High-Resolution Melting Analysis to screen the ST18 Gene functional risk variant for Pemphigus Vulgaris

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Introduction: Pemphigus vulgaris is the most frequent and representative form of autoimmune bullous disease with intraepithelial lesions involving the skin and the Malpighian mucous membranes. Many evidences support the role of genetics factors in PV development.

The *ST18* -497-65050T>C polymorphism (*rs17315309*) exhibits a very strong association in the pathogenesis of PV and could represent a new potential molecular target for the treatment of disease.

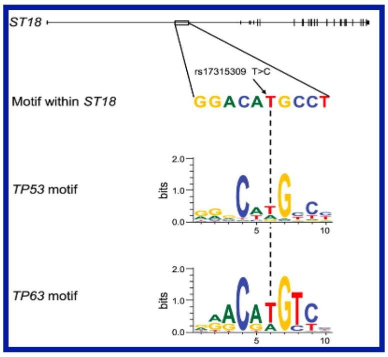

Figure 1: The *rs17315309* results in the substitution of a highly conserved T nucleotide within the consensus binding sites of p53 and p63; *PLoS Genet. 2016.*

The aim of this study is to set up a High Resolution Melting Analysis (HRMA) using the Magnetic Induction Cycler (MIC) (Bio molecular Systems), an innovative magnetic induction rotor-based platform recently become commercially available, to screen the ST18 *rs17315309* variant in patients with PV addressed to our laboratory.

Methods: The method was set-up "*in blind*" on 25 samples, including 5 patients with diagnosis of PV and 20 DNA healthy volunteer. Each samples analyzed by HRM was then genotyped by Sanger sequencing as confirmatory test.

Results: HRMA assay was able to identify easily and unambiguously the *c.-497-65050T*>C genotypes evaluating melting curve shape and melting temperature (T_m) with a full consistency between Sanger results. Heterozygous samples exhibited a unambiguous melting profile while homozygote genotypes for the variant were easily identified by a Tm sfhit of 0.7 ±0.03°C compared to the wild-type ones.

ST18 genotype	**T_m (°C)±SD**
Wild-type	79.97±0.05
Heterozygous	78.78±0.04/80.18±0.1
Homozygous	80.59±0.01

