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Plasma Bin1 Correlates With Heart Failure And Predicts Arrhythmia In Patients With Arrhythmogenic Right Ventricular Cardiomyopathy

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Plasma BIN1 correlates with heart failure and predicts arrhythmia in patients with
arrhythmogenic right ventricular cardiomyopathy

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ABSTRACT

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a disorder characterized by fibrofatty replacement of the right ventricular myocardium and associated arrhythmias that originate from the right ventricle. BIN1 is a membrane-associated protein that is involved in cardiac T-tubule homeostasis and is down-regulated in cardiomyopathy. BIN1 has also been shown to be essential to the differentiation of myoblasts and the induction of skeletal muscle T-tubule invagination.

We hypothesized that BIN1 is released into the circulation and that circulatory BIN1 would provide useful cardiac monitoring data on patients suffering from heart failure in ARVC. The objective was to determine whether plasma BIN1 can measure disease severity in patients with ARVC.

The study presented is a retrospective cohort of 24 patients with ARVC. Plasma BIN1 levels were quantified and analyzed against clinical data to determine its ability to predict cardiac functional status and ventricular arrhythmias. Mean plasma BIN1 levels were decreased in ARVC patients compared to controls (37 ± 1 vs. 60 ± 10, $p < 0.05$). In a cross-sectional analysis, ARVC patients with heart failure had lower BIN1 (15 ± 7 vs. 60 ± 17 in patients without heart failure, $p < 0.05$). BIN1 levels correlated inversely with ventricular arrhythmia burden ($R = -0.46$, $p < 0.05$), and low BIN1 correctly classified patients with advanced heart failure or ventricular arrhythmia (ROC Area under the curve, AUC, 0.88 ± 0.07).

Low BIN1 also predicted future ventricular arrhythmias (ROC AUC 0.89 ± 0.09). In a stratified analysis, BIN1 predicted future arrhythmias in patients without severe heart failure to an accuracy of 82%. In ARVC patients with serial blood draws who had evidence of disease progression during follow up, plasma BIN1 decreased by 63% ($p < 0.05$).

In conclusion, plasma BIN1 correlates with disease severity in patients with ARVC and predicts future ventricular arrhythmias.

ACKNOWLEDGEMENTS

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INTRODUCTION

ARVC

ARVC was first described in 1977 as an anatomical and clinical entity[1] characterized by the replacement of cardiomyocytes by adipose and fibrous tissue that results in recurrent ventricular arrhythmias and right and/or left ventricular dysfunction[2]. Historically, it was originally believed to be the result of a developmental defect in the right ventricular myocardium and was thus originally categorized as a dysplasia[3], though these views have since changed.

The first patients presented with advanced disease, as early disease is usually asymptomatic. These patients were vulnerable to symptomatic arrhythmias originating from the right ventricle, as well as a higher risk of sudden cardiac death. The arrhythmias range from isolated premature ventricular beats to sustained ventricular tachycardia (VT) or fibrillation (VF) , and were most often triggered by effort[4]. Arrhythmogenicity can be present with or without the classic fibrofatty myocardial changes on histology, which occurs transmurally in the right ventricle and localizes to the inflow tract, outflow tract, or right ventricular apex, leading to the progressive loss of right ventricular function. Interestingly, despite being coined a right ventricular disorder, the fibrofatty replacement eventually spreads to the ventricular septum and the left ventricle, resulting in biventricular failure. Left ventricular involvement occurs more frequently than previously thought, with recent reports of up to 76% of cases showing left ventricular pathology[5].

ARVC is a familial disorder, and occurs at a prevalence of approximately 1 in 2000 to 1 in 5000, affecting men more often than women at a ratio of 3:1[6]. It is most often inherited in an autosomal dominant fashion with both reduced penetrance and variable expression, but can occasionally be seen as an autosomal recessive disorder[7, 8]. The majority of ARVC phenotypes are caused by mutations in desmosome function[9], but have also been found to arise from non-desmosomal proteins such as TGF-B3 (a cytokine), RyR2 (which releases calcium from the myocardial sarcoplasmic reticulum), and

transmembrane protein 43 (an adipogenic transcription factor). The following are the known mutations of

ARVC:

Table A. Mutations in ARVC (from OMIM: Online Mendelian Inheritance in Man)

ARVC type	Chromosome/locus	Gene codes	Mode of transmission
ARVC 1	14q23-q24	TGFB-3	Autosomal dominant
ARVC 2	1q42-q43	RyR2	Autosomal dominant
ARVC 3	14q12-q22		Autosomal dominant
ARVC 4	2q32		Autosomal dominant
ARVC 5	3p23	Transmembrane protein 43	Autosomal dominant
ARVC 6	10p12-p14		Autosomal dominant
ARVC 7	10q22		Autosomal dominant
ARVC 8	6p24	Desmoplakin (DSP)	Autosomal dominant
ARVC 9	12p11	Plakophilin-2 (PKP2)	Autosomal dominant
ARVC 10	18q12	Desmoglein-2 (DSG2)	Autosomal dominant
ARVC 11	18q12.1	Desmocollin-2 (DSC2)	Autosomal dominant
ARVC 12	17q21	Plakoglobin (JUP)	Autosomal dominant
Naxos Disease	17q21	Plakoglobin (JUP)	Autosomal dominant

