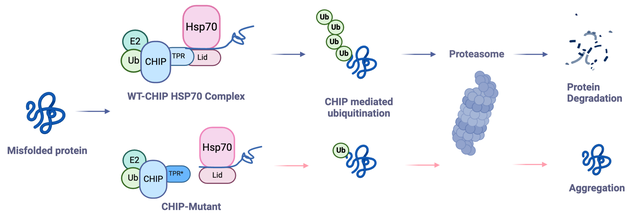
CHIP Mutations and Hsp70 C-terminal Phosphorylation Regulate Specific Binding in the Protein quality Control System

Ivy Peng, Mariah Stewart, Jonathan C. Schisler

ABSTRACT



Background: Protein misfolding and aggregation is a hallmark of various neurodegenerative disorders, such as Alzheimer's Disease, Parkinson's Disease, and Huntington's disease (Zhao, Bi). CHIP's TPR domain interacts with Hsp70 to facilitate proteasomal ubiquitination and degradation of misfolded proteins.

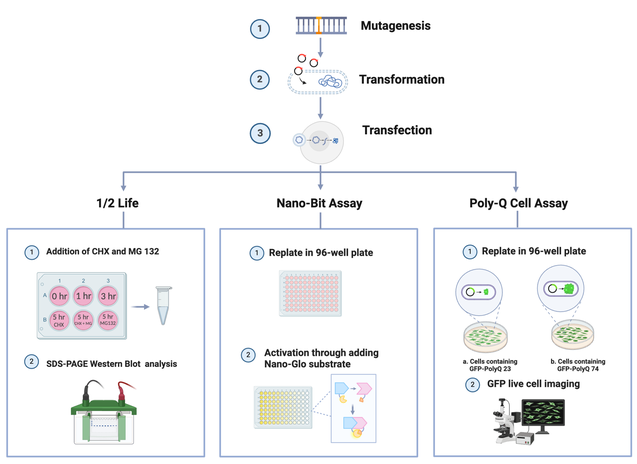
Aim: investigate the relationship between CHIP mutations commonly seen in neurodegenerative disorders and Hsp70 phosphorylation in the process of ubiquitin-dependent proteasomal degradation.

CHIP mutant:
- **G33S**: TPR domain point mutation.
- **P228S**: U-Box domain point mutation.
- **FS**: U-Box domain frameshift mutation.
- **Alt Start**: TPR domain frameshift mutation.

Hsp70:
- **Hsp70A**: preferentially binds with CHIP → ubiquitin dependent degradation
- **Hsp70D**: preferentially bind with HOP → protein integrity

Hypothesis: Decreased binding between CHIP and Hsp70 led to lower rates of proteasomal degradation, longer protein half-life, and more aggregation.

METHODS



½ Life:
CHX: Proteasome inhibitor.
MG132: Protein synthesis inhibitor.

RESULTS

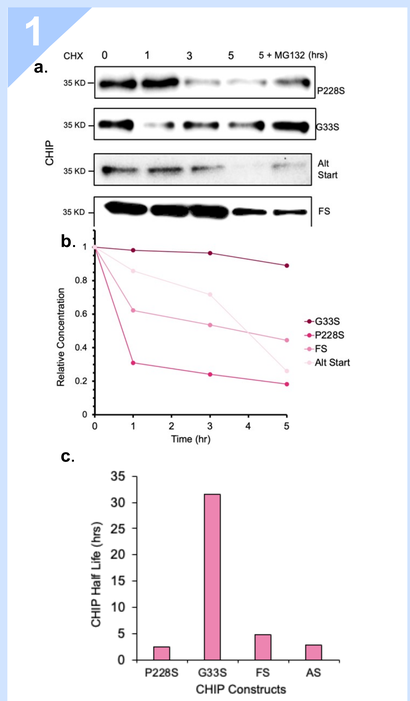


Fig 1. (**a**). Western blot showing degradation of **CHIP mutants** after 1, 3, and 5 hrs cycloheximide addition. (**b**). Scatter plot displaying relative concentration of CHIP mutants. (**c**). Average half-lives of CHIP constructs in association of Hsp70A and Hsp70D.

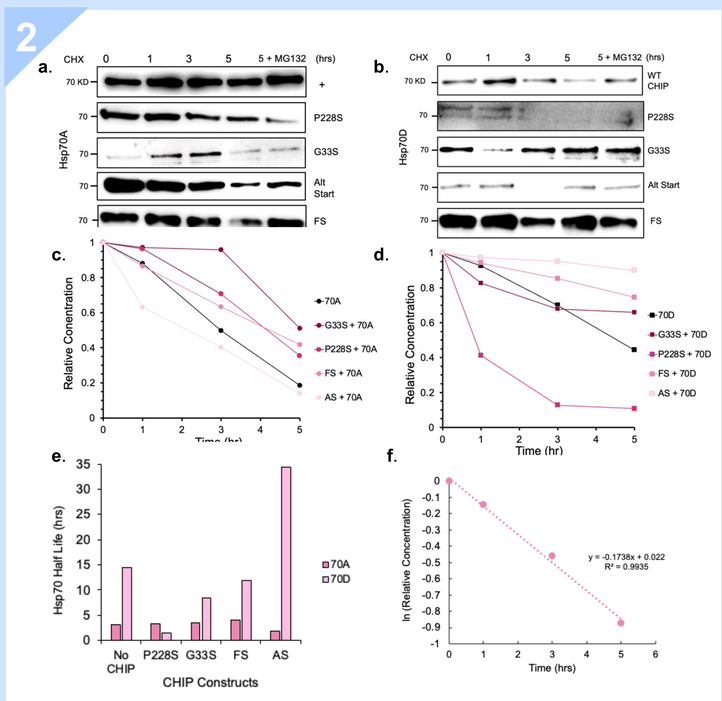


Fig 2. Western blot displaying relative concentration of Hsp (**a**). **70A**, (**b**). **70D** following CHX addition after 1, 3, and 5 hrs; the concentration of Hsp70A in cells without CHIP is included as the positive control. Scatter plot displaying relative concentration of Hsp (**c**). 70A, (**d**). 70D. (**e**). Half-lives of **Hsp70A** and **Hsp70D** in association with WT CHIP and CHIP mutants (P228S, G33S, FS, and Alt Start). (**f**). Sample Half-life calculation.

