SCHOLARLY REVIEW

CRISPR-Cas9 Gene Therapy as a Treatment for Short QT Syndrome

By Willow Shi

Author Biography

Willow Shi is a junior at Emma Willard School in New York. She has had a passion for biology from a young age and aspires to pursue a medical career. She enjoys swimming, playing lacrosse, and dancing in her free time. She is particularly intrigued by the potential of gene therapy as a modern approach to curing diseases.

Abstract

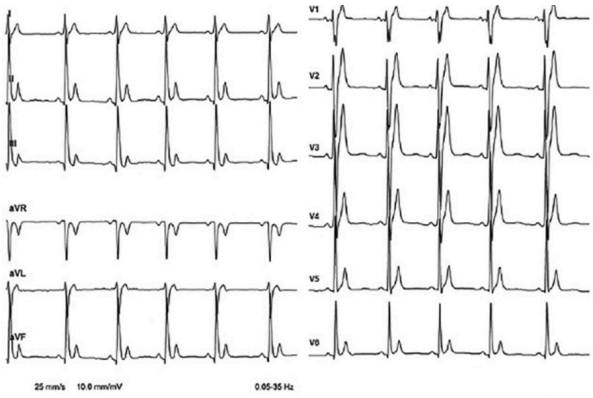
Short QT syndrome (SQTS) is a rare cardiac disease that shortens the heart's repolarization time. This can cause life-threatening arrhythmias, syncope, and atrial fibrillation. It is caused by gain-of-function mutations in the potassium channel genes KCNH2, KCNJ2, and KCNQ1, which increase potassium ion flow and disrupt the heart's rhythm. Current treatments like the implantable cardioverter defibrillator (ICD) and antiarrhythmic drugs manage symptoms but are not curative. The mutations and the disease remain. If the patient stops medication or the ICD malfunctions, they will fall back into a disease state. In addition, with these treatment methods, patients risk having side effects or inappropriate ICD shocks. This paper proposes a gene therapy approach using the CRISPR-Cas9 system to correct mutations in the KCNH2 gene. The strategy aims to correct the repolarization time and, thus, the QT interval. The CRISPR-Cas9 system is used to target the specific mutation and allows for permanent gene correction. Gene therapy is a more sustainable and long-lasting solution compared to pharmacological approaches. This proposed method has the potential to offer a new treatment option for SQTS patients, giving a one-time approach to a chronic genetic condition.

Keywords: Short QT syndrome, Cardiovascular Diseases, Gene Therapy, Genetics, CRISPR, Ion Channels, Adeno-Associated Viruses, Mutations

Introduction

Short QT syndrome (SQTS) is a genetically inherited cardiac disease caused by increased activity in potassium ion channels, disrupting the heart's normal rhythm (Dewi, 2020). The flow of potassium ions increases across the membrane of the cardiac muscle cells, thus altering the repolarization phase of the cellular electrical activity. SQTS causes episodes of syncope, paroxysmal atrial fibrillation, or life-threatening cardiac arrhythmias (Brugada, 2005). It is characterized by an abnormally short QT interval, which refers to ventricular depolarization and repolarization detected on an electrocardiogram (EKG) (Rudic, 2014). Specific voltage waves - P, Q, R, S, T - mark each heartbeat (Ashley & Niebauer, 2004). As blood flows through the heart, it moves from the atrium to the ventricle. Electrical signals initiate contraction of the atria and ventricles, inducing blood flow (Ashley & Niebauer, 2004). The P wave initiates the contraction of the atria, the QRS interval is the electrical signal corresponding to the excitation of the ventricles, and the T wave is the repolarization of the ventricles. The QT interval is the time between the QRS interval and the end of the T wave, which is also the time required for the ventricles to repolarize after depolarization. In healthy individuals, the QT interval for men lasts between 0.35 - 0.45 seconds (350 ms - 450 ms) and 0.36 - 0.46 seconds (360 ms to 460 ms) for women (Rezus, 2015). The ECG of a male with SQTS shows tall T waves and short QT intervals (Figure 1). Males with a QT interval of ≤ 330 ms and females with an interval of ≤ 340 ms are considered to have SQTS, even if they are asymptomatic. Data from over 10,000 adults suggest that the prevalence of a QT interval of less than 340 ms is approximately 0.5%, demonstrating the extreme rarity of SQTS in the global /US population (Rudic, 2014).