Diagnosis has refined significantly over the years. The original diagnostic criteria were published

in 1994 and were based on echocardiographic, histological, electrocardiographic, and family history

findings[10]. These criteria changed recently in 2010, with modifications made to take into account

advances in genetics and diagnostic modalities[11]. The current diagnostic criteria include:

I. Global or regional dysfunction and/or structural alteration as determined by 2D echocardiogram, MRI, or RV angiography

II. Less than 60% residual myocytes by morphometric analysis, with or without fatty replacement of tissue on endomyocardial biopsy

III. Repolarization abnormalities on electrocardiogram in the form of inverted T-waves in the right precordial leads or beyond individuals greater than 14 years of age in the absence of complete RBBB

IV. Depolarization/conduction abnormalities on electrocardiogram in the form of an epsilon wave; i.e., reproducible low-amplitude wave forms between the QRS and T waves

V. History of non-sustained or sustained ventricular tachycardia

VI. Family history of ARVC confirmed in a first-degree relative by the current criteria, by pathologic examination, or identification of a causative mutation

Though our genetic understanding and diagnostic techniques have advanced considerably throughout the years, risk stratification remains fairly primitive. Patients are generally stratified based on individual patient history and presentation, which remains a profound limitation on the prevention of adverse events, especially considering how catastrophically the patients can present. Those who are considered high risk for arrhythmic death include patients who have been resuscitated from sudden cardiac death, very young patients, patients who have syncoped, and left ventricular involvement[12]. Other clinical signs of high risk disease includes signs of right heart failure or left ventricular dysfunction or history of VT[13].

Several invasive indicators have also been described, such as inducible VT, drug failure during several electrophysiologic studies, and RV dilation[14]. The last ten years have seen promising non-invasive stratifiers emerge, including QRS dispersion > 40 ms or QT dispersion > 65 ms. However, even considering these advances, it is still difficult to predict future arrhythmia burden in ARVC patients or

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which of the patients will develop progressive heart failure. A non-invasive test that correlates with heart health and predicts risk of clinical progression would be of considerable clinical utility.

BIN1

Bridging Integrator 1 (BIN1) is a member of the BAR domain superfamily whose gene encodes multiple alternately spliced membrane adaptor proteins[15-17]. It was originally identified in a 2-hybrid screen for proteins that interact with the MYC oncoprotein, and was eventually found to have tumor suppressor activity by inhibiting MYC[18]. It was later described to have structural similarities to the breast cancer-associated autoimmune antigen Amphiphysin (AMPH), including a nuclear localization signal, an SRC homology-3 (SH3) domain, a MYC-interacting domain, and a BAR (Bin1/Amphyphysin/RVS167) domain[19]. The BIN1 gene product expresses in several different tissues in the body, including prostate, brain, bone marrow, peripheral lymphoid tissues, skin, breast, gastrointestinal epithelium, and skeletal myocytes[20]. The gene itself is comprised of twenty different alternatively spliced exons that can produce at least ten different isoforms.

Originally believed to be a gene involved in malignancy and cell cycle control, BIN1 was found to be down-regulated in 14 of 27 carcinoma cell lines and 3 of 6 primary breast tumors[19]. It was later determined that BIN1 had a function in myoblast differentiation: upregulation of the BIN1 protein and mRNA were associated with differentiation of murine myoblasts, whereas BIN1 localized exclusively to the nucleus in undifferentiated cells. These overexpressor-differentiators grew slower than control myoblasts and differentiated rapidly[19]. In differentiated striated muscle, BIN1 is concentrated at T-tubules, and was found to be sufficient to induce tubular plasma membrane invaginations. In summary, a new role for BIN1 has been described in skeletal muscle cells: it is found to express in high levels during the differentiation of skeletal myocytes, and to be sufficient to induce T-tubule formation in non-myocytes[21].

These basic findings have found clinical correlates. In 2011, it was demonstrated that alternative splicing of BIN1 was disrupted in skeletal myocytes derived from patients with myotonic dystrophy[19]. Specifically, a loss of exon 11 correlated with little or no T-tubule formation in culture myocytes; consequently, it was postulated that the phosphatidylinositol 5-phosphate-binding site contained in exon 11 was necessary for BIN1's membrane tubulating activity. Predictably, expressing the abnormal BIN1 variant in mice resulted in abnormal T-tubules and decreased muscle strength.

More recently, BIN1's influence has also been found to extend to cardiomyocytes. Our group has found that BIN1 is necessary for localizing the delivery of L-type calcium channels to T-tubules in cardiac T-tubules[22], a function that is important for maintaining normal calcium transients and myocardial contractility[23, 24]. Furthermore, our studies show that heart failure results in a reduction of the transcription and total BIN1 content in cardiomyocytes[25]. An important structural feature of BIN1 is that it is a membrane attached protein, which makes it susceptible to release from the cell via membrane turnover. Many cells in the body such as platelets[26, 27], leukocytes[28], and endothelial cells[29] release small vesicles of membrane together with membrane-associated proteins into the blood as microparticles. These particles and their associated proteins have the potential for becoming biomarkers of cardiovascular disease[31-33]. The reduction of cardiac BIN1 in failing hearts[25] and its existence as a membrane associated protein[22] prompted us to explore whether BIN1 is available in the blood in patients with failing hearts and whether plasma BIN1 is a direct measure of cardiomyocyte health.

