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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

Background—Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a disorder involving diseased cardiac muscle. BIN1 is a membrane associated protein important to cardiomyocyte homeostasis and is down regulated in cardiomyopathy. We hypothesized that BIN1 could be released into the circulation and that blood-available BIN1 can provide useful data on the cardiac status of patients whose hearts are failing due to ARVC.

Objective—To determine whether plasma BIN1 can measure disease severity in patients with ARVC.

Methods—We performed a retrospective cohort study of 24 patients with ARVC. Plasma BIN1 levels were assessed for their ability to predict cardiac functional status and ventricular arrhythmias.

Results—Mean plasma BIN1 levels were decreased in ARVC patients with heart failure (15 ± 7 vs. 60 ± 17 in patients without heart failure, $p < 0.05$; plasma BIN1 is 60 ± 10 in non-ARVC normal controls). BIN1 levels correlated inversely with ventricular arrhythmia ($R = -0.47$, $p < 0.05$), and low BIN1 correctly classified patients with advanced heart failure or ventricular arrhythmia (ROC Area under the curve, AUC, of 0.88 ± 0.07). Low BIN1 also predicted future ventricular arrhythmias (ROC AUC of 0.89 ± 0.09). In a stratified analysis, BIN1 could predict future arrhythmias in patients without severe heart failure ($n = 20$) with an accuracy of 82 %. In the seven ARVC patients with serial blood samples, all of whom had evidence of disease progression during follow up, plasma BIN1 decreased significantly (decrease of 63 %, $p < 0.05$).

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Conclusions—Plasma BIN1 seems to correlate with cardiac functional status and presence or absence of sustained ventricular arrhythmias in a small cohort of ARVC patients and can predict future ventricular arrhythmias.

Keywords

ARVC (arrhythmogenic right ventricular cardiomyopathy); BIN1 (Bridging Integrator 1); heart failure; ventricular arrhythmia; membrane scaffolding protein; calcium transient

INTRODUCTION

ARVC is a disease characterized by focal or diffuse fibrofatty replacement of the ventricular myocardium, which can result in recurrent ventricular arrhythmias and right and/or left ventricular dysfunction [1]. The disease is familial and several causative genes involved in desmosome function have been implicated [2]. Disease severity and progression can range from asymptomatic disease to severe heart failure and refractory ventricular arrhythmias [3]. Currently, it is difficult to predict future arrhythmia in ARVC patients or which of the patients will develop progressive heart failure. ARVC diagnosis may be aided by electroanatomic mapping [4], or inflammatory markers [5], or inflammatory markers combined with scintigraphy [6]. At present an assay of ARVC heart specific to cardiomyocytes is not available. While risk of cardiovascular death increases with left or right ventricular dysfunction in patients with ARVC [3], there are few tools available to assay functional status of ventricular muscle beyond overall contractility. A blood test that correlates with heart functional status and predicts risk of clinical progression would be of considerable clinical utility.

Bridging Integrator 1 (BIN1) is a member of the BAR domain superfamily whose gene encodes several alternately spliced membrane adaptor proteins [7–9]. We recently discovered that in cardiac myocytes, BIN1 is necessary for localizing delivery of L-type calcium channels to T-tubules [10], a process which is important for maintaining normal beat-to-beat calcium transients and contractility [11, 12]. More recently, we found that the transcription and total BIN1 content in muscle is decreased in human failing hearts [13]. An intriguing property of cardiac BIN1 is that it is a membrane attached protein and may be released in the process of membrane turnover. Many cells in the body such as platelets [14, 15], leukocytes [16], and endothelial cells [17] 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 [18–20]. The reduction of cardiac BIN1 in failing hearts [13] and its existence as a membrane associated protein [10] 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 likely result in reduction of cardiac origin BIN1, which could potentially be reflected in the blood levels of this protein. In addition, ARVC patients typically have a higher functional status with better physical and mental components scores than other patients with genetic cardiomyopathies [21] and have received full clinical work-ups and are followed closely. Also, the occurrence of arrhythmia in these patients presents the opportunity to assess clinical 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 are promising that plasma

BIN1 can assay cardiac functional status, with prognostic insight into both heart failure progression and development of ventricular arrhythmias.

