A novel triadin variant causes a severe clinical CPVT phenotype in two young brothers

Brøndberg AK1, Dait C1, Bjerre J1, Pedersen LN4, Nielsen JC1, Corydon TJ3, Jensen HK1
1 Department of Cardiology, Aarhus University Hospital, Aarhus, Denmark
2 Department of Biomedicine, Aarhus University, Denmark
3 Department of Pediatrics, Aarhus University Hospital, Denmark
4 Department of Molecular Medicine (MOMA), Aarhus University Hospital, Denmark

Purpose

catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare inherited cardiac disorder. Mutations in Triadin (TRDN) is an extremely rare cause of CPVT and is inherited in a recessive manner. To clinically characterize a family with a novel TRDN variant (NM_001251987)c.179T>C (p.Trp60Leu) and to establish functional properties of this variant.

Methods

Genomic DNA purified from blood was used for genetic screening in our targeted next generation sequencing gene panel. TRDN wildtype and variant cDNA has been cloned in a pcDNA3.1 expression vector. Following transient transfection of human cells functional properties of Triadin wildtype and variant proteins have been obtained by means of Western blot analysis, immunostaining and confocal laser scanning microscopy (CLSM).

Clinical presentation

Two brothers (Fig 1, II-1 & II-2) both collapsed with exercise induced cardiac arrest and VF at the ages of three and two years, respectively. The proband (II-1) has received appropriate shock therapy 6 times during exercise or emotional stress within 44 months of observation. He is currently stable on the combination of beta-blocker and flecainide. The youngest brother has not received any ICD therapy within 15 months on beta-blocker therapy.

Figure 1

Conclusion

Patients with a TRDN p.60W>L (HO) variant demonstrated a severe clinical CPVT phenotype. The amount of protein and the rate of protein degradation was similar in WT and TRDN p.60W>L and TRDN p.59T>R. Finally, we found a larger accumulation of degraded triadin protein in TRDN (p.60W>L) compared to WT with CLSM.

Correspondence:
Anders Krogh Brøndberg, MD, anders.krogh@clin.au.dk

Functional Expression

TRDN 32 p.59T>R steady state expression is similar to that of wildtype and p.60W>L triadin. 5 µg Protein extracted from cells transfected with 4 µg of either pcDNA3.1 TRDN 32 WT, pcDNA3.1 TRDN 32 p.60W>L or pcDNA3.1 TRDN 32 p.59T>R was subjected to SDS-PAGE and Western Blotting. A) shows a section of this blot, stained for both triadin and histone H3 (loading control). Triadin variants are seen as single bands of approximately 37 kDa. Band intensities were quantified using Imagej, showing in B). Relative intensities are shown in C). Relative triadin 32 steady state expression appears similar for the three analysed variants.

COS-7 slides were subjected to CLSM. Triadin and KDEL signals appear to co-localize, confirming that triadin WT and p.60W>L variants correctly localize to the ER. Accumulations of triadin were noted in many cells, along with a similar increase in ER signal in these areas. While these accumulations were found within both wildtype and p.60W>L triadin variant transfected cells, a tendency was noticed for these to be larger in triadin p.60W>L cells.

No apparent difference in rate of protein degradation. Rate of degradation of triadin 32 WT, p.60W>L and p.59T>R variants was compared by transfecting COS-7 cells with 1 µg pcDNA3.1 TRDN 32 WT, pcDNA3.1 TRDN 32 p.60W>L or pcDNA3.1 TRDN 32 p.59T>R constructs. Curves show quantification of a single experiment for the amount of each protein.