Our first cohort of patients with failing hearts was that of patients with ARVC. The ARVC population has several advantages. Progressive fibrosis and loss of cardiomyocytes in ARVC, even if subclinical, will result in reduction of cardiac origin BIN1, which could be reflected in the blood levels of this protein. In addition, ARVC patients typically have less non-cardiac involvement in their disease process[34, 35] relative to most heart failure patients, have received full clinical work-ups, and are followed closely. Also, the occurrence of arrhythmia in these patients presents the opportunity to assess clinical

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outcomes distinct from heart failure. Therefore, we tested the hypothesis that plasma BIN1 levels would correlate with disease severity and predict future arrhythmia events in patients with ARVC. The results demonstrate that plasma BIN1 can assay cardiac health, with prognostic insight into both heart failure progression and development of ventricular arrhythmias

STATEMENT OF PURPOSE

The intent of this work is to determine whether serum BIN1 can be used to measure disease severity in patients with ARVC.

HYPOTHESIS AND SPECIFIC AIMS

Similar to how intracellular BIN1 varies with heart failure, plasma BIN1 is predicted to measure overall cardiomyocyte health in ARVC.

The specific aims are to:

1) Determine if BIN1 is detectable human plasma.

2) Determine the relationship between plasma BIN1 levels and ARVC.

METHODS

Patients

The study was approved by the institutional review boards of the hospitals of University of California San Francisco (UCSF) and Johns Hopkins University (Western IRB, with clinical data collected via a Johns Hopkins protocol). All participating patients gave informed consent. The cohort consisted of thirty-one patients with a diagnosis of ARVC. The majority of the patients in this cohort (29/31) were established patients in the electrophysiology clinics at the time of the blood draw. Two patients had blood drawn at the referral visit. Many patients were referred to these clinics by outside cardiologists or after ARVC was diagnosed in a family member. Several patients were hospitalized at UCSF or Johns Hopkins after an initial arrhythmic event. All ECGs and echocardiograms were performed at UCSF and Johns Hopkins. If an MRI, angiogram, or right heart biopsy was performed at an outside facility, the primary data was reviewed at UCSF or Johns Hopkins in order to establish the diagnosis of ARVC.

Patients were included in the study if a diagnosis of ARVC could be confirmed with available data according to the modified International Task Force Criteria[35] by two separate cardiologists blinded to the assay results. Exclusion criteria included inadequate data to confirm diagnosis or presence of ventricular tachycardia (VT) storm or several defibrillation events at the time of the blood draw, which might be expected to cause spurious BIN1 measurement.

Of the initial 31 patients with a possible diagnosis of ARVC, 24 were included in the final analysis. Three (3) patients were excluded based on incomplete data and three (3) for failure to meet diagnostic criteria. One (1) patient with active VT storm and several defibrillation events on the day of the lab draw was also excluded. The patient characteristics are displayed in Table 1.

A control group consisted of 48 age, sex, and BMI-matched healthy controls without diabetes, hypertension or history of cardiac disease. These controls were identified from a large UCSF database containing clinical data and plasma specimens from healthy volunteers. All participants gave consent to future use of these specimens.

Measurement of plasma BIN1

Venous blood samples were drawn between April 2004 and January 2011. Seven patients had two blood samples drawn at separate time points. All follow up blood samples were drawn during routine clinical care. The samples were obtained and centrifuged at 4000 rpm for 20 minutes at 4ºC, and plasma stored in -80ºC freezer for later ELISA analysis.

A commercial ELISA reagent set (BD Bioscience, San Diego, CA) was used to design a highly-sensitive ELISA test to quantify BIN1 in human plasma. This assay system uses two antibodies against human BIN1. A mouse monoclonal anti-BIN1 (1:500 in coating buffer, Sigma, St. Louis, MO) was used as the capture antibody and a primary goat anti-BIN1 antibody (1:500 in 1% BSA, Everest, Clifton, NJ) was used as the detection antibody. BIN1 standards were generated from lysates of 293FT cells overexpressing exogenous human BIN1. A standard curve ($R2>0.98$) was generated to determine the relative amount of BIN1 in each sample. The determined plasma concentration of BIN1 of each individual was normalized to and expressed as percent of BIN1 value in plasma pooled from three 25 year-old healthy male adults. This assay is highly reproducible with an intra-assay variability of < 5%. Plasma BIN1 levels were then measured in duplicate from all ARVC and control samples. For patients with serial blood draws, the earliest sample drawn was used for the cross-sectional analysis.

Measurement of baseline characteristics and outcomes in ARVC patients

All clinical data were obtained by persons blinded to the BIN1 assay results. Baseline New York Heart Association (NYHA) class, RV and LV function, and ventricular arrhythmia history were recorded

for each patient. NYHA class was obtained from chart review, RV and LV function were determined by echocardiography. Right ventricular function was recorded as normal or mild, moderate or severe dysfunction. Ventricular arrhythmia history was obtained from chart review and ICD interrogation data.