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 Hospital 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 sampling. Two patients were originally not patients from the two institutes and had their blood sampled at their initial referral visit at UCSF or Johns Hopkins Hospital. 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 Hospital 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 were 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 [22] by two separate cardiologists blinded to the assay results. Exclusion criteria included inadequate data to confirm diagnosis or presence of persistent ventricular tachycardia despite several defibrillation events at the time of the blood sampling, 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 persistent VT and several defibrillation events with hospitalization for intravenous therapy on the day of the blood sampling was also excluded. The patient characteristics are displayed in Table 1. Of the fifteen patients genotyped for known mutation, 10 were positive for a plakophilin 2 (*PKP2*) mutation and one was positive for a desmoglein 2 (*DSG2*) mutation.

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 obtained between April 2004 and January 2011. Seven patients had two blood samples obtained at separate time points. All follow up blood were sampled during routine clinical care. The samples were obtained and centrifuged at 4000 rpm for 20 minutes at 4°C, and plasma stored in -80°C 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 ($R^2 > 0.98$) was generated to determine the relative amount of BIN1

in each sample that is reported as a dimensionless ratio. The common denominator is plasma pooled from three 25 year-old healthy male adults, divided by 100. 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 samples, the earliest sample 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. For patients diagnosed with ARVC, 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 (the initial diagnosis of ARVC in each patient included MRI imaging, echocardiography was used to track progression). Right ventricular function was recorded as normal or mild, moderate or severe dysfunction as graded by qualitative visual echocardiography, and taken directly from echocardiography reports at each medical center. 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. ICDs that were defined as placed for primary prevention were placed in the absence of history of sustained VT (>30 seconds) or history of aborted sudden cardiac death (hemodynamic collapse associated with sustained ventricular tachycardia/ventricular fibrillation). For patients who had an ICD placed for secondary prevention (n=10), 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). For patients with serial samples, NYHA class, interval arrhythmia history and LV and RV function at the time of the second blood sampling 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 $\pm$ 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 were 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, as evaluated by modified Task Force Criteria [22]. 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 sampling, 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 sampling. 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 are 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).

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). As discussed below, the existence of clinical heart failure accounted for the lower BIN1 of ARVC patients.

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 were not different between control patients without failing hearts, and ARVC patients who did not have heart failure. This suggests BIN1 is not specific for ARVC, but may more generally indicate myocardial health. 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 -0.61 , $p < 0.005$) 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 -0.47 , $p < 0.05$), 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 sampling 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, as revealed and confirmed by subsequent ICD interrogation. $BIN1 < 30$ predicted a high future arrhythmia rate (>0.5 arrhythmia events/year, Figure 3) with a sensitivity 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), 10 of 17 had ICDs placed for primary prevention and 2 of them had recorded ventricular arrhythmias prior to the first blood sampling. 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). The relative risk for high future arrhythmia events for BIN1 <30 is 8.56 (95% CI 1.27–57.26, $p < 0.01$). In comparison, the relative risk for high future arrhythmia events for NYHA class II or greater heart failure at baseline alone is 3.61 (95 % CI 1.25–10.37, $p < 0.05$). Thus low BIN1 is a stronger predictor of arrhythmia than the presence of heart failure.

BIN1 in ARVC patients with serial blood samples

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 (3.8 events/year) and developed new RV dysfunction. At the time of the initial blood sampling, 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 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 (2.1 events/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. In addition, in these healthy controls, plasma BIN1 remains the same when analyzed initially and repeated after two years storage in a -80°C freezer, indicating long term stability in cold storage.

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 are that lower plasma BIN1 levels occur in patients with clinical heart failure, and correlate with a high rate 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 or BMI. In ARVC patients with serial blood samples, plasma BIN1 decreased over time or remained low. Taken together, the data indicate that BIN1 correlates with cardiac functional status and presence or absence of sustained ventricular arrhythmias.

