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Electrocardiographic and Genetic Idiosyncrasies and their Implications for Heart Transplantation

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Electrocardiographic and Genetic Idiosyncrasies and their Implications for Heart Transplantation

By

Erik Vaughn Carter

DISSERTATION

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DOCTOR OF PHILOSOPHY

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Acknowledgments

For the last ten years, I've focused my graduate studies on the study of cardiovascular electrophysiology and cardiogenomics. This body of research has provided me with a vast array of opportunities. As a graduate of the UCSF MS program, I received my master's degree with a focused major in cardiogenomic nursing as a cardiovascular nurse specialist with a minor in nursing education. This education provided me the foundation needed to join the UCSF School of Nursing's PhD program. Once admitted, I continued my scholarly work under the advisement of two great mentors, Dr. Barbara Drew and Dr. Brad Aouizerat: commitment to my research studies was without compromise. In addition, opportunities ranging from an internship at the National Institutes of Health as part of the Summers Genetics Institute, a fellowship in Pathways to Careers in Clinical & Translational Research (PACCTR) program and the Advance Training in Clinical Research certificate program in the department of Epidemiology and Biostatistics have all helped me develop the skills needed to become a exceptional nurse scientist.

Many have been responsible for my gravitation to this moment; Dr. Barbara Drew, Dr. Brad Aouizerat, many in the School of Nursing and School of Medicine, my colleagues who are too many to name but know their roles in my academic growth, my friends inside and outside of UCSF who provided continued support through it all and last but not least my rock and foundation, Dr. Eric Kessell, who provided both personal and professional support during these last few years of my endeavor. I thank you all!

Erik V Carter

Dedication

To those who said that the odds were against me and to those that believed that I would beat those odds!

Prologue

My research interest stems from three decades of cardiac critical care nursing. After honing my cardiac clinical expertise, I have been able to produce a body of research that aims at understanding the intersection of pre/post heart transplant assessment, electrocardiographic evaluation and cardiac ion channel diseases (e.g., Brugada Syndrome), and how these entities interconnect. My path through nursing did not follow the normal trajectory. After completing my licensed vocational nursing certificate during high school, I desired more and pursued my registered nursing degree. This is where my love for cardiac nursing began.

Fast forward two decades to now, as a nursing veteran, my experience has ranged significantly, from telemetry nursing care, nursing administration, out-patient nursing settings, specialty nursing units, electronic intensive care, cardiac surgical intensive care, coronary intensive care and ventricular assist devices, to clinical and didactic teaching in a community college setting as well as a masters program. Needless to say, I would always return to cardiac nursing and although a neophyte in the realm of research, I became quickly fascinated with research that centered on cardiac electrophysiology, genetics, and heart transplantation.

With thoughtful guidance from Dr. Drew and Dr. Aouizerat, I have been able hone my research interests into a thought-provoking and innovative dissertation. The result of this body of research takes a novel approach such as how in the pre/post heart transplantation phases, assessment of electrophysiologic markers and genetic appraisal of cardiac ion channel mutations can aid in the care of those in the heart transplant realm.

Abstract

The intersection of cardiovascular disease, heart transplantation and gene-environment interactions are laying a new landscape in how genes and environmental risk factors are connected. Methods like next gene sequencing and whole genome sequencing, once thought too novel for use in common disease states like heart transplantation are becoming more common in understanding this gene-environment interaction. Cardiac ion channel disease is not assessed routinely during the procurement phase of heart transplantation yet hidden diseases like Brugada Syndrome can occur. Cardiac disease as we once thought is not necessarily inherited, instead we acquire a set of susceptible risk factors to certain environmental influences and this heightened risk is what causes certain disease. This is why certain individuals who live a healthy lifestyle can suffer from a myocardial infarction at a young age yet others who seem immune to the effects of cigarette smoking; poor diet and obesity can live long lives. It is this variation to susceptibility that increases disease. Hence, all humans carry some variation genetically and understanding this variation will ultimately assist in intervention strategies aimed at high-risk individuals (i.e., the heart transplant population). Therefore, further research is required to determine how these variations can affect individuals in the pre-/post-transplant phase.

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CHAPTER 1

Introduction

"Death is the inevitable consequence of life, and the postponement of death until it occurs naturally at an advanced age, after a full life of vigor and good health is the goal of healthcare. The sudden death of a young, apparently healthy individual is the antithesis of this goal." [1] Sudden cardiac death (SCD) claims an estimated 300,000 to 350,000 lives annually in the United States [2]with ~ 5% to 15% of young SCD victims showing no signs of structural abnormalities at autopsy.[3,4] In the setting of heart transplantation, accurate evaluation of the procured donor heart is essential. Additionally, follow-up of recipients during the first year is paramount. Issues of allograft rejection, cardiac injury patterns, environmental issues, arrhythmic conditions and concealed ion channel disease states can occur in a potential donor and potentially place the recipient at risk for SCD, if left unidentified.

The International Society for Heart and Lung Transplantation (ISHLT) has made an unprecedented commitment to organize experts in all areas of heart transplantation in an effort to develop practice guidelines for the care of heart transplant recipients, due to the scarcity of available donors and the overwhelming demand for transplanted organs. The ISHLT guidelines for the care of heart transplant recipients' details certain characteristics and offers recommendations for procuring donor hearts. Current recommendations exist to promote graft survival and focus on areas like donor age, infection, drug toxicities, preexisting cardiac abnormalities, and donor cardiac function. [5] According to the ISHLT, elements of donor age,

infection, and rejection have a vital role in graft survival and recipient morbidity and mortality. [6] Additionally, patients who present with severe acute rejection can experience SCD. Furthermore, we know that the first year following transplantation is a perilous time, when infection and rejection (i.e., acute cellular, T-cell mediated) is highest and can result in poor outcomes and increased mortality. [5] Additionally, recipients require approaches that specifically test procured donors, avoid the sequelae that can develop during the post-transplant phase, and protect against concealed preexisting issues. [5] Yet current methods used to monitor rejection are both invasive and potentially harmful to the new transplanted organ. [7] To date, evaluation of cardiac ion channelopathies is currently not a recommendation of the appraisal process of procuring a donor heart.

Several mechanisms are associated with arrhythmias post heart transplantation. These arrhythmic states can range from sinus bradycardia; conduction-system disease states (e.g. right bundle-branch blocks, progressive conduction disease, atrial fibrillation/flutter, supraventricular tachycardia, sustained/non-sustained ventricular tachycardia). Additionally, asystole and pulseless electrical activity have been reported. For example, in a study of 628 heart transplant recipients, Vaseghi et al. found that SCD represented the mode of death in 35% of their cohort with asystole and pulseless electrical activity being the main mechanisms. [8]. Furthermore, primary arrhythmic death diagnosed when no anatomic source is identified and described in approximately 25% of sudden deaths after heart transplantation. [9,10] This suggests that other factors (e.g. denervation) as well as other post transplant changes of the heart may play a significant role in the formation of arrhythmogenesis and mortality in this population. Additionally, other electrocardiogram (ECG) markers such as the frontal QRS-T angle have been demonstrated to be a powerful risk marker for cardiac mortality, independent of other clinical

markers. In a study by Raposeiras-Roubín et al. the authors demonstrated that a frontal QRS-T angle >90° increases the adjusted risk of all-cause mortality by two-fold (HR 2.18, 95% CI 1.6 to 3.0, p <0.001). [11]

It is evident that noninvasive ECG assessment and predictive genetic markers are needed in both the pre- and post-transplant phase to assure 1) overall survival of the transplanted organ, 2) that non-diseased organs are implanted, and 3) the development of new techniques to assess potential electrocardiographic problems that may arise in the recipient. Additionally, given the common availability of ECG's, their use as a low cost marker for risk stratification and prevention strategies is key. Therefore the following three manuscripts provide a new perspective on the evaluation of procured donor hearts and the evaluation of rejection in the heart transplant recipient and ion channel diseases, like Brugada Syndrome. The approaches reveal novel and provocative concepts in pre-/post-heart transplant evaluation that potentially increase the longevity of scarce transplanted hearts.

Chapter 2 introduces the first paper, a pilot study that was completed as part of receiving funding for a National Institute of Nursing Research (NINR) grant; the NEW HEART Study (Novel Evaluation With Home Electrocardiogram And Remote Transmission-R01NR012003). A donor heart is entirely denervated during transplantation surgery. The absence of parasympathetic activity has profound consequences with most heart transplant patients experiencing higher than normal resting heart rate and significantly reduced heart rate variability. Although some sympathetic and parasympathetic regeneration can occur, it is slow and incomplete. [12,13]

A potential major benefit of allograft denervation is that without the confounding influences of heart rate and autonomic nervous system activity, an observed increase in the QT

interval is likely to indicate abnormal ventricular repolarization due to another cause, such as acute allograft rejection. The long-term goal of the NEW HEART study is to apply novel technology in early detection of donor organ (allograft) rejection to improve patient outcomes following heart transplantation. Recent evidence has suggested that acute allograft rejection after heart transplantation causes an increased QT interval on electrocardiogram (ECG).

The NINR required a feasibility and compliance study of transplant individuals to see if the transplant population could be amenable with adhering to daily and weekly self-administered recording of their ECG. The pilot study, "Feasibility and Compliance with Daily Home Electrocardiogram Monitoring of the QT interval in Heart Transplant Recipients" aimed to (1) determine whether heart transplant recipients could achieve compliance in transmitting a 30-second ECG every day for one month using a simple ECG device and their home telephone, (2) evaluate the ease of device use and acceptability by transplant recipients, and (3) evaluate the quality of transmitted ECG tracings for QT-interval measurement. Our conclusion was that recipients could comply with recording and transmitting daily and weekly ECGs, paving the way for the New Heart study.

Chapter 3 presents an analysis comparing frontal QRS-T (QRS-T_F) angle between heart transplant recipients in the first year following transplant surgery with age- and sex- matched controls from a healthy ambulatory population. QRS-T_F angle is a prognostic indicator for cardiovascular death and is easily obtained from the limb leads of a standard ECG. It is well documented in the literature that ventricular heterogeneity leads to arrhythmogenesis, which results in increased all-cause mortality. [11] It is apparent that heart transplant surgery alters cardiac anatomy and thereby electrophysiological characteristics, yet no studies have evaluated the QRS-T_F angle during the early phase of heart transplantation as compared to that of a healthy

population. QRS-T angle may play a role in quantifying the degree of abnormal repolarization and detect repolarization abnormalities before overt electrocardiographic changes (e.g., T-wave inversion and ST depression). The 12-lead ECG QRS and T-axis are routinely measured following heart transplantation, thus making QRS-T_F angle an easily assessable parameter to evaluate during the early stages of the post-heart transplant phase and in the first year.

Chapter 4 discusses phenotypic and genotypic assessment for the cardiac ion channelopathy, Brugada Syndrome (BRS), prior to heart transplantation surgery. The 12-lead ECG is a well-established gold standard in cardiology and is an assessment tool for evaluating both donor hearts and ion channel disease states. Mutations in genes that encode for cardiac ion channels change the shape of the action potential and these changes can be recorded on the ECG. BRS was first described by Brugada, et al. in 1997 and described a group of individuals who displayed a distinctive diagnostic electrocardiographic pattern that could eventually lead to ventricular arrhythmogenesis and sudden cardiac death. [14]

To date, the only diagnostic tool that authenticates BRS is the 12-lead ECG. Unfortunately, due to BRS's tendency to normalize and conceal, capturing these electrocardiographic changes are often difficult, making BRS elusive; the event rate is unclear. In early 2011, through electrocardiographic evaluation of heart transplant (HTx) recipients, an incidental discovery of an ECG of a cardiac transplant donor revealed the distinctive electrocardiographic pattern of BRS. Therefore, the purpose of chapter 4's case report was to evaluate an organ donor for genetic evidence of BRS and to identify phenotypic and genetic signs suggestive of this cardiac ion channel disease entity.

These manuscripts bring to light the genotypic/phenotypic/environmental complexity that exists in the population of heart transplants and demonstrates how genetic variance and

modification can contribute to SCD and mortality. Furthermore, assessing the clinical picture of heart transplant donor's evaluative practice, as well as that of the post-transplantation management, can assist in increasing survival in this population.

CHAPTER 2

Feasibility and compliance with daily home electrocardiogram monitoring of the QT interval in heart transplant recipients

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BACKGROUND: Recent evidence suggests that acute allograft rejection after heart transplantation causes an increased QT interval on electrocardiogram (ECG). The aims of this pilot study were to (1) determine whether heart transplant recipients could achieve compliance in transmitting a 30-second ECG every day for 1 month using a simple ECG device and their home telephone, (2) evaluate the ease of device use and acceptability by transplant recipients, and (3) evaluate the quality of transmitted ECG tracings for QT-interval measurement.
METHODS: A convenience sample of adult heart transplant recipients were recruited and trained to use the device (HeartOne, Aerotel Medical Systems, Holon, Israel). Lead II was used with electrodes that were easy to slip on and off (expandable metal wrist watch-type electrode for right wrist and C-shaped band electrode for left ankle). Patients used a toll-free number with automated voice prompts to guide their ECG transmission to the core laboratory for analysis.
RESULTS: Thirty-one subjects (72% were male; mean age of 52 ± 17 years; 37% were nonwhite) achieved an ECG transmission compliance of 73.4% (daily) and 100% (weekly). When asked, how difficult they thought it was to record and transmit their ECG by phone, 90% of subjects replied "somewhat easy" or "extremely easy." Of the total 644 ECGs that were transmitted by subjects, 569 (89%) were acceptable quality for QT-interval measurement. The mean QTc was 448 ± 44ms (440 ± 41ms for male subjects and 471 ± 45ms for female subjects). Eleven subjects (35%) had an extremity tremor, and 19 subjects (55%) had 2:1þ left leg edema. Neither of these conditions interfered with ECG measurements.
CONCLUSION: Transplant recipients are compliant with recording and transmitting daily and weekly ECGs.

INTRODUCTION

Approximately 13% of adult heart transplant recipients do not survive to 1 year, and a major cause of death is acute cellular allograft rejection.[15,16] According to the 2009 annual US data published from the International Society for Heart Lung Transplantation Registry, acute rejection occurs in 25% to 35% of transplant recipients within the first year after transplant

surgery.[17] To detect the early stages of rejection so that more aggressive and early immunosuppressant therapy can be initiated, frequent biopsies of heart tissue are performed (typically, weekly or every other week in the first 3 months and then monthly or every other month during the first year). Although endomyocardial biopsy is not a perfect "gold standard" for a correct diagnosis of acute allograft rejection, it is considered the best available test, and thus it is the current standard practice. Unfortunately, endomyocardial biopsy is an invasive and costly procedure that is not without risk.[18,19] If a simple noninvasive biomarker could be identified to detect the early stages of acute rejection, it might be possible to reduce the number of invasive biopsy procedures and to initiate earlier therapy that might prevent death from severe rejection.

In a retrospective analysis by Tenderich et al, six 12- lead electrocardiograms (ECGs) were recorded in 200 heart transplant recipients during the first 3 months after transplant surgery. Prolongation of the QTC interval > 25ms predicted acute cellular allograft rejection with sensitivity of 77% and specificity of 96%. [20] In normal individuals, there are 2 major influences on the duration of the QT interval: heart rate and autonomic nervous system activity. There is an inverse relationship between the heart rate and the QT interval.

In terms of the autonomic nervous system, sympathetic stimulation shortens the QT interval, whereas parasympathetic stimulation lengthens the QT interval. In the denervated cardiac allograft, both influences of heart rate and autonomic nervous system activity are almost entirely removed, so there is little diurnal variation of the QT interval.[21] A potential major benefit of allograft denervation is that without the confounding influences of heart rate and autonomic nervous system activity, an observed increase in the QT interval is likely to indicate abnormal ventricular repolarization due to another cause, such as acute allograft rejection.

