LETTERS

Functional characterization of desmin mutant p.P419S

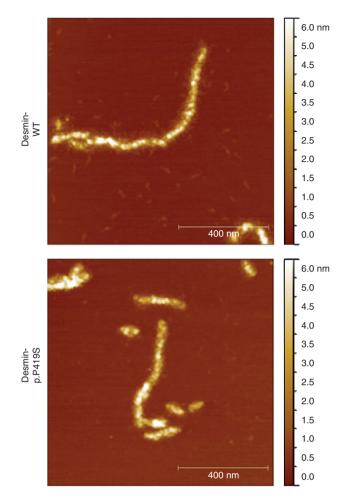
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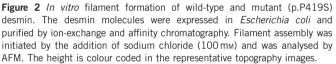
Recently, Hedberg et al¹ identified a DES mutation (p.P419S) in a Swedish family, suffering from myofibrillar myopathy (MFM) in combination with arrhythmogenic right ventricular cardiomyopathy (ARVC), by next-generation sequencing. Originally, a linkage analysis indicated that the genetic defect is located on chromosome 10q22.3 in this family.² The analysis of muscle biopsies of affected patients demonstrated an aggregation of desmin and further proteins.

The same desmin mutation (p.P419S) was identified before by Olivé et al³ in patients suffering from skeletal myopathy or hypertrophic cardiomyopathy, respectively. However, Hedberg et al¹ demonstrated that this mutation did not completely co-segregate within the Swedish family, raising the questions on pathogenesis or penetrance, respectively. Of note, in both studies the desmin mutant p.P419S was not functionally characterized. Hence, it is currently difficult to judge the pathological potential of this variant. Especially, it is unclear whether the DES mutation p.P419S is a sufficient molecular trigger for aggregate formation.

For this reason, we introduced this mutation by site-directed mutagenesis into a desmin construct (pEYFP-N1-Desmin) using appropriate oligonucleotides and transfected H9c2, C2C12 and SW-13 cells with mutant and wild-type desmin-eYFP constructs. The filament or aggregate formation was investigated in cell culture

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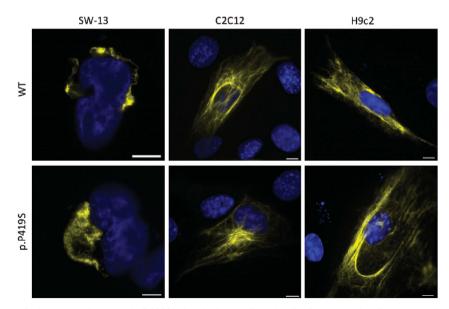


Figure 1 Filament formation of wild-type and mutant (p.P419S) desmin in transfected cells. Representative fluorescence images of transfected SW-13, C2C12 and H9c2 cells expressing desmin-eYFP constructs (yellow) were shown. The nuclei were stained with DAPI. Scale bars represent 10 µm.

and the filament formation of purified recombinant mutant desmin was analysed *in vitro* by atomic force microscopy (AFM), as previously described.⁴

To our surprise, the expression of desmin-p.P419S does not induce an aggregation in either cell line as recently described for other ARVCrelated desmin mutants^{4–6} (Figure 1). The cell culture data were also supported by the AFM analysis virtually yielding undistinguishable desmin filaments between wild-type and desmin-p.P419S *in vitro* (Figure 2). Thus, our data reveal that the desmin mutant p.P419S published by Hedberg *et al*¹ forms filaments *in vitro* and in transfected cells. Consequently, it might be important to look for further molecular triggers, which induce or influence the protein aggregation in the Swedish patients suffering from MFM/ARVC. From our point of view, the next-generation sequencing data of Hedberg *et al*¹ might provide an important basis for further studies, identifying modifier genes or other molecular abnormalities responsible for desmin aggregate formation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Reply to Brodehl et al

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We appreciate the comments by Brodehl et al¹ on our recent article describing a DES mutation in a family with myofibrillar myopathy and arrhythmogenic right ventricular cardiomyopathy.² We would like to clarify that the mutation, p.P419S in the desmin gene (DES), indeed co-segregates with the disease. When we compared the muscle biopsy findings with the presence of the p.P419S DES mutation, desmin storage was found in all investigated family members with the DES mutation but not in those without the mutation. The clinical expression of the disease was highly variable within the family. The original linkage study on this family was based on combined findings from clinical examination, electromyography and muscle biopsy.³ Three of five asymptomatic individuals were incorrectly considered affected by the myopathy based on these investigations. These three individuals showed only mild and unspecific myopathic changes and no desmin storage. Whether these individuals were affected by another mild myopathy remains to be clarified. These results demonstrate diagnostic difficulties with some forms of dominantly inherited muscle diseases, as they can display a wide clinical and morphological variability even within a given family.

In conclusion, despite the report by Brodehl *et al*¹, we believe that the identified desmin mutation is causative for the diseases in our family, as it segregates perfectly with desmin storage in muscle.

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- Hedberg C, Melberg A, Kuhl A, Jenne D, Oldfors A: Autosomal dominant myofibrillar myopathy with arrhythmogenic right ventricular cardiomyopathy 7 is caused by a DES mutation. *Eur J Hum Genet* 2012; 20: 984–985.
- 2 Melberg A, Oldfors A, Blomstrom-Lundqvist C *et al*: Autosomal dominant myofibrillar myopathy with arrhythmogenic right ventricular cardiomyopathy linked to chromosome 10q. *Ann Neurol* 1999; **46**: 684–692.
- 3 Olivé M, Armstrong J, Miralles F *et al*: Phenotypic patterns of desminopathy associated with three novel mutations in the desmin gene. *Neuromuscul Disord* 2007; 17: 443–450.
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Further support for this conclusion is the finding of the same mutation segregating with desminopathy in a Spanish family.⁴

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