It is currently unclear why plasma BIN1 is lower in patients with more severe disease, but one possible explanation is that BIN1 expression correlates with cardiomyocyte content in the heart, and the significant loss of cardiomyocytes in ARVC after fibrofatty replacement

results in significant reductions in the cardiac source of BIN1. Also, diseased cardiomyocytes generate less BIN1 protein [13]. 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. Although the current findings of plasma BIN1 reduction are consistent with our previous findings of myocardial BIN1 reduction in heart failure [13], we cannot exclude the possibility that plasma BIN1 might be released from non-cardiac cells. Future studies that identify cardiac origin BIN1 will be helpful in the future to confirm that plasma BIN1 originates from the heart..

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 that 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 [23]. 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 arrhythmogenicity may include non-desmosomal proteins [24], altered intracellular calcium handling [25, 26] and altered stress-related signaling cascades [27]. 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. First of all, the metabolic characteristics and kinetics of BIN1 in human plasma including its plasma half-life as well as clearance methods are largely unknown. Secondly, the effects of other physiological factors such as physical activity, which might affect intra-individual and inter-individual variations of plasma BIN1, are also not addressed in the current small cohort study. As for the ARVC cohort, all but one of the ARVC patients had ICDs and therefore there may be an association between the presence of an ICD and low BIN1. Given that BIN1 levels did not correlate with the presence of an ICD (Table 2) and BIN1 is diminished in human heart failure [13], the chance that the ICD itself reduces BIN1 is low. Also, as there are several splice variants of BIN1 [28] that have been described in an array of cellular functions [29], our current assay may include detection non-cardiac BIN1. It is possible that a cardiac specific splice variant of BIN1 exists, 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 [30]. Use of larger ARVC cohorts will help validate the clinical utility of a BIN1 assay.

Conclusions

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.

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ABBREVIATIONS

ARVC	arrhythmogenic right ventricular cardiomyopathy
BIN1	Bridging Integrator 1
LV	left ventricle
RV	right ventricle
VF	ventricular fibrillation
VT	ventricular tachycardia
ICD	implantable cardioverter-defibrillator
NYHA	New York Heart Association
BMI	Body Mass Index
GFR	glomerular filtration rate

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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 sensitivity 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 sample, second sample): 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.)

Table 1

Baseline Characteristics of ARVC Patients and Controls.

Characteristics	ARVC Patients (n=24)	Controls (n=48)	P Value
BIN1			
Mean $\pm$ SD for all patients	37.1 $\pm$ 10	60 $\pm$ 10	<0.05
Mean $\pm$ SD for NYHA class II or greater	15 $\pm$ 7		
Mean $\pm$ SD for NYHA class I	60 $\pm$ 17		
Median	17	27	
Age (years)	43 $\pm$ 15*	47 $\pm$ 13	0.470
Gender			
Male	13	26	
Female	11	22	
Body Mass Index (BMI)	25.4 $\pm$ 5.9*	23.6 $\pm$ 1	0.200
History			
Family History Positive	8 (33%)		
Documented Genotype Positive			
Plakophilin 2 (PKP2)	10		
Desmoglein 2 (DSG2)	1		
Arrhythmias			
History of any sustained ventricular	16 (70%)		
Average length of ICD follow up (years)	4.4 $\pm$ 4.3*		
ICD placed	23 (96%)		
Primary prevention	13 (57%)		
Secondary prevention	10 (43%)		
Documented Anti-Arrhythmic Agent			
Class I			
(procainamide 1, mexiletine 1, flecainide 1)	3		
Class II			
(metoprolol 3, atenolol 2, other 3)	8		
Class III			
(sotalol 11, amiodarone 1)	12		
Heart Failure			
NYHA class II or greater	7 (30%)		
NYHA class III or IV	4 (18%)		
Moderate to severe RV dysfunction (two class I, one class III, one class IV)	4 (18%)		

*Mean $\pm$ SD

Table 2

BIN1 Correlation with Baseline Characteristics in ARVC Patients and Controls

Continuous Variables	Spearman's Rho	P Value
Number of ventricular arrhythmias to plasma draw	−0.590	<0.005
Ventricular arrhythmia rate to plasma draw (events/yr.)	−0.460	<0.05
Length of diagnosis	−0.090	0.702
ARVC		
BMI	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

* BMI = Body mass index (kg/m^2), GFR= Glomerular filtration rate