Figure 1
Baseline Electrocardiogram (ECG) Recording (Standard 12-lead ECG, Paper Speed 25 mm/s) of a 25-year-old Male with Short QT Syndrome (Rudic, 2014)



Mutations in KCNH2, KCNJ2, and KCNQ1, can cause SQTS (Badura, 2024). Gain-of-function mutations in these potassium channel genes enhance repolarization, causing abnormal shortening of the cardiac action potential in short QT syndrome (Kim, 2014). The KCNH2 gene expresses a protein that makes up the potassium channel responsible for the rapidly activating rectifier outward current (IKr) (Brugada, 2005). Two different missense mutations in KCNH2 cause the exact amino acid change in the protein, leading to a change in the IKr current. One is a missense mutation with cytosine to guanine substitution at nucleotide 1764. The other is a cytosine-to-adenine substitution at the same nucleotide. Both mutations lead to a substitution of asparagine with lysine at codon 588 (Rudic, 2014). To determine how the mutation N588K alters the potassium channel, Brugada et al. (2005) coexpressed N588K with KCNE2, which encodes a small transmembrane subunit of the KCNH2 channel. The current generated through the mutated channels showed that the mutation abolished the inactivation of the KCNH2 channels, thus increasing the IKr current. In Figure 1, mutation N588K significantly increased the amplitude of the IKr current, which was much larger than wild type past 0 mV. The mutation had similar effects on currents regardless of the coexpression of KCNE2. The current through the mutated channels failed to rectify in various voltages. When examining action potential clamp experiments, outward potassium flow in normal channels demonstrated a typical "hump"-

like waveform due to slow activation in phases 1 and 2 and a rapid increase in current in phase 3 as the inactivated channels recovered. In mutated channels, the currents in all action potential phases were larger. The mutation showed a "gain of function" in the Ikr current, shortening the action potential (Brugada, 2005).

The shortening of the action potential is the root cause of SQTS,, and one of the methods that rectify the action potential is by using drugs. A study evaluated the efficacy of various antiarrhythmic drugs - flecainide, sotalol, ibutilide, and hydroquinone- in prolonging the QT interval to normal ranges. Six patients with SQTS from two different families were included. Four drugs were tested; only hydroquinone prolonged the QT interval to the normal range. This was these patients' most effective pharmacological treatment (Gaita, 2004). Terfenadine and astemizole have also been associated with prolonging the QT interval. Both block the rapidly activating component of the delayed rectifier channel, Ikr-. However, at high concentrations, both terfenadine and astemizole block other cardiac channels, including Na+, Ca2+, and K+ channels (Eva Delpón, 2012). One of the primary concerns of using drugs for SQTS is that many antiarrhythmic drugs are not highly specific. Blocking other cardiac channels can lead to unwanted side effects (Priest, 2015).

Gene therapy, a promising medical approach, can potentially treat or prevent diseases by correcting the genetic mutation causing a disorder. It serves as an alternative to surgery and drugs, offering a permanent solution. Gene therapy can work by replacing a disease-causing gene with a healthy copy of the gene, inactivating a disease-causing gene, or introducing a new or modified gene into the body (U.S. Food and Drug Administration, 2018). The genetic material delivered into the body, either DNA or RNA, leads to changes in the protein produced. Genetic material can be altered in two ways: ex vivo and in vivo. Ex vivo treatment removes a person's cells and delivers the gene therapy material to the cells outside the body, which are then returned to the body. In vivo treatment involves genetic material delivered directly to the person (Children's Hospital of Philadelphia, n.d.). A newer technique in gene therapy, called genome editing, allows scientists to change an organism's DNA. Genetic material can be added, removed, or altered at locations in the genome.

A well-known approach to genome editing is CRISPR-Cas9. It is a two-component system that allows researchers to edit any sequence in an organism's genome. The two components are the guide RNA (gRNA), which recognizes the target sequence, and the CRISPR-associated endonuclease (Cas) that cuts the targeted sequence (Redman, 2016). The gRNA is a small RNA with a short "guide" sequence that binds to a specific target sequence in a cell's DNA. Another component of this technology includes the Protospacer Adjacent Motif (PAM) sequence. This short DNA sequence (3 base pairs) is found in 3-4 nucleotides adjacent to the targeted DNA region and is required for a Cas nuclease to cut. The Cas nuclease will also search for the PAM sequence before cutting. After Cas identifies the correct PAM, it will check the upstream region to see if it matches the guide RNA before making the change (Synthego, 2024b). CRISPR-Cas9 can be an approach for gene therapy treatment of SQTS. This study is aimed at proposing a gene therapy treatment for SQTS.

