MISSENSE MUTATIONS (R1441C, A1442P) WITHIN THE ROC DOMAIN REDUCE THE STABILITY OF LRRK2 PROTEIN

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Introduction: Missense mutations in the LRRK2 (Parkin8) gene have been linked to cases of familial and sporadic Parkinson's disease (PD). We previously reported a novel, but as yet uncharacterised LRRK2 mutant A1442P, located in the proteins' ROC domain and whose contribution to PD is unknown.

Aims: To determine if the A1442P LRRK2 mutant is pathogenic, we expressed the protein in HEK293 cells and compared its behaviour to the relatively common pathogenic ROC domain mutant R1441C, as well as wildtype (WT) LRRK2.

Methods: Mutant and WT LRRK2 were expressed as green fluorescent protein (GFP) fusion proteins by plasmid mediated transfection of HEK293 cultures. Transfected cultures were analysed by western blotting, fluorescence microscopy and flow cytometry up to 72 hours (h) post-transfection. Cell viability was assessed by flow cytometry under both normal and oxidative conditions.

Results: WT LRRK2 protein expression (% GFP transfection rate and fluorescence intensity) increased before plateauing by 72h. By contrast, mutant LRRK2 proteins attained only about 75% of WT expression at 24h before rapidly declining to about 60% of WT expression by 72h post-transfection. Blockade of mutant LRRK2 degradation by proteosomal inhibition increased mutant LRRK2 protein to WT levels. Cell viability was comparable between WT and mutant LRRK2 under normal and oxidative conditions. Expression of WT and mutant LRRK2 proteins gave rise to intracellular aggregates, which were morphologically indistinguishable between WT and mutant LRRK2.

Conclusion: Our findings suggest that ROC domain mutations (R1441C, A1442P) render LRRK2 unstable, thus potentially reducing LRRK2 protein levels below normal.