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Regional variation in *RBM20* causes a highly penetrant arrhythmogenic cardiomyopathy

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Abstract

Background: Variants in the cardiomyocyte-specific RNA splicing factor *RBM20* have been linked to familial cardiomyopathy but the causative genetic architecture and clinical consequences of this disease are incompletely defined.

Methods and Results: To define the genetic architecture of *RBM20* cardiomyopathy, we first established a database of *RBM20* variants associated with cardiomyopathy and compared these to variants observed in the general population with respect to their location in the *RBM20* coding transcript. We identified two regions significantly enriched for cardiomyopathy-associated variants in exons 9 and 11. We then assembled a registry of 74 patients with *RBM20* variants from 8 institutions across the world (44 index cases and 30 from cascade testing). This *RBM20* patient registry revealed highly prevalent family history of sudden cardiac death (51%) and cardiomyopathy (72%) among index cases, and a high prevalence of composite arrhythmias (including AF, NSVT, ICD discharge and sudden cardiac arrest, 43%). Patients harboring variants in cardiomyopathy-enriched regions identified by our variant database analysis were enriched for these findings. Further, these characteristics were more prevalent in the *RBM20* registry than in large cohorts of patients with DCM and titin (*TTNtv)* cardiomyopathy, and not significantly different from a cohort of patients with Lamin A/C associated (*LMNA)* cardiomyopathy.

Conclusions: Our data establish *RBM20* cardiomyopathy as a highly penetrant and arrhythmogenic cardiomyopathy. These findings underline the importance of arrhythmia surveillance and family screening in this disease and represent the first step in defining the genetic architecture of *RBM20* disease causality on a population level.

Background

Autosomal dominant variants in a subset of cardiomyocyte-expressed genes represent a pathologically significant contributor to the etiology of cardiomyopathies. Variants in *RBM20* represent an unusually complex genetic etiology of cardiomyopathy in that *RBM20* controls post-transcriptional splicing of many sarcomeric and calcium handling genes in the cardiomyocyte [1–3]. Loss of function in *RBM20* leads to missplicing of such genes as *TTN, LDB3, CAMK2D*, and *RYR2* in humans and murine models, all of which are independently implicated either in the cause or molecular propagation of ventricular dysfunction or arrhythmia [1,3,4]. *In vivo*, *Rbm20* $^{-/-}$ and *Rbm20*$^{+/-}$ rats develop both dilated cardiomyopathy and ventricular arrhythmias, and most recently, data in isolated cardiomyocytes from *Rbm20* $^{-/-}$ mice demonstrate pro-arrhythmic calcium release from the sarcoplasmic reticulum [4,5].

As such, the study of *RBM20* cardiomyopathy stands at the center of our understanding of disrupted alternative transcriptional splicing in the contractile dysfunction and arrhythmogenesis of heart failure. A small number of pathogenic variants in *RBM20* have been reported from kindred studies [6–9], the majority of which lie within regions of *RBM20* critical to spliceosome binding: the (RS-domain) and RNA recognition motif (RRM). However, a global understanding of variant pathogenicity across the coding transcript of this gene remains elusive. Further, though small cohort and kindred-based studies of *RBM20* cardiomyopathy have been reported, a comprehensive clinical description of this patient population in the broader context of genetic cardiomyopathy has yet to be established.

Here, we compare the genetic distribution of clinically reported cardiomyopathy-associated *RBM20* variants to *RBM20* variants found in the general population, allowing us to define regions of the *RBM20* transcript enriched for cardiomyopathy-associated variants, and therefore more prone to pathogenic variation. We go on to establish an international registry of patients harboring variants in *RBM20* and characterize their clinical features. We compare this registry to large patient cohorts with dilated cardiomyopathy (DCM) and specifically cardiomyopathy due to truncating variants in *TTN (TTNtv) and Lamin A/C (LMNA).*

Methods

The data, analytic methods, and study materials will made available upon request to other researchers for purposes of reproducing the results or replicating the procedure.

RBM20 cardiomyopathy-associated variant database and comparison to population variant frequency

We compiled a database of 403 clinical genetic tests revealing variants in *RBM20* from patients with cardiomyopathy. These variants were collated by the Laboratory for Molecular Medicine at Harvard Medical School and Invitae, Inc, and cross-referenced with reports in ClinVar (as of January 3, 2017). Variants reported by these testing providers in ClinVar were not double-counted. Synonymous coding variants and non-coding variants were eliminated from analysis to focus on those variants with the greatest potential impact on protein function. 171 missense, insertion/deletion and nonsense variants were included. The position of insertion/deletion and nonsense variants was taken as the position at the start of the insertion/deletion, and the position of termination for nonsense variants respectively. Variants were included regardless of their classification by the reporting institution (e.g., as benign, VUS or pathogenic). A full list of included variants is supplied in Supplemental Table 1.

