# FUNCTIONAL INSIGHT INTO THE ROLE OF OPTINEURIN NOVEL VARIANT K489E IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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## Abstract

Mutations in the Optineurin (OPTN) gene have been implicated in amyotrophic lateral sclerosis (ALS). Although optineurin protein (OPTN) is known to regulate autophagy, apoptosis, and other cellular processes, its role in ALS pathology is unclear. Recently, while carrying out a genetic analysis of Indian ALS patients, our group identified a novel mutation K489E in the OPTN gene. Additionally, through whole exome sequencing of blood DNA from 25 Indian ALS patients, we found the same mutation in three Indian ALS patients. This adenine to guanosine missense mutation lies in the ubiquitin-binding domain of OPTN. To identify the molecular mechanism associated with this mutation, we developed an in-vitro cell culture-based model in SH-SY5Y cells bearing the K489E OPTN mutation. We observed that 36.5% more of the K489E mutant cells die compared to the wild type. To ascertain the cause of death in OPTN-K489E cells, we measured the expressions of apoptosis, necroptosis, and autophagy-mediated genes and proteins. We observed alteration in gene expressions of miR-9, REST, CoREST, and BDNF, and their concerted effect on the regulation of miRNA-9-mediated apoptosis. We also observed alterations in the expressions of necroptosis-mediating genes RIPK1, RIPK3, and MLKL compared to the wild type. Our results show upregulation of the expressions of autophagy mediating proteins TBK1, P62, and LC3II. We have also seen the effect of this mutation on its binding partners like TBK1 through immunoprecipitation, confocal microscopy and RT-PCR. In the current scenario, there is no permanent cure for this disease, but researchers are trying continuously to delay the progression of this disease by studying various drugs and vitamins. Similarly, we have also studied the effect of vitamin C and vitamin D on the mutant model of SH-SY5Y. We found an increase in cell survival in the case of the mutant after the addition of vitamin C. We have also focused on how this cell survival is happening in OPTN-K489E-expressing cells.