



Peer-Reviewed Original Research

Damaging Cardiac and Cancer Genetic Variants in the LVAD Population

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Citation: Lowes,B. et al. (2015). " Damaging Cardiac and Cancer Genetic Variants in the LVAD Population," The VAD Journal, 1. doi: <http://dx.doi.org/10.13023/VAD.2015.19>

Editor-in-Chief: Maya Guglin, University of Kentucky

Received: October 8, 2015

Accepted: November 12, 2015

Published: November 12, 2015

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Funding: Not applicable

Competing interests: Not applicable

Abstract

Background

Next generation sequencing technology, coupled with population genetic databases, have made broad genetic evaluation relatively inexpensive and widely available. Our objective was to assess the prevalence of potentially damaging cancer and cardiac gene variants in advanced non-ischemic cardiomyopathy patients.

Methods

Explanted human heart tissue procured at LVAD placement was obtained from the University of Nebraska Medical Center Heart Tissue Bank. Genomic DNA was isolated from tissues and amplified by PCR using targeted amplicon primer pools from an inherited disease panel. Individual libraries were amplified by emulsion PCR on Ion Sphere particles and sequencing was performed on a PGM sequencer (Ion torrent) using the Ion 316 chip. The Ion Torrent browser suite was used to map the reads and call the variants. The identified single nucleotide polymorphisms, insertions, and deletions were then annotated and characterized with ANNOVAR. Non-synonymous mutations with a population frequency of less than or equal to 1% were identified and analyzed utilizing an open source integrative genomics viewer. Amino acid substitution effects on protein function were determined by a bioinformatics algorithm. Myocardial recovery was defined as an improvement in EF to greater than 45% at three months post implant.



Results

Our sample population included 12 males and 2 females with an average age of 49 and an average EF at presentation of 17%. Damaging cardiac gene variants were present in 11/14 patients. Only 1 of the 11 patients with damaging cardiac gene variants improved their ejection fraction to greater than 45% post LVAD. Two of the 2 patients without mutations improved their ejection fraction to greater than 45%, p-value=.04. Nine of the 14 patients in this population had damaging oncogene mutations.

Conclusions

Damaging variants in cancer and cardiac genes are common in end-stage non-ischemic cardiomyopathy patients undergoing LVAD placement. Genetic variation likely contributes to disease progression and cancer risk.

Keywords

Ventricular assist device; genetics; LVAD; cardiomyopathy; oncology; sequencing

Introduction

Heart failure is a complex disease process. Patients are at increased risk for sudden cardiac death, pump failure, and, interestingly, cancer. (1) Genetic variation likely contributes to cancer risk, progressive cardiac remodeling, arrhythmias, as well as poor response to both medical and mechanical interventions. Population based sequencing studies utilizing mutation databases suggest that healthy individuals on average carry about 400 damaging genetic variants and 2 disease causing mutations.(2) The prevalence of deleterious or disease causing mutations in patients undergoing left-ventricular assist device placement is unknown. Non-ischemic cardiomyopathies may be caused by variants in at least 30 genes. Cancer has also been associated with variation in numerous genes as well. These are likely underestimates as disease causing variants are often rare and disease allele discovery continues. It is estimated that the current human population contains billions of mutations that cause or modify disease.(3) Understanding the impact of genetic variation within the advanced heart failure populations will hopefully improve patient selection for advanced heart failure interventions and improve clinical outcomes.

Ventricular assist devices have become standard of care for advanced heart failure patients, both as a bridge to transplantation and as destination therapy.(4, 5) Clinical outcomes of patients with left ventricular assist devices are, however, difficult to predict utilizing demographic, laboratory, and echocardiographic risk scores.(6) Patients who receive ventricular assist devices suffer from the most advanced heart failure, but have an immediate improvement in myocardial systolic and diastolic parameters.(7, 8) These devices effectively improve hemodynamics, quality of life, and survival in appropriately selected individuals.(5, 9) Despite these obvious benefits, myocardial recovery to the



degree that devices can be explanted is rare.(10, 11) Right ventricular failure remains a problem with many of these patients.(12, 13) Unloading the heart with mechanical cardiac support may contribute to progressive myocardial atrophy or these hearts may simply be unrecoverable due to underlying disease processes including genetic abnormalities.(14-18) Cardiac transplantation is a limited option for the majority of these patients, secondary to limitations in donor supply, and malignancy is a major problem in the advanced heart failure population post-transplant.

