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SPEAKER ABSTRACTS

**The identity thief: Silencing of B lymphocyte commitment gene PAX5 is coincident with gene methylation in common variable immunodeficiency**

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**Award:** New Innovator Award

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Common variable immunodeficiency (CVID) is a late-onset humoral deficiency characterized by B lymphocyte dysfunction or loss, decreased immunoglobulin production, and recurrent bacterial infections. Although CVID is the most common human primary immunodeficiency, variability in phenotype, genetic background and age at onset have precluded rapid definitive diagnosis and understanding of its etiology. Causative genetic mutations have been described for <10% of CVID patients; furthermore, family members of CVID patients are usually unaffected. Our laboratory diagnosed CVID in 30 equine patients; these cases manifest with a natural impairment of B lymphocyte differentiation in the bone marrow, and serve as a unique model to identify mechanisms of disease. Several independent lines of evidence revealed the loss of genes and proteins indicative of the pro-B cell differentiation stage in equine CVID patients, including fluorescent immunocytochemistry, bone marrow transcriptome analysis, and immunoglobulin recombination joint quantification. PAX5 expression is a signature of the pro-B cell stage and is essential to B lymphocyte identity. PAX5 expression is significantly decreased or absent in the bone marrow of equine CVID patients.

We hypothesized that aberrant epigenetic regulation caused PAX5 gene silencing, resulting in the late-onset and non-familial manifestation of CVID. Both genome-wide reduced-representation bisulfite sequencing and bisulfite PCR followed by sequencing methods revealed a significant increase in methylation of the PAX5 enhancer region in equine CVID patients ( $p=0.000$ ).

The reversible nature of epigenetic modifications facilitates *in vitro* investigation of their relevance in B lymphopoiesis of both healthy controls and CVID patients. In recent years, our laboratory developed a protocol to differentiate equine hematopoietic stem cells into B lymphocytes *in vitro*. At present, the consequences of demethylation agent azacytidine on B lymphopoiesis are being examined. We further plan to assess effects of histone deacetylase inhibitors, such as valproic acid, on B lymphopoiesis. Successful reversal of PAX5 gene silencing would provide means for rescuing B lymphopoiesis, and significantly, new avenues for patient treatment.

Thus, integrating gene expression with epigenetic status may be a key insight to understanding the onset of CVID and investigating novel therapeutic strategies.