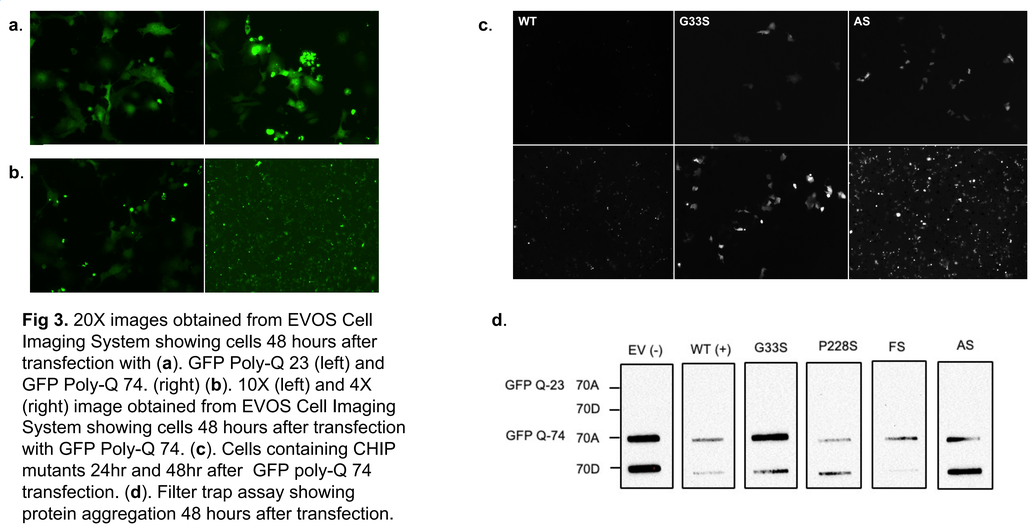
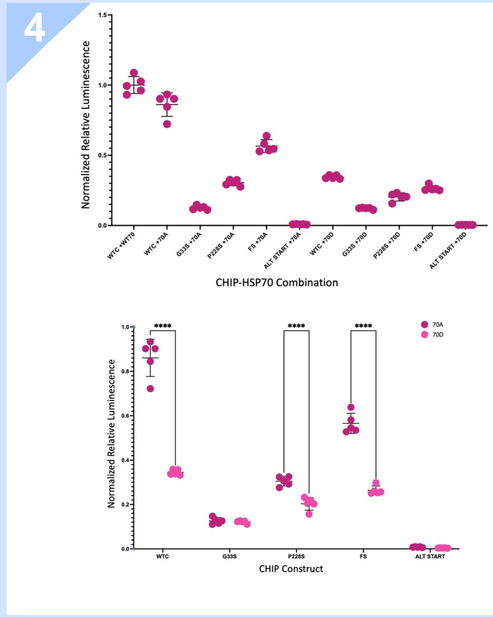


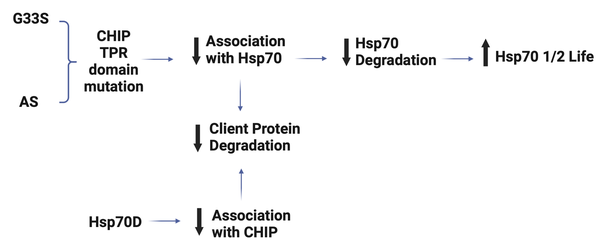
Fig 3. 20X images obtained from EVOS Cell Imaging System showing cells 48 hours after transfection with (**a**). GFP Poly-Q 23 (left) and GFP Poly-Q 74. (right) (**b**). 10X (left) and 4X (right) image obtained from EVOS Cell Imaging System showing cells 48 hours after transfection with GFP Poly-Q 74. (**c**). Cells containing CHIP mutants 24hr and 48hr after GFP poly-Q 74 transfection. (**d**). Filter trap assay showing protein aggregation 48 hours after transfection.

RESULTS



(**a**). Scatter plot showing the percentage fluorescence of CHIP interacting with Hsp70A and Hsp70D, normalized to the positive control (WTC + WT70). The percentage fluorescence differs significantly across all CHIP mutants.
(**b**). A two-way ANOVA test confirmed significant differences in the percentage fluorescence from Hsp 70A and Hsp 70D interaction with WTC ($p < 0.0001$), P228S ($p < 0.0001$), and Frameshift ($p < 0.0001$) .

CONCLUSION



G33S and **Alt Start** CHIP mutations:
- Both occur within the **TPR domain**.
- resulted in **less association** with Hsp70 and **longer Hsp70 ½ lives**.
- Poly-Q assay confirmed **increases in protein aggregation.**

Frameshift and **P228S** CHIP mutations:
- Both occur within the **U-Box domain**.
- resulted in relatively **higher levels of association** with Hsp70 and **shorter** Hsp70 ½ lives.

REFERENCE