No prospective study to date has investigated whether such increases in the QT interval could provide early detection of acute allograft rejection. We plan to conduct a prospective National Institutes of Health- funded clinical trial (1RO1 NR012003) to determine whether daily monitoring of the transplant recipient's ECG using a simple home device would provide an early sensitive and specific biomarker for acute allograft rejection. In preparation for this clinical trial, the current pilot study was undertaken to (1) determine whether heart transplant recipients could achieve compliance in transmitting a 30-second ECG every day for 1 month using a simple ECG device and their home telephone, (2) evaluate ease of device use and acceptability of time required for transmission by transplant recipients, and (3) evaluate the quality of transmitted ECG tracings for QT interval measurement.

MATERIALS AND METHODS

Sample and Setting

In a 3-month period ending in May 2010, we selected a convenience sample from 3 transplant centers: University of California Los Angeles Medical Center, Cedars Sinai Medical Center in Los Angeles, and Columbia University-New York Presbyterian Medical Center in New York City. Institutional review board approval was obtained from these 3 institutions and the University of California, San Francisco (UCSF) Medical Center, which served as the ECG core laboratory for the study. The inclusion criteria were adult heart transplant recipients living independently who were clinically stable. Demographic characteristics are detailed in Table 1.

Characteristics of the 31 subjects enrolled in the New Heart pilot study		
	Men (n = 22)	Women (n = 9)
	Mean ± SD	Mean ± SD
Age (n 31)	55 ± 14 y	54 ± 14 y
	%	%
Ethnicity (non Hispanic) (n 25)	81	78
Ethnicity (Hispanic) (n 6)	18	22
Race (n 31)		
Black	9	45
White	59	33
Asian	14	0
Language spoken at home (n 31)		
English	78	89
Spanish	14	11
Other	9	0
Cause of HF (n 31)		
Ischemic	18	0
Viral/bacterial	0	0
Cardiomyopathy	59	67
Congenital	5	0
Other	18	33
Type of CM (n 31)		
Dilated	50	89
Restrictive	9	0
Hypertrophic	9	0
Nonspecific	32	11
Upper extremity tremor (n 31)		
None	64	67
Barely visible	23	22
1 <3 cm	9	11
5 <10 cm	4	0
Lower extremity edema (n 31)		
None	50	33
1+	27	22
2+	13	45
3+	5	0
4+	5	

HF, heart failure; CM, cardiomyopathy; SD, standard deviation.

Table 1. Demographics and clinical characteristics of subjects

INSTRUMENTS AND PROCEDURE

Home ECG Device

After a thorough search of the available technology, the HeartOne ECG recorder (Aerotel Medical Systems, Holon, Israel) was selected (Figure 1A). The ECG device is pocket size and lightweight, and stores up to four 30-second recordings of a bipolar ECG lead.

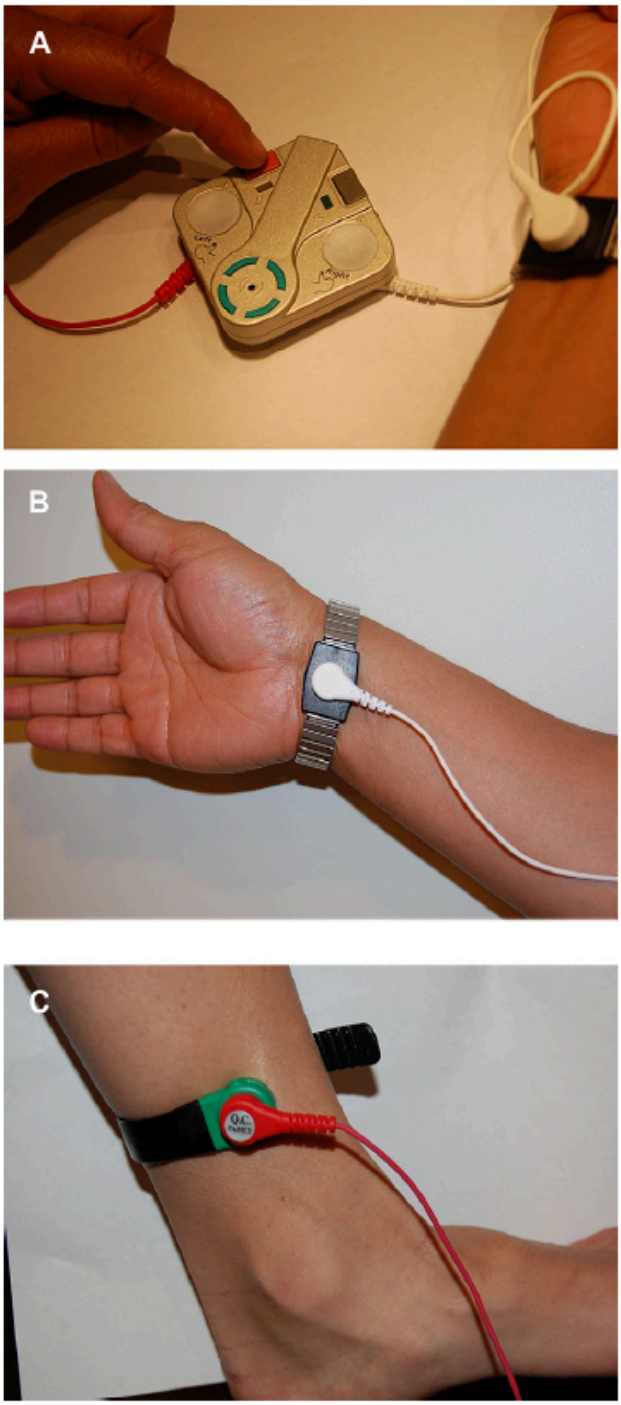

Figure 1. A) HeartOne ECG recorder (Aerotel Medical, Israel). B) Placement of expandable metal wristwatch type electrode for the right wrist. C) Placement of C-shaped electrode for the left ankle.

ECG Lead and QT Measurement

The QT interval is measured from the onset of the QRS complex to the end of the T wave. T waves must be of sufficient amplitude to identify the T-wave end point. Because normal T-wave axis is between 15 and 75 degrees in adults, lead II, which is at the 60-degree axis point, often has the largest amplitude T wave. Lead II requires an electrode on the right wrist and left ankle. We used an expandable metal wristwatch type electrode for the right wrist and a C-shaped electrode for the left ankle, both of which could be easily slipped on and off (Figure 1B and 1C). Subjects were given spray bottles of saline to spray on the electrode site to improve conductance without the need to use more permanent adhesive-type ECG electrodes.

ECG Acquisition and Transmission

At the time of enrollment, site investigators taught each patient how to use the ECG device. Subjects were asked to perform a return demonstration in front of the site investigator and were provided a booklet with detailed photographs of each step of the recording and transmitting process to take home with them. To minimize myopotential noise, patients were instructed to acquire the ECG while sitting quietly with their arms resting on a table/desk or supine in bed. After ECG data acquisition, recipients were instructed to transmit the data trans-telephonically (landline telephone required) using a toll-free number that guided the patient with voice prompts. A small subset (6%) of subjects who had mobile telephones, but no landlines, were allowed to participate in the study after it was confirmed by the investigators that their mobile devices were capable of transmitting an ECG of acceptable quality.

ECG Analysis

The HeartLine Receiving Station (Aerotel Medical Systems) received ECG transmissions and stored each subject's ECGs in a separate file folder that contained all their daily ECGs. QT and

RR intervals were made in a computer-assisted manner. The Aerotel measurement software provides a zooming feature to enlarge ECG waveforms for better visualization. In addition, electronic calipers were provided so that the researcher could select the appropriate waveform onset and offset points and the computer software provided the interval value in milliseconds. The end of the T wave was defined as the intersection of a tangent to the steepest slope of the last limb of the T wave and the isoelectric baseline (Figure 2). [22] One investigator (E.V.C.) made all measurements for the current analysis. Criteria for acceptable QTc measurement were as follows: T-wave amplitude > 100 mV, minimal baseline artifact, non-noisy consecutive RR and QT intervals, and no baseline wander.

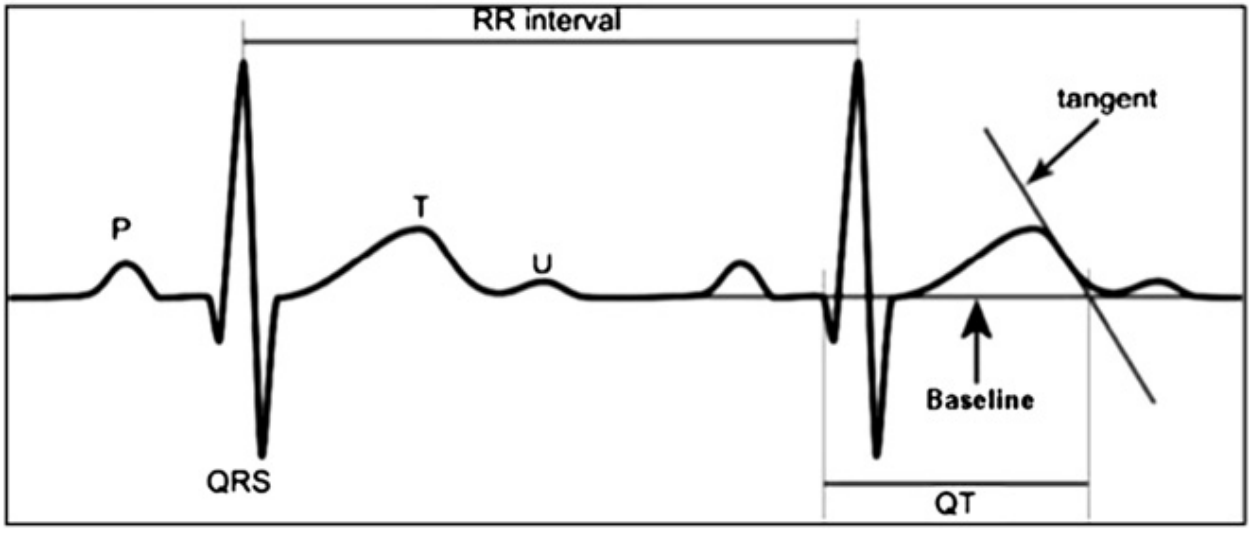

Figure 2. A tangent is drawn to the steepest slope of the last limb of the T wave; the end of the T wave is the intersection of this tangent with the baseline.

Database Management and Statistical Analysis

All sites used a secure web-based data capture site, Research Electronic Data Capture, which is sponsored by a consortium of 118 research institutions, including the institutions involved in our study. Stata 11 (StataCorp, College Station, TX) was used to calculate descriptive statistics (frequencies, means, and standard deviations), chi-squares, and t tests.

RESULTS

Sample Characteristics

At Columbia University, all patients approached (n=15) were invited to participate and agreed to be in the pilot study; at UCLA, 19 of 27 patients who were approached participated. Reasons for nonparticipation were lack of interest (6) and being too busy (2). A total of 34 subjects (24 male, 10 female) were enrolled. After initiation of the study, 2 subjects (UCLA, 1; Columbia, 1) were excluded from the analysis because they were hospitalized and could not transmit ECGs from home. In addition, a third subject (Columbia) decided to withdraw from the study for personal reasons. The remaining 31 subjects comprised the pilot study and had a mean age of 55 ± 13 years. Ethnicity and race reflected the demographics of urban Los Angeles and New York City, with 37% of subjects being nonwhite (Hispanic, 19%; Asian, 12%, black, 27%). Eleven subjects (35.5%) had visible extremity tremor, and 17 subjects (55%) had >1+ peripheral edema involving the left ankle electrode site.

ECG Quality

There were a total of 644 ECGs successfully received by the ECG core laboratory at UCSF during the study period. Because of construction, there was a period of several days in which power outages in the core laboratory precluded ECG transmissions. Of the total 644 ECGs, 569 (89%) were acceptable for QTC measurement (i.e., non-noisy consecutive RR and QT intervals). There were no statistically significant differences in the proportion of acceptable quality ECGs in patients with and without extremity tremor (p=.151) or peripheral edema (p=.212).

The mean QT interval was 415ms in men and 426ms in women. We defined abnormal as a QT interval of > 470ms in women and > 460ms in men. In both sexes, the QT interval was within normal limits without any correction formula used. When using the Bazett correction formula

(bQTc), of the 569 ECG readings read, 415 ECG readings from the male subjects (33%) had bQTc intervals > 460ms and 154 ECG readings from the female subjects (57%) had bQTc intervals > 470ms. This small gender difference in bQTc (~10ms longer in women compared with men) was statistically significant (p=.000).

Patient Compliance and Ease of Device Use

Daily ECG transmission was achieved by 73%, and all 31 subjects (100%) achieved at least a weekly ECG transmission. To the question, "how difficult do you think it was to record and transmit your ECG by phone?" 88.9% answered "somewhat easy" or "extremely easy." Some patients reported that recording their ECG was easy; however, transmitting their ECG by telephone was more difficult because sometimes the receiving computer was unavailable (due to power outages) and repetitive attempts had to be made. In determining willingness to use the HeartOne device every day for 6 months, 89% answered positively. In total, 644 transmissions out of a potential 976 ECG transmissions over the 1-month pilot period were facilitated. During this time, 48 incidences of non-transmission due to power outages and 133 incidences of non-transmissions due to various patient reasons occurred. This represents approximately 18% (non-transmission/expected transmissions) of ECG data loss. Because our primary aim was to test compliance and feasibility, we believe 18% represents a minor loss of data. In addition, each patient provided multiple transmissions; we assume that because of the central limit theory, an increase in the number of transmissions would tend to lead more toward mean bQTc. Therefore, the data described should be considered upper- or lower-bound sample estimates.

DISCUSSION

This pilot study demonstrates that transplant recipients can be compliant in recording their ECGs daily and weekly using a home device. Subjects achieved an approximately 74%

daily and 100% weekly compliance in recording and transmitting their ECGs. The transplant population is especially well suited for applying a novel technology. They are typically younger than other cardiac populations with 86% of adult heart transplant recipients aged 18 to 64 years. Moreover, patients are prescreened to be free of conditions that would prevent adherence to the complex post-transplant regimen, such as cognitive impairment, substance abuse, or psychological disorders. Compliance would have undoubtedly been better in our study if patients had not been asked to transmit their ECGs by landline telephone 100% of the time.

There is a growing trend for Americans to use mobile phones exclusively, especially in urban areas that are well saturated with cell phone towers. Unfortunately, the HeartOne ECG device used in our study is not recommended for use with mobile phones. We did permit 2 study participants to use mobile devices because they did not have a landline. In both cases, mobile telephone transmission did not impede the investigators' ability to analyze the transmitted ECG. Another problem with telephone transmission is that any power surge or outage triggers the receiving computer to shut down until the computer can be rebooted. As a result, patients cannot connect to the receiving computer and have to make repeated attempts to transmit their ECG.

For these reasons, we will use a different ECG transmission system for our National Institutes of Health clinical trial. The new ECG device will automatically seek, find, and upload the ECG by wireless Bluetooth communication to an Internet transmitter located in the subject's home. Subsequently, by using mobile phone technology (subscriber identity module card), the Internet transmitter device will automatically seek, find, and send the digital ECG to a UCSF server via wireless General Packet Radio Services Internet access. Thus, subjects will not have to dial a telephone to transmit their ECG; they will only need to record their ECG, and the rest will

be automatic. The ECG will be sent to a large UCSF server, and investigators in the ECG core laboratory will access the ECG data via a virtual private network.

Although we took measures to reduce noise in the ECG recordings, 12% of the transmitted ECGs were unanalyzable because of issues such as myopotential noise. There are various causes for myopotential noise, with the most likely cause in the present study being due to the tensing of arm/leg muscles while the ECG was being acquired. We measured involuntary muscle movement due to tremor, however, because patients performed these recordings in their homes, we were unable to control all sources of voluntary myopotential noise.

I n future studies, we will ask patients to lie down to record their ECGs. Two conditions that we hypothesized might be associated with a poor-quality signal were lower-leg edema and extremity tremor; however, neither of these conditions was related to poor-quality ECGs. The QT interval in our cohort was shorter than normal (mean 387.63ms, men; 340.32ms, women), and this is in agreement with prior observations in the transplant population. In normal individuals, vagal dominance at rest prolongs the QT interval compared with transplant recipients who do not have such vagal tone. As a result, QT intervals are typically shorter than in normal individuals.

