Genetics inMedicine | CORRESPONDENCE



# Response to Brodehl et al.

We are pleased that Brodehl and colleagues concur with us regarding the utility of functional studies to evaluate genetic variants identified by genome sequencing in patients with dilated cardiomyopathy (DCM).[1,2] Their correspondence provides new data about two missense variants, *RBM20* p.R636C and *DES* p.L398P, that were identified in our familial DCM cohort, and serves to highlight the difficulties that are frequently encountered when interpreting the significance of genetic variants in the clinical setting.

The *RBM20* p.R636C variant is located within the arginine/serine-rich (RS) domain, which is a putative DCM mutation hotspot.[3,4] The p.R636C variant has been associated previously with familial DCM, and two other substitutions at the same amino acid residue, p.R636S and p.R636H, have been shown to segregate with disease in DCM kindreds.[3,4] Notably, there are no missense variants at this site in >60,000 individuals in the Exome Aggregation Consortium (ExAC) population database. Functional evaluation of the *RBM20* p.R636S variant using induced pluripotent stem cell–derived cardiomyocytes from two DCM patients has demonstrated altered sarcomere morphology, together with altered transcriptional profiles of sarcomeric gene isoforms, and abnormalities of calcium handling.[5] Recently, the RS domain was shown to be a critical determinant of nuclear localization of *RBM20* (ref. [6]). In keeping with this, Brodehl and colleagues showed that the p.R636C variant results in marked cytoplasmic accumulation of *RBM20* in transfected HEK293T cells, in contrast to the nuclear distribution seen in wild-type transfected cells.[2]

The *DES* p.L398P variant is located in the coil 2B region of the desmin rod and is a novel variant not previously reported in DCM patients or in the ExAC database. Desmin is an intermediate protein that forms a three-dimensional cytoskeletal scaffolding in heart and skeletal muscle. Functional studies performed by several groups, including Brodehl and colleagues, have shown that DCM-associated *DES* missense variants at p.L398 and in other regions of the desmin protein disrupt normal filament assembly and result in abnormal desmin aggregate formation.[2,7,8]

Taken together, Brodehl et al.'s data convincingly show that both the *RBM20* p.R636C and *DES* p.L398P variants alter normal protein function.[2] However, it needs to be borne in mind that not all function-altering variants are necessarily disease-causing, and that the human genome includes thousands of genes in which loss-of-function variants are completely tolerated. We recently evaluated the prevalence of rare variants in cardiomyopathy-associated genes in a cohort of DCM patients and healthy control subjects.[9] Surprisingly, we found that two-thirds of the asymptomatic control subjects carried rare cardiomyopathy gene variants, with many individuals having high-impact truncating and predicted-deleterious missense variants.[9] We then ranked the cardiomyopathy genes according to the strength of genetic, in vitro, and animal model evidence for roles in DCM pathogenesis, and found that the odds ratio for DCM was increased ninefold for the subset of truncating and predicted-deleterious missense variants that were present in genes that had highest a priori likelihood of disease causation.[9] These data clearly show that effective variant stratification requires information about the variant itself, and the biological role of the specific genes involved.[9] Importantly, both *RBM20* and *DES* achieved group A status in our DCM disease gene ranking.

Do these new functional data change our classification of the *RBM20* p.R636C and *DES* p.L398P variants? Based on the collective weight of genetic and functional evidence linking variants at p.R636 and at neighboring residues to DCM, *RBM20* p.R636C readily meets the American College of Medical Genetics and Genomics (ACMG) criteria for variant pathogenicity.[10] Interestingly, although *RBM20* p.R636C was present in all affected individuals in family KS, it was also present in one unaffected female aged >50 years.[1] Does this apparent lack of segregation mitigate against pathogenicity? In our experience, it is not uncommon in families with deleterious *RBM20* or *TTN* variants to find genotype-positive female carriers who remain completely asymptomatic until late in life. The reason for these age and sex effects on DCM penetrance is as yet unclear. Other factors that may contribute to genotype–phenotype discordance in families include phenocopies due to acquired environmental causes of DCM, second independent disease-causing genetic variants in some individuals, and phenotypic variability due to modifier variants. On the other hand, *DES* p.L398P is a novel variant that segregates completely with DCM, but only in one small family.[1] Although we initially classified this as a variant of unknown significance, the addition of in vitro experimental data upgrades this classification to likely pathogenic (ACMG rule PS3) (ref. [10]). However, as noted by Brodehl and colleagues, *DES* variants that result in in vitro filament assembly defects are not always detrimental in vivo and may show incomplete penetrance in families.[2,8]

Interpretation of genetic testing results is not straightforward, and we agree with Brodehl and colleagues[2] that testing is best undertaken in the setting of a multidisciplinary clinic. Until more knowledge is available, the likelihood of novel variants and variants in novel genes to meet criteria for pathogenicity will be limited. Comprehensive cataloging of genetic variants in healthy and diseased populations,

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functional evaluation of variants, and a better understanding of the roles of genes in cardiomyocyte biology and in DCM pathogenesis, are urgently needed for genome sequencing to realize its potential in genomic medicine. This will require a concerted effort by clinicians and basic scientists alike in the cardiovascular genetics community.

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## DISCLOSURE

M.E.D., K.W., A.L.S., and B.L. are employed by Genome.One, a clinically accredited genetic testing provider that uses genome sequencing. The other authors declare no conflicts of interest.

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