Mutation analysis and evaluation of the cardiac localization of TMEM43 in arrhythmogenic right ventricular cardiomyopathy

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Introduction
A single report has associated mutations in the gene transmembrane protein 43 (TMEM43/LUMA) with arrhythmogenic right ventricular cardiomyopathy (ARVC). No mutations have been reported outside Newfoundland and the immunohistochemical features associated with TMEM43 mutations are unknown. The aims of the present study were to screen a European ARVC cohort for mutations and characterize the myocardial expression of TMEM43, plakoglobin, and emerin in mutation carriers.

Methods
Sixty-five index patients (55 fulfilling 1994 Task Force criteria and 10 borderline cases) of Northern European descent were included in the study. We performed genetic screening, sequence alignments, in silico analysis, family evaluation and immunohistochemistry.

• The patients were screened for mutations in the coding regions and flanking introns of TMEM43 by direct sequencing or LightScanner melting curve analysis. For all identified sequence variants 1300 control alleles were screened.

• Sequence alignments were performed with ClustalW2.

• Family evaluation consisted of clinical evaluation according to a standard protocol and genetic analysis.

• Identified splice variants were analyzed with in silico methods using the Human Splice Finder, NetGene2 and Genscan algorithms.

• Immunohistochemical myocardial analyses for TMEM43, plakoglobin and emerin were performed in gene-positive individuals (n=3) and controls (n=3).

Results
The genetic screening identified heterozygous variants in two families (Figure 1): one novel variant (c.705+7G>A; Family A) of unknown significance and one reported mutation (c.1073C>T, p.S358L; Family B). The two TMEM43-positive probands both fulfilled ARVC criteria and did not have carry disease-causing mutations in DSC2, DSG2, DSP, JUP, PKP2, TGFb3, or have large genomic deletions.

In silico analysis of the c.705+7G>A variant predicted the variant to be damaging in only one out of the three used algorithms. Immunostaining of myocardium with TMEM43-specific antibody showed a concentrated staining of the sarcolemma (Figure 2). No staining of the nucleus was observed. The immunoreaction was reduced in all three TMEM43-positive patients. Immunostaining of a tissue panel for TMEM43 did not show any immunoreaction in the plasma membrane. The observed immunoreaction intensity for plakoglobin was also consistently reduced in all TMEM43-positive patients. All samples investigated displayed a similar staining pattern for emerin.

Conclusions
Two sequence variants in TMEM43 were identified in this Danish ARVC cohort. Immunohistochemical myocardial analysis showed a unique cardiac localization of TMEM43. Furthermore, mutation carriers displayed a reduced immunoreaction for plakoglobin. The TMEM43 gene underlies a distinctive form of ARVC which may share a final common pathway with desmosome-associated ARVC.

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