To quantify each patient's number of arrhythmia events, the following system was used. For patients who had an ICD placed for secondary prevention (n=10) due to history of aborted sudden cardiac death (hemodynamic collapse associated with sustained ventricular tachycardia/ventricular fibrillation) or sustained VT (>30 seconds) observed on Holter monitor or in an inpatient setting, the index event was counted as one event. Once an ICD was placed, (n=23), subsequent appropriate ICD therapy for sustained VT or ventricular fibrillation (VF) was counted as one event (even if several therapies were required for termination), and hospitalization for persistent VT or VT storm was weighted as 1.5 events to account for the more severe clinical manifestation. For patients with serial draws, NYHA class, interval arrhythmia history and LV and RV function at the time of the second lab draw were also recorded. New right ventricular dysfunction was defined as an interval change from normal to any degree of RV dysfunction (mild, moderate, or severe). Worsening right ventricular dysfunction was defined as any increase in RV dysfunction grade.

Statistics

Prism 5 software (GraphPad) was used for all statistical analysis. Data are expressed as mean ± SE. A two-tail non-parametric Mann Whitney Test was used for comparison of BIN1 between the control and ARVC groups. For comparison of BIN1 between heart failure patients and healthy controls, a two-tail student's t-test was used with Welch's correction for significant different variances from the two groups. To test for correlations between BIN1 and baseline continuous variables, Spearman analyses were performed. Non-parametric Receiver-Operator Curve (ROC) analyses was used to determine the sensitivity and specificity of BIN1 values to diagnose patients with severe baseline disease and to test for

prediction of future arrhythmia events. For comparison of serial BIN1 levels within the same patients, a paired two-tail student's t-test was performed.

RESULTS

Patient characteristics and baseline BIN1 levels

There were no statistically significant differences between the 24 ARVC patients and 48 controls with respect to age, gender, or BMI (Table 1). Of the 24 ARVC patients, 21 met at least 2 major criteria and 3 patients met 1 Major and 2 minor criteria for diagnosis. Eight (33%) had a confirmed family history of ARVC, defined as either autopsy confirmation of ARVC or clinically confirmed diagnosis of ARVC in a family member. Of the patients who underwent genotype analysis, 11/15 (73%) were gene positive (Table 1).

At the baseline blood draw, 16 (70%) had at least one ventricular arrhythmia event, 7 (30 %) were NYHA class II or greater, and 4 (18%) were NYHA class III or IV. Four patients (18 %) had moderate to severe RV dysfunction. Twenty-three of the 24 patients had ICDs, with an average length of follow up after ICD placement of 4.4 ± 4.3 years to the time of the initial blood draw. Of the patients with ICDs, 13 (57 %) were placed for primary prevention and 10 (43%) were placed for secondary prevention.

Plasma BIN1 did not significantly correlate with age, sex or BMI in the ARVC or control populations. As the metabolism kinetics and clearance of plasma BIN1 protein is not yet known, we analyzed plasma BIN1 against GFR to exclude the possibility of BIN1 changes with renal dysfunction. We found that BIN1 is not associated with renal function (Table 2). Furthermore, the mean plasma BIN1 level in the ARVC population was 37 ± 1 with a median value of 17, as compared to controls with a mean of 60 ± 10 and a median of 27 ($p < 0.05$) (Table 1).

Cross-sectional analysis of BIN1 in patients with heart failure and arrhythmia

Within the ARVC population, we explored measured BIN1 against the absence (NYHA class I) or presence (NYHA class II-IV) of symptomatic heart failure. In ARVC patients with symptomatic heart failure, the mean BIN1 level was 15 ± 7 (n=7), whereas in ARVC patients without clinical heart failure the mean BIN1 level was 60 ± 17 (n=15), ($p < 0.05$). Results are in Figure 1. Note plasma BIN1 levels

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were not different between control patients without failing hearts, and ARVC patients who did not have heart failure. Spearman analyses were performed to assess for correlation of plasma BIN1 with baseline continuous clinical variables (Table 2). For patients with multiple samples, the first sample was used in this analysis. Plasma BIN1 levels inversely correlated with *number* of accumulated ventricular arrhythmia events (Rho of -0.60, p < 0.01) up to the point of the first plasma measurement (Table 2), but not with the length of ICD follow up, indicating an effect separate from an implanted ICD. To further control for the length of ICD implantation in each patient, the plasma BIN1 was analyzed against the *average* annual number of ventricular arrhythmia events. Consistently, plasma BIN1 inversely correlates with the average annual number of ventricular arrhythmia events (Rho of -0.46, p <0.05), independent of the presence of an ICD.

To assess the ability of plasma BIN1 to distinguish between patients with severe and mild ARVC, a Receiver-Operator-Curve analysis was performed (Figure 2). Severe ARVC was defined as either NYHA class III/IV heart failure or >1 ventricular arrhythmia event, and by default, mild ARVC was defined as NYHA class I/II and ≤1 ventricular arrhythmia event. At a cutoff value of less than 33, plasma BIN1 had an 82% sensitivity and an 82% specificity for predicting NYHA class III/IV heart failure status or >1 ventricular arrhythmia event (ROC AUC of 0.88 ± 0.07). Thus low plasma BIN1 correlates with the occurrence of severe ARVC.

