

## **HEME AND SENSORY NEUROPATHY: INSIGHTS FROM NOVEL MUTATIONS IN THE HEME EXPORTER FLVCR1**

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Hereditary sensory and autonomic neuropathies (HSANs) are a group of clinically and genetically heterogeneous disorders of the peripheral nervous system mainly characterized by impaired nociception and autonomic dysfunction. We previously identified heme metabolism as a novel pathway contributing to sensory neurons maintenance and nociception. Indeed, we reported mutations in the Feline Leukemia Virus subgroup C Receptor 1 (FLVCR1) gene in individuals affected by HSAN. FLVCR1 gene encodes for two heme export proteins, FLVCR1a (plasma membrane) and FLVCR1b (mitochondria), crucially involved in the regulation of cellular heme homeostasis.

Here, we report on two additional patients carrying novel biallelic mutations in FLVCR1 translation initiation codon (TIC) (c.2T>C; p. (Met1Thr), c.3G>T; p. (Met1Ile)). We describe the impact of the c.2T>C; p. (Met1Thr) mutation on protein structure and function in comparison with other previously reported mutations: the c.661C>T; p.(Pro221Ser) missense mutation and the c.1324dup; p.(Tyr442Leufs\_7). We generated the FLVCR1 mutant by site-directed mutagenesis and we overexpressed it in HEK293T cells and in HeLa cells. We found that TIC mutations interfere with translation in two different ways: by lowering levels of translation of wild-type protein and by inducing translation initiation from a downstream in frame ATG. We showed that the TIC mutant correctly localizes on the plasma membrane where it retains, at least in part, heme export activity. Moreover, we demonstrated that the missense mutation does not affect protein localization whereas the frameshift mutation results in ER retention.

The identification of novel FLVCR1 mutations in HSAN reinforces the crucial role of heme in sensory neurons maintenance and pain perception. Moreover, our in vitro findings demonstrate that heme export is not completely lost in HSAN patients and suggest that modulation of FLVCR1 activity may be important for therapeutic purposes.