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

Supplemental Methods

Justification for use of position-specific unique variants rather than frequency in registry and population
The lack of complete patient and haplotype-specific genomic data in the *RBM20* variant database precludes formal assessment for the effect of linkage disequilibrium and relatedness on our findings. To eliminate the possibility that relatedness lead to over-representation of variants due to cascade screening or the same variant in multiple distantly-related families, we counted each variant as a single observation rather than each patient carrying a given variant. For example, if two patients were reported from separate testing companies with the same variant (e.g., c.1901G>A), these were not counted individually, rather that variant was recorded as having been observed in the appropriate population at position c.1901. This was necessary for two reasons: First, because the data was collected anonymously from testing laboratories and ClinVar, we have no way of knowing how many cardiomyopathy patients were tested to reveal the observed variants in *RBM20*. Second, as genetic testing is enriched for related individuals, large kindreds well connected to the healthcare system could bias our results toward areas of the transcript in which large families harbor single variants.

Optimization of window size in gene-wide comparison of regional variant frequency
We first attempted to identify exons particularly enriched for cardiomyopathy-associated variants, however, there was no significant enrichment for cardiomyopathy-associated variants in any exon, leading us to speculate that regional concentration, if it existed, was more likely to be found at a more granular level than exons. We therefore hypothesized that smaller serial sliding windows, agnostic to the location of known protein domains, would be preferable for identification of cardiomyopathy-enriched domains. We avoided dividing the transcript based on known functional domains in order to allow for the discovery of novel impactful regions of the transcript, while preserving assessment of the pathogenicity of known functional domains associated with these windows.

We divided the ~3700 bp coding transcript into windows of 40 and 8 bp each (resulting in 93 and 462 windows respectively) along the length of the transcript. We initially tested these two window sizes (approximately 1/100th and 1/500th the length of the transcript) in order to optimize window specificity with the relatively small size of our population. The 40 bp windows were large enough to ensure enough observations within windows to identify statistically significant enrichment while at the same time preserving spatial specificity, whereas too few 8 bp windows contained observations sufficient to reach a valid conclusion.

Definitions of Clinical Characteristics of the RBM20 *registry*
Presence or absence of arrhythmia was defined as any documented non-sustained ventricular tachycardia (NSVT), atrial fibrillation (AF) whether by chart review, long term rhythm monitoring or device interrogation. Sudden cardiac arrest (SCA) was defined by report of sudden cardiac arrest by chart review (as defined by ACC/AHA/HRS definition[1], and appropriate ICD discharge was defined as either shock or anti-tachycardia pacing appropriate for underlying rhythm (also collected by chart review). For comparison to report of ventricular tachycardia in the previously published DCM cohort, “evidence of ventricular arrhythmia” in the RBM20 cohort was defined as either SCA, appropriate ICD discharge or both.

Ejection fraction by echo and MRI were collected by chart review at each independent center and were not centrally adjudicated. Family history of dilated cardiomyopathy (DCM) or sudden cardiac death (SCD) were defined as reported family history in first, second or third degree relative (i.e., parents, grandparents, aunts, uncles or first cousins). Of probands in the registry, two also harbored likely pathogenic variants in *MYH6 and DES* (as classified by their contributing institution). While these patients both had a family history of cardiomyopathy, they did not have a family history of SCD, nor did they have personal history of arrhythmia including AF, ICD discharge or SCA.

References

1. Al-Khatib SM., Stevenson WG., Ackerman MJ., et al. 2017 AHA/ACC/HRS Guideline for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society. Heart Rhythm 2017. Doi: 10.1016/j.hrthm.2017.10.035.

Supplemental Tables

Supplemental Table 1. Variants included in *RBM20* cardiomyopathy-associated variant database

Contributor	cDNA effect	Protein effect	Exon
ClinVAR	32C>T	Ala11Val	1
ClinVAR	42C>G	Ser14Arg	1
ClinVAR	224C>T	Ser75Leu	2
ClinVAR	304C>T	Arg102Trp	2
ClinVAR	488G>A	Arg163Gln	2
ClinVAR	532C>T	Arg178Ter	2
ClinVAR	533G>C	Arg178Pro	2
ClinVAR	611C>T	Pro204Leu	2
ClinVAR	674C>T	Thr225Ile	2
ClinVAR	1019C>T	Pro340Leu	2
ClinVAR	1040A>G	Tyr347Cys	2
ClinVAR	1156C>T	Gln386Ter	2
ClinVAR	1183C>T	Gln395Ter	2
ClinVAR	1281G>A	Trp427Ter	3
ClinVAR	1348C>T	Arg 450Trp	4
ClinVAR	1603G>A	Val535Ile	6
ClinVAR	1606A>G	Ile536Val	6
ClinVAR	1607T>C	Ile536Thr	6
ClinVAR	1748G>A	Gly583Asp	7
ClinVAR	1769T>G	Met590Arg	7
ClinVAR	1807G>A	Gly603Arg	8
ClinVAR	1867C>T	Arg623Trp	8
ClinVAR	1909A>G	Ser637Gly	9
ClinVAR	1909_1911delAGT		9
ClinVAR	1910G>A	Ser637Asn	9
ClinVAR	1913C>G	Pro638Arg	9
ClinVAR	2014G>A	Gly672Ser	9
ClinVAR	2062C>T	Arg688Ter	9
ClinVAR	2089G>A	Gly697Arg	9
ClinVAR	2158A>G	Lys720Glu	9
ClinVAR	2173G>A	Glu725Lys	9
ClinVAR	2219C>T	Ser740Phe	9