Table1: The evaluation of Melting Temperature (T_m) for all genotypes

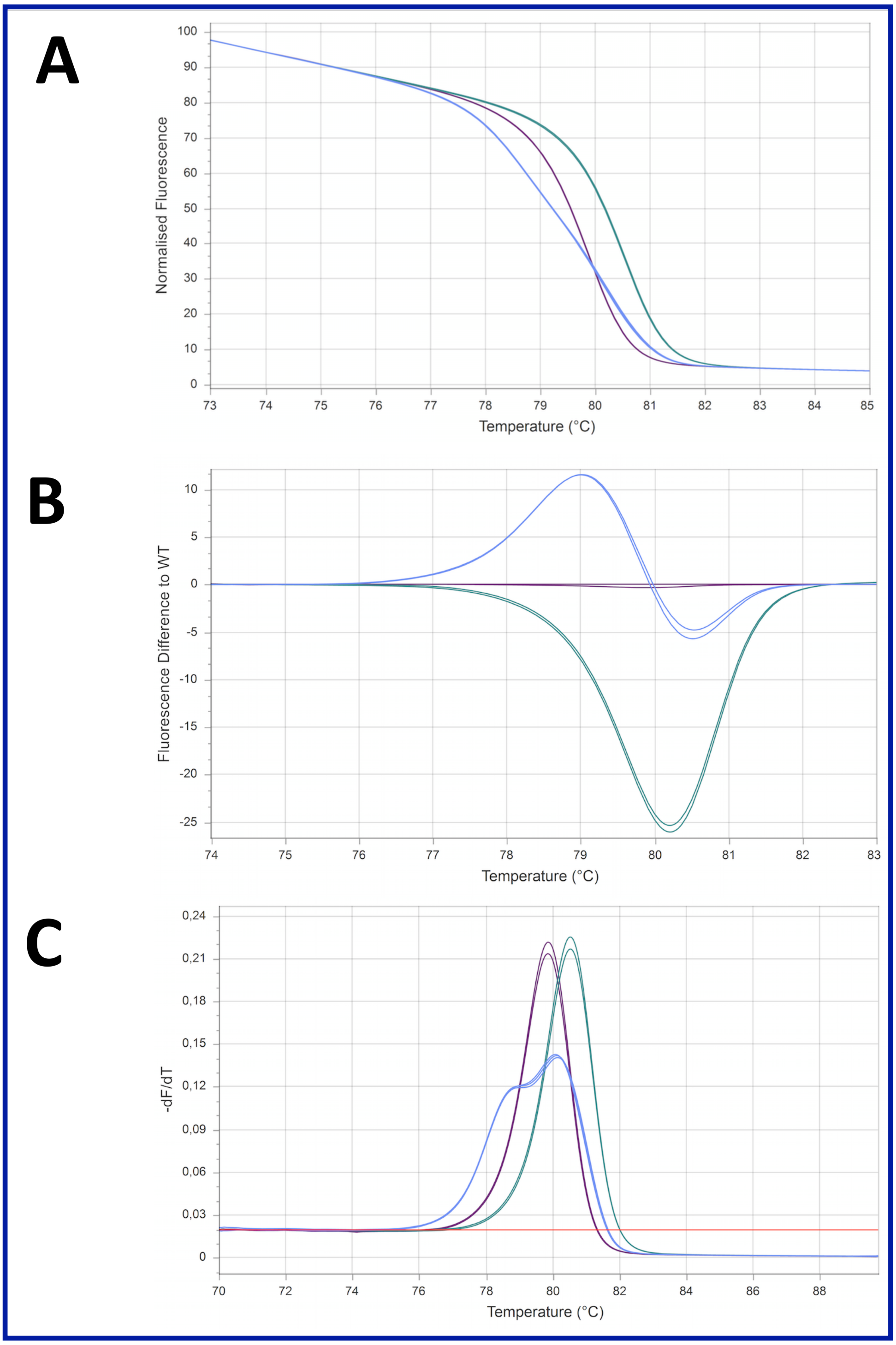

Figure 2: Normalized and Temperature-shifted Plot (A), Fluorescence Difference Plot (B) and Melting Peaks of the three genotypes profiles of *ST18* c.*-497-65050T*>C. Data were reported for WT (violet), homozygous (green) and heterozygous (blue). For the homozygous sample we observed a melting temperature shift of approximately of 0.7°C, while heterozygous profiles show different melting profiles with double peaks in the Derivative Plot.

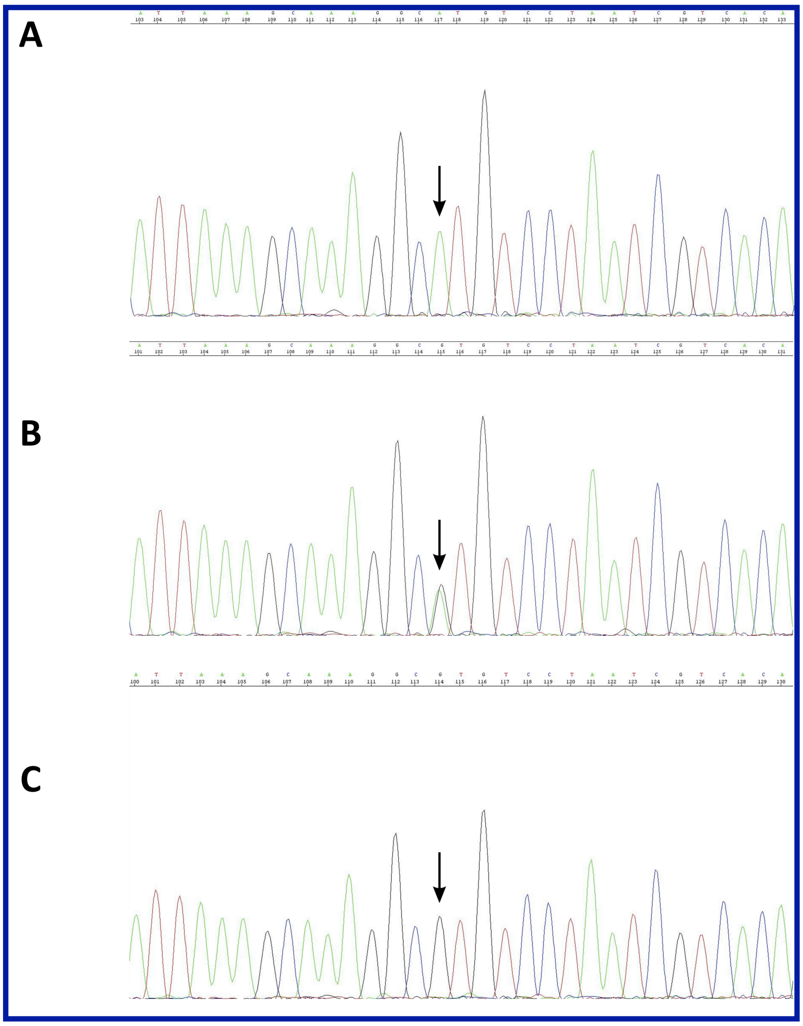

Figure 3: Sanger sequencing results for the target variant *c.-497-65050T>*C (*rs17315309).* Partial chromatograms of the fthree genotypes (A, wild-type; B, heterozygous; C, homozygous;) collected in this study are herein reported. The black arrows note the location of the variant of interest.

Conclusions: We established a highly sensitive and reliable HRMA approach for the identification of ST18 *rs17315309* variant and we showed as it performs with high sensitivity and accuracy in genotyping the variant of interest, being 100% concordant with DNA sequencing. Using of our HRMA approach, which is cheaper, easy and rapid to use, could facilitate large cohort studies on ST18 *rs17315309* variant in order to determine the impact of this variant in the disease development and to promote the innovative molecular treatment, above all in the emerging era of targeted therapy.

References: *Vodo D, Sarig O, Geller S et al. Identification of a Functional Risk Variant for Pemphigus Vulgaris in the ST18 Gene.*PLoS Genet. *2016 5;12(5):e1006008*