Analysis of plasma BIN1 as a predictor of future arrhythmia events

Mean follow up after initial blood draw in the ARVC cohort was 3.3 ± 1.7 years. 23 of the 24 ARVC patients had ICDs. An event was defined as VT or VF requiring termination. BIN1<30 predicted a high future arrhythmia rate (>0.5 arrhythmia events/year, Figure 3) with a sensitively of 83%, specificity of 88% and an accuracy of 85 % (ROC AUC of 0.89 ± 0.09). Of the 23 patients with ICDs implanted, there were only 3 "failures" of the BIN1 test to classify patients in the appropriate high or low risk group. Two patients with BIN1 <30 did not have a high arrhythmia rate, and one patient with a BIN1 >30

continued to have high arrhythmias. Given the observed correlation of BIN1 with baseline heart failure and the known increase risk of arrhythmias in patients with heart failure, a stratified analysis was performed according to heart failure status at baseline. In patients with NYHA class I or II (n=20), BIN1< 30 predicted future arrhythmia event rate with a sensitivity of 83%, specificity of 80 % and an accuracy of 82 % (ROC AUC of 0.82 ± 0.14). In patients with NYHA class I (n=17), 8 of 17 had no history of ventricular arrhythmias and 10 of 17 had ICDs placed for primary prevention. A BIN1<30 predicted a future high arrhythmia event rate with a sensitivity of 82%, specificity 67% and an accuracy of 79% (ROC AUC of 0.76 ± 0.21). NYHA class II or greater heart failure at baseline alone conferred an unadjusted relative risk of 3.61 (95 % CI 1.25-10.37, p<0.05) for high future arrhythmia events (heart failure follow-up are not available).

BIN1 in ARVC patients with serial blood draws

Serial blood samples were available in seven ARVC patients and three controls (Figure 4). Overall, BIN1 values decreased in patients with progressive ARVC. Patient 1 developed new RV dysfunction and had her first episode of VF. Patient 2 developed worsening RV dysfunction, and had multiple VF events. Patient 3 had a continued high arrhythmia burden (3.8 events/year) and developed new RV dysfunction. At the time of the initial blood draw, patient 4 did not meet criteria for ARVC, however during follow up the patient developed new T wave inversion in ECG leads V1-V3 and an epsilon wave in V1, palpitations with a high non-sustained VT burden on Holter monitor. She has since had an ICD placed for confirmed ARVC but has not had sustained VT or VF, heart failure, or ventricular dysfunction. Patient 5 had a decrease in LV function from 70% to 49% and the progression from mild to moderate RV dysfunction. Patient 6 had mild RV dysfunction at baseline, but developed moderate RV dysfunction and 5 separate ventricular arrhythmia events. Patient 7 developed mild RV dysfunction during follow up and has had high ventricular arrhythmia rate (2.1events/year). In sum, patients with clinical progression had marked decreases in their plasma BIN1 levels (decrease of 63%, p<0.05). In

contrast, in serial samples from the three healthy controls (Figure 4), there was no significant change in

BIN1 levels over a two year interval.

DISCUSSION

In this study we describe a novel assay for plasma BIN1 and demonstrate that a single measurement of this protein can categorize disease severity in patients with ARVC and predict future arrhythmias with excellent test characteristics. Our data show that lower plasma BIN1 levels occur in patients with clinical heart failure and correlate with a high burden of ventricular arrhythmias. Furthermore, in patients without heart failure, BIN1 predicts a high future arrhythmia rate with a sensitivity of 82% and a specificity of 67%. Plasma BIN1 is not affected by renal function, age, gender, or BMI. In ARVC patients with serial blood draws, plasma BIN1 decreased over time or remained low. Taken together, the data indicate that BIN1 correlates with cardiac health and inversely with arrhythmogenic potential.

It remains unclear why plasma BIN1 is lower in patients with more severe disease, but one possible explanation is that BIN1 expression correlates with cardiomyocyte or volume in the heart, and the loss of cardiomyocytes in ARVC after fibrofatty replacement results in significant reductions in the circulatory release of BIN1 into the myocardium. An interesting experiment that would suggest this would be to examine chronic circulatory BIN1 levels in patients who have undergone myocardial infarction; presumably, these patients would also have lower BIN1 levels due to decreased numbers of cardiomyocytes, though this idea has yet to be explored. A second possibility is that the cardiomyocytes may simply be producing less BIN1, as it has been demonstrated that diseased cardiomyocytes generate less BIN1 protein overall[25]. A third contributing factor could be that diseased cardiomyocytes may be metabolically quiescent and turn over membrane protein at a slower rate. Cell based studies will be important to determine which of these three possibilities is the major reason that plasma BIN1 is reduced in patients with diseased hearts. A fourth possibility is that the alteration of other serum proteins in chronic heart failure may alter the half-life of circulatory BIN1, either by protein stability or ubiquitination pathways.

An intriguing result of this study is that low BIN1 predicted a risk of ventricular arrhythmia even without the presence of advanced heart failure. We cannot rule out that arrhythmias occurred secondary to the new development of heart failure, but a more likely scenario is that low BIN1 can correlate to myocardium with a heterogeneous distribution of disease which would have a more arrhythmogenic than mechanical consequence. While arrhythmogenicity of ARVC is frequently ascribed to loss of ventricular desmosomal proteins and subsequent impairment of Cx43 based gap junction coupling, heterogeneity of gap junction coupling is critical to the occurrence of unidirectional block and initiation of reentrant arrhythmias[36]. ARVC classically involves non-homogeneous involvement on myocardium, and therefore reduced BIN1 can detect foci of diseased and uncoupled heart while overall contractile function is preserved.

