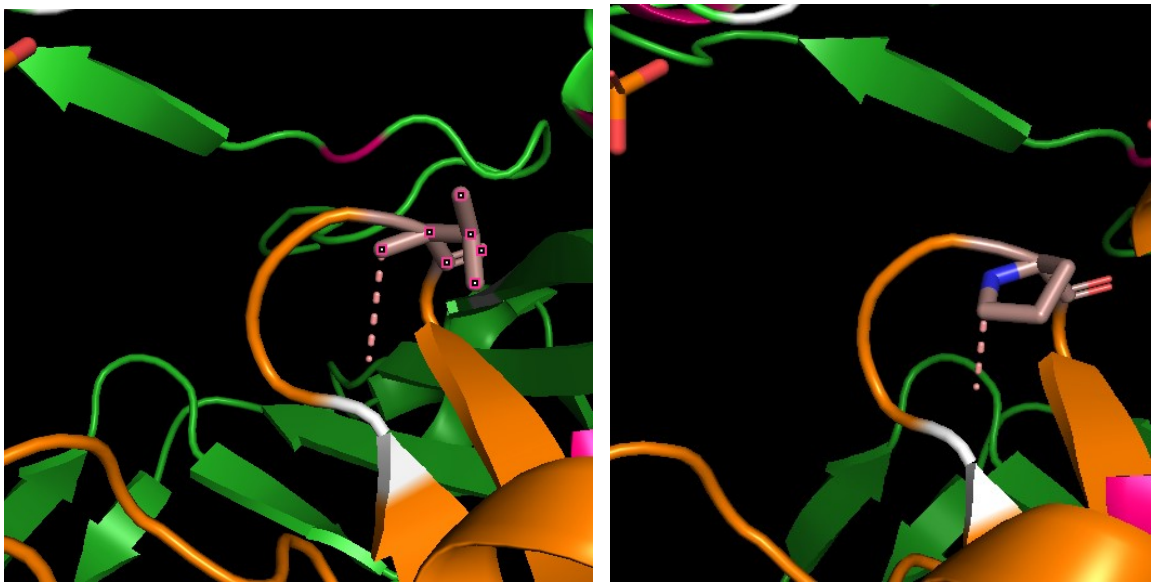


Figure 35 – location of T305 in the WT g-loop (left side); right side: P305 mutant

The change from glycine to proline in the g-loop gate region might influence the gating of the protein, due to the change from a highly flexible side chain to a rigid kink inducing side chain. It is conceivable that this mutation disturbs the conformational changes of the g-loop gate and thus results in a loss of function.

9. Conclusion and discussion

Andersen Tawil syndrome is an inheritable disease, with no specific treatment available. It is rare and occurs in around 1 in 1,000,000 people. Unfortunately, the disease is often not recognized because the symptoms are sometimes not very severe. Common symptoms include arrhythmias, physical changes, and neurological problems.
The inward-rectifying potassium channel Kir2.1 plays an important role in this disease. The mutations influence the binding or gating modulation of PIP2 or channel trafficking. There are significantly more mutations that interfere with PIP2 regulation. As a consequence the channel function decreases or loses its function.
Since the diagnosis of the disease is difficult, and the rarity so far prevented development of specific treatment, only the symptoms, such as arrhythmias, are treated. This means that the cause is not treated, only the effects of the disease. There is probably no other possibility in Andersen Tawil syndrome patients, since only very few patients are affected, but also the mutations on the Kir2.1 are various. One possible task would be that the PIP2 binding is strengthened specifically in Kir2.1 channel in order to normalize the activity of the channel. Alternatively, conductance might be enhanced by development of Kir2.1 specific activators. However, such drugs are currently neither available nor in development. Other channels should not be affected by this treatment, because of side effects. For example, patients with mutation near to the PIP2 binding pocket could receive medication that enhances the binding of PIP2. Detailed studies of the gene in order to make precise statements would be a requirement.

It is important to combine all the results ever collected to provide a good starting point towards development of possible therapeutic approaches. This work summarizes all

important facts about the disease. It makes sense to consider the entire disease properties in order to find the appropriate therapy.

