Molecular exploration of the effect of the PARK7 PD-associated mutation c.192G> C

Zoé Hanss*[1], Ibrahim Boussaad[1], Coline Leghay*[2], Caroline Obermaier[1], Adriana Figueroa-Garcia[2], Amélie Vizeneux , Pierre Semaille[2], François Massart[1], Marie-Christine Chartier-Harlin†[3], and Rejko Krüger‡[1]

[1]Luxembourg Centre For Systems Biomedicine – Luxembourg
[2]Centre de Recherche Jean-Pierre AUBERT Neurosciences et Cancer (JPArc) - U1172 – Université de Lille, Sciences et Technologies, Institut National de la Santé et de la Recherche Médicale : U1172, Université de Lille, Droit et Santé, Centre Hospitalier Régional Universitaire [Lille] – France
[3]Centre de Recherche Jean-Pierre AUBERT Neurosciences et Cancer – Institut National de la Santé et de la Recherche Médicale : U1172, Université Lille 2 - Faculté de Médecine – France

Résumé

Homozygous loss-of-function mutations in *PARK7*, the gene encoding DJ-1, are causative for rare forms of inherited early-onset Parkinson's disease (PD). The DJ-1 protein can act as sensor of oxidative stress, transcriptional regulator of antioxidant genes, glyoxylase and as chaperone. Patient-derived cells carrying the homozygous mutation c.192G> C display specific cellular phenotypes due to DJ-1 loss of function. This mutation was predicted to cause an E64D amino acid change, however, using patient-based material we showed that the c.192G> C mutation causes mis-splicing of the DJ-1 pre mRNA upon which Exon 3 is spliced out. Although the resulting truncated ΔEx3–mRNA is present in the patient derived cells, protein levels of DJ-1 are dramatically reduced to an almost undetectable level. In this study we deciphered the molecular mechanism underlying the loss of protein due to the *PARK7* PD-associated mutation c.192G> C. First, we determined that neither increased proteasomal nor autophagy-lysosomal degradation contribute to the reduction of the mutant DJ-1 levels in homozygous mutation carriers. To exclude that translation is impaired by auxiliary effects during mRNA processing in patient-derived cells we overexpressed cDNA constructs that do not require splicing. Although overexpression of recombinant full length DJ-1 restored protein levels in patient-derived cells, overexpression of DJ-1 ΔEx3–mRNA did not lead to translation into recombinant ΔEx3–DJ-1 protein. To deepen our understanding of the failed translation of DJ-1 ΔEx3–mRNA, we evaluated two essential steps of the RNA processing: polyadenylation and polysomal association. The polyadenylation study showed that ΔEx3–mRNA is slightly less polyadenylated than full length mRNA but this small reduction cannot explain the complete loss of DJ-1 protein in homozygous mutation carriers. Polysomal analyses on sucrose gradients are ongoing to establish the polysome profiles as well as the mRNA levels of DJ-1 c.192G> C mutant compared to DJ-1 in the polysome fractions from patient and control derived cells.

*Intervenant
†Auteur correspondant: marie-christine.chartier-harlin@inserm.fr
‡Auteur correspondant: rejko.krueger@uni.lu

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