Review Article

Timothy Syndrome, A CACNA1C Gene Genetic Mutation, Results In Varying Symptomatic Disease Progressions

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ABSTRACT

Timothy Syndrome is a rare, multisystem genetic disorder originating from mutations in the CACNA1C gene, which encodes the a1C subunit of the L-type voltage-gated calcium channel. Distinct mutations give rise to multiple clinical variants, leading to heterogeneity in presentation, which can include cardiac arrhythmias, neurodevelopmental abnormalities, and congenital defects. This review synthesizes current knowledge of the molecular and pathophysiological mechanisms linking *CACNA1C* dysfunction to the syndrome's clinical manifestations. Insights gained from studying Timothy Syndrome extend beyond the rare disorder, offering a framework for understanding other calcium channel disorders and more prevalent genetic diseases.

Keywords: Timothy Syndrome; Genetic Mutations; Rare Genetic Disease; Differing Phenotypes; Neurological Pathologies

INTRODUCTION

Despite rare genetic diseases afflicting a small number of patients worldwide, there is a strong case for in-depth investigations into their origins to develop a basic understanding of disease progression. A small number of patients may be challenging for experimenters to investigate due to ethical reasons, differing variants of the same disorder, and all the patients not producing the same expected symptoms. Although this may be the case, oftentimes it proves to be essential for experimenters and scientists, as it may expose insights that will be essential for the treatment of other similar afflictions.

Timothy Syndrome (TS), a mutation of the *CACNA1C* gene (1), causes fewer than a hundred children worldwide to endure severe and painful symptoms such as syncope (loss of consciousness), frequent infections, large recharging delays between heartbeats (prolonged QT), seizures, and multiple physical deformities. Symptoms such as those listed previously are present in multiple variants of TS (2). TS can also lead to other diseases such as Autism, Arrhythmias, and Epilepsy (3). This paper aims to offer insights into these causations, as these potential ties are ambiguous to researchers.

TS has multiple prominent variants known as TS1, TS2, among other rarer mutations (2). Despite pathological differences in variants, they originate from the genetic mutation of the *CACNA1C* gene. The *CACNA1C* gene,

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which is almost 300 kb in size, encodes the alpha-1c subunit of the voltage-gated calcium channel $CA_v1.2$ (4). The gene both produces the calcium channel (Figure 1), and aids with the electrical signaling, communication between cells, muscle contraction, and gene regulation. Calcium flow found within these processes is essential for normal physiology. The channel that the gene produces, $CA_v1.2$, is located throughout the body, such as the heart and brain. In the heart, the channel helps to regulate the flow of calcium into cardiomyocytes. In the brain, calcium is involved in processes such as neuron communication and activity.

Timothy Syndrome Type 1

Timothy Syndrome Type 1 (TS1) was originally discovered in 13 individuals as they reported the molecular cause of TS (2). The variant disrupts the 6th transmembrane helix in domain I of the alpha-1c subunit, enabling increased activity in the *CACNA1C* gene (a gain-of-function phenotype). The resulting abnormal gain-of-function calcium flow affects various organs. For instance, the gain-of-function calcium flow results in an increased calcium influx in cardiomyocytes, disrupting normal cardiac electrophysiological signaling. The aberrant calcium handling also contributes to developmental abnormalities such as syndactyly and craniofacial malformations, resulting in diverse downstream phenotypic effects (2).

Timothy Syndrome Type 2

Timothy Syndrome Type 2 (TS2) is caused by abnormal splicing of the *CACNA1C* gene. The *CACNA1C* gene undergoes multiple alternative splicing events; however, TS2 primarily involves differential splicing of exons 8 and 8a (exon 8-containing mRNAs are approximately fourfold more abundant than exon 8a-containing transcripts in tissues most affected by TS) (2). Both exons consist of 104 nucleotides (code for 34 amino acids) and code for Glycine at residue 406. However, mutations can alter the nucleotide sequence, resulting in amino acid substitutions. In 2005, a mutation at residue 406 of exon 8 was identified, resulting in a glycine to arginine substitution (Gly406Arg). The mutation later defined the TS2 variant (2). Children with the Gly406Arg variant are more severely symptomatic compared to children with the same missense variant in exon 8 because of varying expression levels within the brain and heart. For instance, individuals with TS2 do not present with syndactyly and craniofacial manifestations compared to TS1 individuals, but rather exhibit more severe cardiac and neurological symptoms. Additionally, individuals with TS2 die at a younger age due to worsened clinical outcomes, when considering the exon 8 mRNAs' abundance rate compared to exon 8a mRNAs in the brain and heart.

