"RNA Expression in Acute and Chronic Immune Thrombocytopenia"

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This project is the first to utilize RNA sequencing to study molecular differences between patients with acute, self-resolving ITP and chronic ITP. RNA sequencing is a technique that assesses RNA expression, which is the presence and quantity of RNA. RNA expression reflects which parts of the DNA are most actively being used to make proteins; RNA expression is a molecular snapshot of what is happening in a cell at a given time.

This study stems from the hypothesis that acute (defined here as documented spontaneous resolution of symptoms within one year from diagnosis) and chronic ITP are molecularly distinct and could be distinguished on the basis of a unique RNA profile. To identify a characteristic molecular profile of chronic ITP could improve our understanding of the cause of the disease and provide prognostic information that is crucial to therapeutic decision making between patients, their families and medical providers.

RNA sequencing was performed in two cohorts: one, a group of patients with acute ITP, with samples drawn at the time of diagnosis and at the time of disease resolution. The other cohort included samples from chronic ITP patients. RNA sequencing was completed on samples from enrollment and at 3-6 months later in the chronic cohort. In order to assess natural disease progression and not the effect of therapy, patients who received treatment were excluded, with the exception of chronic ITP patients with remote history of therapy such as IVIG at presentation.

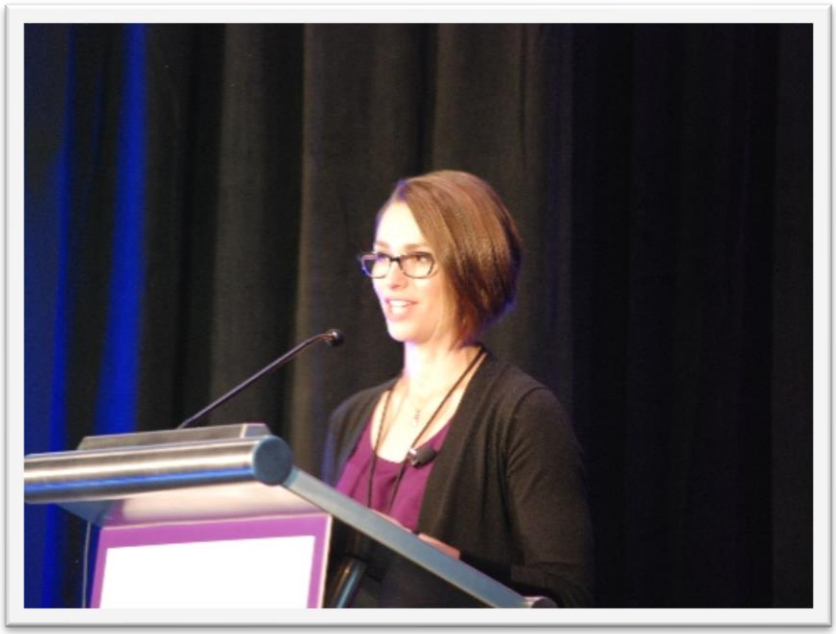
To date, 5 pairs of samples from patients with acute ITP, 7 pairs from patients with chronic ITP and 9 non-ITP controls samples have met rigorous quality control measures and were sent for RNA sequencing.

The term "transcripts" refers to specific copies of RNA. Transcripts which have more copies, or higher expression, are more active in a cell at a given time. Transcripts for which expression levels had a two-fold decrease from the time of presentation to disease resolution in acute patient samples, but stable expression in chronic ITP patients, were highlighted and tested in a pathway analysis. A pathway analysis is a statistical method that references public databases to group transcripts by function or molecular signaling pathway. From our RNA expression data set, pathways involved in systemic lupus erythematosus as well as complement activation were identified ($p<0.001$ and $p=0.012$, respectively). As an autoimmune condition, lupus has been linked to chronic ITP previously and complement, a type of inflammation, has also been implicated in ITP pathophysiology. Demonstrating that transcripts identified by RNA sequencing are a part of pathways with known relevance to ITP validates the use of RNA sequencing to distinguish acute and chronic ITP.

A cluster analysis was then performed to group samples with similar RNA profiles. This analysis was able to separate RNA expression profiles between healthy control patients and those with ITP. For those with acute ITP, some samples from the time of disease resolution clustered with control samples. While less distinct, acute and chronic patients do appear to cluster together as well.

These preliminary findings suggest the initial hypothesis is correct: chronic ITP does have a unique molecular signature and RNA profiling has potential as a prognostic tool. This analysis is currently being improved by adding data from additional patients (4 paired samples from patients with acute ITP and 5 paired samples from patients with chronic disease) as well as performing a normalization to correct for any impact of batch effects. This study is ongoing and we anticipate more specific identification of molecular signaling to distinguish acute and chronic ITP in the near future.

PDSA has designed our research program specifically to prioritize patient priorities and know these two studies will make a significant impact on ITP diagnosis, therapies, and quality of life. If you'd like to make a donation to our research fund, please visit https://www.pdsa.org/pdsa-donation.html.



Dr. Taylor Olmsted Kim presenting her PDSA funded research
"RNA Expression in Acute and Chronic Immune
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