PJP Research Grant Report

Date of report: 12/1/2022 ☐ This is a progress report ☒ This is a final report

Recipient Information

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Grant Details

Project name	Targeted High-Throughput Methylome Analysis of Early and Late Onset Preeclampsia			
Original grant amount & period	$ 138,696	From: 09/2018 (month & year)	To: 08/2020 (month & year)	
Has an extension been granted?	☒ Yes ☐ No	*If yes*, what is the current grant period?	From: 09/2018 (month & year)	To: 06/2022 (month & year)
Dates covered by this report	From: 09/2018 (month & year)	To: 06/2022 (month & year)		

Project Aims & Accomplishments

Short description of project & aims:

The overall goal of this study was to quantify and compare DNA methylation signatures associated with early and late onset preeclampsia in mothers and infant pairs with normal mother-baby pairs as controls. We leveraged DNAs available from The Preeclampsia Registry (TPR) and control DNA samples were obtained from patients recruited at OHSU. High-throughput, next-generation sequencing based methods were applied to measure DNA methylation from three groups of pregnant women: controls, early PE onset (<32 weeks), and late onset (>37 weeks), and infants for each mother in order to identify differential methylation between these groups according to aims below:

Aim 1: To determine DNA methylation profiles in DNA extracted from saliva of women with early- and late-onset preeclampsia compared with epigenetic profiles of age matched women without pre-eclampsia. The different groups will be compared to identify DMRs and identify affected pathways.

Aim 2: a) To determine epigenetic profiles of neonates born to mothers with early- and late-onset preeclampsia and compare with profiles with neonates born to normal stage matched mothers without preeclampsia.
b) To compare epigenetic profiles of non-preeclamptic mothers and mothers with early and late-onset preeclampsia with epigenetic profiles of their offspring.

Did you meet the aims you set out to achieve?
☒ Yes ☐ No

Please explain:
We were able to obtain genome-wide methylation data for all samples that qualified for the study in terms of DNA quality. Overall, we analyzed and compared 6 groups: 1) Early PE (<32 weeks), 2) Late PE Mothers (>37 weeks), 3) Control Mothers, 4) Early PE infants, 5) Late PE infants, 6) Control infants. Each group included 16 samples. After performing differential methylation analysis by comparing different groups, we identified differentially methylated regions (DMRs) and associate them with nearby genes. In total, the most DMRs were found when comparing samples from the mothers, with a total of 720 DMRs versus the 290 DMRs found in the infant comparisons. We observed the highest number of DMRs in the late-PE vs control mothers' comparison, with 165 hypomethylated DMRs (hypo-DMRs) and 104 hypermethylated DMRs (hyper-DMRs). For the early-PE vs control mothers we identified 105 hypo-DMRs and 111 hyper-DMRs. When we compared the late-PE and early-PE infants with the control infants, we observed the highest number of DMRs in the early PE infant vs the control infant samples, with 49 hypo-DMRs and 141 hyper-DMRs. For the late PE infant samples analyzed against the control infant samples, there were 11 hypo-DMRs and 37 hyper-DMRs.
Pathway analysis of significant DMRs using PANTHER identified the cadherin signaling pathway as being the most prevalent between PE mothers compared to control mothers. Cadherin signaling pathways are key to creating cell junctions by using the cadherin proteins that form strong cell to cell adherence bonds. Cadherin pathways mediate calcium ion dependent adhesion, which allows cells to transmit information and is important in embryonic development, cell adhesion, and inflammation. Another pathway identified for the same comparison was the WNT signaling pathways. WNT signaling pathways are important for regulating embryo development, immune cell genesis and can be used for tumor and cancer detection.