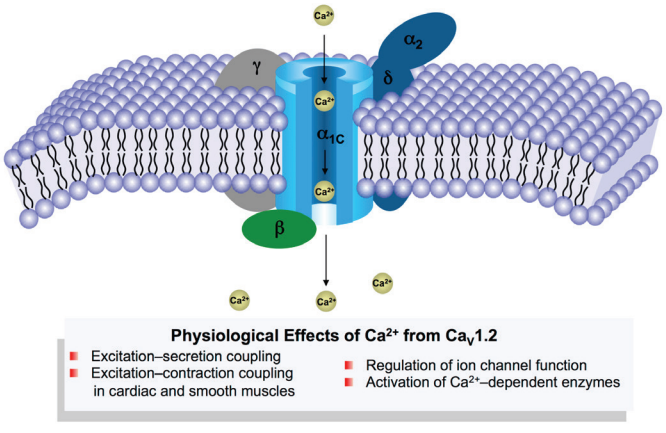

Figure 1. Example of Calcium Channel (5). Calcium Channels span the cellular membrane, facilitating the passive transport of calcium ions. The flow of calcium ions corresponds to subsequent physiological functions.

Atypical Timothy Syndrome

A diverse spectrum of TS variants arises from multiple distinct mutations within the *CACNA1C* gene (2). For instance, analogous to the TS2 variant, a patient was identified with a Gly402Ser substitution, where glycine at residue 402 is replaced by serine. Unlike individuals with TS2, this patient did not exhibit syndactyly but presented with severe neurological impairments. Notably, the patient was diagnosed solely with LQT8 cardiac arrhythmia, without features of a syndromic disorder. Since 2005, at least another 12 missense variants have been identified as TS-causing alleles.

In 2012, an individual with an Ala1473Gly missense variant was identified (2). This individual presented with facial malformations, bilateral hip dislocation, joint contractures in the arms, and syndactyly in the fingers and toes of all limbs. In contrast to previously reported cases, the patient also exhibited severe neurological symptoms, which later progressed to seizures, apnea, and profound neurodevelopmental delay.

Multiple other cases involving different mutations were reported, each affecting different residues and resulting in distinct combinations of symptoms unique to each individual.

SYMPTOMS OF THE SYNDROME

The clinical manifestations of TS exhibit significant variability across different variants and individual patients (5). Primary symptoms of TS include syndactyly (fusion of fingers or toes), prolonged QT interval, arrhythmias, congenital heart defects, immunodeficiencies, endocrinological dysfunction, hypotonia, gastrointestinal issues, facial malformations, frequent infections, hypoglycemia, hypothermia, and seizures (1). The presence and severity of these symptoms often vary according to the specific TS variant present in each individual. For instance, Individual A was diagnosed with TS after a glycine-to-arginine substitution at residue 419 of exon 8 was identified. The individual presented with symptoms such as bilateral hand and foot syndactyly, mild neurodevelopmental delay, prolonged QT interval, and ventricular preexcitation (2). In contrast, an individual with TS1, resulting from mutations in the sixth transmembrane helix of the alpha-1c subunit of the *CACNA1C* gene, may exhibit fewer cardiac and neurodevelopmental symptoms compared to those with mutations such as the Gly419Arg variant (2). Moreover, variants involving mutations in exon 8, rather than 8a, tend to present with more severe cardiac and neurological symptoms, reflecting the approximately fourfold higher expression of exon 8-containing mRNAs in the brain and heart compared to exon 8a transcripts. Individuals with TS exhibit a strong association with neurological disorders (such as Autism, ASD, etc.); however, the precise mechanisms linking TS mutations to the development of these conditions remain incompletely understood.

PATHOLOGIES RELATED TO TIMOTHY SYNDROME

Depending on the individual, the genetic mutation that causes TS can also result in the development of other downstream pathologies. The *CACNA1C* gene is involved in regulating multiple physiological processes, including electrical signaling, communication between cells, muscle contraction, and gene regulation. Given its involvement in numerous physiological processes, dysfunction of the *CACNA1C* gene is likely to have widespread effects, potentially contributing to the development of various secondary disorders. This section summarizes several conditions indirectly associated with TS.

