

0054 - IDENTIFYING AN EPIGENETIC SIGNATURE FOR NOONAN SYNDROME AS A COMPLEMENTARY DIAGNOSTIC APPROACH.

Mode: Research paper

Thematic Unit: Growth

Thematic Unit 2: Rare Diseases

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Background/Objectives: Noonan syndrome is a genetic heterogeneous condition characterized by short stature, congenital heart defects, and distinctive facial features. It is associated with variants in genes involved in the RAS-MAPK pathway. Nevertheless, some clinically suggestive cases remain undiagnosed after standard molecular analysis, suggesting underlying pathogenic mechanisms. In recent years, DNA methylation analysis has emerged as a complementary diagnostic tool in syndromes with clinical and genetic heterogeneity. This study aimed to identify a specific episinature for Noonan syndrome and to provide insights into its molecular pathophysiology.

Methods: We performed genome-wide DNA methylation profiling using the EPICv2 array in patients with RASopathy-related phenotypes and molecular diagnoses involving genes from the RAS-MAPK signaling pathway. Samples underwent quality control assessment using principal component analysis (PCA) to identify outliers and evaluate potential batch effects. Differentially methylated CpG sites (probes) were identified between cases and controls. A Support Vector Machine (SVM) classifier was trained to recognize the Noonan syndrome methylation pattern based on these probes, and Methylation Variant Pathogenicity (MVP) scores were generated to estimate the probability of a sample belonging to the affected group. Model performance was assessed in an independent testing set of 25 additional cases. To evaluate specificity, the classifier was also trained against control samples and samples from other neurodevelopmental disorders.

Results: The cohort included 77 individuals with RASopathy-related phenotypes, predominantly Noonan syndrome (n = 71; 92.2%). Overall, 67 individuals (87.0%) fulfilled definitive Noonan syndrome criteria according to Van der Burgt. The cohort showed broad genetic heterogeneity, with variants most frequently affecting PTPN11 (n = 32; 41.6%), SOS1 (n = 13; 16.9%), and RAF1 (n = 9; 11.7%). Clinically, the cohort showed a highly suggestive profile, with short stature in 77.9%, congenital heart defects in 79.2%, and typical or suggestive facial gestalt in 93.5% of patients. Noonan syndrome samples clustered separately from control samples in heatmap, hierarchical clustering, and multidimensional scaling (MDS) analyses, supporting the presence of a distinct methylation profile. The SVM classifier demonstrated



moderate specificity in discriminating Noonan syndrome cases from control samples and individuals with other neurodevelopmental disorders. Phenotype-based subgroup analyses showed a similar methylation pattern.

Conclusion: Patients with Noonan syndrome showed a distinct DNA methylation pattern, suggesting a detectable episinature. Although the SVM classifier demonstrated moderate specificity, DNA methylation profiling may represent a promising complementary diagnostic tool after further validation in larger and genetically diverse cohorts.

