Pathology and Genetics of Phospholamban R14del Cardiomyopathy

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Phospholamban R14del mutation in patients diagnosed with dilated cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy: evidence supporting the concept of arrhythmogenic cardiomyopathy

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PLN R14del in the Netherlands

- PLN R14del in 39/257 DCM patients (15%)
- PLN R14del in 12/97 ARVC patients (12%)

- Highest mutation rate in Dutch cardiomyopathy cohorts

- Haplotype analysis: PLN R14del is a founder mutation
- origin: northern part of Netherlands, 575-825 years ago

van der Zwaag et al. Eur J Heart Failure 2012
L: postal code map showing the distribution of PLN p.Arg14del mutation carriers
R: postal code map: likely origin of founder haplotype / grandparents’ birthplaces
PLN R14del in the Netherlands

- Highest mutation rate in Dutch cardiomyopathy cohorts

- Surgical path specimens, Pathology, UMC Groningen
  - 13/50 (26%) of heart specimens (explants)
  - 08/33 (24%) of LV apex biopsies (LVAD)
Physiology of phospholamban

- In its unphosphorylated state, PLN inhibits SERCA

- With adrenergic stimulation, PKA phosphorylates PLN, reduces SERCA2 inhibition, and augments Ca handling

- Regulates diastolic function and stroke volume
PLN R14del pathophysiology

- R14 site involved in PLN cellular protein trafficking

- PLN R14del mutation >>
  - Partial inhibition of SERCA
  - Eliminates adrenergic control
  - Impairs dynamic contractility
  - Calcium overload
  - Cardiac fibrosis
PLN R14del impairs cardiac muscle contractility in 3D human engineered cardiac tissues

PLN R14del cardiomyopathy

Variable Clinical Phenotype

Pathology specimens

- Malignant ventricular arrhythmia (ACM)
  - sudden death: autopsy cases
  - some are exercise-related (sports)

- Chronic heart failure (DCM)
  - heart failure: surgical pathology/autopsy cases
  - including left ventricular apex excision (LVAD implants)
  - including DCM heart transplant specimens (explants)
2 risk factors for malignant VA, namely LVEF<45% and sustained or nonsustained VT

Biventricular cardiomyopathy

Compared to desmosomal ACM, the LV is more often involved in PLN R14del.

Groeneweg et al. Am J Cardiol 2013
Biventricular phenotype
Biventricular phenotype
Female 48 yr; p.Arg14del PLN mutation
Biventricular phenotype: DCM and ACM

Courtesy dr Aryan Vink, UMC Utrecht
Biventricular ACM phenotype
Posterolateral LV wall
RV wall
LV and RV fibrofatty change in PLN R14del

Regional LV fibrofatty change

Cardiomyopathy: distinct fibrosis patterns (LV)

- Fibrosis epicardial part
- Fibrosis endocardial part

Sepehrkhouy et al. Heart Rhythm. 2017
Posterolateral wall most distinctive

Phospholamban
- epi > endo: 8
- epi = endo: 1

Desmosomal
- epi > endo: 5

Lamin A/C
- epi < endo: 5

Sarcomeric
- epi = endo: 7

Desminopathy
- epi > endo: 2
- epi = endo: 1

Sepehrkhouy et al. Heart Rhythm. 2017
Distinct fibrosis patterns in cardiomyopathies

- Increased sensitivity to wall stress
  - Desmosomal mutations
  - p. Arg14del Phospholamban
  - Desminopathies?

- Increased energy need
  Classical phenotype of DCM associated with
  - Sarcomeric mutations
  - Lamin A/C mutations
  - Undetermined genotype
PLN cellular localization
PLN aggregation
PLN aggregation

- **Ventricular myocardium**
  - Heart specimens
  - LVAD apex excisions
  - RVEMB (low sensitivity)

- **Atrial myocardium >>>>>>**
PLN-IHC = sensitive & specific

- PLN-IHC is sensitive/specific for PLN R14del
  - In heart specimens (autopsy/explants)
    - 20 PLN R14del cardiomyopathies positive
    - 11 genetic DCM and ARVC cases negative
  - In LVAD apex excisions
    - 9 PLN R14del cardiomyopathies positive
    - 21 genetic cardiomyopathies (DCM) negative

te Rijdt et al. Histopathology 2016, Cardiovasc Pathol. 2017
IHC: loss of cytoplasmic PLN
IHC: perinuclear aggresomes indicative of autophagocytosis
Autophagy in (every) failing heart

Selective autophagy (of PLN) p62 (SQTSM1) and LC3
PLN aggregation and p62
PLN aggregation and LC3
Molecular mechanisms of ACM
Nature Reviews Cardiology 2019

2 - dissociated plakoglobin, 8 - GSK3-β redistribution, 10 - Cx43 loss
Compared to desmosomal ACM, PLN R14del has a different molecular signature. Only in case 1 (PLN R14del & emerin VUS) were molecular alterations of desmosomal ACM observed:

- Loss in plakoglobin in intercalated disks
- No loss of Cx43 in gap junctions
- Reduction of cytoplasmic SAP97
- Junctional redistribution of GSK3-beta

### Table 1
Overview of case characteristics and genetic, clinical and immunofluorescent findings

<table>
<thead>
<tr>
<th>Case</th>
<th>Characteristics</th>
<th>Additional targeted NGS results</th>
<th>Clinical criteria</th>
<th>Immunofluorescence†</th>
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<tr>
<td>1</td>
<td>F, 58</td>
<td>EMD c.166G &gt; A p.(Ala56Thr)</td>
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<td>PKP2 c.2084G &gt; A p.(Arg695His)</td>
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† Immunofluorescence results for all cases show consistent staining patterns.
Distinct molecular signature of PLN vs desmososomal ACM

1. Plakoglobin IF at ICD was negative in 4/5 ARVC cases, positive in 2 DCM cases
2. Plakoglobin IF at ICD was only negative in cases with other gene mutations
3. No reduction of the gap junction protein connexin-43 (Cx43)
4. No loss of synapse-associated protein 97 (SAP97) from sarcomeres
5. No junctional redistribution of glycogen synthase kinase-3 beta (GSK-3β)

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5. No junctional redistribution of glycogen synthase kinase-3 beta (GSK-3β).
PLN R14del cardiomyopathy

- PLN R14del cardiomyopathy is Dutch in origin
- Patient characteristics: SCD and/or HF
- Biventricular ACM phenotype ending in DCM
- Focal PLN aggregation in cardiomyocytes
  - PLN-IHC is sensitive and specific
  - Aggregation is associated with autophagocytosis

- PLN - classic ACM: different molec signatures
Thank you