Autism Spectrum Disorder (ASD)

Given the essential role of the *CACNA1C* gene in electrical signaling and intercellular communication, mutations can disrupt these processes and cause negative alterations in the signaling and efficiency of these processes (4). Scientists from Stanford University School of Medicine analyzed cells from TS patients and proposed that the observed autism spectrum disorder may stem from a gene mutation. This mutation impairs neuronal membrane function, initiating a cascade of downstream effects (7). Proper calcium influx into neurons is critical for action potential generation, and dysregulation of calcium flow disrupts normal neuronal activity. Analysis of brain cells from individuals with Timothy Syndrome revealed a reduction in neurons connecting the cerebral hemispheres, alongside an overproduction of dopamine and norepinephrine. Subsequently, researchers reprogrammed skin cells from TS patients into induced pluripotent stem (iPS) cells for further study. These cells formed neurons, enabling researchers to identify abnormalities that may contribute to autism. Many neurons exhibited abnormally large spikes in calcium channels, suggesting that the impaired channels were unable to shut off properly. Scientists consequently treated the neurons with Rocscovitine, resulting in a decreased proportion of the cells producing the enzyme. The study confirmed that defective calcium channels lead to excessive production of dopamine and norepinephrine, suggesting that some autism-related abnormalities may be amenable to therapeutic intervention.

Additional experiments, including a Mouse model study (Figure 2), were performed to investigate the connection between ASD and TS (8). Researchers created mutant mice exhibiting TS2, but they encountered viability concerns in both the heterozygous and homozygous versions of the mice. To allow the mice to retain an inverted neomycin cassette, enabling them to survive into adulthood, the researchers modified the group accordingly. Using the TS2-neo mice, the researchers phenotyped their behavior and repeatedly showed, "restricted, repetitive… altered social behavior… and enhanced tone-cued/contextual memory following fear conditioning." These observations suggested that the expression of TS mutant channels, under the qualifier that no death is caused, may be enough to cause behavioral abnormalities similar to ASD.

Epilepsy

Mutations in the *CACNA1C* gene significantly

impair calcium channel function, leading to disrupted neurotransmitter release and defective neuronal signaling (6). In TS, excessive calcium influx disrupts neuronal electrical activity, contributing to seizure development. The persistent calcium overproduction observed in Timothy Syndrome patients increases their susceptibility to recurrent seizures (epilepsy), including photosensitive epilepsy. Excessive calcium influx may also result in neuronal hyperexcitability, potentially contributing to the development of Attention Deficit Hyperactivity Disorder.

Long QT syndrome

Long QT syndrome is a cardiac disorder characterized by delayed repolarization of the ventricular myocardium, leading to prolonged myocardial recovery time between beats (7). The electrical conduction abnormality predisposes TS patients to potentially fatal arrhythmias and sudden cardiac arrest (Figure 3). Given that the *CACNA1C* gene encodes voltage-gated calcium channels essential for muscle contraction, mutations in this gene can disrupt normal myocardial contractility, contributing to the cardiac dysfunction observed in Long QT syndrome.

Due to severe cardiac complications, individuals with TS generally have a prognosis of survival until the end of their childhood. Approximately 80% of Timothy Syndrome cases result in mortality due to Ventricular Tachycardia, a rapid ventricular arrhythmia in which the ventricles of the heart beat abnormally fast, leading to cardiac arrest and, ultimately, sudden death.

Syndactyly

Syndactyly is a prominent feature in certain variants of TS, whereas in other variants, it is absent from the individual (7). Syndactyly may result from mutations in the *CACNA1C* gene, which encodes for the creation of the calcium channels. These mutations result in delayed channel closure, leading to increased cellular excitability. Altered calcium influx impairs the regulation of downstream genes, contributing to developmental abnormalities, including craniofacial malformations and syndactyly.

TREATMENT AND ONSET OF TIMOTHY SYNDROME

The diagnosis of TS typically occurs earlier in one's lifetime, either before birth or within infancy (1). Prenatal diagnosis of TS may involve ultrasound assessments of fetal heart rate to detect arrhythmias or abnormalities. Postnatal diagnosis typically involves a combination of tools, including an EKG to examine heart rhythm, imaging tests, genetic tests, cardiology/neurology evaluations, and metabolic blood tests.