The data from the homology model could provide a better understanding of the effects of certain mutations. Only if you know the reason for a cause it can be properly treated. But for further research it would be advisable to generate more homology models with different states of the protein. The current model represents a closed state model only. In the future, models of the open state might be built as well, given the recent publication of such a structure (cite: Zangerl-Plessl et al, JGP 2020). Optimally, however x-ray crystal structures of Kir2.1 preferably in different states and possibly with different disease causing mutants will be needed to address changes on protein structure, caused by ATS mutants in detail. Further, molecular dynamic simulations can have an impact on future research, since they can address for example changes in the selectivity filter (e.g. G144S mutant) and how this mutant might affect conduction and perhaps selectivity. Interestingly, a disease causing mutation at the exact same position has been reported for Kir3.2 channels, causing another very rare disease called “Keppen-Lubinsky” syndrome, which leads to loss of K+ selectivity in these channels. (add ref: https://www-sciencedirect-com.uaccess.univie.ac.at/science/article/pii/S0002929714005175)

10. Bibliography

[1] H. Hibino, A. Inanobe, K. Furutani, S. Murakami, I. Findlay, and Y. Kurachi, “Inwardly Rectifying Potassium Channels: Their Structure, Function, and Physiological Roles,” *Physiol. Rev.*, vol. 90, no. 1, pp. 291–366, Jan. 2010.

[2] S. B. Boyer, P. A. Slesinger, and S. V. P. Jones, “Regulation of Kir2.1 channels by the Rho-GTPase, Rac1,” *J. Cell. Physiol.*, vol. 218, no. 2, pp. 385–393, 2009.

[3] N. D’Avanzo, W. W. L. Cheng, D. A. Doyle, and C. G. Nichols, “Direct and specific activation of human inward rectifier K+ channels by membrane phosphatidylinositol 4,5-bisphosphate,” *J. Biol. Chem.*, vol. 285, no. 48, pp. 37129–37132, 2010.

[4] S. Pegan *et al.*, “Cytoplasmic domain structures of Kir2.1 and Kir3.1 show sites for modulating gating and rectification,” *Nat. Neurosci.*, vol. 8, no. 3, pp. 279–287, 2005.

[5] D. W. Hilgemann, “Regulation of Cardiac Na+,Ca2+ Exchange and,” vol. 273, no. August, pp. 20–23, 1996.

[6] O. Fürst, B. Mondou, and N. D’Avanzo, “Phosphoinositide regulation of inward rectifier potassium (Kir) channels,” *Front. Physiol.*, vol. 4 JAN, no. January, pp. 1–8, 2014.

[7] C. M. B. Lopes, H. Zhang, T. Rohacs, T. Jin, J. Yang, and D. E. Logothetis, “Alterations in Conserved Kir Channel-PIP2 Interactions Underlie Channelopathies,” *Neuron*, vol. 34, no. 6, pp. 933–944, Jun. 2002.

[8] S. Pegan *et al.*, “Cytoplasmic domain structures of Kir2.1 and Kir3.1 show sites for modulating gating and rectification,” *Nat. Neurosci.*, vol. 8, no. 3, pp. 279–287, 2005.

[9] M. Tristani-Firouzi *et al.*, “Functional and clinical characterization of KCNJ2 mutations associated with LQT7 (Andersen syndrome),” *J. Clin. Invest.*, vol. 110, no. 3, pp. 381–388, Aug. 2002.

[10] X. Chen, M. Bründl, T. Friesacher, and A. Stary-Weinzinger, “Computational Insights Into Voltage Dependence of Polyamine Block in a Strong Inwardly Rectifying K+ Channel,” *Front. Pharmacol.*, vol. 11, no. May, pp. 1–9, 2020.

[11] M. Tristani-Firouzi *et al.*, “Functional and clinical characterization of KCNJ2 mutations associated with LQT7 (Andersen syndrome),” *J. Clin. Invest.*, vol. 110, no. 3, pp. 381–388, 2002.

[12] R. Klein, R. Ganelin, J. F. Marks, P. Usher, and C. Richards, “Periodic paralysis with cardiac arrhythmia,” *J. Pediatr.*, vol. 62, no. 3, pp. 371–385, Mar. 1963.

[13] H. L. Nguyen, G. H. Pieper, and R. Wilders, “Andersen-Tawil syndrome: Clinical and molecular aspects,” *Int. J. Cardiol.*, vol. 170, no. 1, pp. 1–16, 2013.

[14] M. Tristani-Firouzi *et al.*, “Functional and clinical characterization of KCNJ2 mutations associated with LQT7 (Andersen syndrome),” *J. Clin. Invest.*, vol. 110, no. 3, pp. 381–388, 2002.

[15] L. Y. Ballester *et al.*, “Trafficking-competent and trafficking-defective KCNJ2 mutations in Andersen syndrome.,” *Hum. Mutat.*, vol. 27, no. 4, p. 388, 2006.

