Adam Suhy
PhD Candidate

“Regulation of Cholesteryl Ester Transfer Protein and Expression of Transporters in the Blood Brain Barrier”

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VITA

June 28, 1985 . . . . . . . . . . . . . . . . . . Born – Clarksburg, WV

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ABSTRACT

Coronary artery disease (CAD) accounts for more deaths in America than any other disease, and places a considerable economic burden on the healthcare system. Statins have successfully reduced the risk of cardiac mortality; however, a residual risk of approximately 20-26% has been observed. Reduced activity of cholesteryl ester transfer protein has been shown to increase the risk of atherosclerotic death in males on statins, supporting a possible genetic basis for a portion of the observed residual risk. SNPs in CETP have been associated with a non-active splice form, increased HDL cholesterol, and allelic expression imbalance in CETP.

The goal of the first portion of this study is to identify the functional SNPs responsible for the differential regulation of CETP splicing and expression through the use of molecular genetics. I use mini-genes and a qRT-PCR based assay to examine the effect of 2 SNPs on alternative splicing. I also investigate the interactions between SNPs residing in an upstream haplotype block with transcription factor binding sites. I demonstrate the effect of 3 candidate SNPs on expression of a luciferase reporter. Additionally, I interrogate RNA-sequencing data to uncover expression and alternative splicing of ABC and SLC transporter proteins in the blood brain barrier using computational tools.

The effect of each candidate SNP (rs5883, and rs9930761) on splicing was apparent in the in vitro system that I assayed. The minor allele of rs5883 significantly increased the amount of alternatively spliced mRNA in HepG2 cells by 1.25 fold (p-value=0.0001), while rs9930761 had no effect. Thus, demonstrating the measureable effect of only rs5883 in regulating the amount of alternative splicing in liver.

In the upstream haplotype block, I found that 3 SNPs interact with putative transcription factor binding sites for factors that are highly expressed in liver. rs17231506 had no effect in HepG2 cells, however, rs247616 caused a significant decrease in luciferase activity, and rs173539 resulted in a significant increase in luciferase activity. Due to the high linkage disequilibrium between these two SNPs, and the association of the minor allele of the SNPs in this haplotype block with decreased allelic expression imbalance, it is apparent that the effect of rs247616 predominates over that of rs173539.

I conclude that the increase in alternative splicing, and thus decrease in CETP activity, is accounted for by the activity of rs5883.
rs5883 should be included in future clinical association studies to verify its effect on clinical outcomes. Additionally, rs247616 as a marker is sufficient to assess the effect on CETP expression due to the upstream haplotype block. Knowledge of a patient’s rs5883 and rs247616 genotype in combination with previously established CETP SNPs will provide an improved prediction of their CETP activity and statin response.

In addition to my work on CETP regulation, I also studied the expression of ATP-binding cassette (ABC) and solute carrier (SLC) transporters in the blood brain barrier (BBB). The BBB is the name given to the virtually impenetrable nature of the blood vessels in the brain and central nervous system (CNS) preventing the passive diffusion of most solutes. This barrier, in combination with specific transporters expressed in the endothelial walls of the vessel, allows for precise control of nutrient and waste influx and efflux. Several diseases, such as Parkinson’s and Alzheimer diseases, associate with genetic variants in certain transporters, indicating a need to understand what transporters are present in the BBB, and thus what solutes or drugs are transported in or out of the BBB.

I used RNA-sequencing data to screen the expression and splicing of all transporters in the ABC and SLC families. Using computational tools, such as LifeScope alignment software and Cufflinks to align and assemble transcripts, I compared the expression of transporters in whole cerebral cortex tissue samples and cerebral cortex samples enriched for brain microvessel endothelial cells (BMEC) from the same individual. I identified nearly 160 transporter genes and pseudogenes that are at least 1.25-fold enriched in the BMEC enriched samples. Sixty-three of these were more than 2-fold enriched, indicating a likely role in the BBB. Many were previously implicated in BBB function, others have known functions in the brain, and some do not have any previous evidence of brain or CNS function. Additionally, I analyzed splice junctions to determine what splice forms are enriched in BMECs.

I’ve shown that RNA-seq can be a powerful tool for screening tissues, such as these, for transporters that have not been shown previously to express in the BBB. Knowledge of BBB transporters can lead to a better understanding of some neurological disorders and improved drug therapies.
RECENT ABSTRACTS AND PRESENTATION

Regulation of CETP expression by upstream polymorphisms,
Suhy, A., Hartmann, K., Papp, A., Sadee, W., OSU/NCH 2nd Genomics Symposium, 2015 (Poster)

Genetic Variants Affecting Alternative Splicing of Human Cholesteryl Ester Transfer Protein
Suhy, A., Hartmann, K., Newman, L., Papp, A., Toneff, T., Hook, V., Sadee, W., OSU/NCH 1st Genomics Symposium 2014 (Poster)

Characterization of Functional Polymorphisms Affecting Splicing of Cholesteryl Ester Transfer Protein

Genetic Regulation of Alternative Splicing in CETP, Research in Progress Seminar, The Ohio State University, 2014


The Genetic Regulation of CETP, Suhy, A., Hartmann, K., Newman, L., Papp, A., Sadee, W. The Ohio State University Research Day, 2013 (Poster)
Characterization of Functional Polymorphisms Affecting Splicing and Expression of Cholesteryl Ester Transfer Protein
Suhy, A., Hartmann, K., Newman, L., Papp, A., Wang, D., Sadee, W.
The Ohio State University Research Day, 2012 (Poster)

DHLRI Research Day, 2011 (Poster)
RECENT PUBLICATIONS


In review:

Suhy, A., Hartmann, K., Papp, A. C., Wang, D., Sadee, W. Regulation of CETP expression by upstream polymorphisms: Reduced expression associated with rs247616.

AWARDS AND HONORS

2010 – The Ohio State University Fellowship

FUTURE PLANS

I am currently planning to pursue a teaching post-doc followed by a teaching position at a university.
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