Comparison of this cardiomyopathy-associated *RBM20* variant database to general population variation in *RBM20* was achieved by comparison to variants reported in 123,136 exomes and 15,496 genomes in the Genome Aggregation Database (gnomAD). The gnomAD database was used instead of the Exome Aggregation Consortium (ExAC) because mean coverage of *RBM20* is greater in gnomAD (~40X) compared to ExAC (<10X) [10]. We limited this analysis to the number of unique variants rather than patients (details included in Supplemental Methods). In all, we identified 171 unique cardiomyopathy-associated variants and 604 unique variants in the general population (Figure 1).

We divided the ~3700 bp coding transcript into windows of 40 bp each, creating 93 windows along the length of the transcript (for details of window size optimization, see Supplemental Methods). We then compared the fraction of unique variants within each of these windows (# unique variants falling in a window / # total unique variants in respective database) between the cardiomyopathy and general population groups. We then identified windows with high odds of containing cardiomyopathy-associated variants.

International RBM20 Patient Registry

An international registry of patients with *RBM20* variants was assembled from pre-existing registries at 8 inherited cardiomyopathy centers (University of Sydney, Australia, University of Trieste, Italy, Johns Hopkins University, USA, University of Colorado, USA, Brigham and Women's Hospital at Harvard University, USA, The Ohio State University, USA, University of Heidelberg, Germany and Stanford Center for Inherited Cardiovascular Disease, USA). Data were collected, stored and shared in a de-identified manner in accordance with the institutional review board (IRB) at each contributing center by retrospective chart review. (Please see Supplemental Methods for description of clinical characteristic definitions.) Where information could not be collected, it was marked as missing, and that patient was excluded from analysis for that variable.

The *RBM20* patient registry was assembled independently of the *RBM20* variant database described above, and the two datasets are not combined in our analyses. However, variants discovered in registry patients may have been included in the database due to the small number of cardiovascular genetic testing providers in the US and the ability of testing providers and care teams to post novel variants in ClinVar. These 74 patients were diagnosed independently by their respective centers of excellence for genetic cardiomyopathy, and therefore, specific sequencing tests used varied by center. In general, disease-specific panel-based testing was used for diagnosis in probands and variant specific sequencing was used for cascade screening.

Dilated Cardiomyopathy (DCM), Titin truncating variant (TTNtv), and LMNA cardiomyopathy cohorts

To better understand the clinical characteristics of *RBM20* cardiomyopathy in the context of other causes of DCM, *RBM20 r*egistry data was compared with prevalence data from three large existing cohorts: one of all comers with DCM (N=633), one with *TTNtv* (N=83) cardiomyopathy patients,[11] and one with *LMNA* cardiomyopathy patients (N=87)[12] to serve as a comparison to known arrhythmogenic cardiomyopathy.

Statistical Analysis

RBM20 patient registry statistics were compared using the Fisher Exact test for discrete variables and Student's T-test for continuous variables. Statistical significance for odds ratios in variant database analysis was determined using a Fisher Exact test due to sample size <100 per window and was corrected for multiple testing using a Benjamini-Hochberg false discovery rate (FDR) set at 20% [13–15]. For multiple comparisons of clinical observations, a Bonferroni correction was used. These corrections for multiple testing were

used separately for these respective purposes. All statistical comparisons were performed using Stata® 14.

Results

Comparison of RBM20 sequence architecture between cardiomyopathy patients and the general population identifies high confidence regions of pathogenicity in exons 9 and 11

We identified two windows enriched for cardiomyopathy-associated variants compared to population variants after correction for multiple testing. These windows occurred at positions c.2721– 2760 (exon 11), and c.1881–1920 (exon 9, including the RS-domain), where the odds of inclusion of a cardiomyopathy-population associated variant were higher than that of a variant from the general population (Figure 1). The c.1601–1640 window (in exon 7) is the third most confidently ranked pathogenic window, and encodes a section of the RRM domain. It also encompasses the V535I variant reported by Li et al. in a single individual with a strong family history of DCM [7]. The significance value of this region (p=0.025) did not meet our gene-wide multiple testing criterion for statistical significance, but this was the third most highly ranked window. A full list of windows ranked by statistical probability of enrichment with cardiomyopathy variants is included in Supplemental Table 2.