Next generation sequencing technologies involve parallel sequencing of millions of DNA fragments simultaneously allowing for whole genome or targeted sequencing of individuals. Familial cardiomyopathies, rhythm disorders, and cancer all display high degrees of heterogeneity, necessitating the sequencing of the coding region of multiple genes. Advances in sequencing technologies have made genetic testing faster, cheaper, and more readily available. Bioinformatic algorithms have been used to describe the functional significance of these variants. These include annotating single nucleotide variants and insertions/deletions for their effects on genes, reporting their conservation levels, calculating their predicted functional importance scores, retrieving allele frequencies in public databases, and implementing a 'variants reduction' protocol to identify a subset of potentially deleterious variants/genes.

Non-ischemic cardiomyopathy is a common and important cause of heart failure worldwide. It is the leading indication for cardiac transplantation or ventricular assist device placement. The main aim of this study was to assess the prevalence of damaging or disease causing variants in cancer or cardiac genes in a cohort of advanced non-ischemic cardiomyopathy patients undergoing LVAD placement.

Methods

Myocardial tissue was obtained from the Nebraska Cardiovascular Biobank at the University of Nebraska Medical Center (UNMC). These patients were undergoing LVAD implantation at the time of tissue procurement. LVADs were implanted as bridge to transplantation or as destination therapy. Patients were selected for non-ischemic dilated cardiomyopathies. Cardiac function was determined at baseline and three month follow-up by echocardiography. This study was approved by the IRB at UNMC.

Next Generation Sequencing: Genomic DNA was isolated from tissues and amplified by PCR using targeted amplicon primer pools from an inherited disease panel. Individual libraries were amplified by emulsion PCR on Ion Sphere particles and sequencing was performed on a PGM sequencer (Ion torrent) using the Ion 316 chip. The Ion Torrent browser suite was used to map the reads and call the variants. The identified single nucleotide polymorphisms, insertions, and deletions were then annotated and characterized with ANNOVAR. Non-synonymous mutations with a population frequency of less than or equal to 1% were identified and analyzed utilizing an open source integrative genomics viewer. Amino acid substitution effects on protein function were determined by a bioinformatics algorithm. Genomic DNA isolation kit utilized All prep DNA/RNA



mini kit from Qiagen, Valencia, CA. Inherited disease panel primers, DNA library preparation kit, OT2 emulsion PCR kit and sequencing kit were purchased from Ion torrent from Life technology, Carlsbad, CA.

Variant Classification: According to the Association for Clinical Genetic Science recommendations, we utilized a stepwise approach to recording our variants, Figure 1. We annotated our results to sort out synonymous gene mutations leaving only non-synonymous mutation results. In our next step of evaluation, we utilized ClinVar, an open database of greater than 30,000 mutations, to assess for documented or known pathogenic gene mutations. If the variant was documented to be clearly pathogenic or clearly benign, we reported these results as such.