CONCLUSIONS

Adult heart transplant recipients are compliant with recording daily and weekly ECGs. Direct Internet transmission of ECGs from a patient's home to an ECG core laboratory rather than telephone modem transmission would likely improve patient compliance further. Ankle edema and side effects of immunosuppressive drugs that can cause tremor are common but do not interfere with ECG quality. Whether an increase in the QT interval measured daily will prove

to be an early and sensitive, specific biomarker for acute allograft rejection in the first 6 months after transplantation will be the subject of an ongoing clinical trial.

CHAPTER 3

A Comparison of QRS-T Angle in Heart Transplant Recipients and an Ambulatory Population

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Introduction: Frontal QRS-T (QRS-T_F) angle is a prognostic indicator for cardiovascular death and is easily obtained from the limb leads of a standard electrocardiogram (ECG). A widened QRS-T_F angle represents a larger discordance between depolarization and repolarization, and ventricular dyssynchrony. Heart transplantation surgery alters cardiac anatomy and thereby certain electrophysiological characteristics (e.g., conduction). To date, no studies have evaluated the QRS-T_F angle during the early phase of heart transplantation. The aim of this study was to compare QRS-T_F angles between heart transplant recipients in the first year following transplant surgery with age- and sex- matched controls from a healthy ambulatory population.
Methods: A post-surgery standard 12-lead ECG was acquired from heart transplant recipients as part of an ongoing clinical trial, the New Heart study (Novel Evaluation With Home Electrocardiogram And Remote Transmission-NH). Excluded from analysis were heart transplant ECGs that were =<7 days from surgery. Transplant ECGs were compared with 12-lead ECGs matched by age and sex from a large ambulatory population from the Veterans Affairs (VA) Health Care System. The ratio of cases to controls was 1:5. The frontal QRS-T_F angle, defined as the absolute value of the frontal QRS and T axes, was classified as normal (0°-50°), borderline (51°-100°), or abnormal (101°-180°).
Results: The sample consisted of 222 heart transplant recipients (NH) and 1110 age- and sex-matched controls (VA). The mean age was 55±13years. Mean QRS axis was greater in the NH vs. VA group. The mean QRS-T_F angle was greater in the NH vs. the VA group, with a difference in the proportions of normal compared to borderline/abnormal QRS-T_F angles. There was a significant association between group and abnormal QRS-TF angle (NH > VA). NH QRS-T_F angle was greater than VA QRS-T angle by 23.5°. Lastly, an incidental finding of a greater QTc_F interval was observed in the NH cohort (8.9 milliseconds).
Conclusion: A statistically significant finding of a 23°-greater QRS-T_F angle was observed in the NH group compared to age- and sex- matched patients in the VA sample. In addition, an incidental finding of a QTc_F interval difference was observed between the two groups. Whether the difference in QRS-T_F angle and QTc_F interval differences observed in the NH group is a significant clinical finding will be a subject of the ongoing clinical trial.

INTRODUCTION

Spatial QRS-T angle is measured from the three X, Y, and Z leads of a vectorcardiogram or mathematically derived with a computer algorithm from the readily available standard 12-lead electrocardiogram (ECG). On the ECG, the QRS complex represents ventricular depolarization and certain ECG irregularities and can reflect structural anomalies (e.g., ventricular damage and hypertrophy) whereas others can reflect cardiac ion channel disease. Similarly, the ST segment and T wave represents ventricular repolarization. T wave morphologic abnormalities may be suggestive of cardiac ion current instability (e.g., channelopathies, pharmacologic) as well as structural anomalies (e.g., ischemic changes). [23]

A growing body of research suggests that a wide spatial QRS-T angle carries a poor prognosis for cardiac and all-cause mortality.[23] Studies linking wide spatial QRS-T angle to mortality have been reported in post-menopausal women [24], HIV-infected individuals [25], chronic heart failure patients [26], coronary bypass recipients [27], elderly individuals [28], and patients with acute coronary syndrome. [29] Hence, the spatial QRS-T angle is considered a powerful marker of electrocardiographic arrhythmogenesis and a marker of death in the general population. [23,30,31]

In current clinical practice, vectorcardiograms are not routinely recorded, and the spatial QRS-T angle derived by computer algorithms from a standard ECG might be inherently different in width of the angles. A readily available frontal QRS-T (QRS-T_F) angle obtained from the six frontal-plane limb leads of a standard ECG has been shown to be a clinical substitute for spatial QRS-T angle as a prognostic indicator for cardiovascular death and all-cause mortality. [29,32,33] This easily obtainable, clinician-friendly approach to calculating frontal QRS-T_F angle measures the difference between the QRS axis and T wave axis from a standard 12-lead ECG. [29,32,34,35] The structural changes associated with the surgery of heart transplantation (e.g., anastomosis of new

heart and suture line) may modify depolarization/repolarization currents thus changing QRS and T axes. To date, no studies have evaluated the QRS-T_F angle in patients during the early phase of heart transplantation compared to an ambulatory healthy population.

The aim of this study was to examine whether QRS-T_F angles differs between heart transplant recipients in the first year following transplant surgery and age and sex-matched controls of a healthy ambulatory population.

METHODS

Study Population

Subjects included transplant patients from the NEW HEART (Novel Evaluation With Home Electrocardiogram And Remote Transmission) (NH) Study and an age and sex-matched comparison group from a large (n>40,000), healthy ambulatory population collected from the Veterans Administration (VA) Health Care System in Palo Alto, CA. The ratio of transplant cases to ambulatory controls was 1:5. We excluded from analysis heart transplant ECGs that were =< 7 days from surgery. A final sample of 1332 subjects was then included in the analysis (NEW HEART transplant patients, *n*=222; VA ambulatory patients, *n*=1110).

Electrocardiographic measurements

QRS-T_F angle was calculated as the internal angle of the absolute difference in the frontal plane QRS and T wave axes, as measured by the electrocardiograph's computer algorithm (GE Healthcare electrocardiographs at all study sites). If the calculated QRS-T_F difference exceeded 180 degrees, then the QRS-T_F angle was calculated as 360° minus the absolute value of the difference between the QRS and T axes. The QRS-T_F angle was calculated as described above and classified as normal (0° to 50°), borderline (51°to 100°), or abnormal (101°to 180).

Statistical Analysis

Statistical analyses were performed using SAS 9.4 (SAS Institute Inc. 2014.) Descriptive statistics were used to report demographic and clinical information. Continuous data were presented as means ± SD; mean and interquartile values were presented if SD was greater than mean value (e.g. QRS axis and QRS-T_F), QRS axis and QRST-$_F$ were transformed to check for adequate distribution of values, and categorical data were expressed as proportions (%) using the $\chi 2$ test to compare the two groups. The correlation between group assignment and QRS-T_F angle was checked with Pearson's correlation. A two-sample Student's t-test assuming equal variances using a pooled estimate of the variance was performed to test the null hypothesis that there was no difference in QRS-T_F angle between the transplant and ambulatory groups. If variances differed, then the Satterthwaite variance estimator was utilized. A linear regression model was utilized to test relationships between the two study groups with QRS-T_F controlling for the effect of ethnicity. A p value < 0.05 was considered statistically significant.

RESULTS

Patient Characteristics

Baseline characteristic and demographic data are shown in Table 1. The average age was 55.14 years (SD±12.6), and females comprised 30% of the sample. There was a positive correlation between the NH and the VA groups and QRS-T_F angle (r=0.21). The NH group had a higher percentage of Hispanics than the VA group (21% versus 6%, $\chi 2$ =59.8, p<0.0001). As shown in Table 1, transplant patients (with denervated hearts) had the expected shorter PR and decreased uncorrected QT intervals as well as faster heart rates compared with their ambulatory counterparts.

Table 2. Clinical and electrocardiographic characteristics of the NH and VA cohorts.
*PR interval *(NH:n=213; VA: n=1213 due to absence of PR interval); **QRS-T_F square root transformation*; p <0.05

Variables	Transplant Cohort (n=222	Ambulatory Cohort (n=1110	*P* value	*95% CI*
Demographics				
Age (mean, years)	55±13	55±13	N/A	N/A
Hispanic n=109, (8.2%)	62(21%)	47(6%)	**< .0001**	**N/A**
Female n=396 (30%)	66, (30%)	330(30%)	N/A	N/A
Electrocardiographic Data				
PR interval(msec)	145.9±23.9	158.4±26.8	**<.0001**	**(8.9, 16.1)**
QRS duration(msec)	90.4±17.3	93.0 ±15.7	**.026**	**(0.3, 4.9)**
QT interval(msec)	364.3±41.5	391.2±40.3	**<.0001**	**(21.1, 32.8)**
QT_{cF} (msec)	421.6±38.24	412.7±25	**.0010**	**(-14.1, -3.6)**
QRS Axis	44.65°±50.8	29.57°±38.9	**<.0001**	**(-22, -8)**
T wave Axis	55.2°±48.0	41.8°±39.5	**.0005**	(-21.0,-5.9)
Heart rate (beats per minute)	94.67±13.87	72.80±15.75	**<.0001**	**(-24, -20)**
QRS-$T_{F\ Adjusted}$	59.74°±47.21	36.22°±38.35	**<.0001**	**(-30.2, -17)**
**QRS-T_FSQRT	7.00°± 3.28	5.28°±2.87	**<.001**	**(1.28, 2.13)**

Proportions of NH and VA groups are detailed in Table 2.

Table 3. Heart transplant and ambulatory groups in the categories of normal, borderline and abnormal.

SAMPLE *n*=(1332)	Frontal QRT-T Angle		
	Normal 0° to 50° *n* (%)	**Borderline (51° to 100°) *n* (%)**	**Abnormal (101° to 180°) *n* (%)**
Heart Transplant recipients	(114) 51.35	**(64) 28.83**	**(44) 19.82**
Ambulatory outpatients	**(860) 77.48**	(160) 14.41	(90) 8.11

Due to the large variance of QRS-T_F angle between the two groups, the median and IQR were calculated and are displayed in Table 3. QTc_F (Frederica) interval was slightly longer (mean, 9 msec longer) in transplant versus ambulatory subjects (t=-3.31, p=0.0010, 95%CI=-14, -3.6). Mean QRS axis in the transplant group was 44.6° vs. 29.6° in the VA group (t = - 4.2, p<0.0001). Mean T axis in the transplant group was 55.2° vs. 41.8° in the VA group (t = -3.50, p=0.005). A t-test showed that the mean QRS-T_F angle differed between the two groups: QRS-T_F angle was greater in the NH vs. VA group (T=-8.01, p<0.001, 95%CI -30.16, -16.89).
In addition, QRS-T_F was skewed to the right, therefore, we transformed values of both groups into a square root and performed a t-test (t=3.33. p, 0.001) with no change in significance noted after transformation (Table 4).

Table 4. Transformed square root of the QRS-T_F angle compared by group assignment

QRS-T_F Angle	**Mean**	**SD**	**Median**	**Q1**	**Q3**	**IQR**
Transplant cohort	7.00	3.28	6.92	4.58	9.38	4.8
Ambulatory cohort	5.28	2.87	4.79	3.16	6.78	3.18

Lastly, we performed a Wilson rank-sum (Mann-Whitney) non-parametric test, which does not assume distribution normality. Group assignment and QRS-T_F angle were positively correlated, (Pearson's r (1332)= .21, $p < 0.001$). The results indicate that the median QRS-T_F angles between the NH group and the VA group are significantly different (U=0.34, p<0.0001, r=0.21) giving the decision to reject the null hypothesis that there is no difference between the two groups (Table 2).

QRS-T_F angle was then dichotomized into a normal group (0°-50°) and an abnormal group (51°-180°) values. Chi-square analysis was used to compare the two study groups on the proportion that were abnormal. A greater percentage of the NH group were abnormal (48.6%) than in the VA group (22.5%), $\chi 2$=64.3, p<0.0001.

Although ethnicity did differ between the two study groups, it was not significantly related to the QRS-T_F angle (r = .03, p = .254). Despite this non-significant relationship with the outcome, the difference between the NH and VA groups in the QRS-T_F were again analyzed controlling for ethnicity and no effect of controlling for ethnicity was found. The magnitude of the difference between the groups remained the same.

DISCUSSION

This is the first study to analyze QRS-T_F angle in heart transplant recipients early after transplant surgery and to compare them with a healthy ambulatory cohort. Key findings from our present study demonstrate there is a statistically significant difference in QRS-T_F angle between a post-heart transplant group and an ambulatory group. A greater proportion of the ambulatory cohort had normal QRS-T_F angle (77%) compared with the transplant cohort (51%). This difference is most likely due to the remodeling of the heart following transplant surgery and some studies support a frontal QRS-T angle increase with increased mortality. [36-38]

In addition, the QRS axis was greater in the NH vs. VA group (44.65° vs. 29.58°) and T-wave axis differed between the two groups (NH=55°vs. VA=41°). Golshayan et al. described mean QRS axis changes ranging from 34° (6 months) to 58° (5 years) in a 62-member heart transplant cohort. [39] An earlier paper from our group in this population described ECG abnormalities in the first year following transplant surgery. [40] This group found a QRS axis abnormality in 23% of the sample, with 13% of deviation in the frontal plane right axis. In the current study, a higher percentage (~29%) of frontal plane right axis deviation was observed. Whether this difference is associated with greater risk for mortality is unknown and will be a subject of the ongoing clinical trial.

Two prior studies have demonstrated that a spatial QRS-T angle wider than 100° is associated with cardiac disease and an increase in cardiovascular mortality [23,30] Additionally, Aro et al. determined that a frontal QRS-T_F angle ≥ 100° elevates the risk of death through arrhythmogenesis, mainly through an alteration of the T wave. [41] Lastly, QRS-T_F angle differences in recent heart transplant recipients may be either an indicator of structural surgical abnormalities that can affect depolarization or repolarization sequence alterations. [36-38,41]

Cardiac arrhythmias are usual in the orthotopic heart transplant recipient, especially in the early postoperative period of recovery. Several mechanisms are responsible for these cardiac arrhythmias, ranging from surgical trauma, ischemic times during organ preservation, surgical suture line, and early rejection. [42] Additionally, bradyarrhythmias [43], supraventricular arrhythmias (e.g., atrial fibrillation) [44,45], conduction disturbances, and sustained ventricular tachycardia[46] all have been reported in the literature in the heart transplant population. Widened QRS-T_F angles in the HTx population have not been reported which is interesting given the

QRS-T_F angle reflection of alterations of repolarization progression, thus a widened QRS-T_F angle can represent a large disparity between depolarization and repolarization currents.

Given that the conduction disturbance of new right bundle branch block (RBBB) is the most common abnormality occurring in heart transplantation with up to 70% of heart transplant recipients exhibiting an incomplete right bundle branch block, conduction disturbances effects on the QRS-T_F angle have become of interest. [47,48]

In a study by Zhang et al., the authors sought to evaluate the usefulness of electrocardiographic QRS-T angles with versus without bundle branch blocks to predict heart failure (HF) using data from the Atherosclerosis Risk in Communities Study. The authors presented findings of an increased risk of HF in individuals with RBBB where only the frontal plane QRS/T angle was measured. A QRS-$T_F \geq 55°$ significantly increased the risk more than two-fold for incident HF [*HR*: 2.29, $p< 0.001$ (*95% CI*: 0.84-5.64)].

In the current analysis, we combined the QRS-T_F angles into two categories: normal and borderline/abnormal. This analysis revealed that the NH group had a statistically significant greater proportion in QRS-T_F angle resulting in the conclusion that there is an association that exists between the particular group assignment and QRS-T_F angle.

An incidental finding was that the QTc_F interval was slightly longer (9ms longer) in transplant versus ambulatory subjects (t=-8.57, p<0.0001, 95%CI=-25, -11.9). During heart transplantation surgery, the donor heart is completely denervated, giving rise to higher resting heart rates, as well as reduced heart rates. Although, reinnervation occurs, it is variable in each patient and non-uniformed.[49] Similarly, some studies have correlated sympathetic reinnervation and QTc interval with an increase in ventricular arrhythmogenesis and increased mortality. [50] Therefore, autonomic denervation may account for several electrophysiological findings in HTx

recipients. The slightly longer QTc we observed in the transplant cohort may be due to acute allograph rejection in some patients. In the ongoing NEW HEART study, we are investigating whether daily monitoring of the transplant recipient's ECG using a simple home device with direct internet transmission to an ECG Core Laboratory will provide an early and sensitive, and specific biomarker for acute allograft rejection based on QTc lengthening. Therefore, this finding will be a focus of the ongoing analysis.