Together, these three windows encode two previously characterized functional domains of the RBM20 protein (the RS domain and the RRM, Figure 1), as well as an area of exon 11 where a familial variant has previously been identified, but for which no function has been identified [8]. Only one of the 8 previously reported cardiomyopathy variants was not encompassed in the three mostly confidently predicted windows (R716Q), but was found to be in a window where population and cardiomyopathy associated variants are equally prevalent. On further examination, the analysis window in which the corresponding variant (c.2147G>A) lies is enriched both for cardiomyopathy associated and general population variants, indicating that it may represent an area of high tolerance to genetic variation and not a site for pathogenic variation (purple indicator arrow, Figure 2). Prior evidence for the potential pathogenicity of this variant includes a reporter assay for splicing of the PEVK segment of *TTN* in which this variant displayed altered splicing similar to variants in the RS domain. [16] This large family of 43 individuals was originally described in 2010[7], at which time, 17 of its members were noted to carry the c.2147G>A variant. 10 of 17 of these family members also had DCM. One family member with mild disease (sick sinus syndrome and mild DCM easily reverse with medical therapy) did not carry the variant. Therefore it was felt to segregate with disease (though with low penetrance), and the familial DCM was attributed to this variant in *RBM20*. Of note, 8 members of this family also carried a previously reported variant in *LMNA* [7,17], 3 of whom also had DCM, and one of whom died suddenly. Taken together, our population-level genetic data and this family-level data cast some doubt on the pathogenicity of this variant and highlight the utility of the integrated model incorporating population and patient variation.

Cardiomyopathy associated with RBM20 variants has an arrhythmogenic, highly penetrant phenotype

74 total patients are included in the RBM20 registry. 44 of these patients were index cases, while the remaining 30 patients were identified through cascade screening (Table 1). Patients in this registry harbored variants distributed across the length of the *RBM20* transcript and included those inside (N=30) and outside (N=44) highly predicted windows in exons 9 and 11 from our variant database analysis (Figure 2).

We first examined the clinical characteristics of this registry as a whole (N=74, Table 1). Among index cases alone, was a high prevalence of family history of both cardiomyopathy and sudden cardiac death (72% and 51%, respectively). We also noted a young age at diagnosis among index cases (40±12 years). In the complete registry, there was also a high prevalence of sudden cardiac arrest (SCA, 8%), and appropriate implantable cardiac defibrillator (ICD) discharges among those patients with ICDs (28%). This was coupled with a high prevalence of nonsustained ventricular tachycardia (NSVT) and atrial fibrillation (AF) (36% and 17%, respectively). The mean PR interval was 169±33 ms (mean±SD) and 4 patients displayed first degree AV block (PR>220 ms, ranging between 230 ms and 260 ms). Mean left ventricular ejection fraction (LVEF) was 40±17% (mean±SD). Forty-eight percent of patients had an LV end diastolic dimension (LVEDD) greater that 5.7 cm. Cardiac magnetic resonance (CMR) imaging from the *RBM20* registry reports delayed gadolinium enhancement in 11/22 patients (50%) who had undergone MRI. This represents a risk factor for arrhythmia in multiple types of cardiomyopathy [18–20]. Five patients in the registry carried a diagnosis of hypertrophic (HCM) by chart review and 1 carried a diagnosis of left ventricular non-compaction in the absence of LV dilation or reduced LV systolic function (Table 1).

Arrhythmia and family history of sudden death are enriched in patients harboring RBM20 variants within high confidence likely-pathogenic regions

We sought to examine the clinical consequences of variation specific to the likely pathogenic regions identified from our population level analysis above. We hypothesized that patients harboring variants in the two statistically predicted pathogenic regions (c.2721– 2760 in exon 11 and c.1881–1920 in exon 9) would have higher prevalence of family history and arrhythmia outcomes compared to patients harboring variants outside of likely pathogenic regions. We identified 30 patients in the registry with variants lying inside these high confidence, likely pathogenic regions (high likelihood regions, Figure 2). We found that index patients with variants in high likelihood windows were more likely to have a family history of SCD (93% vs. 51% in the overall registry, p=0.002) than index patients with variants lying outside of these regions (Table 1). They were also more likely to personally have an arrhythmia (66% vs. 43% p=0.002), in particular non-sustained ventricular tachycardia (NSVT, p=0.009) and atrial fibrillation (p=0.04), though these did not remain statistically significant after correction for multiple comparisons (Bonferroni corrected α = 0.002, Table 1). Lastly, patients with variants in high likelihood regions had significantly shorter PR intervals (150±21 ms vs. 169±33 ms, p=0.0003).