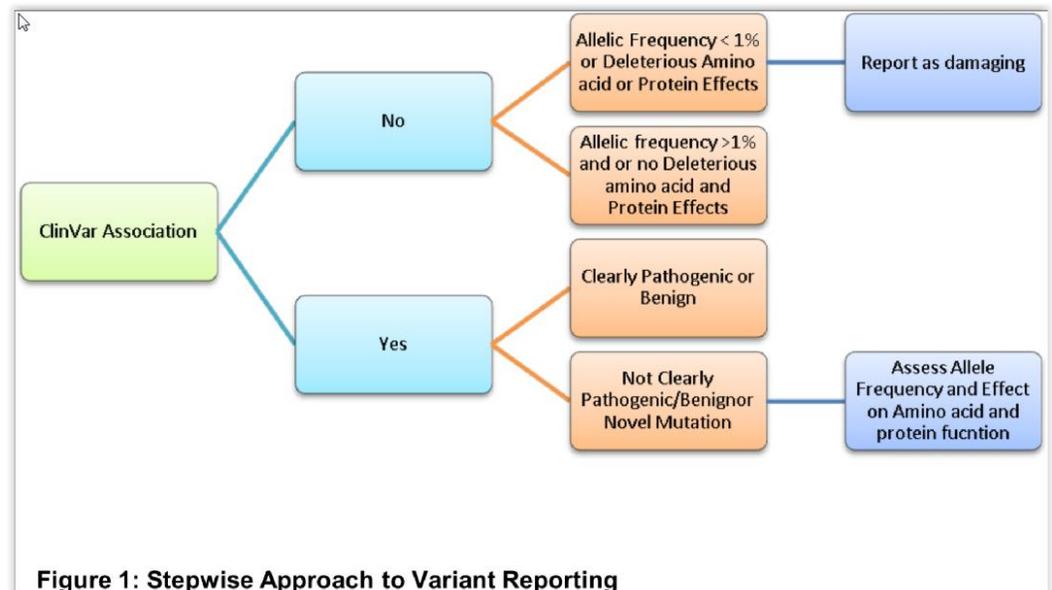


Figure 1: Stepwise Approach to Variant Reporting

Mutations that were novel or did not meet ClinVar criteria, as either clearly pathogenic or clearly benign, were investigated further. Mutations fulfilling the following internationally recommended criteria were considered likely to be pathogenic: 1) non-synonymous variant causing an amino acid change in a residue that is highly conserved among species and predicted to significantly damage the protein structure or function, and 2) the variant was rare in control populations utilizing the 1000 Genomes Project, the National Heart, Lung and Blood Institute Exome Sequencing Project and the Single Nucleotide Polymorphism Database with a cutoff allelic frequency of < 1%. Co-segregation analysis was not performed as our study collected tissue in an anonymous fashion.

Results

Patient Characteristics: Our sample population included 12 males and 2 females with an average age of 49 years and an average LVEF at presentation of 17%.



Baseline patient characteristics are shown in Table 1. Damaging cardiac gene variants were present in 12/14 patients in Table 2. Only 1 of the 12 patients with damaging cardiac gene variants improved their ejection fraction to greater than 45% post LVAD. Four of the 12 patients had the same damaging mutation in the SCN5A gene. Two of the 2 patients without mutations improved their ejection fraction to greater than 45%, p-value=.04.

Table 1: Patient Characteristics

Gender	M	12	87%
	F	2	14%
Family History	Y	6	43%
	N	7	50%
	Unknown	1	7%
Race	Caucasian	11	79%
	AA	2	14%
	Hispanic	0	0%
	Asian American	1	7%
Hypertension	Y	10	71%
	N	4	29%
Diabetes	Y	6	43%
	N	8	57%
Chronic Kidney Disease	Y	1	7%
	N	13	93%

Two of our patients had gene variants associated with ion channels, RYR2 and SCN5A. SCN5A or sodium channel, voltage gated, type V, alpha subunit belongs to a family of genes that provide instructions for making sodium channels. The phenotypic associations of this gene include Brugada syndrome, Romano-Ward syndrome and the sick-sinus syndrome. The RYR2 gene is also termed ryanodine receptor 2 (cardiac). This protein is responsible for the makeup of calcium ion channels. Phenotypic associations include catecholaminergic polymorphic ventricular tachycardia, as well as arrhythmogenic right ventricular cardiomyopathy (ARVC). Other gene variants included those at MYH7, MYH6, DSG2, DSC2 and DES. All are associated with the makeup of the sarcomere and cytoskeleton, and are associated with a range of cardiomyopathies including ARVD10, and various dilated cardiomyopathies.