Lastly, there were more Hispanics in the NH group vs. the VA group ($\chi 2=59.8$, $p<0.0001$) although racial effect on QRS-T_F angle was not significant. The demographic difference was due to the location of one of the NH study sites (Columbia University, New York), which has a slightly larger Hispanic population than the nation as a whole (17.6% vs. 16.3%). [51]

LIMITATIONS

Several limitations warrant consideration. First is that the QRS-T_F angle derived from a standard ECG is not considered a more powerful substitute for the spatial QRS-T angle, VCG-derived planar method, which is superior and more powerful in its diagnostic yield when detecting cardiac disease. [52] Frontal plane angles are easily calculated, whereas a spatial QRS-T angle requires an additional electrocardiographic dimension to allow the mean spatial QRS vector to be derived, as does the mean spatial T vector. [53] In our study, the decision to use the QRS-T_F angle was solely based on availability and ease. Additionally, we only had physical 12-lead ECG images from the NH cohort, and ECG parameters from the VA cohort were retrieved from an existing dataset.

We controlled for confounding issues by using a matched dataset, matching patients on demographics (age and sex); however, it was not possible to match on ethnicity and the

transplant group had a significantly larger Hispanic population. As a result, we controlled for ethnicity with a regression model, which confirmed that race did not result in a significant difference in QRS-T_F angle. Lastly, given the large percentage of RBBB in the heart transplant population, RBBB could be a possible confounder to a widened QRS-T angle when exploring any outcome analysis of HTx recipients if a widened QRS-T is observed.

CONCLUSION

Although there was a statistically significant difference in QRS-T_F angle observed between the transplant and ambulatory groups, it is unclear whether the 23° difference in QRS-T_F angle is clinically significant. We hypothesize that these differences may be the result of structural changes within the heart transplant cohort due to the heart transplant surgery. Given the high prevalence of RBBB in the transplant population, the effect on the QRS-T_F angle becomes an even more interesting ECG index to observe in this population. Widened QRS-T_F angle is a known marker for increased risk for SCD, whether this risk is consistent within the heart transplant population remains to be studied. Nevertheless, this ECG assessment may warrant further evaluation in the cohort of heart transplant recipients.

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CHAPTER 4

Cardiac Ion Channel disease in a Heart Donor-A Genetic and Phenotypic Analysis

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BACKGROUND: Sudden Cardiac Death (SCD) is a common cause of mortality, with a yearly incidence rate of one per 1000 affected individuals in the United States [54] Cardiac ion channel gene mutations leading to arrhythmogenesis (e.g., ventricular fibrillation) such as is seen in Brugada Syndrome (BRS), have also emerged as a potential substrate for SCD in structurally normal hearts. [55] [56]Mutations in genes that encode for cardiac ion channels, change the shape of the action potential, and these changes can be observed on the surface 12-lead electrocardiogram (ECG). In early 2011, through ECG evaluation of heart transplant (HTx) recipients, an incidental discovery of an ECG of a cardiac transplant donor revealed the distinctive electrocardiographic pattern of BRS.
METHODS: Through the use of the case report format, the purpose of this study was to evaluate an organ donor for evidence of BRS and to identify phenotypic and genetic indications suggestive of this cardiac ion channel disease entity. The clinical records of the donor, including electrocardiographic and pharmacologic records, were assessed.
RESULTS: Several distinct genetic variants in known BRS susceptibility genes were identified using next-generation sequencing (NGS) and verified by cycle sequencing. Missense mutations included CACNA1C gene intronic and exonic region and SCN1B gene. Synonymous mutations included CACNB2, CACNA2D1, SCN5A, HCN4, and KCNH2 genes. The effect of these mutations on the cardiac ion channels have been reported and include several phenotypic effects, ranging from first-degree AV block, intraventricular conduction delay, right bundle branch block, sick sinus syndrome and death through ventricular arrhythmogenesis.
CONCLUSION: The results suggest that the interaction between genetic variation, environmental effects and phenotypic expression is multifaceted and requires extensive clinical and genetic data evaluation in the population of heart transplantation.

INTRODUCTION

Cardiac transplantation has become the established therapeutic modality for patients with end-stage heart failure caused by ischemic heart disease or idiopathic dilated cardiomyopathy when end-stage heart disease is refractory to medical or surgical therapeutics. [5] If left untreated,

heart failure can lead to sudden cardiac death (SCD) triggered by paroxysmal ventricular tachycardia or ventricular fibrillation. [5]

SCD refers to an unexpected death from a cardiovascular cause in a person with or without preexisting heart disease. [57] The yearly incidence of SCD in western societies is one per 1000 individuals and is usually a result of ventricular fibrillation. [57,58] Cardiac channelopathies comprise a group of heritable conditions also responsible for arrhythmias and SCD. This spectrum of diseases occur in the absence of overt structural heart disease, occurring with activity, nocturnally or when sedentary. This has been demonstrated specifically in the cardiac ion channel disease entity known as Brugada Syndrome (BRS) (Figure 3).

BRS is thought to be responsible for 4% to 12% of unexpected SCDs and for up to 20% of all SCDs in individuals with apparently structurally normal hearts. [59,60] Individuals with BRS are often asymptomatic and BRS is known to cause SCD in individuals less than 40 years of age with structurally normal hearts.[61] Additionally, the BRS ECG may appear as a result of BRS phenocopy, defined as the absence of true BRS despite the presence of the characteristic of the Type-1 ECG findings (Figure 4.) [62] These BRS ECG changes can occur due to metabolic conditions (e.g., hyperkalemia, hyponatremia, hypercalcemia), ischemic states (e.g., right coronary artery occlusion), mechanical compression (e.g., CPR), myocardial and pericardial disease (e.g., Chagas' disease, myotonic dystrophy), and other miscellaneous conditions (e.g., Ebstein's anomaly). True BRS is only diagnosed with an ECG displaying spontaneous ST-segment elevation in one or more precordial leads $V_{1\text{-}3}$ or with the use of an unmasking agent (i.e., sodium channel blocking drug) to reveal a Type-1 BRS ECG pattern.

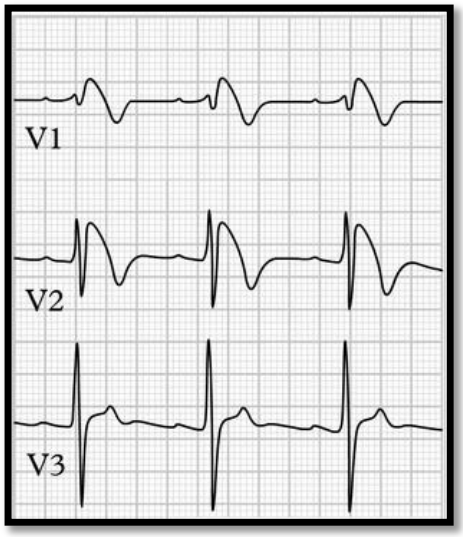

Figure 3. Typical Brugada Syndrome ECG: Type-1 coved ST-segment in V_{1-3}

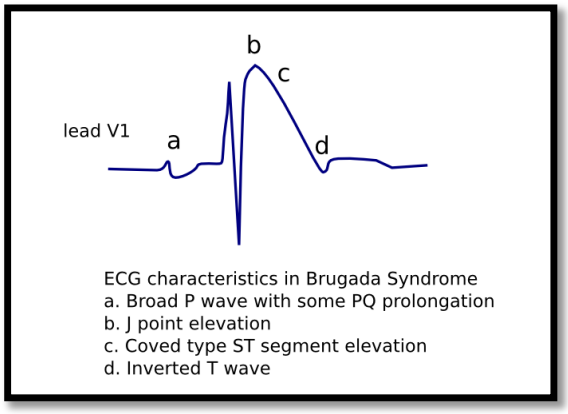

Figure 4. Diagnostic ECG characteristics of BRS.

In a recent article by Baranchuk et al., the authors state that most BRS ECG phenocopies arise from cardiac sodium blocking effects or structural anomalies.[62]

The use of the 12-lead surface ECG is well established in cardiology in the assessment of risk for cardiovascular mortality and SCD. [63] Given the common availability of ECGs, the use of the 12-lead ECG, as a low cost marker for risk stratification and prevention strategies is key. Additionally, the 12-lead electrocardiac measures of the PR interval, QRS interval, QT interval, early repolarization sign (ERS), and frontal QRS-T angle >90° have been used as risk markers for all-cause mortality, cardiac mortality, and SCD. [23,57,64,65]

In individuals with structurally normal hearts, the mechanism resulting in SCD is poorly understood and is due in part to undefined genetic factors and environmental influences. It has been reported in the literature that 17% to 42% of individuals with structurally normal hearts diagnosed with BRS, present with syncope or SCD as a consequence of a ventricular arrhythmia at some time during their lives. [60,66-69] Additionally, spontaneous daily fluctuations observed on ECGs may contribute to the elusiveness of BRS ECG ST-segment and QRS abnormalities. [66]

To date, genetic variations in 17 genes are known to cause some form of BRS by impacting ion channel function and the action potential. These ion channel genes include five sodium channels, six potassium channels, four calcium channels and two sodium-related channels; the BRS Subtypes, genes, ion current affected, functional effects and estimated frequency in population identified in Table 5.

Table 5. BRS Subtypes, genes, ion current affected, functional effects and estimated frequency in population

BRS Subtype	Gene name	Gene product	Ion current affected	Functional effect	Estimated frequency in population
BRS1	*SCN5A*	**$Na_v1.5$**	Na^+ channel (fast)- I_{Na}	Decrease in gene function	15% to 30% [70]
BRS2	*GPD1-L*	**G3PD1L**	Na^+ channel (fast)- I_{Na}	Decrease in gene function	Unknown [71]
BRS3	*CANA1C*	**$CA_v1.2$**	Ca^{2+} channel (slow inward, L channels)- I_{Ca}	Decrease in gene function	6% to 7% [72]
BRS4	*CACNB2*	**$Ca_v\beta2$**	Ca^{2+} channel (slow inward, L channels)- I_{Ca}	Decrease in gene function	4% to 5% [72]
BRS5	*SCN1B*	**$Na_v\beta1$**	Na^+ channel (fast)- I_{Na}	Decrease in gene function	1% to 2% [73]
BRS6	*KCNE3*	**MiRP2**	K^+ channel (transient outward)-I_{to}	Increase in gene function	Less than 1% [74]
BRS7	*SCN3B*	**$Na_v\beta3$**	Na^+ channel (fast)- (I_{Na})	Decrease in gene function	Unknown [75]
BRS8	*HCN4*	***HCN4***	Na^+ (pacemaker current)-I_f	Unknown	Unknown [76]
BRS9	*KCNJ8*	**Kir6.1**	K^+ channel (ATP-sensitive)-K_{ATP}	Increase in gene function	Unknown [77]
BRS10	*CACNA2D1*	**$Ca_v\alpha2\delta$-**	Ca^{2+} channel (slow inward, L channels)- $\boldsymbol{i_{Ca}}$	Unknown	Unknown [78]
BRS11	*RANGRF*	**MOG1**	Na^+ channel (fast)- I_{Na}	Decrease in gene function	Unknown [79]
BRS12	*KCNE5*	**MiRP4**	K^+ channel (transient outward)-I_{to}	Increase in gene function	Unknown [80]
BRS13	*KCND3*	**$K_v4.3$**	K^+ channel (transient outward)-I_{to}	Increase in gene function	Unknown [81]
BRS14	*KCNH2*	**hERG1**	K^+ channel (delayed rectifier)-I_{Kr}	Increase in gene function	Unknown [82]
BRS15	*SLMAP*	***SLMAP***	Na^+ channel (fast)- I_{Na}	Decrease in gene function	Unknown [83]
BRS16	*TRMP4*	***TRMP4***	Ca2+-activated non-selective cationic	Increase and decrease in function	6% (based on the original cohort consisting of 248 BRS cases) [84]
BRS17	*SNC2B*	**$Na_v\beta2$**	Na^+ channel (fast)- (I_{Na})	Decrease in gene function	Unknown [85]

Gene Name-***SCN5A***: sodium channel, voltage-gated, type V, alpha subunit; ***GPD1-L***: glycerol-3-phosphate dehydrogenase 1-like; ***CANA1C***: calcium channel, voltage-dependent, L type, alpha 1C subunit; ***CACB2***: calcium channel, voltage-dependent, beta 2 subunit; ***SCN1B***: sodium channel, voltage-gated, type I, beta subunit; ***KCNE3***: Potassium Voltage-Gated Channel, Isk-Related Family, Member 3; **SCN3B**: sodium channel, voltage-gated, type III, beta subunit; **KCNH2**: potassium voltage-gated channel, subfamily H (eag-related), member 2; ***KCNJ8***: Potassium Inwardly-Rectifying Channel, Subfamily J, Member 8; ***CACNA2D1***: calcium channel, voltage-dependent, alpha 2/delta subunit 1; ***RANGRF***: RAN guanine nucleotide release factor; ***KCNE5***: Potassium Voltage-Gated Channel, Isk-Related Family, Member 1-Like**;** ***KCND3***: Potassium Voltage-Gated Channel, Shal-Related Subfamily, Member; ***HCN4***: hyperpolarization-activated cyclic nucleotide-gated potassium channel 4; ***SLMAP***: Sarcolemmal-Associated Protein; ***TRMP4***: Transient Receptor Potential Cation Channel, Subfamily M, Member 4; **SCN2B**: Sodium Channel, Voltage-Gated, Type II, Beta Sub unit Gene Protein-**$Na_v1.5$**: Voltage-gated sodium channel 1.5; **G3PD1L**: Glycerol-3-Phosphate Dehydrogenase 1-Like Protein; **$CA_v1.2$**: Voltage-sensitive calcium channel 1.2; **$Ca_v\beta2$**: beta subunit of voltage-dependent calcium channel; **$Na_v\beta1$**: Sodium voltage-gated, type I, beta subunit; **MiRP2**: MinK-Related Peptide 2; **$Na_v\beta3$:** Sodium voltage-gated, type 3, beta subunit; **hERG**: Human Ether-A-Go-Go-Related Potassium protein; **Kir6.1**: Inward Rectifier K(+) Channel Kir6.1; **$Ca_v\alpha2\delta$-1**: Calcium channel alpha-2/delta1 protein; **MOG1**: multicopy suppressor of Gsp1 protein**;** **MiRP4**: potassium channel, voltage-gated, isk-related subfamily; **$K_v4.3$**: Potassium Voltage-Gated Channel Subfamily D Member3; **HCN4**: hyperpolarization-activated cyclic nucleotide-gated potassium channel protein; **SLMAP**: Sarcolemmal-Associated Protein; **TRMP4**: Transient Receptor Potential Cation Channel, Subfamily M, Member 4; **$Na_v\beta2$**: Sodium voltage-gated, type I, beta subunit2

MATERIALS AND METHODS

Clinical phenotype

The following heart donor's clinical and demographic characteristics (e.g., acquired hospital, California Transplant Donor Network (CTDN) records), computed tomography (CT-scan), echocardiograph (ECHO) were evaluated and the all surface 12-lead ECG's were analyzed (three in total). Additionally, recipient post transplant records were evaluated and several surface 12-lead ECG's were analyzed. The California Transplant Donor Network (CTDN) received signed authorization for use of stored blood collected during explantation for future research studies. An inquiry regarding internal review board (IRB) approval was sought through the University of California, San Francisco Committee on Human Research. The UCSF IRB stated that no IRB approval was required for case studies involving just one subject.

