AMPLICON-BASED NGS: AN EFFECTIVE APPROACH FOR THE MOLECULAR DIAGNOSIS OF EPIDERMOLYSIS BULLOSA

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Background: Epidermolysis Bullosa (EB) is caused by mutations in genes that encode proteins belonging to the epidermal-dermal junction assembly. Due to the extreme clinical/genetic heterogeneity of the disease, the current methods available for diagnosing EB involve immunohistochemistry of bioptic samples and transmission electron microscopy followed by single candidate gene Sanger Sequencing (SS), which are labour intensive and expensive clinical pathways.

Objectives: According to the recently published recommendations for the EB diagnosis and treatment, the assessment of the mutational landscape is now a fundamental step for developing a comprehensive diagnostic path. Next-Generation Sequencing (NGS) via the parallel ultra-deep sequencing of many genes represents a proper method for reducing the processing time and costs of EB diagnostics.

Methods: We developed an EB disease-comprehensive AmpliSeq panel to accomplish the NGS on the Ion Torrent PGM platform. The panel was performed on ten patients with known genetic diagnoses and was then employed in eight family trios with unknown molecular footprints.

Results: The panel was successful in finding the causative mutations in all ten of the patients with known mutations, fully confirming the SS data and providing proof of concept of the sensitivity, specificity, and accuracy of this procedure. In addition to being consistent with the clinical diagnosis, it was also effective in the trios, identifying all of the variants, including ones that the SS missed or de novo mutations.

Conclusions: The NGS and AmpliSeq were shown to be an effective approach for the diagnosis of EB, resulting in a cost- and time-effective 72-hour procedure.