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Final Report on the outcome of the ESC First Contact Initiative Grant

Dear Sir or Madam,

I was honored to receive the ESC First Contact Initiative Grant in 2014. With this final report I have the pleasure to inform you on the outcome of the initiative, which from my perspective has been highly rewarding and very successful. Let me thus take the opportunity to express my gratitude for selecting me.

The ESC First Contact Initiative Grant made it possible to join the laboratory of Dr. Patrick Ellinor in the Cardiovascular Research Center of the Massachusetts General Hospital (MGH) in Boston for a period of 6 weeks from May 1st to June 13th 2014. Dr. Ellinor's laboratory is working on atrial fibrillation and has a long history of performing human genetic studies and functional genomics in zebrafish and mouse models. A recent genome-wide association study (GWAS) revealed six new genetic susceptibility loci for atrial fibrillation (AF). Although there is strong evidence for an association between these six loci and atrial fibrillation, the mechanism through which genetic variation at these loci ultimately lead to atrial fibrillation is unknown. To elucidate a functional role and a potential mechanism leading to atrial fibrillation knockout mouse models are used.

During my short-term fellowship I was trained by an experienced post-doctoral fellow in Dr. Ellinor's laboratory in performing several techniques necessary for a cardiac characterization of knockout mouse models. These techniques include non-invasive electrocardiography (ECG), telemetry recording, echocardiography, and invasive electrophysiological studies. After a week of intensive training and practicing I had the chance to contribute to a very promising project. One of the six susceptibility genes mentioned above was knocked out in a mouse model. Since homozygous knockout mice were not viable we used heterozygote knockout mice for the project. At the age of 3 months a first cardiac characterization was performed but showed no differences between knockout and wildtype mice in regard to ECG or echocardiographic parameters. Invasive electrophysiologic studies did not show any increased susceptibility to

arrhythmias. However, telemetry analysis could demonstrate significant changes in ECG parameters in older mice: at the age of 7 months P wave duration and PR interval start to differ from wildtype mice with the difference further increasing over time.

My first task, therefore, was to confirm the phenotype seen in older mice. I used four mice at the age of 6,5 months and implanted a telemetry transmitter. Animals were anesthetized with isofluorane (1-2% in 700 ml O2/min., dose to effect) following induction in a chamber containing 5% isoflurane IH. Mice were intubated using a 22G angiocath and put on small animal ventilator CWE. Ventilation parameters were: tidal volume 0.25 mL, resp. rate 110 br/min., insp. Time 0.3 sec. The Isoflurane vaporizer driven by an oxygen source with flow meter was connected to the scavenging unit. The anesthetic vapor was administered to either an induction chamber or to the mouse via the modified Bain circuit masks inner tube. Excess anesthetic vapor was scavenged away from the mouse via the outer tube of the mask. The excess anesthetic vapor was drawn through a canister of activated charcoal, which absorbed the excess anesthetic gas. Additionally, all experiments were performed in a fume hood to further reduce the exposure to research personnel. Rectal temperature was continuously monitored and maintained within 37-38°C using a heat pad and heat lamp.

The telemetry device (or loop recorder, figure 1) was implanted into the abdominal cavity. The implant site was prepared by removing fur using hair removal. Multiple coats of Betadine were applied to the abdomen and chest to disinfect the surgical area. Local anesthetic was applied to the implant site. Sterile drapes were then be applied to the surgical field. Pre-sterilized instruments were used for the procedure. An abdominal incision was made and access gained into the peritoneal cavity. The telemeter was inserted into the peritoneal cavity. The peritoneal incision was closed with Prolene sutures, ensuring that the two telemeter leads remain extraperitoneal. The telemetry transmitter has 2 leads (electrodes). The 2 leads were tunneled to the modified lead II position. The leads were anchored to the pectoral muscle using a suture. The wound in the abdominal fascia was subsequently closed. Afterwards mice were individually housed in standard cages that were placed onto a receiver platform enabling continuous recording of the ECG over several weeks. Finally, I could confirm the findings from the previous group of mice: at the age of 7 months heterozygote mice showed significant differences in P wave duration and PR interval compared to wildtype mice.

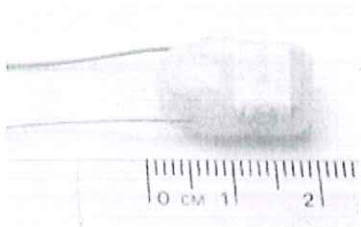


Fig. 1. Telemetry transmitter (DSI Datascience International, St. Paul, MN, USA)

Next, I performed echocardiography on mice at the age of 8-9 months. Echocardiography was performed on unanesthetized mice since studies have shown that anesthetics may decrease cardiac function and lead to artifacts in echocardiographic measurements. The animal's chest was shaved and ultrasound transmission gel was applied to the animal's chest wall to fill the space between the transducer and the chest wall. The mice were gently held while a transthoracic cardiac ultrasound was performed using a General Electric Vivid Five cardiac ultrasound equipped with a high frequency transducer. Unfortunately, I could not identify any differences in cardiac anatomy or function between heterozygote and wildtype mice.

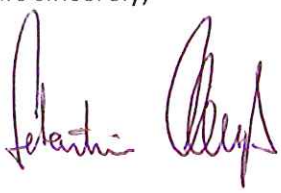
Finally, invasive electrophysiological studies were performed on mice at the age of 8-9 months. Animals were anesthetized with isofluorane as described above. The operative field was shaved, cleaned with 10% betadine and 70% ETOH. Rectal temperature was continuously monitored and maintained within 37-38°C using a heat pad and heat lamp. Dissection was carried down to the level of the internal jugular vein, and the SciScience octapolar EP catheter was inserted and advanced to the right heart. Using the Medtronic stimulator, OctalBioamp and AD systems data acquisition system, electrophysiology studies was conducted. Unipolar and bipolar electrogram recordings were obtained, amplified and filtered. A standard electrophysiological pacing

protocol was used including extrastimulus and burst pacing maneuvres. Subcutaneous needle ECG leads were inserted subcutaneously in the skin along the upper and lower chest to allow for simultaneous ECG recording for leads I, II, and III. I performed electrophysiological studies on 8 heterozygote and 8 wildtype mice but could not find any significant difference regarding sinus node recovery time (SNRT), conduction properties, or refractoriness (atrial, AV, ventricular). Furthermore, heterozygote knockout mice did not show increased susceptibility to atrial or ventricular arrhythmias using extrastimulus or burst pacing maneuvres.

In summary, heterozygote knockout mice did show a very interesting phenotype: older mice differ significantly from wildtype mice in regard to P wave duration and PR interval suggesting abnormalities in atrial and/or atrioventricular conduction. However, echocardiography and electrophysiological studies did not show a clear phenotype. Further experiments are planned to further evaluate these heterozygote knockout mice including parasympathetic/symphatethic blockade during invasive EP studies and optical mapping on the atria of these mice.

The outcome of my stay in Boston was highly positive in all respects. First, I was able to contribute promising results to a very interesting project. Second, I acquired knowledge and skills that will enable me to continue research on mouse models in the future. Third, I was very well accepted in Dr. Ellinor's laboratory and had a phantastic time!
In summary, the concept of the First Contact Initiative Grant turned out to be highly successful. I thus again would like to take the opportunity to express my most sincere thanks to the ESC.

Yours sincerely,



Sebastian Clauss