Table 2: Damaging Cardiac Variants

Table 2 LVAD Damaging Cardiac DNA Variant Results							
Patient	Gene	Function	Chromosome	SNP Database #	Allele Ref - > ALT	Residue Change	
1	PSEN2	missense	chr1	rs58973334	G - A	R [Arg] ⇒ H [His]	
	SCN5A	missense	chr3	rs1805124	T - C	H [His] ⇒ R [Arg]	
2	DSG2	missense	chr18	rs121913013	G - A	V [Val] ⇒ M [Met]	
3	RYR2	missense	chr1	NA	A - C	K [Lys] ⇒ Q [Glu]	
4	CASQ2	missense	chr1	rs28730716	T - C	H [His] ⇒ R [Arg]	
	DES	missense	chr2	NA	C - T	R [Arg] ⇒ W [Try]	
	SCN5A	missense	chr3	rs1805124	T - C	H [His] ⇒ R [Arg]	
5	ANK2	missense	chr4	rs35530544	C - A	L [Leu] ⇒ I [Ile]	
	SCN5A	missense	chr3	rs1805124	T - C	H [His] ⇒ R [Arg]	
	DSG2	missense	chr18	rs2230234	A - G	I [Ile] ⇒ L [Leu]	
	CALR3	missense	chr19	rs142951029	T - C	K [Lys] ⇒ R [Arg]	
6	RYR2	missense	chr1	rs34967813	A - G	Q [Glu] ⇒ R [Arg]	
7	VCL	missense	chr10	NA	A - G	E [Glu] ⇒ G [Gly]	
	SGCD	missense	chr5	rs45559835	G - A	R [Arg] ⇒ Q [Gln]	
8	AKAP9	missense	chr7	NA	A - T	D [Asp] ⇒ V [Val]	
	DSC2	missense	chr18	rs61731921	T - G	R [Arg] ⇒ Q [Gln]	
10	SCN5A	missense	chr3	rs1805124	T - C	H [His] ⇒ R [Arg]	
	CACNB2	missense	chr10	rs58225473	T - G	D [Asp] ⇒ E [Glu]	
11	DES	missense	chr2	NA	A - C	Q [Glu] ⇒ P [Pro]	
12	ANK2	missense	chr4	rs28377576	T - C	V [Val] ⇒ A [Ala]	

Damaging oncogene mutations were present in 9 of the 14 patients in Table 3. These included the RET proto-oncogene, as well as NF1, a tumor suppressor gene. Other variants were identified in genes responsible for DNA repair. These include WRN, a gene that is responsible for an enzyme that can function as both a helicase and an exonuclease. This gene has the ability to unwind DNA, remove damaged segments and repair it. Phenotypic associations include multiple types of cancers. It is also interesting to note that this gene can be subject to methylation, a process where chemical modification of a gene is acquired over time via the addition of methyl groups. When the gene undergoes excessive methylation or hypermethylation, it is rendered non-functional. Other DNA repair genes include the FANCG gene, as well as the MLH1 gene.



Table 3: Damaging Oncogene Variants

Table 3 Damaging Cancer Gene Mutations							
Patient	Gene	Function	Chromosome	SNP Database #	Allele Ref - > ALT	Residue Change	
1	FANCC	missense	chr9	rs1800367	G - A	V [Val] → M [Met]	
	RET	missense	chr10	NA	G - A	V [Val] → M [Met]	
2	MLH1	missense	chr3	rs63751684	C - T	P [Pro] → L [Leu]	
3	MLH1	missense	chr3	rs1799977	A - G	I [Ile] → L [Leu]	
4	MLH1	missense	chr3	rs1799977	A - G	I [Ile] → L [Leu]	
	PTCH1	missense	chr9	rs73527759	C - G	D [Asp] → H [His]	
5	APC	missense	chr5	rs150973053	A - G	S [Ser] → G [Gly]	
6	MLH1	missense	chr3	rs1799977	A - G	I [Ile] → L [Leu]	
7	MSH2	missense	chr2	rs41295182	T - G	L [Leu] → R [Arg]	
	MLH1	missense	chr3	rs1799977	A - G	I [Ile] → L [Leu]	
8	MLH1	missense	chr3	rs1799977	A - G	I [Ile] → L [Leu]	
	TSC 1	frameshift	chr9	rs118203724	(-) - T	N [Asn] → K [Lys]	
	BMPR1A	missense	chr10	rs11528010	C - A	P [Pro] → T [Thr]	
9	TP53	missense	chr17	rs1042522	C - G	P [Pro] → R [Arg]	