That five patients in this registry expressed a hypertrophic cardiomyopathy phenotype is uncharacteristic of prior reports of a dilated phenotype associated with *RBM20* pathogenic variants [7,8,21] (Table 1). Therefore, we examined whether these patients' variants were associated with high confidence likely pathogenic regions. None of these HCM patients harbored variants in the top three regions of the gene predicted to be pathogenic by our analysis, which also encompasses the RS and RRM domains (these variants occurred at residues 374, 429, 545, 888 and 1089).

RBM20 cardiomyopathy has increased penetrance and higher prevalence of arrhythmias compared to other causes of cardiomyopathy

To better understand the clinical characteristics of *RBM20* cardiomyopathy in the context of other causes of DCM, *RBM20 r*egistry data was compared with prevalence data from two large existing cohorts. To compare RBM20 cardiomyopathy patients at the time of evaluation to other patients with DCM, we first compared RBM20 index cases (N=43) to a large cohort of all-comers with DCM (N=633) [11]. Further, as *RBM20* is thought to control *TTN* splicing, we also compared these patients to a cohort of *TTNtv* cardiomyopathy patients, specifically (N=83) [11].

We found family history of DCM and SCD to be more common among *RBM20* registry index patients (N=43) than in the DCM cohort (N=633, p<0.0001 for both comparisons) and the TTNtv cardiomyopathy cohort (N=83, p<0.0001 for both comparisons) (Table 2). A combined metric of evidence of sustained ventricular arrhythmia (VA) (reported personal history of SCA or appropriate ICD discharge) in the *RBM20* registry was more common than reported sustained VT in the DCM population and TTNtv cardiomyopathy cohort (*RBM20*: 20.0%, DCM 2.2%, *TTNtv* 1.2%, p<0.0001 for both comparisons, Table 2). NSVT was also more common in the *RBM20* registry (*RBM20*: 36.0%, DCM 10.6% (p<0.0001), *TTNtv* 19.3% (p=0.03) Table 2). Atrial fibrillation prevalence was not different between *RBM20* and DCM or *TTNtv* cardiomyopathy groups. The average age of patients in the DCM cohort (54±14 years) and TTNtv cohort (49±13 years) was significantly older than the *RBM20* registry (40±15 years, p<0.0001 vs. DCM and p=0.0008 vs *TTNtv* cardiomyopathy, Table 2).

As we found evidence of increased ventricular arrhythmia in the *RBM20* cardiomyopathy index cases, we also compared them with *Lamin A/C (LMNA)* cardiomyopathy patients from a recently published cohort [12]. *LMNA* cardiomyopathy is an inherited dilated cardiomyopathy with a high burden of ventricular and atrial arrhythmias, observed even in the absence of significant LV remodeling [12,17]. We found that, at initial evaluation, *RBM20* cardiomyopathy displayed similar rates of evidence of ventricular arrhythmia (VA) compared to documented VA in the LMNA cohort. There were trends toward less atrial fibrillation in the *RBM20* index patients (OR=0.4 [0.2,0.9], p=0.03), and increased NSVT (OR= 2.1[1.3,6.7], p=0.01), but these were not statistically significant after correction for multiple comparisons (Bonferroni corrected α= 0.0027, Table 2). Family history of SCD and DCM in index cases was also similar between *RBM20* and *LMNA* cardiomyopathy index cases (Table 2). We additionally hypothesized that arrhythmia in *RBM20* cardiomyopathy might occur in the absence of significant LV remodeling, as has been observed with *LMNA*

cardiomyopathy[12,22]. Indeed, of 12 *RBM20* cardiomyopathy patients with evidence of sustained ventricular arrhythmia (appropriate ICD discharge and/or SCA), 4 patients (33%) had an LVEF≥45% at the time of evaluation.

Discussion

Here, we provide a comprehensive view of genetic variation and clinical characteristics of *RBM20* cardiomyopathy, defining it as a highly penetrant, arrhythmogenic cardiomyopathy. We have: (i) used population and clinical genetics data to define the regional architecture of *RBM20* variant pathogenicity, confirming the RS domain as a pathogenic domain and identifying a new likely pathogenic region in exon 11, (ii) assembled an international registry of patients with variants in *RBM20* to define the clinical characteristics of this disease, (iii) demonstrated that patients within the *RBM20* registry harboring variants in predicted pathogenic regions have an increased prevalence of family history of SCD, and increased personal history of arrhythmia compared to those with variants outside these regions and (iv) contextualized these findings in comparison to a large cohort of DCM patients, as well as *TTNtv* cardiomyopathies, finding a higher incidence of arrhythmias and more frequent family history of DCM and SCD in the *RBM20* registry, similar to a large cohort of *LMNA* cardiomyopathy patients.

