

## VU university medical center



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Dear Council Members,

Thank you for awarding me the ESC First Contact Initiative Grant which provided me with the opportunity to travel to the University of Arizona and collaborate with Professor Henk Granzier.

My visit to Arizona has been an enriching experience, allowing me to take my research project one step further. During the internship I have learnt to determine cardiac titin composition and phosphorylation on titin specific analysis gels and western blots, methods which I will further introduce in the laboratory in the VUMC Amsterdam. In addition to experimental work, I have actively been involved in scientific meetings and lectures.

Please find enclosed a brief report on the experimental work performed during my 6 week internship at the University of Arizona.

I sincerely appreciate your support, which allowed me to perform research in a top institute in the USA. This was indeed a great opportunity to learn new techniques and take my research one step further.

Yours sincerely,

Silvia Rain



Report

The purpose of my visit to the University of Arizona was to extend my research on right ventricular diastolic stiffness in patients with pulmonary arterial hypertension and to unravel the molecular mechanisms involved in this process. Idiopathic pulmonary arterial hypertension is a fatal disease due to rapid deterioration of right ventricular function. Recently we have demonstrated severe increase in right ventricular diastolic stiffness in rats and patients with pulmonary arterial hypertension. However, the molecular mechanisms determining the increase in right ventricular diastolic stiffness remain obscure.

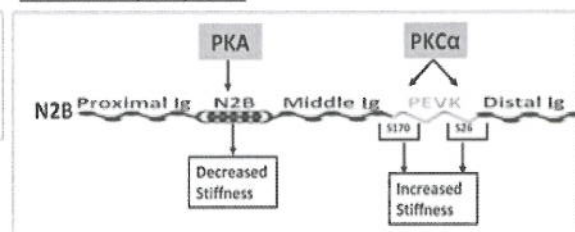
The giant cytoskeletal protein titin is considered the main determinant of left ventricular stiffness and diastolic dysfunction. Professor Henk Granzier and his research group are leading in titin research and its determinant role in muscle stiffness. Increased right ventricular diastolic stiffness in patients with pulmonary arterial hypertension may be caused, at least in part, by increased cardiomyocyte stiffness caused by alteration in titin composition or phosphorylation. Titin stiffness can be altered by either:

1. shift in titin isoform composition (e.g. a shift from a compliant N2BA to a stiffer N2B isoform would increase passive stiffness).
2. titin phosphorylation (e.g. decreased PKA phosphorylation and/or increased PKC phosphorylation would increase stiffness).

1. Titin Isoform Shift



2. Titin Phosphorylation



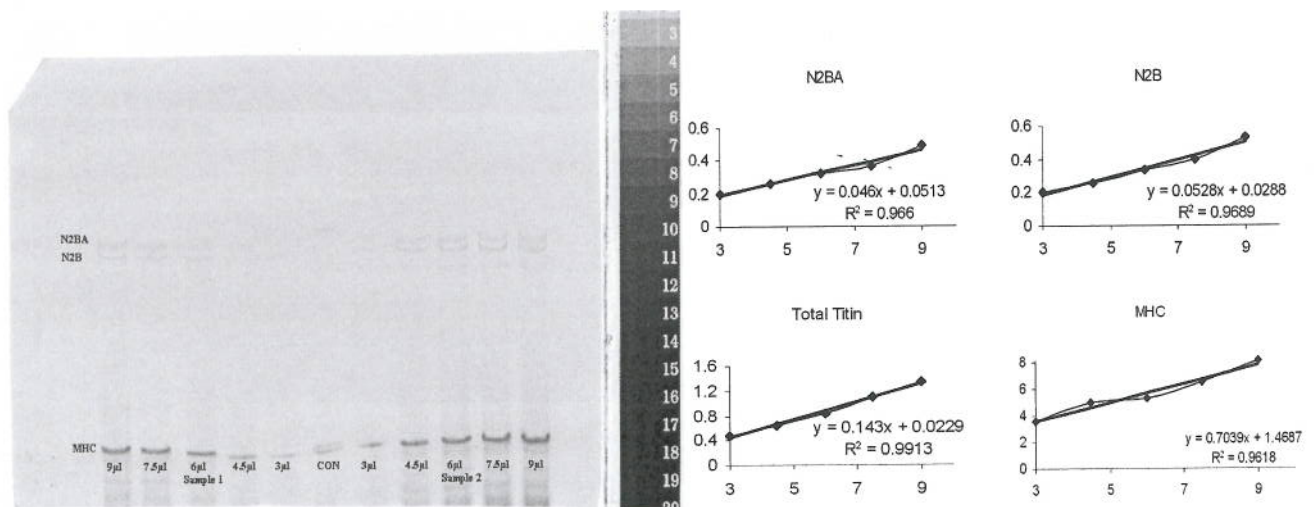


During my internship, I was able to learn specific techniques for titin detection and quantification, as well as perform PKA and PKC phosphorylation assays.

### 1) Titin isoform composition

Right ventricular tissue samples from patients with pulmonary arterial hypertension and healthy controls (ns=20, 9 control and 11 pulmonary arterial hypertension transplant pieces) and rats (ns=15, 5 control, 5 right ventricular hypertrophy and 5 right ventricular failing) and were solubilized using 8M urea buffer with specific protease inhibitors. After solubilization, initial 1% Agarose gels were run to calculate even sample dilutions. Further on, analysis gels were run in order to quantify titin isoform composition.

Slopes of stiff N2B and compliant N2BA isoform were calculated for increasing loading volumes (3µl – 9 µl) and the N2BA/N2B ratio was obtained by dividing the corresponding slopes.

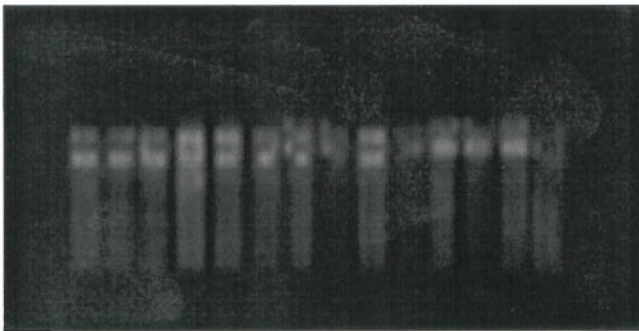




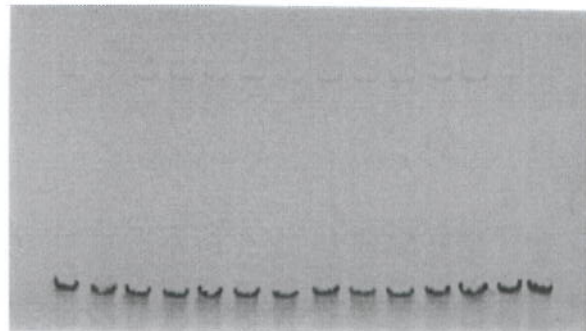
## 2) Titin phosphorylation

Western blot analysis using specific antibodies targeting PKA and PKC (S26 and S170) mediated titin phosphorylation sites was performed.

Western Blot



Ponseau S



Further directions:

At present, I am setting up the protein analyses methods to separate titin isoforms and perform titin phosphorylation assays in the laboratory for Physiology at the VUMC, Amsterdam.

## References

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