Furthermore, reduction in BIN1 may correlate with arrhythmogenesis independent of Cx43 expression. As a T-tubule associated protein, BIN1 is not a component of the cardiac desmosome. It is increasingly recognized that ARVC arrhythmogenecity may include non-desmosomal proteins[37], altered intracellular calcium handling[38, 39] and altered stress-related signaling cascades[40]. Thus BIN1-detectable pathologic remodeling of the cardiomyocyte (heterogeneous or otherwise) may indicate pathologic cardiomyocyte remodeling prior to loss of cardiomyocyte contractile function.

Our study has several potential limitations. There exist several splice variants of BIN1[41] that have been described in an array of cellular functions[42], and our current assay may include detection other non-cardiac BIN1 as well. However, the total plasma BIN1 was reduced in patients with clinical heart failure/ventricular dysfunction and/or electrophysiologic instability, indicating a cardiac health dominated BIN1 plasma measurement. Our primary concern was whether BIN1 from skeletal myocytes were confounding our results; as such, we controlled for BMI, age, and gender. We have not precluded the possibility that other confounding variables may exist. A potential solution to the specificity of BIN1

detection is that a cardiac specific splice variant of BIN1 may exist, which would substantially increase the utility of the test as a marker for cardiovascular health.

With regard to the limitations from the study population, the ARVC cohort used in this study is more severely affected compared to other larger described cohorts[34]. Use of larger ARVC cohorts will help validate the clinical utility of a BIN1 assay.

Our findings indicate that a single measurement of BIN1 in blood can categorize disease severity and predict future arrhythmia events in patients with ARVC. A well-designed prospective study of a larger group of patients will be necessary to validate this assay in ARVC disease monitoring and use in guiding treatment strategies.

FIGURE LEGEND

Figure 1. Plasma BIN1 is lower in ARVC with HF. Plasma BIN1 (mean±SE) is significantly lower in ARVC patients with symptomatic heart failure (15±7, NYHA II-IV, N=7), as compared to ARVC patients without heart failure (60±17, NYHA I, n=15). P<0.05. (Plasma BIN1 is expressed as percent of BIN1 in plasma pooled from three healthy 25 year old males.)

Figure 2. Plasma BIN1 predicts disease severity. The Receiver-Operator-Curve analysis indicates that low plasma BIN1 predicts severe ARVC defined as either NYHA class III/IV or >1 ventricular arrhythmias. Plasma BIN1<33 (indicated by red arrow) had an 82% sensitivity and an 82% specificity for predicting patients with NYHA class III/IV heart failure or >1 ventricular arrhythmia event (ROC AUC of 0.88, SE +/- 0.07, P<0.01).

Figure 3. Plasma BIN1 predicts future ventricular arrhythmias. The Receiver-Operator-Curve analysis indicates that low plasma BIN1 predicts high future ventricular arrhythmias. Plasma BIN1<30 (indicated by red arrow) predicted a high future arrhythmia rate (>0.5/year) with a sensitively of 83%, specificity of 88% and an accuracy of 85 % (ROC AUC of 0.89, SE +/- 0.09, P<0.01).

Figure 4. Plasma BIN1 decreases with ARVC progression. Serial plasma BIN1 values are shown in controls (black circles, n=3) and in ARVC patients (red squares, n=7). The plasma BIN1 remained similar in healthy controls (mean±SE from 65±20 to 72±28, NS) while experienced a significant reduction (63% decrease) in ARVC patients during disease progression (mean±SE from 80±32 to 30±17, p<0.05). BIN1 values for ARVC patients were as follows (first draw, second draw):   patient 1 (237, 130), patient 2 (145, 130), patient 3 (74, 35), patient 4 (52, <5), patient 5 (33, 16), patient 6 (10, 8), patient 7 (8, 9). (Plasma BIN1 is expressed as percent of BIN1 in plasma pooled from three healthy 25 year old males.)

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Table I: Baseline Characteristics of ARVC Patients and Controls

Characteristics	ARVC Patients	Controls	p Value
	(n= 24)	(n=48)	
BIN1(mean, median, SD)			
Mean	37.1 +/-10	60 +/- 10	<0.05
Median	17	27	
Age (yrs)*	43 +/- 15	47+/- 13	0.470
Sex			
Male	13	26	
Female	11	22	
Body Mass Index *	25.4 +/- 5.9	23.6+/1	0.200
History			
Family history positive	8 (33%)		
Rhythm			
History of any sustained ventricular arrhythmias	16 (70%)		
Avg. length of ICD follow up *	4.4 +/- 4.3		
ICD placed	23 (96%)		
Primary prevention	13 (57%)		
Secondary prevention	10 (43%)		
Heart Failure			
NYHA class II or greater	7 (30 %)		
NYHA class III or IV	4 (18%)		
Moderate to severe RV dysfunction	4 (18%)		

* Plus-minus value is the mean +/- SD.