Identification of likely pathogenic regions of RBM20 highlights the power of population-level genetic data to illuminate structure-function relationships

We, and others, have previously demonstrated structure-function relationships in larger genes with frequent genetic variation [23,24]. These analyses rely on the premise that areas of genes enriched with variants in diseased patients (compared to the general population) represent regions of low functional tolerance to genetic variants, and thus high probability of pathogenicity. Careful mechanistic validation of highly predicted pathogenic regions in genes of interest remains critical. Kindred-based studies of *RBM20* variants in the US and Europe have harnessed the disease-modeling power of induced pluripotent stem cell derived cardiomyocytes to better understand the downstream effects of familial *RBM20* variants on RNA splicing, myocyte contractility and calcium cycling, specifically in the RS domain of exon 9, independently identified by our analysis as well [8,9,25–27]. The transcript-wide pathogenicity data for *RBM20* generated by our study adds to this body of work because it incorporates population-scale data, which can improve diagnostic power in rare disease, as well as increase our understanding of structure-function relationships at the protein level.

Clinical characteristics of RBM20 registry underline arrhythmia risk for patients with pathogenic variants in RBM20

Our data draw attention to unique clinical characteristics and risks for patients with *RBM20* variants as compared to other patients with a personal or family history of DCM. They are supported by a recent report showing deranged calcium handling in the *Rbm20*$^{-/-}$ mouse, coupled with results from a smaller cohort of *RBM20* cardiomyopathy patients with increased ventricular arrhythmias compared to *TTNtv* cardiomyopathy [5]. Our findings underline the imperative of family screening in this group, especially as some of the ICD discharge and sudden cardiac arrest was observed prior to the onset of severe LV

dysfunction. Though current guidelines in DCM point to severe and irrecoverable LV dysfunction or personal history of arrest as indications for ICD placement [28], a small number of studies have shown that some genetic cardiomyopathies carry a significantly greater risk of malignant arrhythmia than the general population with DCM. Patients with *LMNA* cardiomyopathy display a highly progressive disease with high rates of significant ventricular arrhythmia. This prompted a recent revision to the ACC/AHA/HRS guidelines with a Class IIa recommendation for ICD implant in patients with LMNA variants and two or more risk factors (nonmissense variant, male sex, EF <45% or NSVT) [12,22]. As we establish here, *RBM20* cardiomyopathy is more highly penetrant with increased arrhythmogenicity than *TTNtv* cardiomyopathy and DCM without a genetic diagnosis, with ventricular arrhythmia rates at evaluation not significantly different from an *LMNA* cardiomyopathy cohort.

As we find that *RBM20* cardiomyopathy is more similar to *LMNA* cardiomyopathy with respect to these characteristics, we suggest that these patients should be viewed with clinical concern similar to other arrhythmogenic cardiomyopathies or to catecholaminergic polymorphic ventricular tachycardia (caused by *RYR2*, whose splicing is affected by *RBM20)*, in particular with respect to their capacity for early life-threatening arrhythmia [29]. Longitudinal data will be required to make specific recommendations regarding risk stratification for SCD in *RBM20* cardiomyopathy beyond depressed EF alone, and this is clearly of urgent importance. At present, we would suggest that early and aggressive arrhythmia monitoring in patients carrying likely pathogenic or pathogenic variants in *RBM20* with or without severe ventricular remodeling is critical to identify patients at risk for life threatening arrhythmia.

Study limitations

Our study, focused on rare disease, is limited by small sample size and the inter-relatedness of study subjects. However, we believe this population represents a significant proportion of patients known to harbor *RBM20* variants internationally, as it represents a culmination of data from 8 major inherited cardiovascular disease centers in the world.

Conclusion

Taken together, our findings define the clinical syndrome of *RBM20* cardiomyopathy as a highly penetrant, arrhythmogenic cardiomyopathy. We have prioritized discrete regions of the *RBM20* transcript that are likely critical to the pathogenesis of *RBM20* cardiomyopathy and defined the clinical characteristics of the largest reported multicenter international cohort of these patients to date, including prominent arrhythmogenesis and high penetrance of cardiomyopathy and sudden death. These findings lay the foundation for examination of longitudinal outcomes in this registry, investigation of the novel putative *RBM20* functional domain in exon 11, and, more broadly, for defining the role of *RBM20* and alternative splicing in the biology of heart failure and arrhythmia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Clinical Impact