ClinVAR	2231A>T	Asn744Ile	9
ClinVAR	2287G>A	Glu763Lys	9
ClinVAR	2299A>C	Lys767Gln	9
ClinVAR	2303C>G	Ser768Trp	9
ClinVAR	2338G>C	Gly780Arg	9
ClinVAR	2359G>A	Glu787Lys	9
ClinVAR	2393C>T	Pro798Leu	9
ClinVAR	2501dupA	Asp834Glufs	9
ClinVAR	2588C>G	Pro863Arg	10
ClinVAR	2662G>A	Asp888Asn	11
ClinVAR	2736C>A	Asp912Glu	11
ClinVAR	2869C>G	Leu957Val	11
ClinVAR	2986G>T	Asp996Tyr	11
ClinVAR	3023G>C	Arg1008Pro	11
ClinVAR	3046G>A	Gly1016Ser	11
ClinVAR	3047G>C	Gly1016Ala	11
ClinVAR	3067G>T	Asp1023Tyr	11
ClinVAR	3331G>T	Val1111Leu	12
ClinVAR	3464T>C	Val1155Ala	13
ClinVAR	3496T>C	Cys1166Arg	13
ClinVAR	3632C>T	Pro1211Leu	14
ClinVAR	3677 A>G	Lys1226Arg	14
Invitae	138_149dupGCCGCCCCAGCC	Gln49_Pro52dup	1
Invitae	247C>A	Leu83Ile	2
Invitae	299T>G	Leu100Arg	2
Invitae	352A>G	Thr118Ala	2
Invitae	434G>A	Gly145Asp	2
Invitae	442G>A	Gly148Ser	2
Invitae	613C>A	Gln205Lys	2
Invitae	710G>T	Gly237Val	2
Invitae	761C>T	Ser254Leu	2
Invitae	1100G>A	Arg367Gln	2
Invitae	1160C>T	Ala387Val	2
Invitae	1451C>T	Thr484Ile	5
Invitae	1511C>T	Ala504Val	5
Invitae	1543G>A	Gly515Arg	6
Invitae	1552C>T	Arg518Cys	6
Invitae	1766G>A	Arg589Gln	7
Invitae	1784A>G	Lys595Arg	7
Invitae	1900C>T	Arg634Trp	9
Invitae	1901G>A	Arg634Gln	9
Invitae	1922G>A	Arg641Gln	9
Invitae	1988G>A	Gly663Asp	9

Invitae	2018G>A	Arg673Gln	9
Invitae	2069C>T	Pro690Leu	9
Invitae	2131C>T	Arg711Cys	9
Invitae	2551G>A	Ala851Thr	10
Invitae	2565A>C	Glu855Asp	10
Invitae	3076G>A	Glu1026Lys	11
Invitae	3119C>G	Ala1040Gly	11
Invitae	3245T>G	Leu1082Arg	11
Invitae	3268A>T	Ile1090Phe	11
Invitae	3330C>G	Tyr1110*	12
Invitae	3331G>A	Val1111Met	12
Invitae	3545G>A	Arg1182His	13
Invitae	3564G>C	Arg1188Ser	13
Invitae	3584C>A	Ser1195Tyr	14
Invitae	3595G>A	Glu1199Lys	14
LMM	56C>G	Pro19Arg	1
LMM	128_130dupAGC	Gln43_Pro44insGln	1
LMM	131C>A	Pro44Gln	1
LMM	136C>T	Pro46Ser	1
LMM	364C>A	Gln122Lys	2
LMM	441C>A	His147Gln	2
LMM	448G>A	Ala150Thr	2
LMM	517C>A	Pro173Thr	2
LMM	529A>T	Thr177Ser	2
LMM	530C>T	Thr177Ile	2
LMM	530C>G	Thr177Arg	2
LMM	536G>A	Gly179Asp	2
LMM	544C>A	Pro182Thr	2
LMM	680G>T	Gly227Val	2
LMM	695G>A	Gly232Asp	2
LMM	746G>T	Gly249Val	2
LMM	850G>A	Gly284Arg	2
LMM	925G>A	Gly309Arg	2
LMM	1013T>C	Met338Thr	2
LMM	1024C>A	Pro342Thr	2
LMM	1051G>C	Asp351His	2
LMM	1093G>A	Gly365Arg	2
LMM	1190A>C	His397Pro	2
LMM	1259A>G	Lys420Arg	2
LMM	1286T>C	Leu429Pro	3
LMM	1329C>G	Phe443Leu	3
LMM	1364C>T	Ser455Leu	4
LMM	1418C>T	Ala473Val	4