Genetic Analysis

An aliquot of stored blood was received from a UCSF lab that maintains and stores blood samples of donors from the CTDN. Extraction of genomic DNA from peripheral blood leukocytes of the heart transplant donor was completed using Puregene DNA Isolation System (Invitrogen, Carlsbad, CA). The donor specimen was submitted to Expression Analysis Inc. (Durham, NC) for whole exome sequencing (WES). Sequencing data were converted from raw data into a variant call format (.vcf) file, which is a text file that compresses meta-information about each nucleotide position in the genome that is variant (i.e., single nucleotide polymorphism (SNP). Sample data (i.e., the .vcf file) were then uploaded to QIAGEN's (IVA) software (www.qiagen.com/ingenuity) for whole exome analysis (WEA). Ingenuity Variant Analysis version 3.1.20150224 is a comprehensive search engine that examines for a series of putative functional effects (i.e. non-synonymous changes). This software and associated knowledge

database were used to determine causal variants in the donor's blood. Our focus was the evaluation of known gene variants associated with BRS, which currently includes 17 known genes.

Several of the sequence variants among the 17 candidate genes identified by the IVA software were excluded from analysis after compairing variants to public databases: for example the National Center for Biotechnology Information (dpSNP), 1000 Genomes, and the National Heart, Lung, and Blood Institute (NHBLI) databases. The exclusion criteria included the use of IVA filtering to eliminate common and non-deleterious variants based on 1) confidence (variants that are of potential low quality, n=71997 with n=14202 genes that were excluded), 2) biological context (ontology for diseases, phenotypes, genes, domains, pathways, processes of BRS, n=67 variants with n=15 genes that were excluded), and 3) pharmacogenetics (variants that are predicted or observed to impact drug response, metabolism or toxicity based on literature evidence, n=60 with 11 genes that were excluded).

Candidate gene mutations identified by IVA were verified by cycle sequencing. The Pearson format (FASTA) sequence is a text-based format for representing either nucleotide sequences or peptide sequences. The FASTA sequence of each selected target was analyzed using the National Center for Biotechnology Information (NCBI) Primer Basic Local Alignment Search Tool (BLAST) to design primers flanking each candidate gene mutation. Optimal efficiency of the polymerase chain reactions (PCR) was achieved through the titration of reaction components to minimize the formation of undesired (secondary) products. Excess primers and nucleotides were removed using ExoSAP-IT (USB Corp., Cleveland, OH). Once the excess nucleic acid removal procedure was completed, PCR products were submitted to the UCSF Genomic Core Facility (GCF) for direct sequencing using BigDye terminator sequencing chemistry (Applied

Biosystems, Carlsbad, CA) to sequence coding regions and exon boundaries for all selected gene targets. The resulting sequencing chromatograms were analyzed using Sequencher® version 5.2 sequence analysis software, (Gene Codes Corporation, Ann Arbor, MI USA http://www.genecodes.com). Chromatograms were produced using Sequencher 5.2 sequence analysis software, Gene Codes Corporation, Ann Arbor, MI USA and were visually examined and compared with the reference sequences available from NCBI.

RESULTS

Donor characteristics

The heart transplant donor was a 35-year-old Hispanic Caucasian male with no prior medical history. The subject was found unconscious and unresponsive near his home, after a two day period, with multiple abrasions noted on trunk and lower extremities. He was last seen drinking on Friday evening and was returned home by police after being found roaming streets in undergarments. Family states that patient drank alcohol on weekends but were unsure as to type and quantity. Family was unaware of any illict drug use. Family thought patient to be sleeping most of the following day (Saturday) and attempts to awaken on Sunday failed. Family states he looked as if he was hallucinating then his body stiffened and he became unresponsive to stimuli. Family called 911, where he was then transferred to an outside hospital for initial care and was later transferred to Stanford Hospital and Clinics for continued neurologic care where a confirmed diagnosis of an intracranial (subarachnoid) hemorrage (ICH) was made as per CT-scan. Systolic blood pressure on admission was 200 mmHg and temperature was 38°C. No hypothermic measures were appropriated. Weight was 75kg and height was 173cm (i.e., body mass index (BMI) was 25.2). No cardiac arrest, cardiopulomonary resusitation (CPR), or cardiac defibrillation was listed as attempted in medical records.

Donor ECG data

Electrocardiographic measures of three surface 12-lead ECGs are detailed in Table 6. In Figure 5, a coved Type-1 BRS ECG pattern is observed with marked early repolarization sign (ER) and a large J-wave in both the precordial and inferolateral leads. Additionally, on the initial ECG (Figure 5) a right arm/left leg limb lead reversal (e.g., Lead*s I, II, III and aVF*) were completely inverted and lead *aVR* remained upright. Both the coved Type-1 BRS ECG and limb lead reversal in subsequent ECGs vanished. (Figure 6 and 3c) Additional ECG abnormalities included a prolonged QTc and ERS. (Figure 5 and Figure 6)

Table 6. Electrocardiographic measures of all three surface 12-lead ECGs. *RA/LL limb lead reversal noted

ECG #	Heart Rate (bpm)	PR interval	QRS duration	QT interval	QTc interval	QRS Axis	T Axis	QRS-T angle
* **1**	89	152ms	122ms	416ms	477ms	N/A	N/A	N/A
2	89	144ms	110ms	420ms	512ms	65°	79°	14°
3	103	140ms	82ms	336ms	440ms	63°	246°	180°

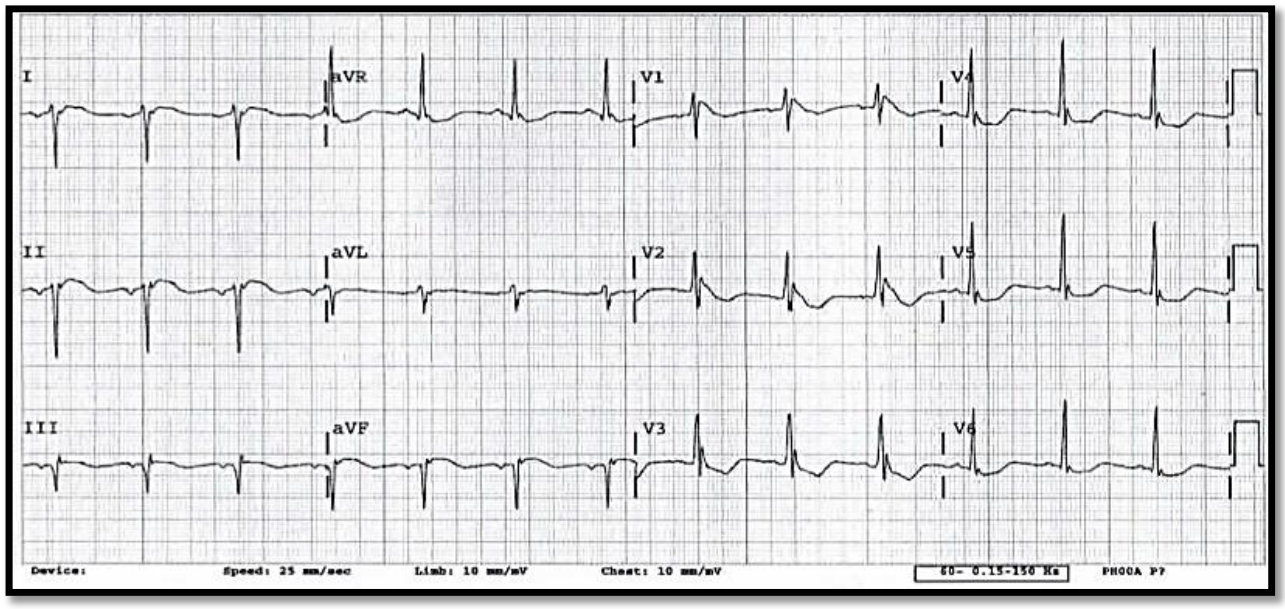

Figure 5. Precordial leads V1 and V2 with characteristic Type-1coved pattern of BRS. Of note, RA/LL limb lead reversal is noted (Lead*s I, II, III and aVF*) QTc measure prolonged: Automated reading 477ms. Over read: 482ms

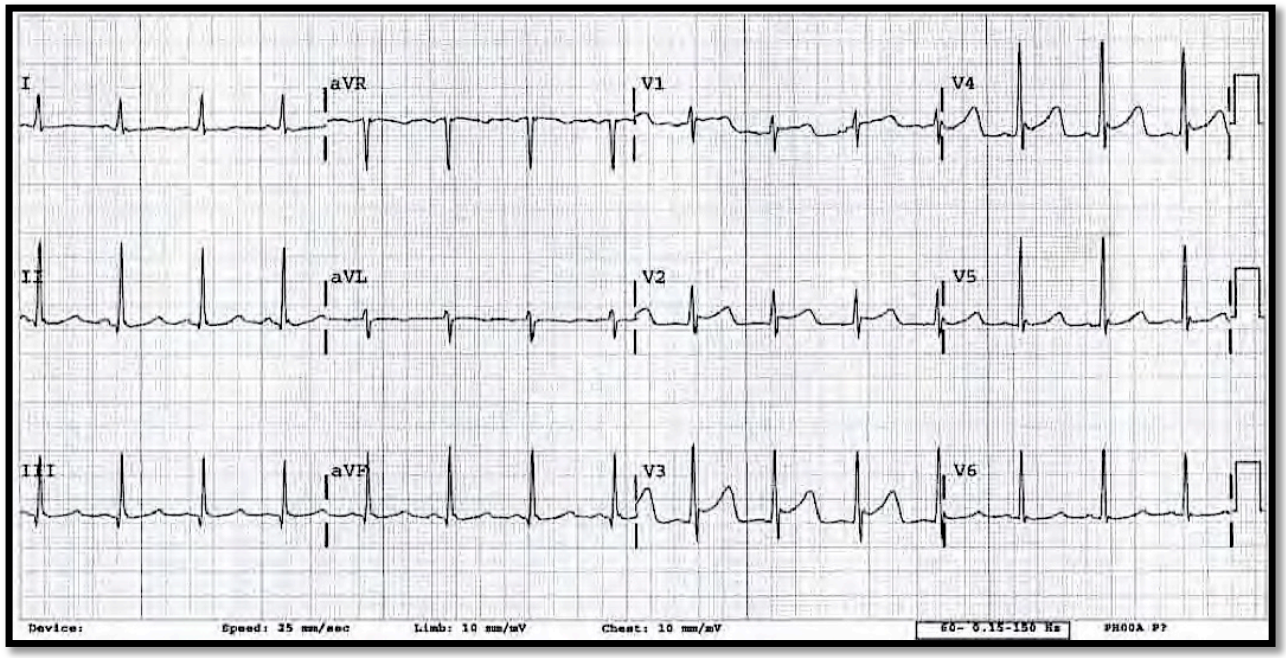

Figure 6. BRS ECG coved Type-1 pattern absent. ER as evidenced by ST segment elevation in the precordial and inferolateral leads without reciprocal ST segment depression. J point notching and slurring are present in the same leads.

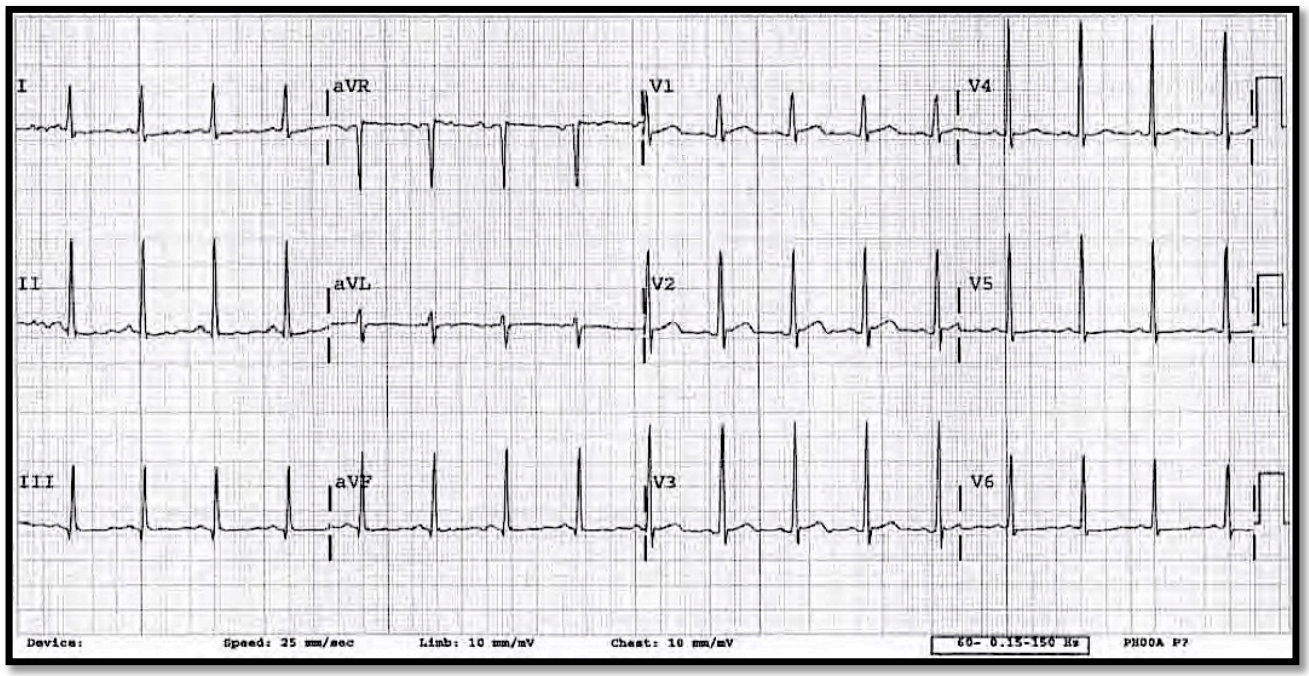

Figure 7. Sinus tachycardia with possible right ventricular hypertrophy pattern (dominant R wave in V_1 > 7mm tall or R/S ratio > 1mm)

Donor CT Scan

A non-contrast and CT angiogram CT-scan revealed an intraventricular hemorrage with a large amount of intraventricular blood, right >left, with blood extending into the right thalamus and associated hydrocephalus. Diffuse sucal effacement and herniation, which may have attributed to ventricular enlargement and cerebral swelling.

Donor ECHO data

Echocardiography revealed an estimated ejection fraction of 68%, Normal LV size, LV diastolic dimension of 4.9cm, LV systolic dimension of 2.7 cm, LV posterior wall thickness of 1cm, percent LV shortening of 46%, RV dimension of 2.5cm and septal wall thickness of 0.9 cm.

Donor Laboratory values

Significant laboratory values included: white blood cell count-16,000/mm2, hematocrit-52%, platlets-231/mm3, sodium- 135/mEq/L, potassium-3.1mEq/L, Blood Urea Nitrogen-29mg/L, creatinine-1.3mg/dl and glucose-159mg/dl. A urine drug toxicity test identified no apparent illicit drugs or alcohol.

Donor Pharmacologic data

Patient was started on systemic prophalatic antibiotics (e.g., Zosyn, imipenem). All pharmacologics that were used during the evaluative stage of the heart transplantation are listed in Table 7.

Table 7. Summary of Medication Administration from Admission through Organ Procurement that patient received. Highlighted drug (in grey) has been associated with affects on cardiac ion channels (e.g. sodium channel: SCN5A; L-type calcium channels: CACNA1C, CANA2B and CACNA2D1). **Propofol infusion completed 24 hours before BRS ECG recorded*

Medication	Peak Dose	Duration
Dopamine	10 mcg/kg/min	30 minutes
Neosynephrine	200mcg/kg/min	60 min
Esmolol	200mcg/kg/min	5 hours
Nipride	3mcgs/kg/min	3 hours
DDAVP/Pitressin	0.04mcgs/hr	5 hours
Ativan	2mg	Given once
Mannitol	50gm	Given once
Zosyn	3.375gms	Every 8 hours
Levoquin	750mg	Daily
Solumedrol	1500mg	Every 8 hours
Decadron	4mg	Given Once
Fentanyl	50mcg	Given Once
***Propofol**	50mcg/kg	Given once
Succinylcholine	180mg	Given Once

Donor CTDN appraisal

Despite aggressive critical care management (e.g., intubation and ventilator support, external ventricular drainage insertion, pharmaceutical managment) patient became terminal. Patient continued to show evidence of increase hydrocephalus with temporal and cerebral herniation

along with development of diabetes insipidus. Immediate family was notified of grave status and were in agreement to cease any heroic measures. Brain death was confirmed using brain death criteria, and continued supportive care and listed as an organ donor. The Santa Clara County coroner was notified. Patient was made a coroners case with permission to donate organs and perform autopsy with the only restriction on the case being nothing was to be retrieved from above the neck. Cause of death was listed as cerebralvascular/stroke with mechanism of death being intracranial hemorrage.