Table 2: BIN1 Association with Baseline Characteristics in ARVC patients

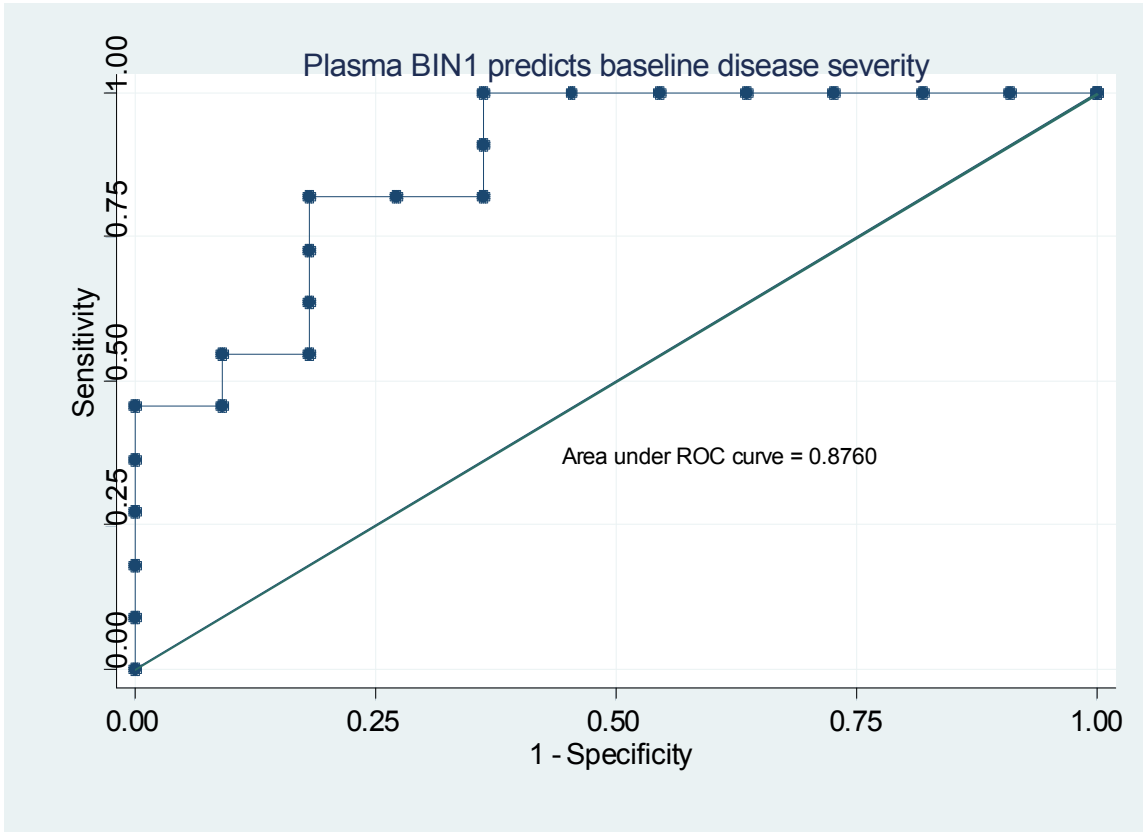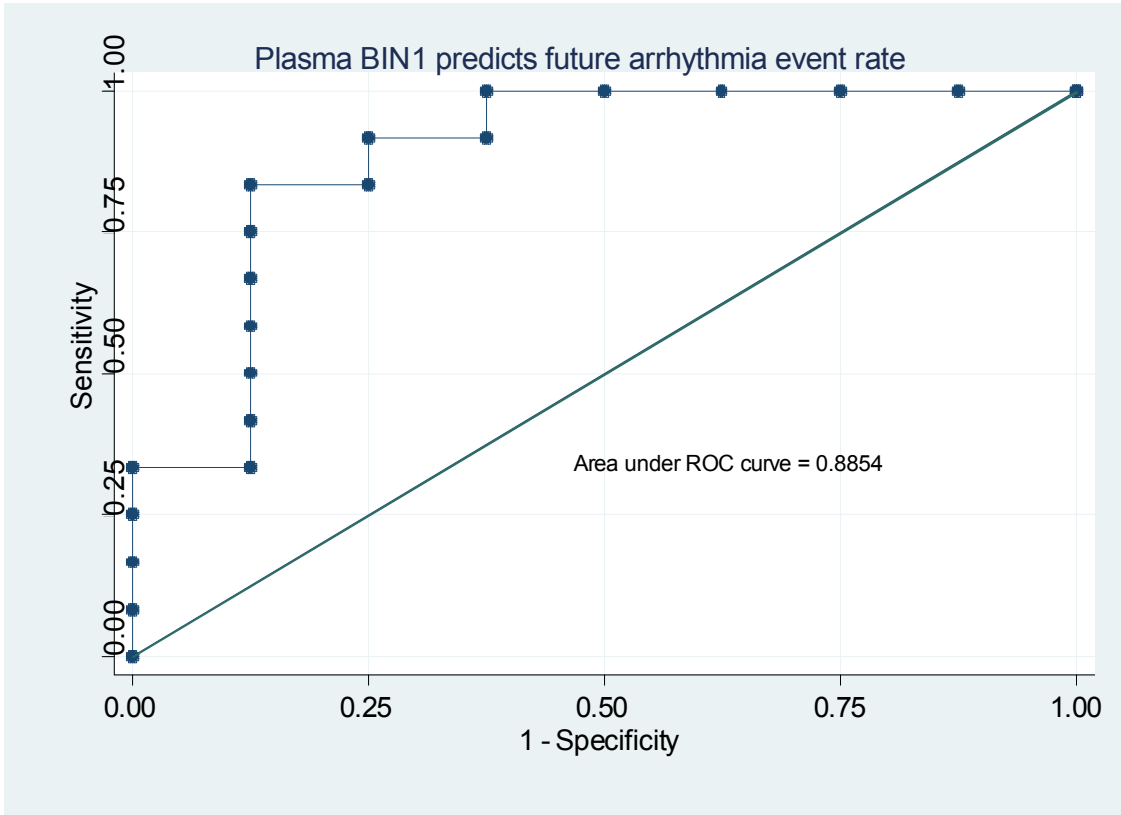| Continous Variables	Spearman's Rho	p Value
Total ventricular arrythmias to time of plasma draw	-0.590	<0.005
Ventricular arrhythmia rate to time of plasma draw (events/yr.)	-0.460	<0.05
Length of diagnosis	0.090	0.702
ARVC		
Body Mass Index	0.050	0.702
Age	-0.250	0.249
GFR	0.079	0.781
Controls		
BMI	0.019	0.902
Age	-0.138	0.350
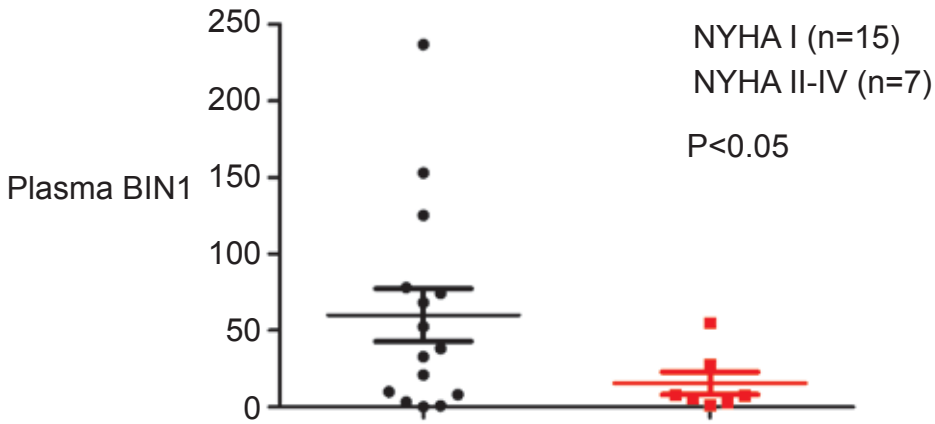
Figure 1