Treatment of Timothy Syndrome is often incredibly challenging for caretakers, as there is no cure for the

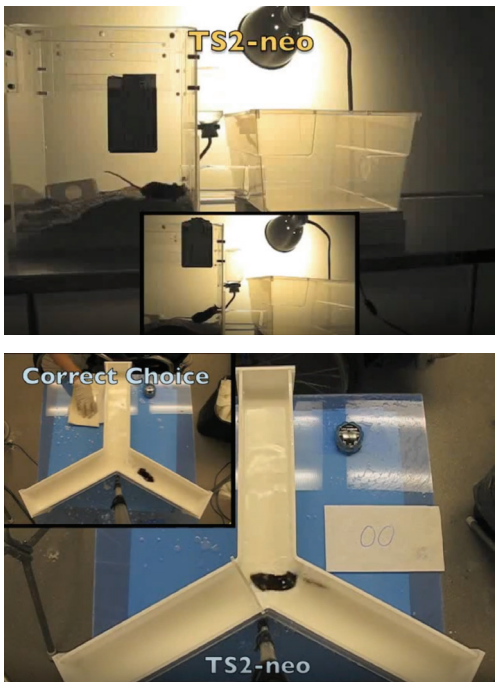

Figure 2. A water Y-maze forced training trial for mouse (8).

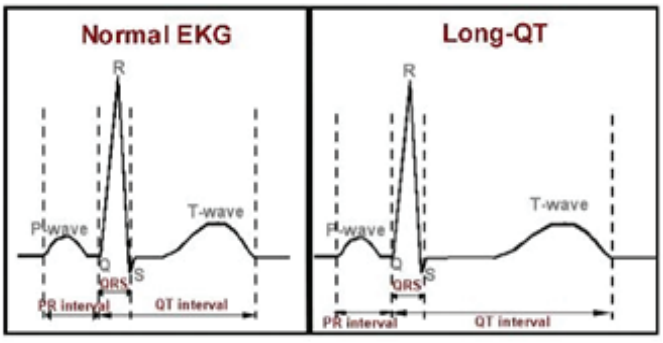

Figure 3. Example of prolonged QT interval. Comparison of an EKG from a normal heart and one from a patient with Long QT syndrome. The QT interval is exaggerated as a result of improper calcium channel activity (9).

syndrome itself, and numerous risks can lead to life-threatening complications (1). Treatment primarily focuses on preventing life-threatening symptoms that affect the heart, as arrhythmias and cardiac arrest are leading causes of mortality in TS patients. Therapeutic interventions include beta-blockers and either an implantable cardioverter-defibrillator (ICD) or a pacemaker to regulate the heart rate. Additional treatments target non-cardiac symptoms; for instance, surgical correction may be considered for syndactyly, and special education programs can support patients with learning disabilities.

While several treatments address the diverse symptoms of TS, many carry associated risks and potential complications. Many of the therapeutic treatments can increase the risk of cardiac complications that TS patients are predisposed to.

Many scientists consider switching the *CACNA1C* exon from exon 8A to 8 a viable therapeutic strategy (10). Scientists have developed antisense oligonucleotides to decrease the number of exon 8A in human cells from being included in vitro and during transplantation. Through this research, they have discovered that the switch from exon 8A to 8 using ASOs assisted in fighting the defects in patient-derived cortical organoids and migration in forebrain assembloids. Scientists also found that a single intrathecal ASO administration can restore the calcium changes within a Timothy Syndrome patient and in vivo dendrite traction of patient neurons. This highlights that shifting *CACNA1C* expression from 8A to 8 is a potential treatment for TS1.

In addition to other treatment strategies, scientists are exploring gene editing software such as CRISPR/Cas 9 technologies to correct genetic mutations, thereby targeting the root cause of the disorder (10). This approach eliminates the increased risks associated with current treatments by addressing the underlying cause, rather than only managing the symptoms the patient experiences.

CONCLUSION

TS is an extremely rare disorder that affects only about 100 individuals worldwide and exists in several variants, depending on the location of the underlying genetic mutation. Despite its rarity, studying TS offers valuable insights into the mechanisms of other complex diseases that are challenging to investigate in depth. The disorder predisposes affected individuals to various comorbidities, including autism spectrum disorder (ASD), epilepsy, and attention deficit hyperactivity disorder (ADHD), which appear linked through the disruptions in calcium channel function. The *CACNA1C* gene, mutated in TS, encodes critical calcium channels; mutations in this gene impair calcium signaling, leading to diverse pathological processes. The complexity and phenotypic heterogeneity arising from different exon-specific mutations facilitate a deeper understanding of genotype-phenotype relationships and guide the development of targeted therapeutic strategies. Furthermore, the comparatively small and well-defined TS population offers a unique opportunity to monitor disease progression and molecular changes with precision. Patient groups for TS provide essential support due to the rarity of the disorder, especially as most research on pathologies focuses on more common conditions. Collectively, these factors underscore the critical importance of continued research and experimentation in understanding and treating TS and its variants.

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CONFLICT OF INTERESTS

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