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EDITORIAL COMMENT

Mutations of Splicing Regulator RBM20 in Atrial Fibrillation*

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Already the most common arrhythmia, atrial fibrillation (AF) is increasing in prevalence as the population ages and as risk factors like obesity, hypertension, and diabetes become more prevalent. Although these modifiable risk factors are major contributors to the development of AF, extensive investigations have also uncovered a genetic contribution. Genome-wide association studies identified more than 130 loci, each with small effect sizes, associated with AF, leading to the development of promising polygenic risk scores.[1] In contrast, only a handful of loci harboring variants with effect sizes large enough to cause familial AF are known. Of these variants, loss-of-function mutations in titin (*TTN*) account for more than 7% of AF identified in early-onset AF.[1] Thus, the report in this issue of *JACC: Basic to Translational Science* by Vad et al[2] showing an association between loss-of-function variants in RNA-binding motif protein 20 (*RBM20*) is a notable addition to the understanding of the genetic underpinnings of AF.

RBM20, an RNA-binding protein important in regulation of messenger RNA (mRNA) splicing, recognizes a consensus UCUU RNA motif through its RNA-recognition motif (RRM) domain and is almost exclusively expressed in cardiomyocytes.[3,4] Among the mRNAs for which RBM20 regulates alternative splicing are transcripts encoding key cardiomyocyte structural proteins important for contraction, like titin, or ion transport proteins, such as the $Ca_V1.2$ Ca^{2+} channel (*CACNA1C*) and the ryanodine receptor (*RYR2*). Alternative splicing of titin and the regulatory role of RBM20 in this process have been well studied. Titin is the longest polypeptide in nature, and its gene contains the largest number of exons. During heart development, alternative splicing is crucial for a shift of *TTN* mRNA from longer to shorter isoforms. Dysregulation of *TTN* splicing in the context of *RBM20* mutations has been linked to a severe dilated cardiomyopathy (DCM), often accompanied by malignant ventricular arrhythmias.[5] Here, Vad et al[2] discovered similar but distinct consequences of *RBM20* mutations for AF (**Figure 1**).

Querying the Danish National Patient Registry and 2 Norwegian hospital registries for subjects with early-onset AF (<50 years of age) and without evidence of other cardiovascular disease, Vad et al[2] recruited 531 patients (83.4% male; median age at AF onset: 30 years). Three (0.56%) subjects had rare (minor allele frequency: <0.01%; not in ClinVar or gnomAD) loss-of-function truncation variants compared to 0.04% in control individuals. Vad et al[2] also interrogated the UK Biobank, in which they replicated the association between *RBM20* loss-of-function mutations and AF, identifying additional *RBM20* variants associated with AF. Moreover, among a subset of UK Biobank participants with cardiac magnetic resonance imaging data, *RMB20* loss-of-function mutations correlated with abnormal atrial anatomic or functional parameters, thus further supporting an association between *RBM20* loss-of-function mutations and AF. The authors' inclusion criterion of subjects without evidence of other cardiovascular disease is important and intriguing. DCM, with which loss-of-function mutations in *RBM20* has been previously associated, can lead to AF (and vice versa). Further, AF appears even more prevalent among DCM patients with *RBM20* mutations compared to an