Figure 2
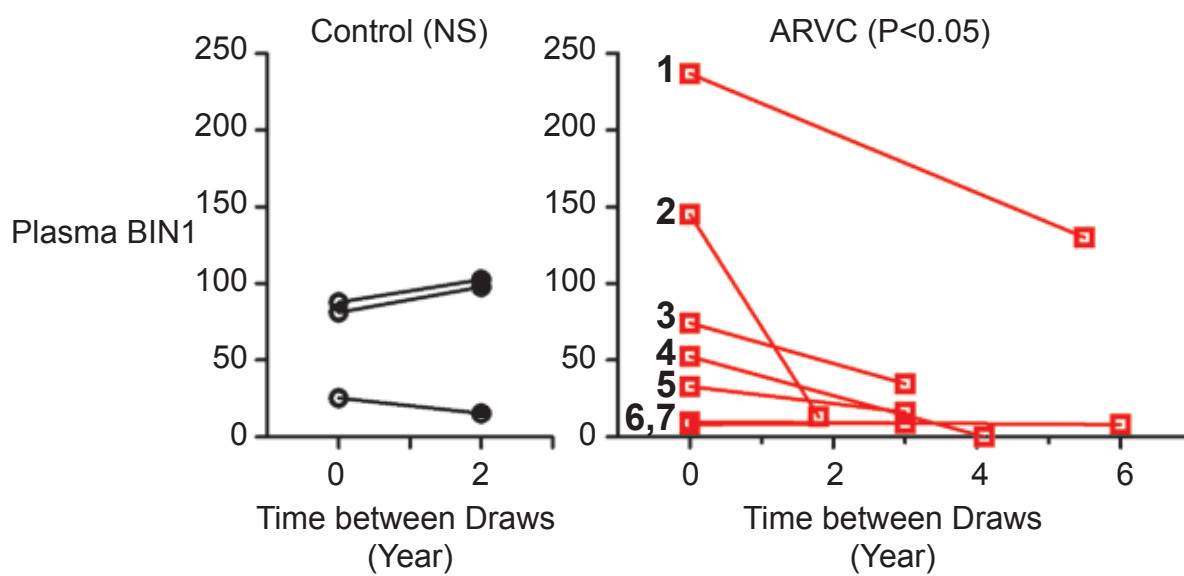
Figure 3

Plasma BIN1 decreases with ARVC progression

Figure 4