- What is New?
  - Largest international description of the clinical features of *RBM20* cardiomyopathy to date
  - Draws attention to unique clinical characteristics and arrhythmic risk for patients with *RBM20* variants as compared to other patients with a personal or family history of DCM
  - First population-based regional analysis of variant-disease association across the length of the *RBM20* transcript
- What are the clinical implications?
  - Immediately clinically impactful information about regional variant pathogenicity can be applied to variants of uncertain significance in a clinical setting
  - Implications for arrhythmia monitoring and family screening in patients with *RBM20* variants thought likely to contribute to disease

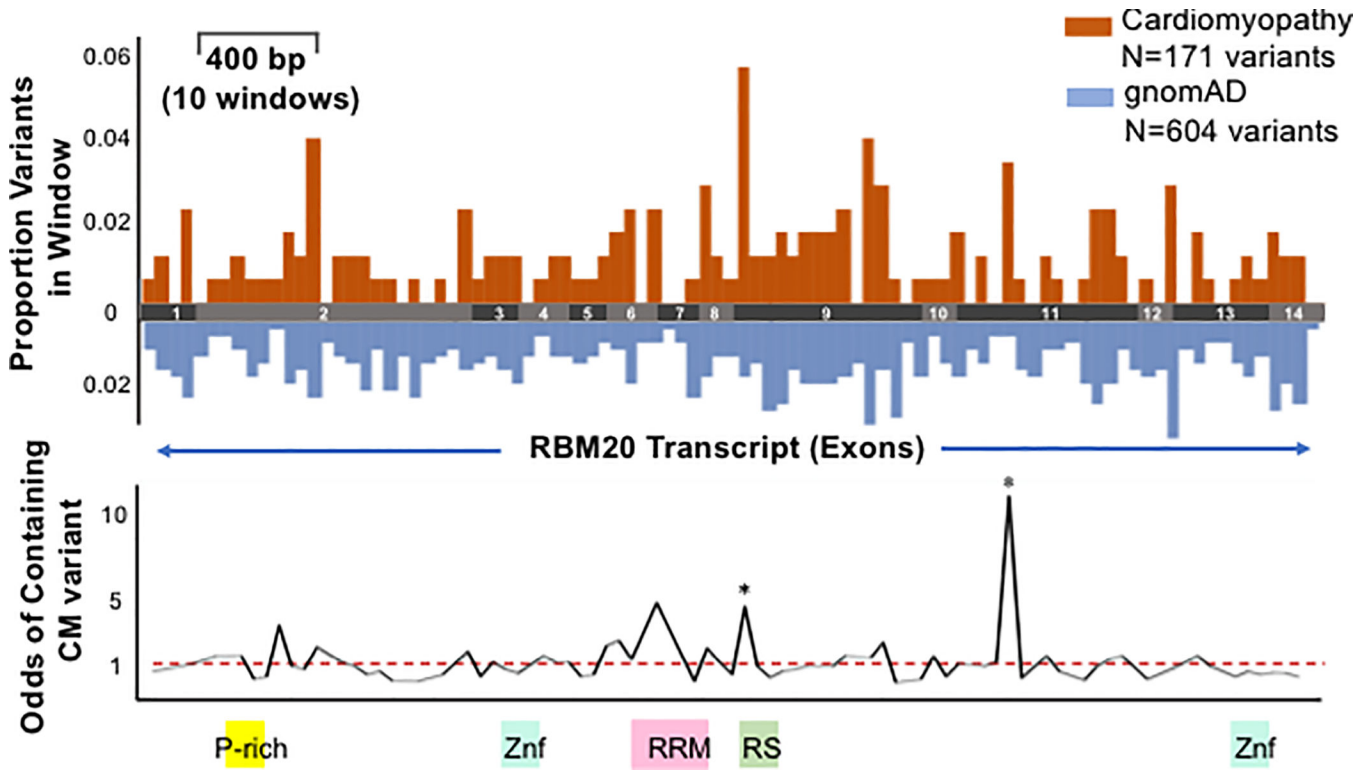

Figure 1. Regional likely pathogenic genetic variation in *RBM20*.

Comparison of regional frequencies of cardiomyopathy-associated variants found on genetic testing to those observed in the population (gnomAD) illuminates two regions enriched for cardiomyopathy-associated variants, and therefore more likely to contain pathogenic variation. The coding transcript of RBM20 is divided into ~100 windows of 40 bp each and the odds of cardiomyopathy-associated versus population variants lying within that window (versus any other window) are displayed. Orange bars denote percentage of cardiomyopathy-associated variants in each window, blue bars denote percentage of population variants per window. * denotes windows with statistically significant odds of containing cardiomyopathy-associated variants after adjustment for multiple testing. Red dashed line denotes OR of 1.