Donor Genetic Analysis

During an investigation of donor ECGs from the California Transplant Donor Network (CTDN) to determine ECG abnormalities in brain dead donors and to link these abnormal ECG characteristics with the recipient's outcome,[86] one of the donor ECGs was found to display a BRS Type-1 ECG pattern. Given the presentation of the case (Type-1 BRS ECG), BRS susceptibility genes were examined for potential causal variants, based on National Center for Biotechnology Information (NCBI) Genetic Testing Regristry (GTI). The 17 genes examined were *SCN5A, GPD1L, CACNA1C, CACNB2, SCN1B, KCNE3, SCN3B, KCNH2, KCNJ8, CACNA2D1, RANGRF, KCND3, KCNE5, HCN4, SLMAP, TRPM4,* and *SCN2B*. A total of 22 sequence variations were identified by IVA software among seven of the known 17 genes identified. Three variations were found to be artifacts (i.e. the rare allele set as the reference allele in the published reference genome used (dpSNP) for variant call analysis was likely the common allele). Excluded variants artifacts are indicated by italicized text in Table 8. Intronic variants were only listed if they occurred within nucleotides at the exon-intron boundary.

Table 8. Table displaying gene target, chromosomal location, rs position, DNA allelic Δ/mRNA position, protein variant, amino acid position, translation impact, SIFT prediction/score, PolyPHEN classification and mRNA accession number.

Gene Target	Chromosomal Location	rs Position	DNA Allelic Δ/mRNA Position	Protein Variant	Amino Acid position	Translation Impact	SIFT Prediction/Score	PolyPHEN Classification	mRNA Accession #
CACNA1C	**12**	**2791130**	**C=>T**	**Pro=>Leu**	**1868**	**Missense**	**Tolerated/0.46**	**Benign**	**NM_199460.3**
CACNA1C	12	2791132	A=>G	Met=>Val	1869	Missense	Tolerated/0.39	Benign	NP_955630.3
CACNA1C	12	2721137	C=>T	Phe=>Phe	1282	Synonymous	None	Benign	NM_001129827.1
CACNA1C	12	2788879	G=>A	Thr=>Thr	1835	Synonymous	None	Benign	NM_199460.3
KCNH2	7	150648198	A=>G	Tyr=>Tyr	652	Synonymous	None	Benign	NM_000238.3
KCNH2	7	150648789	T=>G	Lue=>Lue	564	Synonymous	None	Benign	NM_000238.3
KCNH2	7	150649531	G=>A	Phe=>Phe	513	Synonymous	None	Benign	NM_000238.3
KCNH2	**7**	**150649603**	**C=>A**	**Ile=>Ile**	**489**	**Synonymous**	**None**	**Benign**	**NM_000238.3**
HCN4	15	73614834	*T=>C*	Pro=>Pro	1200	Synonymous	None	Benign	NM_005477.2
HCN4	**15**	**73621946**	*G=>A*	**Leu>Leu**	520	**Synonymous**	**None**	**Benign**	**NM_005477.2**
SCN1B	**19**	**35524824**	**T=>C**	**Leu=>Pro**	**210**	**Missense**	**None**	**Benign**	**NM_199037.3**
CACNB2	**10**	**18828371**	**C=>T**	**Tyr=>Tyr**	**567**	**Synonymous**	**None**	**Benign**	**NP_963890.2**
SCN5A	**3**	**38622467**	**T=>C**	**Glu=>Glu**	**1061**	**Synonymous**	**None**	**Benign**	**NM_001160160.1**
SCN5A	3	38592406	*A=>G*	Asp=>Asp	1801	Synonymous	None	Benign	NM_001099405.1
SCN5A	3	38674712	*T=>C*	Ala=>Ala	29	Synonymous	None	Benign	NM_001160160.1
CACNA2D1	**7**	**81588636**	*G=>A*	**Pro=>Pro**	**1038**	**Synonymous**	**None**	**Benign**	**NM_000722.2**

Seven of the 17 gene variations (bold text in Table 5) were evaluated and confirmed by cycle sequencing (Figure 8): *SCN5A (synynomous mutations: c.5553T=>C, p.Asp1786Asp).); SCN1B(missensnse mutation: c.762T=>C, p. Leu210Pro); CACNA1C(missense mutations: c.5881C=>T, p. Pro1868Leu; CACNB2 (synynomous mutation:c.2151C=>T, p.Tyr512Tyr); CACNA2D1(synynomous mutation: c.3370C=>T, p.Pro1038Pro); KCNH2 (synynomous*

mutation: c.1868C=>A, p.Ile489Ile), and *HCN4 (synynomous mutation:c.4594A=>G, p.Pro1200Pro).* Chromatograms of selected gene variants from cycle sequencing are shown in Figure 8.

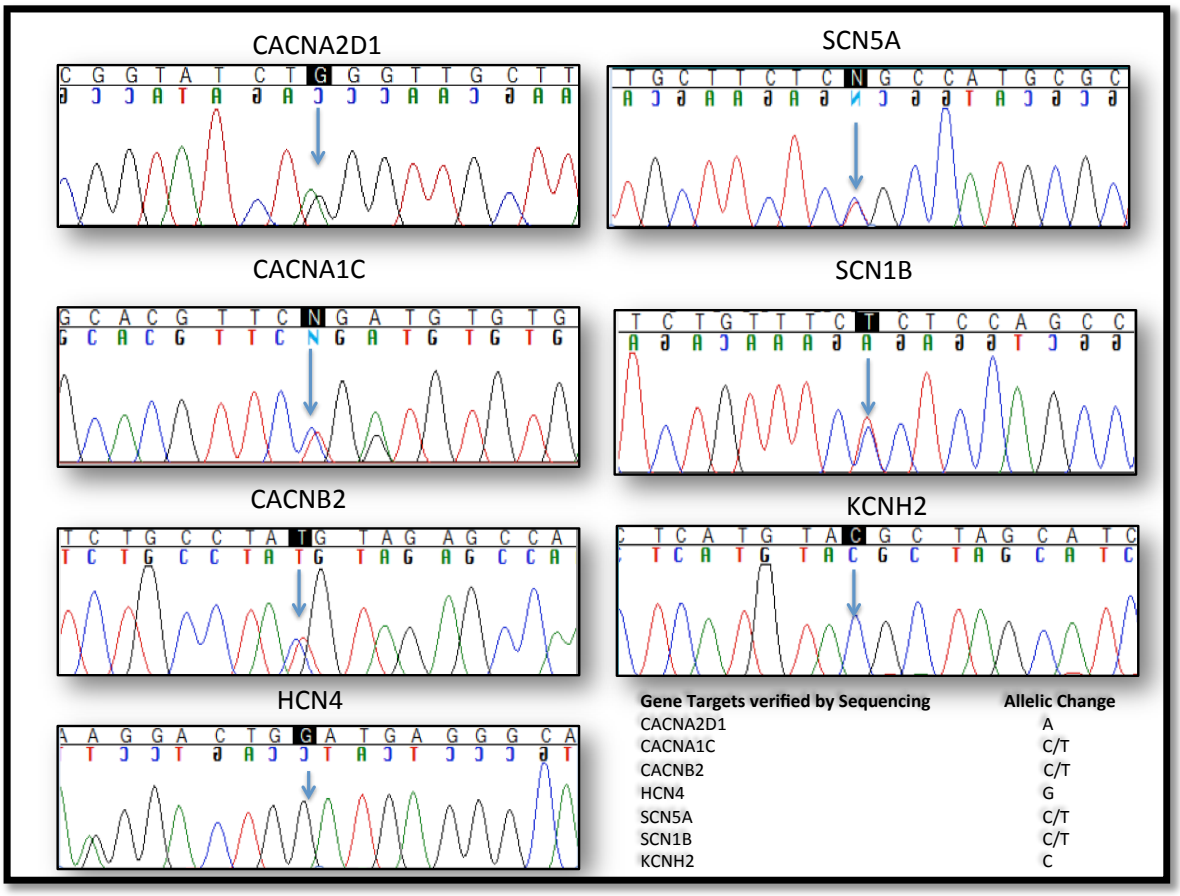

Figure 8. Chromatagram fragments of selected gene targets verifed by sequencing of donor gDNA. Arrows indicate area of interest. DNA sequencing key: Guanine (G) = Black; Cytosine (C) = Blue; Thymine (T) = Red and Adenine (A) = Green

Recipient Status

Heart transplant recipient was a 57-year-old Caucasian male who is currently approximately eight years post-transplant. Initial follow-up was through Kaiser Permanente, but is now being followed by transplantation services at Stanford Hospital and Clinics. A review of progress notes (from 2011) revealed the recipient experienced an episode of a "funny sensation" in his anterior chest, which was similar to his pre-transplant symptoms of ventricular tachycardia,

although not captured by event monitor, later associated with anxiety. Subject also, reported occasional vertigo, which was attributed to dehydration. The one-week post transplantation ECG is depicted in Figure 9. The most recent ECG available of the transplant recipient is depicted in Figure 10.

Recipient records indicate that the Stanford transplant coordinator confirmed donor was positive for BRS although no BRS drug challenge or family history of symptomology substantiates this diagnosis. An electrophysiologist was consulted, and since there has been no BRS ECG signs or syncope, advised no intervention, however, recipient was advised to treat fevers immediately as they could exacerbate the BRS ECG. Additionally, recipient extrapolated ECG Axes measurements and corresponding frontal QRS-T Angles are seen in table 9.

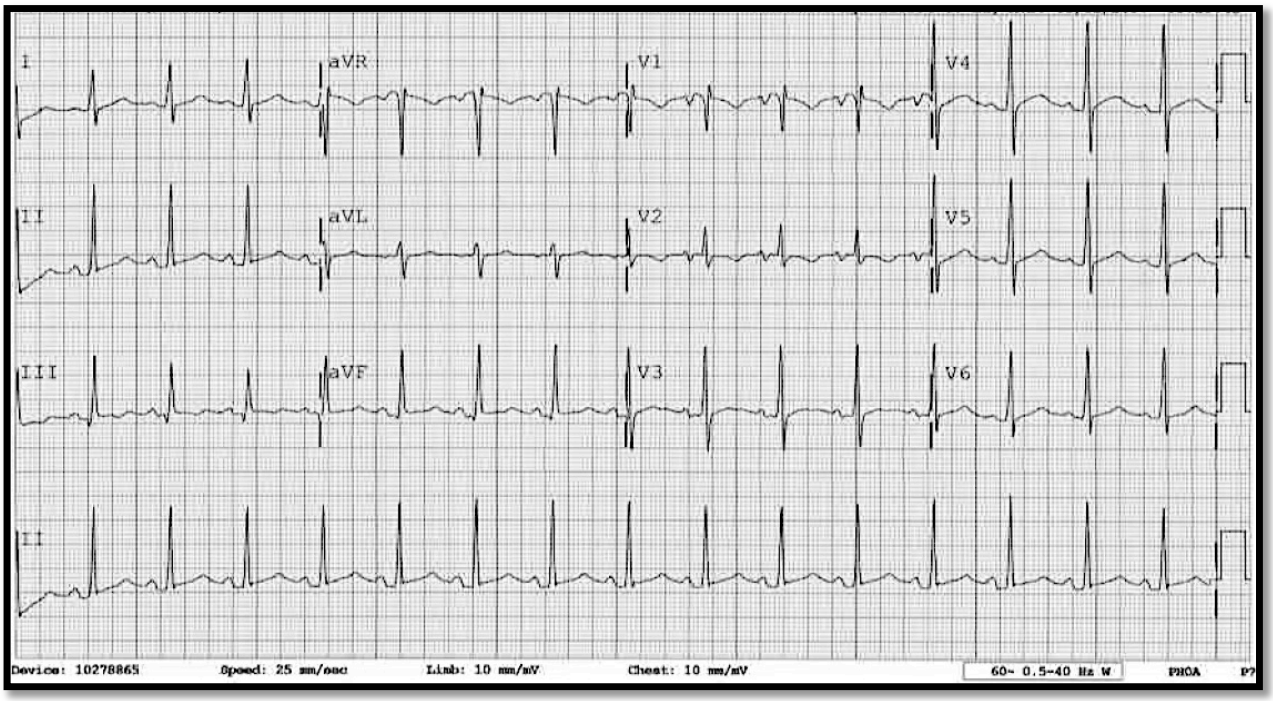

Figure 9. Post-transplant ECG one week post-surgery: sinus tachycardia with left atrial abnormality and a prolong QTc of 511ms.

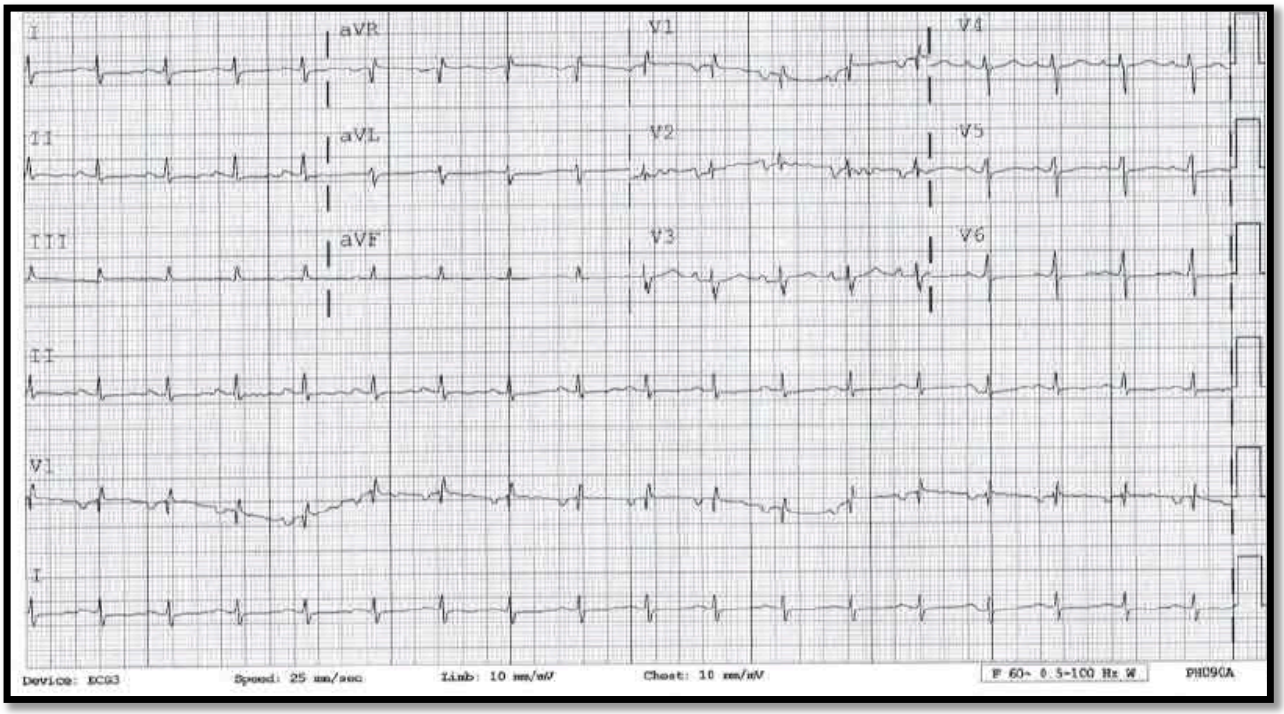

Figure 10. Post-transplant recipient most recent ECG shows sinus tachycardia of 107, left atrial abnormality, incomplete right bundle branch block (QRS with RSR 'configuration and QRSD-82ms), possible right ventricular hypertrophy (R/S ratio > 1mm in V_1), and early repolarization and borderline right axis deviation (QRS axis 89°, (+) forces in Leads III and aVF, slightly (-) forces in Leads I and aVL

ECG Date	QRS Axis	T Axis	Absolute QRS-T_F Angle
4/22/2008	58°	72°	14°
4/21/2009	45°	77°	32°
4/20/2010	59°	92°	45°
4/12/2011	76°	118°	42°
8/27/2011	69°	127°	58°
2/23/2012	97°	150°	53°
2/27/2012	72°	183°	110°
3/09/2012	89°	129°	40°

Table 9. Recipient extrapolated ECG Axes measurements and corresponding frontal QRS-T Angles

DISCUSSION

In the setting of heart transplantation, assessment of the donors ECG occurs as part of the procurement process yet assessment of the donor for specific cardiac ion channel mutations does not typically occur. Currently, evidence does not exist in the literature to support the prevalence

of transplanted hearts that feature ion channelopathies. While cardiac ion channelopathies are pathological and can lead to increased mortality, no published case of a transplanted heart with cardiac ion channelopathy leading to an adverse recipient outcome such as SCD has been found in the literature.