LMM	1459G>A	Val487Met	5
LMM	1494C>A	Ser498Arg	5
LMM	1519G>A	Gly507Arg	5
LMM	1529T>C	Phe510Ser	6
LMM	1538G>A	Arg513Gln	6
LMM	1633G>A	Val545Ile	6
LMM	1769T>A	Met590Lys	7
LMM	1779A>T	Arg593Ser	7
LMM	1816G>A	Val606Met	8
LMM	1906C>T	Arg636Cys	9
LMM	1906C>A	Arg636Ser	9
LMM	1907G>A	Arg636His	9
LMM	1913C>T	Pro638Leu	9
LMM	1958C>T	Thr653Ile	9
LMM	1973C>G	Ser658Cys	9
LMM	2017C>T	Arg673Trp	9
LMM	2108G>A	Arg703Lys	9
LMM	2116C>A	Pro706Thr	9
LMM	2147G>A	Arg716Gln	9
LMM	2161G>T	Ala721Ser	9
LMM	2183_2185delAAG	Glu728del	9
LMM	2201G>A	Arg734Gln	9
LMM	2213C>T	Pro738Leu	9
LMM	2286_2287insA	Glu763ArgfsX38	9
LMM	2303C>T	Ser768Leu	9
LMM	2303G>C	Trp768Ser	9
LMM	2318A>G	Lys773Arg	9
LMM	2333C>T	Ala778Val	9
LMM	2338G>A	Gly780Arg	9
LMM	2357A>G	Asp786Gly	9
LMM	2452G>T	Ala818Ser	9
LMM	2565_2570delACAGGA	Gln856_Glu857del	10
LMM	2679T>G	Ser893Arg	11
LMM	2723T>C	Leu908Pro	11
LMM	2727_2741del	Thr910_Val914del	11
LMM	2728A>C	Thr910Pro	11
LMM	2737G>A	Glu913Lys	11
LMM	2746G>A	Glu916Lys	11
LMM	2761A>G	Ile921Val	11
LMM	2855C>T	Thr952Ile	11
LMM	2887A>G	Lys963Glu	11
LMM	3004C>G	Leu1002Val	11
LMM	3022C>T	Arg1008Trp	11

LMM	3031G>A	Ala1011Thr	11
LMM	3115C>T	Pro1039Ser	11
LMM	3170G>A	Arg1057Gln	11
LMM	3261_3262delinsG	Ser1087ArgfsX17	11
LMM	3265C>G	Pro1089Ala	11
LMM	3265C>A	Pro1089Thr	11
LMM	3373G>A	Glu1125Lys	12
LMM	3500G>T	Gly1167Val	13
LMM	3623C>T	Ala1208Val	14
LMM	3667G>C	Glu1223Gln	14

Supplemental Table 2. Regional Odds of Presence of Cardiomyopathy Associated Variants: 40-basepair windows ranked by statistical significance.

Last Position of 40 bp Window	**Number of gnomAD Variants in Window** (of 604 variants)	**Number of RBM20 CM Database Variants in Window** (of 171 variants)	**Odds Ratio**	**95% CI Upper Bound**	**95% CI Lower Bound**	**p-value**	**FDR 20%**
1920	8	10	4.627329	13.69374	1.610866	0.001786	**0.00212766**
2760	2	6	10.94545	111.3944	1.926553	0.002009	**0.00425532**
1640	3	4	4.798403	32.98772	0.80121	0.045823	0.00638298
560	11	7	2.300998	6.610314	0.742993	0.08915	
2360	7	5	2.568847	9.525956	0.633437	0.150517	
1800	8	5	2.243976	7.889678	0.569084	0.174615	
1520	4	3	2.678571	15.97456	0.387998	0.184471	
120	8	0	0	1.683808	0	0.210864	
2840	8	0	0	1.683808	0	0.210864	
2400	14	1	0.247899	1.653967	0.005829	0.211719	
1040	7	4	2.042772	8.139503	0.432811	0.2715	
2320	15	7	1.676016	4.45548	0.567835	0.295199	
1480	3	2	2.370809	20.84058	0.196268	0.305305	
2240	8	4	1.784431	6.753425	0.388109	0.310795	
920	6	0	0	2.254026	0	0.347903	
2280	6	0	0	2.254026	0	0.347903	
3160	7	0	0	1.928344	0	0.357769	
3240	7	0	0	1.928344	0	0.357769	
440	1	1	3.547059	278.87	0.044925	0.392828	
3360	6	3	1.779762	8.430651	0.284807	0.421815	
800	10	1	0.349412	2.489807	0.008005	0.471428	
880	11	1	0.317112	2.212647	0.007325	0.480367	
1760	11	1	0.317112	2.212647	0.007325	0.480367	
1560	9	4	1.5835	5.75688	0.351704	0.498229	