[16] G. Andelfinger, A. R. Tapper, R. C. Welch, C. G. Vanoye, A. L. George, and D. W. Benson, “KCNJ2 Mutation Results in Andersen Syndrome with Sex-Specific Cardiac and Skeletal Muscle Phenotypes,” *Am. J. Hum. Genet.*, vol. 71, no. 3, pp. 663–668, Sep. 2002.

[17] L. Zhang *et al.*, “Electrocardiographic features in Andersen-Tawil syndrome patients with KCNJ2 mutations: Characteristic T-U-wave patterns predict the KCNJ2 genotype,” *Circulation*, vol. 111, no. 21, pp. 2720–2726, 2005.

[18] Y. Kuroda *et al.*, “Flecainide ameliorates arrhythmogenicity through NCX flux in Andersen-Tawil syndrome-iPS cell-derived cardiomyocytes,” *Biochem. Biophys. Reports*, vol. 9, no. December 2016, pp. 245–256, 2017.

[19] A. A. M. Wilde, “Andersen-Tawil syndrome, scarier for the doctor than for the patient? Who, when, and how to treat,” *Europace*, vol. 15, no. 12, pp. 1690–1692, 2013.

[20] M. Xia *et al.*, “A Kir2.1 gain-of-function mutation underlies familial atrial fibrillation,” *Biochem. Biophys. Res. Commun.*, vol. 332, no. 4, pp. 1012–1019, Jul. 2005.

[21] S. Bendahhou, E. Fournier, S. Gallet, D. Ménard, M.-M. Larroque, and J. Barhanin, “Corticosteroid-exacerbated symptoms in an Andersen’s syndrome kindred,” *Hum. Mol. Genet.*, vol. 16, no. 8, pp. 900–906, Apr. 2007.

[22] N. M. Plaster *et al.*, “Mutations in Kir2.1 Cause the Developmental and Episodic Electrical Phenotypes of Andersen’s Syndrome,” *Cell*, vol. 105, no. 4, pp. 511–519, May 2001.

[23] C.-W. Lu, “Functional and clinical characterization of a mutation in KCNJ2 associated with Andersen-Tawil syndrome,” *J. Med. Genet.*, vol. 43, no. 8, pp. 653–659, Aug. 2006.

[24] H. Hibino, A. Inanobe, K. Furutani, S. Murakami, I. Findlay, and Y. Kurachi, “Inwardly Rectifying Potassium Channels: Their Structure, Function, and Physiological Roles,” *Physiol. Rev.*, vol. 90, no. 1, pp. 291–366, Jan. 2010.

[25] C. M. B. Lopes, H. Zhang, T. Rohacs, T. Jin, J. Yang, and D. E. Logothetis, “Alterations in Conserved Kir Channel-PIP2 Interactions Underlie Channelopathies,” *Neuron*, vol. 34, no. 6, pp. 933–944, Jun. 2002.

[26] S. G. Priori *et al.*, “A Novel Form of Short QT Syndrome (SQT3) Is Caused by a Mutation in the KCNJ2 Gene,” *Circ. Res.*, vol. 96, no. 7, pp. 800–807, Apr. 2005.

[27] H. Tang and P. D. Thomas, “PANTHER-PSEP: Predicting disease-causing genetic variants using position-specific evolutionary preservation,” *Bioinformatics*, vol. 32, no. 14, pp. 2230–2232, 2016.

[28] Y. Haruna *et al.*, “Genotype-phenotype correlations ofKCNJ2 mutations in Japanese patients with Andersen-Tawil syndrome,” *Hum. Mutat.*, vol. 28, no. 2, pp. 208–208, Feb. 2007.

[29] S. Bendahhou, M. R. Donaldson, N. M. Plaster, M. Tristani-Firouzi, Y.-H. Fu, and L. J. Ptácek, “Defective Potassium Channel Kir2.1 Trafficking Underlies Andersen-Tawil Syndrome,” *J. Biol. Chem.*, vol. 278, no. 51, pp. 51779–51785, Dec. 2003.

[30] S. Bendahhou, M. R. Donaldson, N. M. Plaster, M. Tristani-Firouzi, Y. H. Fu, and L. J. Ptácek, “Defective Potassium Channel Kir2.1 Trafficking Underlies Andersen-Tawil Syndrome,” *J. Biol. Chem.*, vol. 278, no. 51, pp. 51779–51785, 2003.