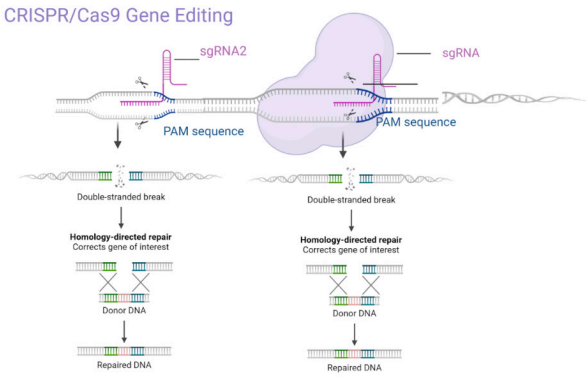
Methodology

The most common length for a guide RNA (gRNA) is around 20 nucleotides (nt). This ensures a high level of specificity in the gene since the 20 nt sequences will unlikely appear elsewhere (Asmamaw, 2021). A shorter gRNA increases the likelihood of the sequence appearing elsewhere on the gene, causing the Cas9 enzyme to cut in the wrong place. A longer gRNA sequence means more nucleotides are needed to match the DNA sequence. Nucleotides in the guide RNA will self-hybridize if it's too long. The Cas9 enzyme can tolerate mismatches between the guide RNA and the target DNA, especially towards the 5' end. The tolerance allows Cas9 to bind and cut DNA at sites that are not a perfect match, increasing the probability of being off target (Lee, 2021).

Chop Chop was used to design my gRNA sequences. The two gRNA sequences that can be used in the proposed gene therapy treatment are ACATGGACTCACGCATCGGCTGG and eCACAACCTGGGCGACCAGATAGG. Having two gRNA sequences increases the specificity of the CRISPR by targeting two adjacent sites. Both gRNAs need to bind to their sequences for Cas9 to cut, reducing the probability of off-target effects. The mutation site is between the two gRNA sequences. A

template DNA sequence and the CRISPR components will be included, including 50 base pairs upstream and downstream of the cut sites. The template DNA sequence is identical to the region surrounding the cut site but will include the corrected base at the mutation site. Figure 2 shows a diagram of the CRISPR system.

Figure 2.
Diagram of CRISPR treatment (Biorender)



Adeno-associated viruses (AAV) are non-enveloped viruses that can be engineered for specific functions in gene therapy applications. Other viral vectors have unwanted properties, such as their propensity to cause cancer, which has limited current use to only particular applications (Naso, 2017). AAVs have not been linked to any human disease despite the high prevalence of these viruses in the human population. Its low immunogenicity and cytotoxicity have made it an ideal choice for in-vivo delivery of CRISPR components. Another advantage of using these vectors is that there are several serotypes of AAV for different tissue tropisms, making tissue-specific delivery easier (Mengstie, 2022). AAV9 is a leading vector for cardiac gene therapy because it has the best cardiac transduction specificity and can be expressed stably and efficiently (Zhang, 2022).

Results

The proposed gene therapy treatment for SQTS starts with a guide RNA. A guide RNA of 20 base pairs will be designed to bind to the region of nucleotide 1764 of the KCNH2 gene where either one of the mutations occurs. The recognition of the mutation site is also facilitated by the PAM sequence, located 3 to 4 nucleotides downstream of the mutation. The Cas9 will cut the DNA at the location specified by the guide RNA, and the existing segment will be replaced with a customized DNA sequence that reverts the adenine to cytosine substitution or guanine to cytosine substitution. An AAV9 injected into the heart will carry the CRISPR components and a corrected template DNA sequence. The expected outcome of the gene therapy for SQTS would be to increase the action potential phase, thus elongating the QT interval.

Discussion

In recent years, CRISPR has been used to edit genomes in almost all organisms, from human cells to tiny microorganisms. This new technology has opened a new pathway for preclinical and clinical research in treating various diseases (Liu, 2021). For example, CRISPR has proved to be an effective treatment for phenylketonuria (PKU), an autosomal recessive liver disease in which the phenylalanine hydroxylase (PAH) enzyme results in decreased phenylalanine metabolism. An AAV carrying a single-base gene editor that converts C-G base pairs to T-A base pairs restored PAH expression. It increased the reduced level of phenylalanine in blood delivered to a mouse model. 63% of the mRNA had the corrected base sequence, confirming the effectiveness of this gene-editing system for PKU (Li, 2023).

For patients with SQTS, the first-line therapy is the implantable cardioverter defibrillator (ICD). A problem with ICDs comes from one of SQTS's main features on the ECG. The T wave that closely follows the R wave can sometimes be over-sensed, provoking an inappropriate shock from the ICD (Dewi, 2020). The high prevalence of atrial fibrillation also presents a clinical challenge for appropriate ICD programming. A high rate of inappropriate ICD therapies of up to 64% was reported in a large pediatric cohort of SQTS patients, accentuating the need for sophisticated and durable ICD devices. Even though ICD remains the primary therapy for SQTS, pharmacological treatment may also be helpful (Rudic, 2014).

