Preeclampsia Foundation Vision Grant
Mid-Year Progress Report
January 2014-June 2014

Awardee: Mandy J. (Bell) Schmella, PhD, RN
Institution: Magee-Womens Research Institute
Project Title: Mechanisms Modulating the Association between the Endoglin Pathway and Preeclampsia

Non-Technical Abstract of Accepted Proposal: Soluble endoglin (sENG) is a protein that's increased in the blood of most women with preeclampsia. We do not know why it's increased or how it contributes to preeclampsia, even weeks before women get sick. This study is designed to clarify the role that sENG plays in preeclampsia. We will investigate if differences in the genetic code of the endoglin gene and related genes account for increased sENG in women with preeclampsia. Because Vitamin D and oxygen may affect sENG levels, we will also explore the effects of different Vitamin D doses and oxygen levels on sENG in cell culture.

Specific Aims:
1. Demonstrate that our genetic association findings are present in other populations from the Global Pregnancy CoLaboratory (CoLab).

   Aim #1 Progress: After Institutional Review Board approval from the University of Pittsburgh was granted and Material Transfer Agreements were executed, I received de-identified DNA samples from Dr. Melissa Wilson at the University of Southern California and Dr. Annetine Staff at Oslo University Hospital Biobank in Norway. During procurement of the samples, it was noted that the DNA quantity of some of the samples was likely too low for downstream applications. As such, it was decided to conduct whole genome amplification on all samples. This has the advantage of ensuring that a consistent template is used across all samples for iPLEX genotyping. Whole genome amplification kits have been ordered/received and we anticipate that the samples will be amplified and sent to the University of Pittsburgh Genomics and Proteomics Core Laboratory for genotyping by the end of summer 2014. iPLEX assays for genotype data collection have been designed, ordered, and received.

2. Examine the genomic region tagged by *ENG* rs11792480 and rs10121110 to identify causal variants that are contributing to PE, using the same subjects studied in my dissertation.

   Aim #2 Progress: To address this aim, I conducted focused genotyping of four single nucleotide polymorphisms (SNPs) (rs10987759, rs35400405, rs45605432, and rs17557600) within the genomic region tagged by *ENG* rs11792480 and rs10121110. I had proposed to collect genotype data for these four SNPs via TaqMan® allelic discrimination, however; I was only able to use this technology to collect genotype data for rs10987759 and rs17557600. Genotype data for rs35400405 and rs45605432 were collected by PCR-RFLP and sequencing,

respectively.

A preliminary analysis of the genotype data for these four SNPs appears to indicate that none of the four SNPs are associated with the development or susceptibility to preeclampsia. Given that the subjects studied in the Vision grant are those previously analyzed in my dissertation, we have maintained the same pregnancy outcome classification for this research aim. Preeclampsia was thus defined by the presence of new onset hypertension with accompanying incremental systolic and diastolic blood pressure increases in a previously normotensive woman after 20 weeks gestation, proteinuria, and hyperuricemia. Healthy women that did not manifest the preeclampsia criteria and did not have a history of conditions that would increase the risk of preeclampsia (e.g., diabetes, renal disease) were classified as healthy controls. A detailed description of the criteria that we used to define preeclampsia and healthy controls can be found in our previous publication (Bell et al., 2013).

At present, I am reviewing the literature and available databases (e.g., UCSC Genome Browser) to see if I can identify any new SNPs, CNVs, or VNTRs within the genomic region tagged by *ENG* rs11792480 and rs10121110 that may shed light onto the association between the *ENG* gene and preeclampsia.

3. Explore mechanisms underlying associations between the ENG pathway and PE.
   A) Test the relationship of pregnancy plasma levels of sENG & TGFβ1 to *ENG* pathway genotypes and pregnancy outcome.

   Aim #3A Progress: Plasma soluble endoglin concentrations have been measured with the Human Endoglin/CD105 Quantikine ELISA from R&D systems in N=102 pre-delivery samples (≤48 hours pre-delivery or at the time of clinically evident disease) collected from white cases that were 1:1 frequency matched to N=102 white controls at the same gestational age time point (±3weeks). This comprised a subset of subjects studied in my dissertation project. The same phenotyping classification system described above was also used for this aim. Preliminary analysis has indicated that median maternal plasma sENG concentrations are significantly higher in women with preeclampsia compared to healthy controls (30.4 ng/mL vs. 8.0 ng/mL, p<0.001). I am currently working with a statistician to analyze the association between sENG protein levels and *ENG* pathway genotypes.

   B) Assess the modulatory effects of Vitamin D on sENG release from human trophoblast (HTR-8) and endothelial cells in culture under basal ("normoxic": 8% O_2) and stimulated (patient serum; hypoxic: 1% O_2) conditions.

   Aim #3B Progress: During our initial preliminary experiments with the HTR-8 cell line, we noted that sENG release into the conditioned media was extremely low. After some research, we decided to switch to a different cell line. Human JAR cells, a placental choriocarcinoma cell line, have been shown to release

measurable amounts of sENG (Valbuena-Diez et al., 2012). At present, we are in the process of writing experimental protocols, becoming familiar with the characteristics of this new cell line, and carrying out preliminary experiments.

References:

Bell, M. J., Roberts, J. M., Founds, S. A., Jeyabalan, A., Terhorst, L., and Conley, Y. P. (2013). Variation in endoglin pathway genes is associated with preeclampsia: A case-control candidate gene association study. *BMC Pregnancy & Childbirth, 13*(82).

Valbuena-Diez, A. C., Blanco, F. J., Oujo, B., Langa, C., Gonzalez-Nuñez, M., Llano, E. et al. (2012). Oxysterol-induced soluble endoglin release and its involvement in hypertension. *Circulation, 126*, 2612-2624.