ECG Findings (Donor)

This case report analyzed the genomic DNA of a transplant donor who exhibited the ECG phenotype of BRS. The etiologic clinical significance of BRS is that it predisposes an individual to risk for SCD due to polymorphic idiopathic ventricular tachycardia or ventricular fibrillation through an apparent inactivation of ion currents and a dominance of outward transient voltage gradients.[59] Some of the key ECG changes noted in Figure 11 that are suspected include, a Type-1 coved BRS ECG pattern in the precordial leads of V_1 and V_2, and, an intraventricular conduction delay (122ms). Additionally, a QTc (Bazett's correct) of 482ms (manual measurement) and ER sign in two contiguous inferolateral leads were also identified. ER sign is a common precordial ECG pattern characterized by J-point and ST-segment elevation in 2 or more contiguous leads. [87] Although ER in the precordial leads has been shown to be benign there is evidence that ER sign in the inferior and lateral leads can be associated with idiopathic ventricular fibrillation (IVF) or in conjuction with other concurrent cardiac disease states (i.e., BRS). [88-90] Given that the ER sign ECG patterns > 1mm in the inferior/lateral leads occurs in 1%–13% of the general population, and in 15%–70% of idiopathic IVF cases, this becomes another interesting finding. The prolonged QTc noted in Figure 6 is of interest as LQTS is associated with a variety of enviornmental substrates including fever, pharmacologics and genetic mutations: one in particular shares a gene mutation with the SCN5A gene as LQTS 3 and causes a gain of function in the sodium channel. Although the focus of this case report was

determining the influence of BRS on the ECG, it would be prudent to note the prolonged QTc and ER sign as being additive to the overall scenario of this case report. In addition, understanding that BRS is based on the abnormal ECG morphologies, these changes are often times transient and hidden and manifest through a range of disease and environmental substrates. The non-contrast CT and CT angiogram showed an intraventricular hemorrhage (IVH) and was listed as a non-traumatic bleed. The report states that there was poor opacification of the dural venous sinuses, which raises the question of extensive dural venous sinus thrombosis. (i.e., trauma). The CT report also states that an insufficient amount of contrast dye was used and therefore was an inadequate study.

Pharmacologic Effects (Donor)

The evaluation of pharmacologics is necessary in this case report, as loss-of-function mutations in the setting of arrhythmogenic agents are responsible for the vast majority of BRS incidents. [59,91,92] BrugadaDrugs.org maintains a list of drugs that patients diagnosed with BRS should avoid. Pharmacologic use during intensive care and donor evaluative course is detailed in Table 7. A urine drug toxicity test identified no apparent illicit drugs or alcohol. As noted in Table 7, propofol and sodium nitroprusside were used during the evaluative stage of the heart transplantation. Propofol' s effect on sodium channels is well documented in the literature. [93-102] In addition, sodium nitroprusside, a potent antihypertensive, affects calcium ion channels through a means of channel inhibition often binding to L-type calcium channels (CACNA1C, CACNB2, and CACNA2D1). [103]

The ability of BRS to express spontaneously contributes to phenotype unique expression, adding to BRS complexity. Certain cardiovascular pharmacologics [91], pyrexia [104-114], antipsychotics [91,115-117], anesthetics .[102,118-124] and illicit drugs [125-132] can have a deleterious effect on cardiac ion

channel function and therefore the ECG phenotype expression. In addition, other disease processes can mimic the ECG phenotype of BRS. Additionally, Baranchuk et al. developed a fundamental concept relevant to the study of congenital forms of BRS mimicry. BRS mimicry is defined as BRS Type l electrocardiographic changes that resemble those of the real genetic form of the disease [123] and was first observed by Riera et al. in a case that involved use of the drug propofol. [62] Lastly, a frontal QRS-T angle of 180° of the donor's ECG was observed in Figure 11. Some studies have shown frontal QRS-T angles > 95° can place an individual at risk for SCD, cardiac mortality or all-cause mortality. [29,133,134]

Genomic Findings (Donor)

We validated seven of the 17 known BRS candidate genes for genomic mutation. Results of the specfic cycle sequencing (PCR) are detailed below:

SCN5A

SCN5A encodes for the α subunit of the cardiac sodium channel and is responsible for the initial upstroke observed; phase 0 of the cardiac action potential. Abnormalities leading to a lack of gene expression cause a decrease in the sodium current and an accelerated inactivation of the channel. [135] In addition, due to the decrease in sodium current, a larger increase in the transient outward current occurs during phase 1 of the action potential.

Mutations of the SCN5A have been linked to other cardiac conduction abnormalities like Long QT Syndrome (LQTS 3) type 3, sick sinus syndrome and conduction disease. [136-138]Furthermore, the SCN5A gene mutation that causes LQTS 3 has a more severe prognosis with a reduced response to beta-blockers thus requiring protection of an ICD.[139] Additionally, SCN5A mutation expressions vary in family members, which may reflect an enviormental contribution in how mutation expression can occur (i.e., variable expressivity). However, SCN5A mutations only

account for 20% to 30% of BRS patients, and not all individuals who display the characteristic BRS ECG have the SCN5A gene mutation. [140]

In the course of our DNA analysis of the SCN5A sodium gene, the following known SNP was identified: rs1805126. However, the SNP variation was a synonymous point mutation with no change in the protein. While the synonymous SNP should not impact on protein function, it may influence mRNA processing and/or half-life, resulting in changes in the level of SCN5A produced.

SCN1B

The sodium channel, voltage-gated, type 1, beta subunit (*SCN1B*) is comprised of three coding exons, mainly encompassing the Purkinje fibers of the conduction system and play a role in conduction of cardiac impulses. Ricci et al. focused on the minor BRS susceptibility gene *SCN1B* whose alternatively spliced mRNAs encode the β1 subunit of the voltage-gated sodium channel (NM_001037, NP_001028, isoform A) and its soluble β1b isoform (NM_199037), which modulates sodium channel function. In the donor case, the SNP at position *rs35524824* resulted in a missense point mutation that changes the protein stucture *(p. Leu210Pro)* (NM_001037). This SCN1B mutation has been associated with BRS as well as with other cardiac arrhythmias and familial epilepsy.[141]

Althought the predictive PolyPhen database classified this mutation as benign, this prediction tool is not comprehensive and does not take into account potential gene-environmental interactions, which may be operant for the case. Of note, the donor presented to the admitting hospital with intraventricular hemmorragic stroke showing a possible link to an associated neuropathologic connection due effect of this gene mutation.

L-TYPE CALCIUM CHANNELS-LTCC (CACNA1C, CACNB2, and CACNA2D1)

CACNA1C encodes for a number of isoforms of the pore-forming α1 subunit of the long-lasting (L-type) voltage-gated calcium channel. This gene causes a depolarization of the myocytes and an influx of calcium, which assists in maintaining the shape of the action potential dome. In addition, this gene product enables a pairing of both excitation and contraction release of calcium from the sarcoplasmic reticulum (SR).

CACNA1C mutations are known to cause calcium leaks, which can cause arrhythmogenic inducibility. The genetic analyses identified two synonymous and two missense point mutations in the CACNA1C gene at the SNP position (rs2721137) and SNP position (rs2788879), however, the SNP variations were synonymous point mutations with no change in the protein. Again, influence on the mRNA processing and/or half-life may be altered, changing the amount of CACNA1C produced. The SNP position (rs2791132) resulted in two missense point mutations *(p.M1821V, p.M1869V)* and SNP position (rs2791130) resulted in two more missense point mutations *(p.P1820L, p.P1868L),* altering the protein structure. The PolyPhen tool predicted the protein changes to be benign. The SIFT function predication, a sequence homology-based tool that's sorts intolerant from tolerate amino acid substitutions and predicts a proteins phenotypic effect labeled both protein changes as tolerated with a score of 0.39 and 0.46. SIFT scores ranges from 0 to 1. The amino acid substitution is predicted damaging if the score is <= 0.05, and tolerated if the score is > 0.05.

CACNB2 contributes to the function of the calcium channel by increasing peak calcium current, shifting the voltage dependencies of activation and inactivation, modulating G-protein inhibition and controlling the alpha-1 subunit membrane targeting. [142] The genetic analyses identified

synonymous point mutations at SNP position (rs78828371) with no protein alterations *(p.Y501Y, p.Y512Y, p.Y513Y, p.Y515Y, p.Y519Y, p.Y529Y, p.Y539Y, p.Y543Y, and p.Y567Y.)*

CACNA2D1 affects the L-type calcium channel and a mutation of this channel can lead to both phenotypic expression of BRS, a short QT (<360 msec for males), idiopathic ventricular fibrillation, and ERS. The analysis revealed a synonymous point mutation with no change in protein structure (*p.P1038P*).

KCNH2

KCNH2 encodes for the pore-forming subunit of a rapidly activating-delayed rectifier potassium channel. This gene plays an essential role in the final repolarization of the ventricular action potential. [143] In our current analysis, point mutations in the KCNH2 consisted of the following synynomous mutation*, c.1868C=>A, p.Ile489Il,* a previously reported variant. Even though these synonymous mutations in the coding region cannot affect the protein structure, these mutations can affect the thermodynamic stability of mRNA secondary structures [144] and RNA degradation. [145]

HCN4

HCN4 contributes to the action potential through the repolarization of ventricular myocytes. Mutations of this gene can cause abnormal splicing, as seen in a BRS positive individual. [146] In our donor's genetic analysis, a point mutation in the HCN4 at SNP position (rs 73614834) included a synynomous mutation *(p.Pro1200Pro)* which has previously been reported in the literature. Although this gene mutation is associated with the rare LQTS 6 subtype and bradycardia, the event that led to the donor's demise is non-documented and unknown therefore postulation as to a bradyarrhythmic event is difficult to validate yet seemingly plausable given the donor's hospital presentation.

Diagnosis of BRS is not done solely based on genetic mutation testing. Molecular genetic testing confirms a genetic diagnosis and may complement clinical diagnosis.

BRS Diagnosis

Currently, the Genetic Testing regristry (GTR) has a listing of various laboratories offering clinical genomic and pathology services that provide genetic testing of various cardiac ion channel disease states, using next generation sequencing (NGS) and massively parallel sequencing techniques. For example, in the Brugada Syndrome Gene set offered by Genomics and Pathology services located at Washington University in St.Louis, testing for eleven BRS candidate genes and variants is possible. Candidate genes included in the assay are CACNA1C, CACNB2, GPD1L, HCN4, KCND3, KCNE3, KCNJ8, PKP2, SCN1B, SCN5A and SCN3B. All but SCN3B and PKP2 were part of the NGS performed in our initial testing. Diagnosis is ultimately based on other clinical characteristics as well as sodium channel blockers challenge to express the BRS ECG.[147] This is relevant because several physiologic and pharmacologic situations can produce the BRS ECG.

The disease displays incomplete penetrance and variable expressivity, which reflects heterogeneity and the influence of environmental and physiological factors. [136,147] In the current analysis, we discovered that pharmacologic agents that have been associated with the BRS ECG (e.g. propofol, nitroprusside) were administered to the donor, although time between propofol dose and BRS ECG was 24 hours. Propofol half-life ranges from two to three minutes up to 184 to 480 minutes in poorly perfused tissues. [148]Nitroprusside was utilized for approximately 3 hours as per hospital records. Half-life of nitroprusside in the circulatory system is approximately two minutes. Use of nitroprusside did not reach time or level that would increase development of toxicity.[149]

Cardiac and ECG changes in cerebral hemorrhage are not uncommon and have been described in the literature. [150-152] Cardiac arrhythmias or repolarization abnormalities occur after stroke in approximately 60 to 70 percent of patients and may have important prognostic implications. [151,153]Also, repolarization abnormalities may account for the superfluous number of arrhythmias and SCD, following acute neurologic disorders. [154-156] Given the donors CT result of atraumatic ICH, consideration should be given to the ECG repolarization abnormalities observed.

Given that this individual was a heart transplant donor who was mechanically and pharmacologically stable for explantation, poor tissue perfusion was not suspected and drug clearance was adequate based on laboratory values. Additionally, for the genetic analysis, after filtering erroneous variants, we selected seven specific BRS candidate genes to verify with cycle sequencing. Taking into account the young age and sex of the donor, variant genes associated with BRS and the phenotypic manifestation one could consider our finding a novel but potentially rare case report. In addition, the theory of somatic mutations occurring during fetal development can offer an explanation as to why germinal cell mutation is not the only theoretical reason for the occurrence of BRS.

With multiple risk alleles verified by cycle sequencing co-occurring, each with modest impact, there is potentially for susceptibility to the effects of propofol induced BRS ECG. Propofol exerts a dose-dependent blockade of whole cell sodium current and induces a hyperpolarizing shift in the voltage-dependence of the inactivation of sodium currents. [157] Propofol has also been found to inhibit cardiac L-type calcium channels, attenuate beta-adrenergic signal transduction, and augment acetylcholine receptor activity. [158-160]This is of importance given that our evaluation revealed both sodium and calcium genetic variations, although time from last dose to phenotypic expression of BRS ECG was greater than 24 hours.

Lastly, the ECG findings of prolonged QT interval and ER sign, and the subsequent cycle sequence validation of variants in specific genes known to be responsible for these phenotypes, supports the complexity of cardiac ion channel diseases and the necessity of increased surveillance in the heart transplant population. ERS is characterized by J-point elevation manifested either as terminal QRS slurring (the transition from the QRS segment to the ST segment) or notching (a positive deflection inscribed on terminal QRS complex) associated with concave upward ST-segment elevation and prominent T waves in at least two contiguous leads. [161]Additionally, prolonged QT > 460ms in males is considered an abnormal finding with prolongation > 500ms placing an individual at risk for development of torsades de pointes. These changes were observed in two of the donors ECGs and one recipient ECG. Lastly, a QRS-T angle of 180° was noted.

Two prior studies have demonstrated that a spatial QRS-T angle wider than 100° is associated with cardiac disease and an increased cardiovascular mortality [23,30] Additionally, Aro et al. determined that a frontal QRS-T_F angle ≥ 100° elevates the risk of death through arrhythmogenesis, mainly through an alteration of the T wave. [134] The frontal QRS-T angle of 180° in the donor is of interest as angles >95° have shown negative outcomes in selected populations. [29,133,134] In viewing the recipients available 12-lead ECGs, frontal QRS-T angles have ranged from 14° to111° since transplantation. Whether QRS-T_F angle widening in heart transplantation is pro-arrhythmogenic or a product of the event of transplant surgery and possibly reversing with time, remains to be seen.

There is ECG evidence of ST elevation, prolonged QT, frontal QRS-T angle and ER, which are potentially arrhythmogenic and can lead to SCD. Whether the prolonged QT is of relevance is questionable as the prolongation was not noticeable in a subsequent ECG done later that day

(Figure 7) and may have been related to an electrolyte imbalance or pharmaceutical agent use. In addition, although ER in the precordial leads is considered benign, ERS in the inferior and lateral leads is not. Additionally, the ST segment elevation is suspect as the ECG high pass filter was set to 0.15 and should be 0.05. The incorrect setting can cause ST segment distortion.