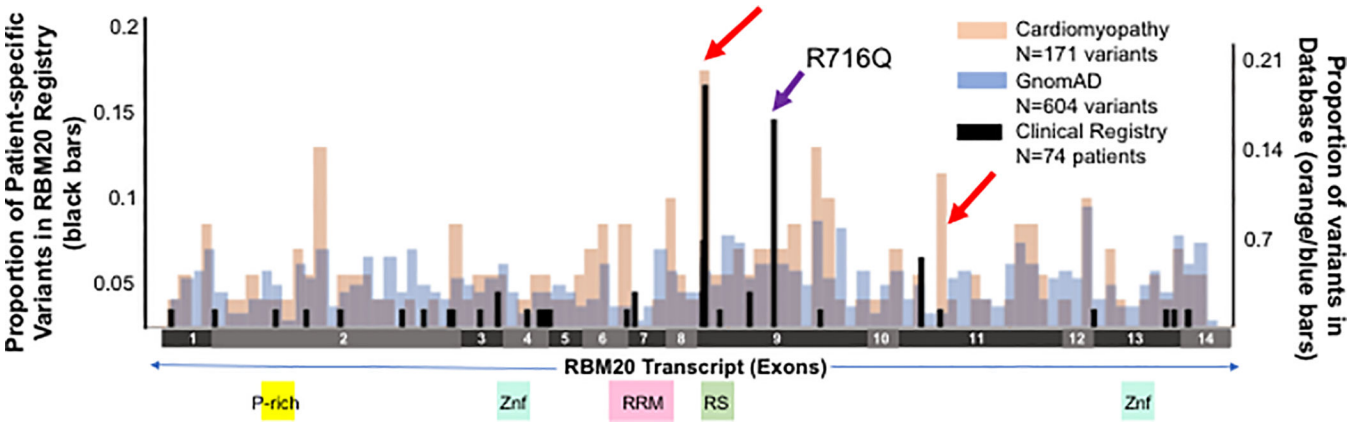

Figure 2. Distribution of variants observed in *RBM20* registry patients.

Black bars represent proportion of *RBM20* registry patients (N=74) with variants in each window ("patient-specific variants" left axis). Position for nonsense and insertion/deletion variants is represented as the first location of change in the transcript sequence. Orange and blue bars represent proportion of variants in each window in cardiomyopathy-associated and population (gnomAD) variant databases respectively (right axis). Red arrows indicate predicted pathogenic regions. Purple arrow indicates region containing previously reported p.R716Q variant lacking high level evidence for pathogenicity that lies outside highly predicted pathogenic regions.

Table 1.

Baseline Clinical Characteristics of the patients in the RBM20 registry

	All *RBM20* Registry Cases (N=74)	Cases Carrying Variants in Predicted Windows (N=30)	Odds Ratio [CI] p-value
Index Cases (vs. identified by cascade screening) (%)	44/74 (59)	14/30 (47)	2.4 [0.9, 7.1] p=0.06
Current Age (years, mean±SD)	46±17	42±16	p=0.17
Age at diagnosis (years, mean±SD) (index cases only)	37±15 40±15	34±14 40±12	p=0.14 p=0.98
HCM phenotype (%)	5/74 (7)	0/30 (0)	0.0 [0.0,1.1] p=0.07
Family History SCD (%) (index cases only)	22/43 (51)	13/14 (93)	11.4 [1.9, 117.2] p=0.002
Family History DCM (%) (index cases only)	31/43 (72)	12/14 (86)	†p=0.06
Any Arrhythmia (%)	25/58 (43)	16/24 (66)	5.6 [1.6, 20.4] p=0.002
NSVT (%)	21/59 (36)	14/26 (54)	4.3 [1.2, 16.0] p=0.009
Atrial Fibrillation (%)	10/58 (17)	7/23 (30)	4.7 [0.9, 30.8] p=0.04
SCA (%)	5/60 (8)	2/26 (8)	0.9 [0.1, 8.2] p=1.00
ICD implant (%)	36/61 (59)	15/25 (60)	1.1 [0.3, 3.5] p=1.00
Appropriate ICD discharge (%) (of patients with ICDs)	9/32 (28)	6/15 (40)	2.9 [0.5, 21.9] p=0.25
LVEF by Echocardiography (%)	40±17	39±15	p=0.67
LVEDD (mm, mean±SD)	56±12	55±13	p=0.51
Dilated (LVEDD>57mm) (%)	33/69 (48)	13/28 (46)	0.9 [0.3, 2.7] p=1.00
LVEF by CMR (%, mean±SD)	45±17 (N=22)	51±14 (N=7)	p=0.21
LV Volume Index by CMR (ml/m^2, mean±SD)	117±50 (N=20)	98±14 (N=7)	p=0.29
RVEF by CMR (%, mean±SD)	50±16 (N=11)	53±9 (N=5)	p=0.52
RV Volume Index by CMR (ml/m^2, mean±SD)	101±59 (N=11)	90±22 (N=5)	p=0.60
Delayed Gadolinium Enhancement (CMR) (%)	11/22 (50)	3/7 (42)	0.7 [0.1, 5.6] p=0.64
PR duration (ms, mean±SD)	169±33	150±21	p=0.0003
QRS duration (ms, mean±SD)	110±27	102±19	p=0.09
LVAD (%)	1/74 (1)	1/30 (3)	†p=0.45
Transplant (%)	5/74 (7)	2/30 (7)	1.0 [0.1, 9.1] p=0.97
Death (%)	3/74 (4)	3/30 (10)	†p=0.06