The four drugs tested in the study mentioned previously were flecainide, sotalol, ibutilide, and hydroquinone. The QT interval was measured at speeds of 25 and 50 mm/s. The interval was then corrected for heart rate. At high heart rates, the interval will shorten as a direct result of increased heart rate.

This logic also applies to low heart rates. Resting heart rate is different for everyone, so the corrected QT interval can be used to compare individuals. The QT interval of patients recorded after treatment was compared with the predicted QT interval (QTp) (QT/QTp%). The QTp was calculated using a formula proposed by Rautaharju et al. The QT interval was significantly prolonged if the QT/QTp% exceeded 80%. Hydroquinidine markedly prolonged the QT interval, which entered the normal range. The only drug that produced a QT/QTp% of over 100% (Table 3). Flecainide produced a slight QT increase in the four patients to which it was administered. The other two drugs, sotalol and ibutilide, caused no changes in QT interval. Even though hydroquinone increased QT interval, two patients were discontinued from the drug in the study due to side effects of diarrhea. In addition, the drug was administered three times a day, making it unfeasible in the long run. Other class III antiarrhythmic drugs, including d-sotalol and nifekalant, were also sporadically reported to be effective in normalizing the QT interval. The pharmacological therapy for patients with SQTS is still poorly defined due to the lack of large-scale evidence (Rudic, 2014).

Table 3.
Drugs and Electrocardiographic Modifications (Gaita et al., 2004)

Family	Pt #	Age, Gender	Drug	Route	Dose	Rhythm	HR	QRS (ms)	QT (ms)	QTc (ms)	QT/QTp(%)
1	1	35, M	none	#	#	sinus	66	80	270	283	68
	1		flecainide	i.v.	2 mg/kg	sinus	62	90	300	305	74
	1		flecainide	oral	100 mg b.i.d.	sinus	60	100	320	320	78
	1		sotalol	oral	80 mg b.i.d.	sinus	63	80	260	266	65
	1		ibutilide	i.v.	1 mg/kg	sinus	60	80	290	290	71
	1		hydroquinidine s.r.	oral	250 mg t.i.d.	sinus	60	100	380	380	93
2	31, F		none	#	#	sinus	86	80	250	299	71
	2		flecainide	i.v.	2 mg/kg	sinus	80	90	240	277	66
	2		flecainide	oral	100 mg b.i.d.	sinus	83	100	240	282	67
	2		sotalol	oral	80 mg b.i.d.	sinus	63	80	280	287	70
	2		ibutilide	i.v.	1 mg	sinus	67	80	280	296	71
	2		hydroquinidine s.r.	oral	250 mg t.i.d.	sinus	85	100	360	428	102
3	6, M		none	#	#	sinus	76	70	250	281	67
	3		sotalol	not tolerated							
	3		flecainide	oral	25 mg b.i.d.	sinus	68	90	280	298	72
	3		hydroquinidine	not tolerated							
4	67, F		none	#	#	sinus	72	120	270	296	71
	4		flecainide	i.v.	2 mg/kg	sinus	73	150	320	350	84

4		hydroquinidine s.r.	oral	500 mg b.i.d.	sinus	83	120	370	435	103
5	40, F	none	#	#	sinus	72	100	280	307	73
5		sotalol	i.v.	50 mg	sinus	81	100	260	302	72
5		hydroquinidine s.r.	oral	500 mg b.i.d.	sinus	66	100	380	380	96
6	15, M	none	#	#	sinus	66	85	260	273	66
6		hydroquinidine s.r.	oral	500 mg b.i.d.	sinus	95	100	320	403	95

The symbols b.i.d. = twice a day; HR = heart rate; i.v. = intravenous; s.r. = slow release; t.i.d. = 3 times a day. QTp = QT predicted # = none.

Conclusion

Gene therapy, especially using the CRISPR-Cas9 system, addresses the genetic mutations in KCNH2 that cause SQTS. The treatment is a permanent solution rather than only treating the symptoms. It can potentially provide a once and for all treatment. This is important for hereditary diseases like SQTS, which require lifetime management. Pharmacological treatments only work at the symptom level, so they need to be taken continuously. Drug efficacy may also diminish over time if tolerance is developed. Another benefit of gene therapy is that it has fewer side effects because it explicitly corrects the mutation. Drugs often impact more than one ion channel, which can lead to unwanted effects. The only treatments currently are drugs and the ICD, which both come with higher risks than using CRISPR.

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