Genetics inMedicine | CORRESPONDENCE

Functional analysis of *DES*-p.L398P and *RBM20*-p.R636C

With great interest, we have read the study of Minoche et al.[1] comparing genome sequencing with panel sequencing for genetic testing in patients with dilated cardiomyopathy (DCM). The authors demonstrate remarkably that genome sequencing is able to identify more genetic variants in affected DCM patients when compared with panel sequencing, which is at present routine practice in genetic cardiovascular diagnostics. One important consequence of this paper is that genome sequencing should be more frequently used not only in research but also for clinical diagnosis.

However, the study of Minoche et al. demonstrates convincingly that the bottleneck for the transfer of genetic information to clinical practice is the interpretation of genetic sequence variants, which still remains challenging. Up to now, the majority of identified variants were classified as variants of unknown significance (VUS),[1] which is currently a general common finding in cardiovascular genetics. Thus, the relevant questions especially after genome sequencing are:

How can pathogenic variants be differentiated from benign variants?
How do specific sequence variants affect the clinical progression of cardiomyopathies?

The authors point out in their study that functional testing in vitro and in vivo could help solve these problems in specific cases. We support this conclusion of the authors.

In this context, the two variants *DES*-p.L398P (c.1192C>T) and *RBM20*-p.R636C (c.1906C>T) aroused our special interest. Whereas *RBM20*-p.R636C was classified as a pathogenic variant, *DES*-p.L398P identified in a different patient was classified as a VUS.[1]

RBM20 encodes the RNA-binding motif protein 20, which is involved in the splicing of several transcripts of important cardiac genes like *TTN* or *RYR2* (ref. [2]). Most of the pathogenic *RBM20* variants are localized in the arginine/serine (RS) rich domain.

DES encodes the muscle specific intermediate filament protein desmin, which is important for the structural integrity of cardiomyocytes. Most of the previously described *DES* variants are missense or small deletion variants affecting the filament assembly leading in consequence to an abnormal cytoplasmic desmin aggregation.

Because our group is interested in the pathomechanisms of both genes, we investigated the effects of the reported variants in vitro. Whereas the wild-type form of RBM20 was localized in the nuclei, RBM20-p.R636C was retained in the cytoplasm revealing a severe mislocalization in vitro (Figure S1, Supplements). Thus, the in vitro data suggest a pathogenic potential of this variant. Desmin-p.L398P formed in transfected HEK293T cells abnormal cytoplasmic aggregates, whereas the wild-type desmin assembles into regular intermediate filaments in vitro (Figure S1, Supplements). Desmin filaments are assembled in a step-wise process by coiled-coil desmin dimers (Figure S1, Supplements). Proline residues prevent hydrogen bonds within the peptide bonds of α-helices forming the desmin backbone. Consequently, many missense *DES* variants leading to a substitution against proline are pathogenic. Therefore, the desmin aggregate formation of desmin-p.L398P in cell culture reveals a pathogenic potential.

These data demonstrate that the regular localization of RBM20 as well as the filament formation of desmin are severely affected by these variants suggesting their pathogenic impact. However, even results from in vitro test systems, which might assist in classification of sequence variants, should be interpreted with care, i.e., the variant *DES*-p.L136P clearly shows a severe and dominant filament formation defect in vitro but the pedigree reveals an incomplete penetrance of this variant, leaving open questions.[3]

In conclusion, we emphasize the need of functional assays even in the context of deep genetic analyses using genome sequencing. However, the interpretation and transfer of functional data for the classification of pathogenicity in clinical genetics needs deeper insights into molecular and cellular pathomechanisms. Therefore, we are convinced that this needs—especially in the clinical setting—an interdisciplinary approach by clinicians, geneticists, bioinformaticians, and basic scientists.

ELECTRONIC SUPPLEMENTARY MATERIAL

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DISCLOSURE

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