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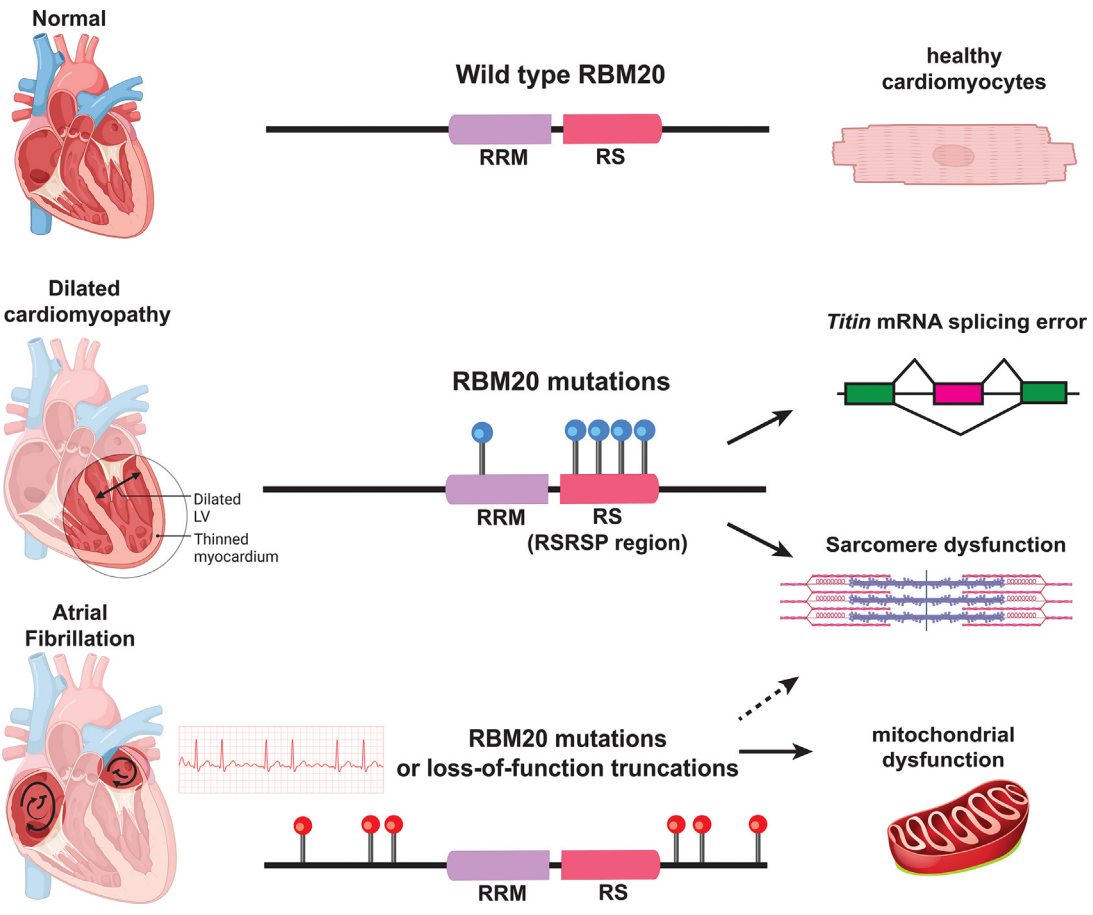
FIGURE 1 Consequences of *RBM20* May Be Location-Dependent



Normal heart function relies on RBM20, a key splicing regulator protein in cardiomyocytes. Previous studies of *RBM20* mutations have focused on dilated cardiomyopathy (DCM), in which aberrant *RBM20* function causes dysregulated splicing of the titin mRNA and consequent sarcomere dysfunction. These DCM-associated mutations are highly enriched in a 5-residue RSRSP region in the arginine and serine-rich domain (RS domain), with additional rare mutations in other regions such as the RRM domain. Vad et al[2] uncovered a new association of *RBM20* mutations and AF. These AF-associated mutations, which affect splicing of transcripts encoding sarcomeric proteins, are all outside of the RS or RRM domains. Different from previously characterized *RBM20* mutations associated with DCM, these mutations can cause loss-of-function truncation of the RBM20 protein and downstream defects in mitochondrial and sarcomere functions. LV = left ventricle; mRNA = messenger RNA; RBM20 = RNA-binding motif protein 20; RRM = RNA-recognition motif.

overall DCM cohort.[6] Thus, the careful selection of subjects with early-onset AF and without other cardiovascular disease may remove that confounder. Subjects with *RBM20* mutations may initially present with either DCM or AF even though some will eventually develop both. That the patients studied by Vad et al[2] developed AF in the absence of DCM implies that the *RBM20* mutations leading to AF and DCM may be distinct subsets.

