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Ihr Zeichen: Unser Zeichen: Munich, February 6, 2011

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

Dear Sir or Madam,

With great delight did I receive notice of the acceptance of my application for the ESC First Contact Initiative Grant on September 9, 2010. 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.

My journey to Dr. Patrick Ellinor's laboratory in Boston started on Sunday, October 31, 2010 with a flight from Munich to Boston. My work in the laboratory started on Monday, November 1st, 2010 in the Cardiovascular Research Center of the Massachusetts General Hospital, located in the Charlestown Navy Yard. The first day of my stay in Boston was used to introduce me to the personal of the laboratory, to familiarize me with laboratory specific rules and regulations and to help orientate myself in the new environment. The remainder of the first day was used to move into my temporary apartment. I was able to pre-organize a furnished short-term housing in relatively close proximity to the laboratory.

The actual research project started the next morning, when the Munich DNA samples, as announced in my First Contact Initiative Grant application, arrived in the Boston laboratory. Following an initial inspection, 2,436 DNA samples were successfully transferred. The next two weeks were used to process these DNA samples in order to prepare them for genotyping. To do so, each sample's DNA concentration was measured by spectrophotometry using a Nanodrop 1000 instrument. The DNA concentration in almost all samples turned out to be of high quality as assessed by the measurement spectra, the 260 / 280 nm ration, and the DNA concentration, which was in the range of 150 to 497 ng/µl. Following the measurements, out of the stock DNA

samples dilution aliquots were prepared yielding a DNA concentration of 50 ng/µl as intermediary step. In a subsequent dilution step, the final DNA concentration of 1 ng/µl was established. All DNA samples were available in single tubes. In order to prepare the samples for genotyping, the single tubes were arranged in 96-sample matrices mimicking the distribution of standard 96-well plates, and a volume of 150 µl DNA solution was transferred. Subsequently, each four of the 96-well plates were arranged mimicking the distribution of a 384-well genotyping plate, and a total of 5 µl DNA solution was pipetted into each well. Upon finalization of DNA transfer into the genotyping plates, the DNA solution was allowed to dry.

In the meantime, single nucleotide polymorphisms (SNPs) were selected for genotyping at the genomic region around the gene *KCNN3*. As detailed in my application, *KCNN3* encodes a calcium-activated potassium channel, expressed in the heart, which became of interest in the context of atrial fibrillation, when a genome-wide significant association signal was detected in Intron 1 of the gene. For the current approach, SNPs were selected to fine-map the genetic architecture of the region around *KCNN3*. Particular emphasis was put on comprehensive coverage of all common variation at the genomic locus in order to identify potential independent association signals. The selection process included the use of various software tools and web-based analysis of SNPs and region. Specifically, we exploited information from the UCSC genome browser, the HapMap project, the NCBI dbSNP database, the SNPper database, and the Broad Institute's software tool SNAP. Following SNP selection, the software Assay Design was used to create genotyping assays. The software is a product of Sequenom, a company which was chosen for subsequent genotyping by the means of Matrix-Assisted Laser Desorption / Ionization Time-Of-Flight mass spectrometry (MALDI-TOF). Following successful creation of a genotyping assay, we send our samples to the hospital affiliated genotyping core laboratory for further processing.

Genotyping results were received back after approximately 3 weeks. After standard quality control measures were applied to the results, we proceeded to statistical analysis of our results. The approach of choice was a logistic regression model with atrial fibrillation as the outcome. The SNP genotypes were considered as predictors, and further covariables for adjustment included age at blood draw, sex and hypertension. Our analysis yielded several highly significant results. Currently, we are waiting for confirmation results of further study samples from other centers to substantiate our findings.

The remainder of my stay in Boston under the First Contact Initiative Grant, and while waiting for the genotyping results was used for an introduction to a multitude of methods and model systems related to atrial fibrillation research. For instance, I was introduced to high-resolution melting curve analysis to screen for mutations and other unknown rare variants in candidate genes for pathophysiological involvement in the arrhythmia. The underlying concept is that if DNA is heated up ("melted") under controlled conditions and at a steady temperature ramp, all DNA strands with the same sequence will show the same melting pattern. If the sequence is

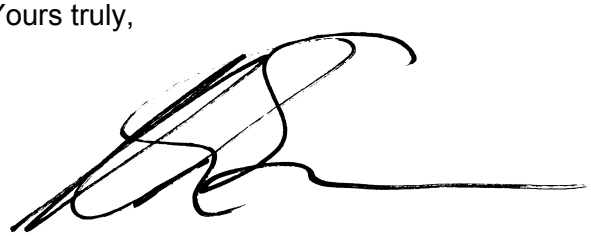
altered by genetic variants, the melting pattern will be visibly different and the suspicious samples can be further analyzed by subsequent sequencing.
In addition, I gained first insight into the promising new technique of next generation sequencing, and whole-exome sequencing in particular. One immense task is to handle the incredible amount of date generated and to dissect promising new identifications of variants from known polymorphisms and noise. The demands are thus particularly high from a biostatistical point of view.

The outcome of my stay in Boston was highly positive in all respects. First, we were able to generate promising results that will transfer into a publication. Second, I was extremely well accepted in Dr. Ellinor's laboratory. Immediate plans were made to prepare a longer-term research fellowship as a postdoc in his laboratory. First clarifications with my current employer at the Grosshadern University Hospital were initiated, and it turned out that thanks to the outstanding support of my mentors in Munich, almost no hurdles had to be taken. After a short flight back to Munich after almost 6 weeks in Boston, I organized pending visa issues and administrative tasks. Towards the end of last year, I returned to Boston, were I am working since in Dr. Ellinor's research laboratory. I am currently funded through Dr. Ellinor, but an own grant has been approved by the German Heart Foundation, which will continue to cover my salary during my stay in Boston.

The amount of 2,500 EUR awarded by the ESC were spent as follows:
720.28 EUR for the flight to and from Boston, departing from Munich.
The remainder of the money was used for housing. The duration of my stay in Boston was 6 weeks. I had arranged short-term housing at a rate of 375 USD / week, which was equivalent to approximately 1650 EUR for the duration of the stay. The remaining, approximately 130 EUR were used to cover incidental local expenses like local transportation, etc.

In summary, the concept of the First Contact Initiative Grant turned out to be highly successful in my case, and directly led to an intensified contact. I thus again would like to take the opportunity to express my most sincere thanks to the ESC.

Yours truly,



Dr. Moritz Sinner