Discussion

Our work suggests that damaging cardiac gene variants are highly prevalent in this advanced heart failure population. In patients undergoing LVAD therapy for non-ischemic dilated cardiomyopathy, 86% have underlying genetic damage to cardiac genes. Cardiomyopathy remains a leading etiology for cardiac transplantation as well as placement of left ventricular assist devices. In 2012, over 50% of cardiac transplants were done for cardiomyopathies. The cardiomyopathies are complex heterogeneous disorders. Previous studies have suggested that between 20 and 48% of patients with idiopathic dilated cardiomyopathies have familial disorders.(19, 20) (21) For dilated cardiomyopathies alone, mutations in over 40 different genes have been described. Within each gene many different mutations may occur, hence many mutations are relatively rare or unique within a family. Historically, cardiomyopathies have been classified and risk stratified according to clinical features. Advances in genetic sequencing now allow for broad testing and better classification within large populations.

While further studies are needed to characterize genetic interactions with clinical outcomes, our results suggest those patients with damaging cardiac gene variants have poor myocardial recovery with LVAD therapy. Of interest is our observation that many of our patients have multiple cardiac or oncogenic mutations, which suggest a multiple hit hypothesis in development of dilated cardiomyopathy. Utilizing genetic sequencing and bioinformatics algorithms, it may be feasible to identify poor responders to medical or device therapy earlier in their disease process. Transitioning these patients more rapidly towards



mechanical support or transplantation may improve outcomes long term. Conversely, identifying patients who could recover sufficient cardiac function with LVAD therapy, with appropriate ancillary medical therapy, could decrease the need for cardiac transplantation and improve utilization of medical resources. Guideline directed medical therapy for heart failure may improve myocardial recovery but this therapy is not without risks and the majority of patients do not respond. Genetic sequencing may further allow us to personalize heart failure treatment algorithms. As an example, a high proportion of patients in this study had damaging mutations in SCN5A. A positive response to Flecainide, a sodium channel blocker, has been observed in cohorts with damaging SCN5A mutations.(22) This is not a drug that we routinely use in the setting of advanced heart failure.

Damaging oncogene variants are also highly prevalent in this advanced heart failure population. Heart failure patients are at increased risk for developing cancer for reasons that are unclear. Advanced heart failure patients who undergo cardiac transplant are also at high risk for developing cancer. This may be related to inflammation, medications, or underlying genetic disorders. Our results suggest that that this patient population may also have a high prevalence of damaging variants in oncogenes. We may have an opportunity to “intervene” in our patient’s care earlier in both their cancer and cardiac disease processes. Cardio-Oncology is evolving as a specialty. The main focus has been on the diagnosis and management of cardiovascular diseases in patients with cancer. Cancer surveillance guided by genetic testing could be equally as important in this population of patients with heart failure.

Our study is not without limitations. This is a single center observational study. Our population was small in size and utilized targeted gene panels to sequence only genes previously associated with inherited diseases. This was done in an effort to look for gene variants that have the best supporting evidence for disease association, but we may miss other gene variants in this process. It is also of note that the concept of “variation” implies that there is variation from a reference genome, i.e., the human genome project. Variation does not always translate to disease causation. Variants of uncertain significance are found every day and add to the expanding knowledge of the human genome.

Conclusion

Damaging cardiac gene variants are common in non-ischemic dilated cardiomyopathy patients with advanced heart failure. The presence of damaging gene variation likely portends a poor prognosis and non-response to both medical and mechanical interventions. The absence of such mutation may predict response and possibly even recovery. Damaging variation in oncogenes is also a common finding in this population. This may help explain the finding of increased cancer prevalence in advanced heart failure. Larger, more controlled studies are needed to help guide therapeutic decisions and increase the precision of the care delivered to this most important population.



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