Treatment of these cardiac ion channelopathies ranges from pharmacologic, life-style modification to device implantation. In the setting of heart transplantation, previous studies of pacer implantations have had mixed results. [162,163] Vakil et al. studied the effects of pacemaker implantation in heart transplant recipients and concluded that pacer maker implantation did not affect SCD negatively. Whether the use of an intracardiac defibrillator (ICD) in post transplant recipients would reduce the occurrence of SCD in this population is yet to be determined. Given the proarrhythmic effects of ICD placement (electrical storm), this treatment modality may be challenging. Lastly, given the young age of the donor, sex, structurally normal heart and the insidious nature of the event, BRS ECG, as well as LQT and ERS as per ECG, cardiac ion channelopathies are suspected.

STUDY LIMITATIONS

There are several limitations of this case report. With the occurrence of multiple variations of unknown significance in a single case, definitive assignment of causal genetic risk is difficult. In non-synynomous point mutations, the amino acid change could have a deletrious effect on the protein structure and function. Although several synyomous mutations were identified, with synynomous point mutation changes, there is no change in protein structure, however, ribonucleic acid (RNA) stability could be influenced which would result in an increase or decrease in protien function. Additionally, caution must be taken in drawing a causal connection in the heart transplant population as a whole (i.e., this case report may be a unique

and singular event). Also, data were retrospectively collected; thus, other valuable information such as family history was unobtainable. Lastly, information bias is possible: we pursued our investigation through presentation of qualitative reporting of both genetic and phenotypic data; controlling our subjectivity through our interpretation of the data.

CONCLUSIONS

This accidental and serendipitous finding provided an opportunity to use this case-reporting, in-depth narrative to present our research. Examination of a BRS type ECG of a heart donor whose organ was transplanted into a recipient was assessed. After verifying that the ECG was a type 1 BRS ECG, we utilized genetic analysis to determine if there was a relationship between the ECG phenotype and the BRS-candidate genotype. Several mutation variations among the seven genes verified through cycle sequencing were identified in the 17 known BRS genes panel. We verified missense as well as synonymous mutations in the donor's blood. Although, synonymous mutations do not alter protein structure, they could possibly have deleterious effects on the production of mRNA. These results suggest, along with the other clinical findings, that there could potentially be an interaction between the several genetic mutations, the BRS ECG phenotype expression and environmental factors, although genetic results should be approached with caution, especial in this case given the *n* of one.

The results presented above detail a clinical consequence that may have been a product of both genetic mutation as well as the influence of unknown environmental effects and genetic modification. Consequently, our results suggest that the interaction between genetic variation, environmental effects and phenotypic expression is multifaceted and requires collection of extensive clinical and genetic data in the population of heart transplantation.

Subsequent steps in this research trajectory would include follow-up with the donor family given BRS heritability. Questions as to issues of syncope or SCD in other family members may hold information, as these could be diagnostic clues of hidden BRS, thereby averting further untimely premature deaths in the donor's family. Additionally, the current recipient of the donor organ should be monitored closely for expression of BRS (i.e., syncopal episodes, arrhythmogenesis or ECG manifestations of other cardiac ion channel diseases) given these results.

In review of the transplant recipient's medical records, BRS was reported although no diagnostic testing was completed. There were reports of vertigo and "funny anterior chest" sensations reminisce of the transplants previous experiences with VT, yet there was no evidence of the BRS ECG. Electrophysiology was consulted and suggested no other treatment other than surveillance. Additionally, issues of bioethics should be explored although not within the current scope of this manuscript. Ethical issues are exceedingly significant given this new and uncharted territory in the realm of heart transplantation and genetics.

SUMMARY

A donor's heart was found to display the Type-1 coved BRS ECG found during a review of transplant donors' ECGs. The decision to analyze the DNA of the donor's stored blood was made and completed. In that analysis, genetic mutations in several of the 17 genes known to be responsible for the phenotypic ECG expression of BRS were determined and included two sodium channel mutations, three calcium channel mutations and two potassium channel mutations. Although several gene mutations were synonymous, thereby adverting no change of protein structure, the deleterious effects on the production of mRNA may occur during

transcription. Nevertheless, whether the BRS ECG occurrence is genetic or acquired remains unclear.

Would it be advantageous to utilize genetic testing when presented with a positive BRS ECG in the donor retrieval phase, prior to explantation of the heart, in an attempt to uncover potential genetic mutations? Given the small pool of donors, would including a genetic assessment represent a negative change in the cardiac transplantation paradigm making receiving an organ more difficult? During the procurement process, what would be the decision if a donor was to present with one or several of the 17 genetic mutations known to cause BRS? These are novel questions that need addressing within the heart transplant population. BRS is a life-threatening disease in which arrhythmogenesis can lead to SCD if not addressed. Most individuals with BRS are asymptomatic, but given the elusive nature of BRS presentation, it is a difficult disease to predict. With the exponential decrease in time to perform genetic testing and lower sequencing costs, it is not inconceivable that genetic testing, specifically of cardiac ion channelopathies, during the procurement phase of heart transplantation may become a routine part of future procedures. [164]

ICDs are the only treatment option available for those diagnosed with BRS. In addition, given the positive ECG signs of prolonged QT, frontal QRS-T angle > 95° and ERS found on the donors 12-lead ECG, use of the ICD may be appropriate. Literature has shown that ICD use can be effective in some groups in preventing SCD. [165-170] Lastly, there has been postulation that in the setting of VF, the use of radiofrequency ablation of ventricular ectopy by ablating the substrate in the RVOT can prevent VF inducibility in the BRS population. Unfortunately, this concept, although novel, is in its infancy stages and requires additional studies.

CHAPTER 5

CONCLUSION

The purpose of this dissertation was to investigate various assessment modalities in both the donor and recipient phases of HTx. Currently, HTx is the only treatment for end-stage heart disease. [5,163] Concerns surrounding heart transplant survival typically place emphases on rejection, infection and SCD with SCD contributing to approximately 10% of total HTx deaths. [163] Several studies point to estimates as high as 39% of SCD in the HTx population. [10,171-175] What incites these deaths remains a puzzle, given that during the donor procurement phase, issues related to atherosclerosis and structural heart disease are ruled out, suggesting other mechanisms may be occurring. As such, assessments of ion channel diseases in the donor and alternative electrophysiologic evaluations are seemingly appropriate and novel.

Arrhythmogenesis is common in HTx, with arrhythmias ranging from sinus bradycardia due to sympathetic denervation, pharmacological effects and injury to the sinus node, [176,177] atrial fibrillation, [178] atrial flutter, [179]and ventricular arrhythmias, and contribute to approximately 25% of SCD when no structural cause can be identified. [10,173] Consequently, other unrecognized pathologies, which have not been formally documented, may be the problem.

The first study sought to investigate feasibility and compliance of a convenience sample of heart transplant recipients to (1) determine whether heat transplant recipients could comply with transmitting a 30-second ECG every day for one month, using a simple ECG device and their home telephone, (2) evaluate the ease of device use and acceptability by transplant recipients, and (3) evaluate the quality of transmitted ECG tracings for QT-interval measurement. This pilot study was part of a larger study aimed at using electrocardiographic markers as a proxy for acute allograft rejection. In the feasibility study, subjects achieved

approximately 74% daily and 100% weekly compliance in recording and transmitting their ECGs. We concluded that adult heart transplant recipients are compliant with daily and weekly ECG transmissions from their homes to an ECG core lab via telephone transmission. Whether an increased QT interval measure will be an early and sensitive biomarker will be the subject of the larger ongoing clinical trial.

In chapter 3, we discussed use of the frontal QRS-T angle as a prognostic indicator for SCD, cardiac mortality, and all-cause mortality in the area of heart transplant. To date, this is the first study to analyze the QRS-T_F angle in heart transplantation with comparison to an ambulatory population. Multiple studies have made use of the prognostic value of the QRS-TF angle in several different populations. [29,32,34,35] Our results showed that between the two groups tested, the heart transplant group had a wider QRS-T_F angle when compared to the ambulatory population.

The demographic was largely Hispanic within the heart transplant population although this fact did not have a significant impact on the results. Despite this non-significant relationship with the outcome, as an extra precaution, the difference between the two study groups in the QRS-T_F was again analyzed controlling for ethnicity and no effect of controlling for ethnicity was found: the magnitude of the difference between the groups remained the same. QRS-T_F angle in the NH group is warranted for the clinician given the effect of QRS and T wave axes change due to heart transplant surgery. Furthermore, with literature supporting a two-fold increase in cardiac mortality with QRS-T_F angles greater than 90°, this measure may be of significance in the HTx population given the structural changes that occur with the surgery. Whether these findings can be generalized to the broader HTx population remains to be seen. However, the availability of the 12-lead ECG QRS and T-axes are easily calculated making

QRS-T_F angle an easily assessable parameter to evaluate during the early stages of the pre-/post-heart transplant phase.

In the last and final paper, we evaluated genetic and phenotypic data for a HTx donor with suspected BRS, an uncommon autosomal dominant genetic disease with incomplete penetrance and variable expressivity stemming from mutations in cardiac ion channels in the cardiac conduction system. Furthermore, discovery of a prolonged QT interval and an early repolarization sign (ERS) in the precordial and inferior leads were noted in a subsequent ECG. These findings support the level of complexity of a gene-environment hypothesis. Additionally, the impact of environmental and pharmacologic substrates adds to the complexity of cardiac ion channel disease.

Our results detail a clinical consequence that may have been a product of genetic mutation, as well as influenced by unknown environmental effects and genetic modification. Given that this individual was a heart transplant donor, being mechanically and pharmacologically stable for explantation, poor tissue perfusion was not suspected and drug clearance was adequate. Additionally, after filtering erroneous variants, we selected seven specific BRS gene sequence anomalies to verify with cycle sequencing. Taking into account the young age and sex of the donor, the nature of the event, variant genes associated with BRS, and the phenotypic manifestations on ECG, one could consider our finding a novel and rare case report. In addition, the theory of somatic mutations occurring during fetal development can offer an explanation as to why germinal cell mutation is not the only theoretical cause for the occurrence of BRS. Additionally, the identification of co-occurring, multiple risk alleles verified by cycle sequencing, each with modest impact, may have resulted in a susceptibility to the effects of propofol-induced BRS. Consequently, our results suggests that the interaction between

genetic variation and environmental effects on phenotypic expression is multifaceted and requires approaches that take into account collection of extensive clinical and genetic data in the population of heart transplantation.

With the addition of SCD to overall mortality in heart transplantation, new and novel approaches to assessment and treatment modalities become important factors within this population. With the emergence of more rapid and inexpensive whole genome and whole exome sequencing analyses, considerable improvement in cost and feasibility of sequencing candidate genes in the heart transplant population may become plausible.

In summary, this unique case report presents a clinical situation that supports genetic and environmental effects of ion channel in heart transplantation, which could possibly lead to SCD and increased mortality. Being the only option for end-stage heart failure, heart transplantation requires astute assessment during the procurement phase so as to prevent specific ion channel diseases that may be hidden in the donor and become detrimental later in the heart recipient.

Implications for Donor

In this dissertation we have observed the genetic and electrophysiologic effects that weigh upon the donor procurement phase as well as the potential sequelae on recipient outcomes. SCD occurs in approximately 1 in 150 adult heart transplant recipients yearly with approximately 10% all-cause mortality. [162,163] The assumption in procuring donor hearts is that the heart is free from any structural anomalies making the organ suitable for transplantation. Assessment occurs with echocardiography (ECHO), measuring ejection fraction and cardiac indices (i.e., cardiac output). The 12-lead ECG is also utilized to evaluate the electrical system of the heart and can be useful when electrocardiographic changes are discovered. Unfortunately, some cardiac ion channel diseases are not readily discovered, given their elusive presentation

(i.e., BRS). In addition, the medical management of these cardiac ions channels requires specific therapeutics (e.g., pharmacologic, device implantation). Currently, the literature does not support cardiac ion channel disease as an absolute contradiction to cardiac transplantation.

Whole genome sequencing, exome sequencing, and clinical exome sequencing are beginning to transform the idea of genetic testing in health care. In a recent study by Lee et al., clinical exome sequencing (CES) was utilized to assess individuals with rare genetic disorders. In this sample of patients with undiagnosed, suspected genetic conditions, trio-CES was associated with higher molecular diagnostic yield than proband-CES or traditional molecular diagnostic methods. [180] Proper data interpretation is key (i.e., assessment of other clinical findings), taking input of genetic information and other effects (i.e., environmental) that are additive to the clinical picture. Although further testing is needed to validate their findings, this approach offers promise in the heart transplant realm. With rapidly declining costs, genetic testing in the procurement phase of heart transplantation may increase our ability to discover cardiac ion channelopathies early in the heart transplant phase in the near future.

Implications for Recipient

Given that the donor heart has been transplanted in a recipient, the discussion of future treatment modalities is paramount. In the HTx realm, pacemakers are implanted in 4% to 17% of HTx patients for issues like bradyarrhymias and asystole. [163,181-183] Additionally, the use of ICD has become the primary therapeutic modality for the secondary prevention of SCD and, in some groups of patients, for primary prevention (i.e., cardiac ion channel diseases). Currently, the use of ICD's in HTx realm, centers around the level of EF. In a study by Vakil et al., 10% of their patient population had an EF of < 40% after heart transplantation and was associated with a 3.6-fold higher risk of SCD, therefore inducing speculation that an ICD may prevent SCD in the

heart transplant population. It would not be prudent though to ignore the complications that could arise from ICD therapy, which includes proarrhythmia, lead complications, and impingement on quality of life. The deleterious effects of ICD (i.e., depression, anxiety, fear of device discharge and failure) affect the quality of life. It then becomes apparent that considerate and well thought out decisions regarding the use of ICD therapy in the HTx population is not to be taken lightly. Although, beyond the scope of this paper, future conversations regarding recipient surveillance are imperative, especially given the positive genetic and phenotypic results noted in this case presentation.

Future Directions

The ultimate goal of this dissertation was to understand the electrophysiologic and genetic peculiarities observed in heart transplantation. These idiosyncrasies can present pre-/post transplant, arising from unknown or concealed ion channel disease states, to new electrocardiographic assessment techniques (i.e., prolonged QT interval assessments for rejection, pre-/post-transplant QRS-T_F angle assessment). Increasing heart transplant surveillance to include genetic testing and adding electrocardiographic indices to expose issues of SCD, offers a novel and provocative stance in the overall care of heart transplant donors and recipients. Lastly, the ethical issue surrounding the cardiac ion channel findings in a heart transplant donor that was transplanted is new and unfamiliar territory in heart transplantation. The only known case that involved BRS and heart transplantation involved an individual who was transplanted for the issue of BRS electrical storms, refractory to medical management and ICD use. [184] Although, this case was presented over a decade ago, improvements in both technology and suppression of thyroid storm in BRS have improved. Furthermore, the question of whether our

findings should be presented to the family of the donor given cardiac ion channel mode of inheritance is a poignant.

Given that our findings clearly demonstrate expression of three cardiac ion channel ECG phenotypes (ERS, prolonged QT and BRS) and other ECG markers (frontal QRS-T angle >95 °), this may be judicious. As this is a post mortem assessment, what is the responsibility of the clinician upholding the privacy of the donor? Disclosure of information after a family member's death is normally avoided unless a legally authorized representative gives explicit permission. Yet, disclosing this information may allow the prevention of future issues with SCD within the donor family.

Although, we used a targeted gene panel for BRS, the advent of whole genome sequencing (NGS) and whole exome sequencing (NES) is producing incidental findings that may be relevant in the larger picture. Whether these variations fall into a specific category (e.g., pathogenic known/deleterious, variants of unknown significance or, benign) or whether a combination of these variants when co-expressed with an environmental substrate can produce negative sequela are important concerns that generate much debate, especially with the ever-growing move from targeted gene panels to whole genome and whole exome sequencing. We are optimistic that the use of these modalities can be clinically supportive in both the pre-/post-heart transplant patient management.

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