3080	9	4	1.5835	5.75688	0.351704	0.498229	
240	2	1	1.770588	34.17236	0.029838	0.527144	
280	2	1	1.770588	34.17236	0.029838	0.527144	
1280	2	1	1.770588	34.17236	0.029838	0.527144	
2520	2	1	1.770588	34.17236	0.029838	0.527144	
2000	13	2	0.538006	2.41391	0.058427	0.542003	
1000	4	0	0	3.393948	0	0.581045	
2640	4	0	0	3.393948	0	0.581045	
3320	4	0	0	3.393948	0	0.581045	
200	5	0	0	2.710279	0	0.591777	
840	5	0	0	2.710279	0	0.591777	
1240	5	0	0	2.710279	0	0.591777	
320	4	2	1.775148	12.49603	0.159162	0.618285	
2880	4	2	1.775148	12.49603	0.159162	0.618285	
3120	4	2	1.775148	12.49603	0.159162	0.618285	
640	5	2	1.417751	8.748432	0.133818	0.653114	
1120	5	2	1.417751	8.748432	0.133818	0.653114	
1320	5	2	1.417751	8.748432	0.133818	0.653114	
1360	5	2	1.417751	8.748432	0.133818	0.653114	
1840	5	2	1.417751	8.748432	0.133818	0.653114	
2720	5	2	1.417751	8.748432	0.133818	0.653114	
680	6	2	1.179487	6.67172	0.1154	0.691313	
1960	6	2	1.179487	6.67172	0.1154	0.691313	
2680	6	2	1.179487	6.67172	0.1154	0.691313	
360	8	1	0.438235	3.309946	0.009819	0.692118	
2480	8	1	0.438235	3.309946	0.009819	0.692118	
3200	8	1	0.438235	3.309946	0.009819	0.692118	
3000	9	1	0.388889	2.843536	0.008821	0.700056	
2600	8	3	1.330357	5.617975	0.224798	0.714389	
480	9	3	1.180556	4.801863	0.203299	0.733081	
2120	9	3	1.180556	4.801863	0.203299	0.733081	
2160	9	3	1.180556	4.801863	0.203299	0.733081	
2200	9	3	1.180556	4.801863	0.203299	0.733081	
3680	12	2	0.583826	2.662827	0.062907	0.745826	
160	11	4	1.291236	4.4343	0.295969	0.752423	
3040	12	4	1.181637	3.963322	0.274155	0.762646	
960	5	1	0.704706	6.361082	0.014813	1	
1440	5	1	0.704706	6.361082	0.014813	1	
1880	5	1	0.704706	6.361082	0.014813	1	
3560	5	1	0.704706	6.361082	0.014813	1	
80	7	2	1.009298	5.357961	0.101404	1	
520	7	2	1.009298	5.357961	0.101404	1	
1160	7	2	1.009298	5.357961	0.101404	1	
2080	7	2	1.009298	5.357961	0.101404	1	
1680	1	0	0		0	1	
3720	1	0	0		0	1	
2800	7	1	0.501681	3.951525	0.011067	1	
720	10	2	0.702959	3.346267	0.074233	1	
3520	8	2	0.881657	4.475896	0.090408	1	

40	4	1	0.882353	8.992929	0.017813	1	
760	4	1	0.882353	8.992929	0.017813	1	
2920	4	1	0.882353	8.992929	0.017813	1	
1200	9	2	0.78238	3.832357	0.081539	1	
3640	9	2	0.78238	3.832357	0.081539	1	
600	3	0	0	4.53231	0	1	
1600	3	0	0	4.53231	0	1	
1720	3	0	0	4.53231	0	1	
2440	3	0	0	4.53231	0	1	
2960	3	0	0	4.53231	0	1	
3440	3	0	0	4.53231	0	1	
3280	17	5	1.040043	2.992977	0.295571	1	
3600	13	3	0.811813	3.003051	0.146745	1	
3400	3	1	1.178431	14.7794	0.022318	1	
2040	12	3	0.880952	3.316867	0.157768	1	
400	6	1	0.586275	4.88565	0.012672	1	
1080	6	1	0.586275	4.88565	0.012672	1	
1400	6	1	0.586275	4.88565	0.012672	1	
2560	6	1	0.586275	4.88565	0.012672	1	
3480	6	1	0.586275	4.88565	0.012672	1	