**Analysing Missense SNPs in *PRKN* as Genetic Modifiers of Machado–Joseph Disease**

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**ABSTRACT:**

**Background:** Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative spinocerebellar ataxia caused by a polyglutamine-coding CAG repeat expansion in the *ATXN3* gene. While the CAG repeat length negatively correlates with the age at onset, it explains only about 50% of its variability. Despite extensive efforts to identify contributing genetic factors, robust candidate genes with a plausible impact on the molecular pathogenesis of MJD remain scarce. In this study, we analysed the role of missense single nucleotide polymorphism (SNP) variants in the *PRKN* genein a large cohort of MJD patients. The *PRKN* gene encodes parkin, an E3 ubiquitin ligase associated with Parkinson’s disease and a known interaction partner of ataxin-3, the MJD protein, which functions as a deubiquitinase.

**Methods:** High-resolution melting analysis and Sanger sequencing were used to genotype three selected missense SNPs in the *PRKN* gene in DNA samples from over 900 MJD patients. A multivariate linear regression model was employed to assess the impact of these genotypes on the age at onset. To investigate functional consequences on MJD molecular pathogenesis, we utilized an overexpression cell model. Using protein biochemistry-based methods and cell viability assays, we examined effects on soluble and aggregated protein levels, protein-protein interactions, and mitophagy – the autophagic removal of damaged mitochondria

**Results:** The V380L variant in parkin was identified as a relevant factor, reducing the age at onset by three years in homozygous carriers. Functional analysis in the MJD cell model revealed that parkin V380L did not alter soluble or aggregated ataxin-3 levels but reduced their protein-protein interaction. Furthermore, the presence of parkin V380L disrupted mitophagy, compromising cell viability.

**Conclusions:** The V380L variant in parkin acts as both a modulator of parkin function and a genetic modifier of MJD, negatively influencing its molecular pathogenesis and accelerating the age at onset of the disease.

***Comment:***

This work has recently been published in *Acta Neuropathologica*:

Weber, J.J., Czisch, L., Pereira Sena, P. *et al.* The parkin V380L variant is a genetic modifier of Machado–Joseph disease with impact on mitophagy. *Acta Neuropathol* **148**, 14 (2024). https://doi.org/10.1007/s00401-024-02762-6