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POSTER ABSTRACTS

301.VASCULATURE, ENDOTHELIUM, THROMBOSIS AND PLATELETS: BASIC AND TRANSLATIONAL

Multiplexed Functional Assessment of Glanzmann Thrombasthenia Variants

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High throughput genome and exome sequencing tests are used to identify disease-causing genetic variants, including those that cause inherited platelet disorders (IPDs) like Glanzmann Thrombasthenia (GT). However, most variants identified in clinical tests are classified as variants of unknown significance (VUS), lacking sufficient evidence to guide informed clinical decision-making. This frequent result of a VUS complicates clinical diagnosis and can be a source of uncertainty, distress, and inequity for patients.

Functional studies that demonstrate a damaging effect of variants are considered strong evidence of pathogenicity but are missing for most variants. However, individually testing the function of all possible missense changes in IPD genes is impractical. Multiplexed assays of variant effect (MAVEs) have been developed for select diseases to simultaneously measure the functional effect of all possible nucleotides in a specific genomic region. However, MAVEs have typically been employed in cell lines, and often utilize over-expression systems, which don't account for the proper cellular and genomic context needed to assess variants in IPDs.

In this study, we use CRISPR/CAS9 mediated homology directed repair (HDR) in CD34+ cells to create MKs with GT mutations in the gene ITGA2B and show that they model GT patient platelet phenotypes. We then implement a MAVE in CD34+ derived MKs to evaluate the impact on αIIb expression of a library of all possible single nucleotide changes for 12 nucleotides surrounding a type I GT variant. This was done by introducing a pooled library of HDR donors containing a mixture of test variants into CD34+ cells. This resulted in a pool of endogenously edited MKs, each containing one of 32 different nucleotide changes. These MKs were sorted into CD42+ and CD41+ or CD41- populations and analyzed using high throughput sequencing to assess variant enrichment.

Analysis of sequencing reads indicated distribution of variant containing reads across all 12 nucleotides and incorporation of all 48 different HDR donors (32 changes, 12 identity replacements). Numerous indels were also identified. Our results identified two distinct clusters of variants: those supporting a damaging effect on expression, and those likely having no effect on expression. Notably, we observed that all nonsense variants and indels resulting in a stop codon clustered in the CD41- enriched population, whereas all silent variants clustered in the CD41+ enriched population. To classify variants more precisely, we used an expectation-maximization (EM) algorithm based on the distribution of read counts between the CD41+ and CD41- populations. This identified 12 missense, 3 nonsense, and 30 indel variants as abnormal, compared to 24 missense and 10 silent variants as normal with respect to CD41 expression. Agreement of our results with computational assessments of variant effects supported the findings.

In conclusion, we developed an approach for the multiplexed assessment of GT variants, enabling the simultaneous evaluation of the functional effect of at least 32 different variants within a specific region of ITGA2B. This opens the door to assessing

the functional impact of all possible nucleotide changes across the entire ITGA2B coding region and lays the foundation for similar investigations in other IPD genes.

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