HCM: Hypertrophic cardiomyopathy, DCM: Dilated Cardiomyopathy, SCD: Sudden cardiac death, SCA: sudden cardiac arrest, NSVT: Nonsustained Ventricular Tachycardia, LVEF: LV ejection fraction: LVEDD: LV end diastolic dimension, RVEF: RV ejection fraction, CMR: Cardiac Magnetic Resonance, LVAD: Left Ventricular Assist Device. Where sample size is less than stated registry size, this indicates lack of available data on a number of subjects.

† indicates insufficient observations in a single group to accurately report odds ratio. Bolded p-vales indicate those that remain significant after correction for multiple comparisons (p-value<0.003).

Table 2.

RBM20 cardiomyopathy is highly penetrant and arrhythmogenic cardiomyopathy

	***RBM20* CM (Index cases) (N=43)**	**DCM (N=633)**	**Odds Ratio [CI] p value**	***TTNtv* CM (N=83)**	**Odds Ratio [CI] p value**	***LMNA* CM (N=87)**	**Odds Ratio [CI], p value**
Age at evaluation (yrs, mean ±SD)	40±15	54±14	p<0.0001	49±14	p=0.0008	43±14	p=0.35
Family History of DCM (%) (*RBM20* Index cases only)	31/43 (72)	87/633 (13.7)	16.0 [8.0, 32.8] $p=2.4\times10^{-16}$	26/83 (31)	5.6 [2.5,12.7] $p=1.5\times10^{-5}$	46/87 (52.9)	1.3 [1.0,1.8] p=0.04
Family History of SCA (%) (*RBM20* Index cases only)	22/43 (51)	95/633 (15)	5.9 [3.1,11.2] $p=1.3\times10^{-7}$	12/83 (15)	6.2 [2.6,14.5] 2.0×10^{-5}	38/87 (44)	1.4 [0.6,2.8], p=0.46
Atrial Fibrillation (%)	9/41 (22)	140/633 (22.1)	1.0 [0.5,2.1] p=0.90	23/83 (28)	0.7 [0.3,1.8] p=0.52	37/87 (43)	0.4 [0.2,0.9], p=0.03
Evidence of Sustained VA (%) *	10/40 (25)	14/633 (2.2)	14.7 [6.0,36.0] $p=1.9\times10^{-7}$	1/83 (1)	27.3 [3.4,223.0] $p=5\times10^{-5}$	18/87 (21)	1.2 [0.6,2.4], p=0.65
NSVT (%)	17/39 (43)	67/633 (10.6)	6.5 [3.3,12.9] $p=5.1\times10^{-7}$	16/83 (19)	3.4 [1.4,7.5] p=0.008	18/87 (21)	2.1 [1.3,6.7], p=0.01

CM = Cardiomyopathy, SCA = Sudden Cardiac Arrest, ICD = Implantable Cardiac Defibrillator, NSVT = Nonsustained Ventricular Tachycardia, DCM = Dilated Cardiomyopathy, LVEF = Left ventricular ejection fraction, LVEDD = Left ventricular end diastolic dimension, SD = standard deviation. VA= ventricular arrhythmia Data from DCM and *TTNtv* cohorts have been previously reported (8). Where N is indicated as less that total cohort for a given variable, data was not available for some individuals.

* Defined as sustained VT or VF on monitoring for DCM, *TTNtv* and *LMNA* and as SCA and/or ICD discharge for *RBM20*. Bolded p-values remain statistically significant after correction multiple comparisons (p<.0027).