Indeed, although not explicitly stated in the report, the newly identified *RMB20* mutations associated with AF appear to be different from previously characterized DCM-associated mutations, most of which are heterozygous missense mutations located in an Arg-Ser-Arg-Ser-Pro (RSRSP, 634-638) region of the arginine and serine-rich domain (RS domain).[4] In contrast, of the more than 10 novel *RBM20* mutations identified by Vad et al[2] that are associated with early-onset AF, none is located in the RSRSP region or the adjacent RRM domain (Figure 1). This may imply distinct consequences for RBM20-mediated splicing control for a mutation within the RSRSP region

compared to a mutation outside of the RSRSP region and that these different splicing consequences differentially confer either DCM or AF, respectively. Supporting this hypothesis, a mouse model with an RSRSP mutation in *Rbm20* developed severe DCM and AF, whereas an *Rbm20* frameshift mutation—similar to the mutations identified in the early-onset AF cohort—displayed a less severe phenotype.[7] Even among the 10 AF-associated mutations studied here, the authors' titin splicing reporter assay (eg, Figure 5C in Vad et al[2]) revealed different consequences depending on the type of mutation. The truncation mutations (generated by early stop codons) generally have major consequence for *TTN* mRNA splicing, whereas the missense mutations (all outside of the RSRSP region) do not alter the splicing pattern. Although *TTN* is a major AF-associated locus, it will be interesting to determine whether dysregulated *TTN* splicing is the main consequence of *RBM20* mutations associated with AF, potentially with a high-throughput sequencing approach in the rodent model, and whether the AF vs DCM associated mutations differentially affect *TTN* splicing.

Vad et al[2] investigated additional mechanisms by which *RBM20* mutations might cause AF. Using an unbiased screen to uncover transcripts that displayed alternative splicing in *Rbm20*$^{+/-}$ or *Rbm20*$^{-/-}$ rat atria, they identified several sarcomeric protein transcripts known to be *Rbm20* targets, including transcripts for tropomyosin 1 and titin. Additionally, the investigators also observed a differential atrial gene expression pattern (between wild type mice and mice with *Rbm20* deletions) that suggested that the *Rbm20* loss-of-function sequence variants affect mitochondrial function. Consistent with that finding, they observed impaired mitochondrial function in *Rbm20*$^{-/-}$ rat atria. Altered mitochondrial gene expression and respiration appear to be novel consequences for *RBM20* variants. Whether these consequences are downstream of sarcomere dysregulation, as implied by the investigators, remains to be defined. We speculate that these mitochondrial consequences could alternatively or additionally result from aberrant splicing of *IMMT* (encoding inner membrane mitochondrial protein), a known RBM20 target.[8]

This multimodal study providing evidence that mutations in *RBM20* may cause early-onset AF also raises additional questions for future investigation. Most prominently, why did these subjects present with AF and not DCM? The apparent segregation of DCM subjects (who bear mutations in RBM20's RSRSP region) from AF subjects (in whom the *RBM20* mutations appear to reside outside of the RSRSP region) is especially intriguing. As noted by the authors, however, the number of subjects studied here is small, so whether this segregation will hold as more early-onset AF cases with *RBM20* mutations are identified is not known. Alternatively, because AF is more common among DCM subjects with *RBM20* mutations compared to a general DCM cohort, perhaps AF only develops in a subset of patients with *RBM20* mutations and who have specific AF clinical risk factors or a polygenic inheritance pattern associated with an increased risk for AF. This hypothesis could be tested, for example, by calculating an AF polygenic risk score in these subjects or looking for a correlation with traditional AF risk factors. Although it is notable that the investigators' exclusion criteria for other cardiovascular disease would suggest that the subjects studied here developed AF exclusively (ie, and did not manifest DCM), it would be interesting to follow these subjects over time to determine if they are at an increased risk of developing DCM. Further, DCM is highly penetrant among families with *RBM20* mutations associated with DCM. Is AF similarly penetrant in families with these novel *RBM20* mutations? Assessing first-degree genotype-positive relatives of the subjects studied here for DCM and/or AF could be revealing. Finally, combining investigations of DCM and AF subjects may yield new insights into critical roles for *RBM20*.

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