Have there been any significant changes to the original project plan over this reporting period? If so, how well have the project team managed these changes?
The most challenging aspect of the project was to identify DNA samples whose quality was sufficient for performing the methylation analysis. We had to reduce the sample size to allow balanced groups for the comparisons. Eventually we decided on a sample size of 16. There were other challenges to the study as well. We were very pleased to use samples from the Preeclampsia registry. However, as the registry does not contain samples from control women we had to expand the project to include obtaining samples from our own patients including getting IRB approval etc. In addition, not all of the samples obtained from the registry were usable. Some of the samples were too small to yield enough DNA to be analyzed. Our laboratory was severely affected by Covid-19 policies at OHSU. Our laboratories were completely shut down for a long period of time. In addition, some personnel left for other opportunities. Nevertheless, we are very excited that we were able to overcome these barriers and obtain highly reliable data that will benefit our understanding or the epigenetic underpinnings of different subtypes of preeclampsia. Again, we appreciate the patience of the Foundation during our difficult times.

Has the project faced any new ethical issues in the past year? If so, how well has the project addressed these?
N/A

What did you accomplish during this reporting period?
We gathered all the samples, including the ones at OHSU, from which we extracted DNA. We generated next-generation sequencing libraries and submitted them for sequencing. We analyzed the sequencing data to perform differential methylation analysis and then characterized the genes and pathways in the differentially methylated regions.

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What new information does this contribute to the field of preeclampsia?
This work reveals some epigenetically modified genes that are associated with people who had preeclampsia or in the case of neonates were exposed to the preeclamptic condition of their mothers. Another Important finding relates to epigenetic differences between early and late onset disease.

Although all the assays and the initial comparisons are completed, we are working to study the Individual genes that were related to DMRs.

Have you or do you plan to present this information at any meetings? Have you or will you submit a manuscript for publication?

We have a publication in preparation that will be submitted in January.

What other impact has receiving this award had on you, your career, colleagues, and/or the field of preeclampsia?

This study will contribute significantly to the field of preeclampsia by demonstrating that epigenetic signatures of this disease can be identified in the saliva of mothers who have had preeclampsia. These data will open the way to new studies to possibly identify some biomarkers for risk. Thus, these data show that women who have had preeclampsia have DMRs not found in non-preeclamptic mothers. One note of caution is that the DMRs in mothers who had preeclampsia may have been present before the pregnancy which would suggest a predisposition marker. It is not possible to know when those regions of DNA were modified – before pregnancy, during pregnancy or after pregnancy.

Please provide a plain language summary of findings. This will be listed on the Preeclampsia Foundation website. *(350 words or less)*

We know little about how genes cause a person to acquire preeclampsia. There is evidence that some people have a genetic predisposition. However, most non-infectious diseases are more related to harmful factors in the environment--like poor diet, stress, and toxins. These stressors lead to chemical modifications to a person's DNA through a process called epigenetics that changes the way that genes are turned on or off. Epigenetic changes are hereditable changes that do not affect the DNA sequence and can switch genes on and off. DNA methylation is one of the chemical modifications known to impact gene activity. Here we investigated if patterns of DNA methylation differ in women who experienced preeclampsia either in the early stage of their pregnancy (<32 weeks) or later (>37 weeks) in comparison with women who experienced a normal pregnancy. We additionally analyzed the infants from these pregnancies.

We observed that regions that are differentially methylated between the preeclampsia mothers and the controls land nearby or within genes that are important for embryonic development, cell adhesion, and inflammation (*e.g.*, the cadherin pathway). Moreover, several genes identified through these comparisons are expressed in tissues relevant to preeclampsia (i.e. brain, heart, kidney, and placenta). Interestingly, some of these genes are directly related to the production of lipids and increased lipid production is known to be associated with the progression of preeclampsia. Finally, we also identified differences in methylation between infants born from mothers with PE and controls, although they were fewer than those observed in the mothers and did not retrieved any pathway enrichment.

Overall, our study demonstrates that there are detectable differences in DNA methylation between mothers experiencing early, late preeclampsia or no complications during pregnancy. As this study was performed on DNA extracted from saliva, this opens the way to future studies aimed at identifying possible biomarkers of risk and help preventing this dangerous disease.

*Please email this completed Word document **and** a financial report to eleni.tsigas@preeclampsia.org*
If you have any questions, please email or call 321-421-6957