Final Report for the Short-Term Visit from 6th of February until 31st of March 2012 between CRP CoGIE (IP4) and CRP EpiGENet (IP6)

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Methylation Analysis of the CHRNA7 gene in patients with Rolandic Epilepsy

Purpose and summary of the study

The purpose of the Short Term Visit to the group of Dr. Thomas Sander at the Cologne Center of Genomics was to investigate the methylation status of the plausible candidate gene CHRNA7, by focusing on the CpGs in the promoter region in patients with Rolandic Epilepsy. The group of Dr. Sander is working on genetically regulated methylation sites predisposing to idiopathic generalized epilepsy. Epigenetic and genetic analysis methods are well established in his group providing me a great opportunity to increase my knowledge. The relevance for an epigenetic study of the CHRNA7 gene was supported by the fact that CHRNA7 was repeatedly associated with epilepsy but no causative genetic variations are found so far.

My research question was to look if epimutations such as hypermethylation of the promoter region, which clearly affect gene transcription and could result in decreased expression of CHRNA7, could be a plausible mechanism influencing susceptibility of Rolandic Epilepsy.

For this purpose I have analyzed the methylation status of CHRNA7 in bisulfite converted genomic DNA of peripheral whole blood cells of 53 patients with Rolandic Epilepsy (mean age 18 years, 29 males/24 females) and 43 neurologically normal controls (mean age 24 years, 20 males/23 females). Bisulfite conversion was performed according to manufacturer's recommendations (EZ DNA Methylation Kit, Zymo Research). PCR amplification and pyrosequencing were performed using PyroMark CpG Assays (Qiagen, Hilden, Germany), which quantitatively assessed the methylation status of 15 CpGs in the CHRNA7 promoter region (chr15:32322123-32322462), around 300bp upstream of the transcriptional start point.

Quantitative pyrosequencing showed that the analyzed CpGs were unmethylated in patients and controls. Therefore, there was no difference in the methylation pattern between patients and control pointing out no epimutations at this region. Regarding this results it is not reasonable to continue this pilot-project with a larger sample size. Comparison with the methylation status of CpGs present at the Illumina Infinium HumanMethylation 450K Chip used by the group of Dr. Thomas Sander will be possible in May. These upcoming data will address the question whether differentially methylated CpG sites exist in the CHRNA7 regions which have not been covered by our pilot study.

Further work carried out during the visit

Besides my main project I was helpfully supervised in generating a genome-wide SNP genotyping dataset of our case-control discovery samples comprising 235 Rolandic Epilepsy patients and 200 population controls, using the HumanOmniExpressExomeBeadChip. Moreover, I have carried out an initial genome-wide association analysis. I have learned to perform SNP and sample quality control, correction for population stratification (principal component analysis using EIGENSTRAT), correction for cryptic relatedness (GRR) and logistic regression analysis for performing a case-control association study using PLINK. In addition, I have performed CNV calling using PennCNV. One of the most important aspects of this short term visit clearly was the huge benefit of strengthening our personal ties and to facilitate future cooperation with the group of Dr. Thomas Sander.

Future collaboration

By integrating association signals of the genome-wide association analysis in RE patients (CRP CoGIE IP4) with the genome-wide assessment of differentially methylated CpG sites in blood cells and brain tissue of epilepsy patients (CRP EpiGENet IP6, Dr. Thomas Sander), we will be able to address the question whether RE-associated SNPs increase risk of RE by an allele-specific regulatory effect on methylation and expression of positional candidate genes. Moreover, we could test the validity of our findings by comparison with the data obtained for common IGE syndromes.