

To the ESC Council Members
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Groningen, the Netherlands, 5 January 2013

ESC First Contact Initiative Grant Report

Host: Professor J.E. Saffitz, Chairman, Department of pathology, Beth Israel Deaconess Medical Center, Harvard Medical School in Boston, USA.

Period: July-October 2012

Dear Council members,

Thank you for awarding me the ECC First Contact Initiative Grant. This grant gave me the opportunity to visit the renowned research group of Professor Jeffrey E. Saffitz at the department of Pathology in the Beth Israel Deaconess Medical Center, Harvard Medical School in Boston, USA.

In 1896, as part of their missionary charter, Methodist deaconesses founded Deaconess Hospital to care for the city's residents. In 1916, Beth Israel Hospital was established by the Boston Jewish community to meet the needs of the growing immigrant population. In 1996, these two great institutions, merged to form Beth Israel Deaconess Medical Center. Nowadays, the BIDMC is one of the four teaching hospitals of Harvard Medical School.

Research in the laboratory of Professor Saffitz focuses on cell-cell communication between cardiac myocytes, particularly as it pertains to arrhythmogenic cardiomyopathies. ARVC is a primary heart muscle disorder characterized by a high incidence of ventricular arrhythmias. Early in the disease's course, before the manifestation of right ventricular myocyte loss and fibro-fatty replacement, there is a sub-clinical 'concealed phase'. This presents a major diagnostic and clinical management challenge because arrhythmias and sudden death may arise in the absence of significant clinical and pathological changes. As in only 40-50% of patients with ARVC a mutation in one of the desmosomal proteins can be identified and mutation analysis is not widely available, it would be of great clinical importance to identify an alternative way to diagnose ARVC in an early stage of the disease.

My PhD project at the department of Genetics at the University Medical Center Groningen in the Netherlands focuses partly on the elucidation of factors that are important for risk stratification in patients with inherited cardiomyopathies. Defining a clinical biomarker to diagnose ARVC or to define patients at a high risk for developing a potential life threatening event is one of my main research topics.

In the following paragraphs I will discuss the details of the project and some preliminary results.

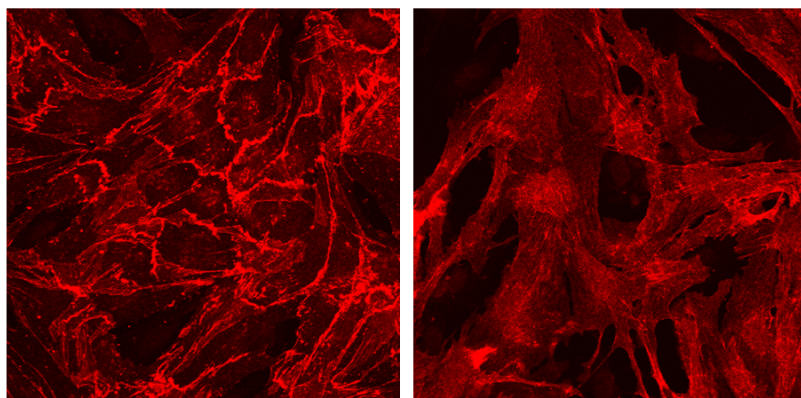
Desmosomal mutations implicated in ARVC lead to abnormal responses to mechanical stress, which promote changes in the sub-cellular distribution of plakoglobin (γ -catenin). Plakoglobin is a desmosomal protein that links adhesion molecules at the intercalated disk to the cytoskeleton. Recently, Asimaki *et al.* performed immunohistochemical analysis on cardiac tissue samples from patients with ARVC¹. They showed that the immunoreactive signal levels for plakoglobin were either absent or reduced at intercalated disks in patients fulfilling the Task force criteria, even in the absence of a

specific mutation in a desmosomal gene. Redistribution of plakoglobin from junctional to intracellular pools inhibits pro-myogenic canonical Wnt/ β -catenin signalling. This finally results in the perturbation of Ca^{+2} homeostasis and the replacement of myocytes by fibro-fatty tissue.

Furthermore, they observed that incubation of primary cultures of normal neonatal rat ventricular myocytes (NRVMs) with serum of ARVC patients causes a dramatic loss of the plakoglobin signal from the cell junctions with redistribution to nuclei. This is also associated with gap junction remodelling or reduced immunoreactive signal for the major ventricular gap junction protein Connexin43 at cardiac intercalated discs. In addition, increased cardiac myocyte apoptosis was observed when NRVMs were incubated with serum of ARVC patients.

We concluded that one or more proteins in the serum of ARVC patients must be responsible for this phenomenon and that this is specific for patients with ARVC. When serum of ARVC patients is incubated at a high temperature or treated with proteinase K (both resulting in destruction or inactivation of all proteins), neither plakoglobin redistribution nor myocyte apoptosis occurs. Serum from patients with other diseases, including end-stage heart failure, sepsis and serious inflammatory conditions, has no effect on junctional protein distribution in NRVM cultures. One of the main goals of my research project was to identify the responsible protein or peptide in the serum of ARVC patients. This might provide new insights into disease mechanisms in ARVC, it may explain exacerbations following exercise and suggest new therapeutic approaches to limit or prevent adverse outcomes.

As abundantly expressed proteins such as albumin and Immunoglobulin G (IgG) make up approximately 75% of the total protein in serum, the first step was to deplete the albumin from the serum samples. Then, I fractionated the samples by size. To conclude which fraction contains the protein of peptide of interest, primary cultures of NRVMs were incubated for approximately 24 hours with the fractionated serum. Subsequently, these cultures were immunostained for the desmosomal protein plakoglobin. If expression of plakoglobin is reduced at the cardiac intercalated disk, we concluded that our 'protein of interest' must be present in that fraction (see figure 1).



A.

B.

Figure 1. A. Immunofluorescence images of neonatal rat ventricular myocytes incubated with fractionated serum of ARVC patients. In A. plakoglobin is expressed at the intercalated disk, whether in B. plakoglobin is redistributed to the nucleus.

Ongoing studies are directed to identify a potential biomarker for ARVC. In the near future we will perform, in collaboration with the proteomics core facility, a spot down Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF) assay and digestion of samples to do bottom-up proteomics and peptide sequencing for

definitive protein ID. If we are able to identify a biomarker, it is necessary to perform thorough functional studies in ARVC patients. At our clinical genetic department many patients with ARVC (with and without desmosomal gene mutations) are known, which provide a great opportunity to continue our collaboration.

This 3.5 months visit provided me with a detailed understanding of basic cardiovascular research. During my stay I had the opportunity to learn different new techniques as cell culturing methods, immunohistochemical staining techniques, and contemporary methods in proteomics. In addition to the experimental work, I have actively been involved in research meetings and scientific lectures at the BIDMC.

I sincerely appreciated your support, which allowed me to perform research in a top institute at the USA. It has been essential for a deeper understanding of the pathophysiology of ARVC. This research project, under supervision of an eminent researcher, was an excellent opportunity for my personal development as well as for our departments, as I'm convinced that this research project formed a thorough basis to continue collaboration.

Yours sincerely,

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References

1. Asimaki A, Tandri H, Huang H et al. A new diagnostic test for arrhythmogenic right ventricular cardiomyopathy. *N Engl J Med* 2009;360:1075-1084.