Poster

From Uncertainty to Molecular Mechanism: Missense Mutations in *BRAF* and *MAP2K1* in Cognitive Disorders

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The RAS/MAPK signalling pathway is one of the most extensively studied pathways, mainly due to its fundamental role in the regulation of cell cycle, proliferation, or senescence. Mutations in RAS/MAPK are primary drivers of cancer [1]. They also contribute to developmental syndromes known as RASopathies. These syndromes are associated with body malformations of different severity and with impaired cognitive function. Individuals with these syndromes often experience intellectual disability (ID) and autism spectrum disorder (ASD) [2, 3]. In this study, we investigate *de novo* recurrent single-point missense mutations in the *BRAF* and *MAP2K1* genes (encoding kinases BRAF and MAP2K1), found in individuals with ID and ASD. They are currently classified as variants of unknown significance (VUS) [4]. It is crucial to investigate whether and how they impact the protein function, as some variants in RASopathies are known to increase while decreasing the kinase activity.

The variants were selected from large sequencing studies [4] using an initial dataset of all missense variants found in ID/ASD individuals. We considered the number of affected individuals with each variant and the presence of secondary mutations in other genes. Further, the variants were cross-validated for their association with ID or ASD with ClinVar Miner (https://clinvarminer.genetics.utah.edu/), SysNDD (https://sysndd.dbmr.unibe.ch/) and SFARI database (https://gene.sfari.org/).

We cloned the wild-type BRAF (BRAFwt), wild-type MAP2K1 (MAP2K1wt), and the corresponding VUS-containing variants. We express and purify the proteins for assessment of kinase activity and for investigating the effect of VUS on 3D structure with X-ray crystallography. This study aims to elucidate the pathogenicity of the novel mutations and the molecular mechanisms by which they lead to ID/ASD. This will help to improve diagnostics and find tailored treatments targets.

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