



Impaired Autophagic Flux And Chaperone-mediated Autophagy In Fibroblasts From Familial Alzheimer's Disease Cases

#0217

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- The dysregulation of autophagy and CMA observed in fibroblasts from FAD patients highlights the cellular disturbances associated with familial Alzheimer's disease. These findings may provide valuable insights into drug testing, facilitate early diagnosis, and aid in identifying novel biomarkers for AD.

INTRODUCCION

The most prevalent cause of dementia is Alzheimer's disease (AD), which is histopathologically characterized by intracellular neurofibrillary tangles composed of the hyperphosphorylated tau protein and extracellular Amyloid plaques composed of the Amyloid- β peptide (A β). Alzheimer's disease is classified into two categories: familial AD (FAD), also known as an early-onset family disease (EOAD), which accounts for 1% of cases, and sporadic late-onset (LOAD) disease, which accounts for over 99% of cases. Autosomal dominant mutations in the APP, PS1, and PS2 genes are the cause of FAD. Our group and others have detected increased expression of macroautophagy markers and changes in signaling pathways associated with cellular stress, autophagy, lysosomes, and tau phosphorylation in fibroblasts from FAD patients with a PS1 mutation (Lopez-Toledo et al., 2022). Furthermore, macroautophagy and chaperone-mediated autophagy (CMA) impairments are associated with neurodegenerative diseases.

AIM

To evaluate Macroautophagy and Chaperone-Mediated Autophagy (CMA) activity in fibroblasts from patients with Familial Alzheimer's disease (FAD) compared to control fibroblasts.

METHODS

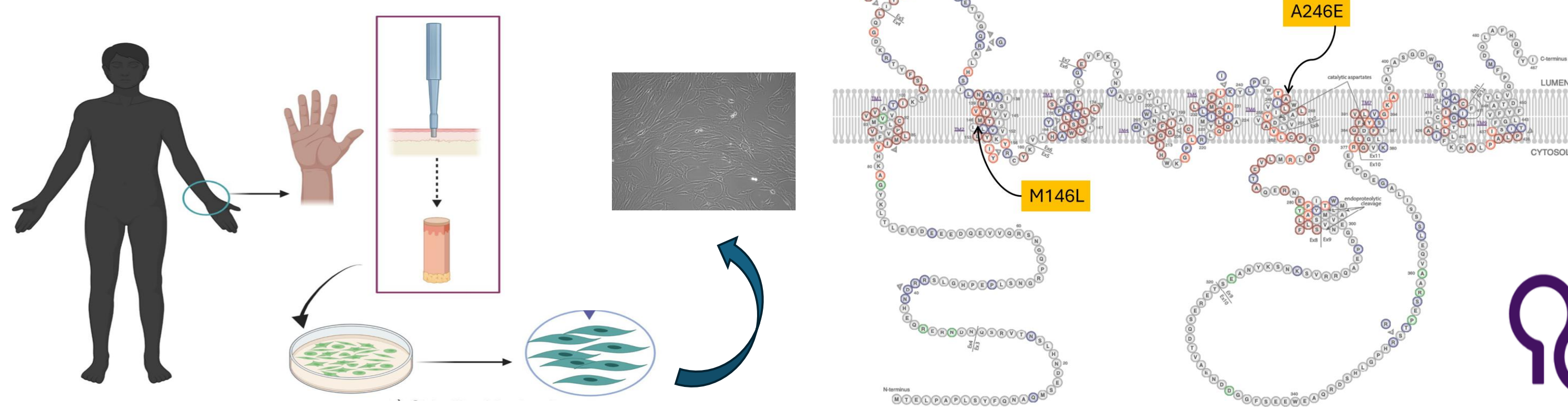
Skin Fibroblasts from FAD patients and non-affected individuals were obtained from the Coriell Institute (New Jersey, USA) repository and cultured in Earl MEM salts medium with 15% non-inactivated FBS. The macroautophagy and CMA markers in these cells were characterized by Western blot. Moreover, autophagy was induced by starvation and chloroquine (CLQ) addition to evaluate the autophagic activity and autophagic flux with Cyto-ID assay and PremoTM Autophagy Tandem Sensor, respectively.

Table 1. Fibroblast lines from elderly controls and FAD patients..

patients.	Internal ID	Age	Sex	Clinical Diagnosis	Mutation	Family
NA1	69	Female	CONTROL (Non-Affected)	NA	NA	Italian-1079
NA3	64	Female				Italian-1079
NA4	36	Male				Canidien-964
NA6	57	Male	CONTROL (Non-Affected)	NA	NA	Canidien-964
AD1	41	Male	FAD	PS1 (M146L)	Italian-1079	
AD2	53	Male	FAD	PS1 (M146L)	Italian-1079	
AD5	56	Male	FAD	PS1 (A246E)	Canidien-964	
AD6	56	Male	FAD	PS1 (A246E)	Canadiense-964	

Table 2. Fibroblast lines from healthy young individuals

Internal ID	Age	Sex	Clinical Diagnosis	Mutation	Family
NA4	36	Male	CONTROL (Non-Affected)	NA	Canidien-964
NA7	11	Female	CONTROL (Non-Affected)	NA	NA
NA8	24	Male	CONTROL (Non-Affected)	NA	NA
NA9	11	Female	CONTROL (Non-Affected)	NA	NA
NA10	2	Female	CONTROL (Non-Affected)	NA	NA



RESULTS

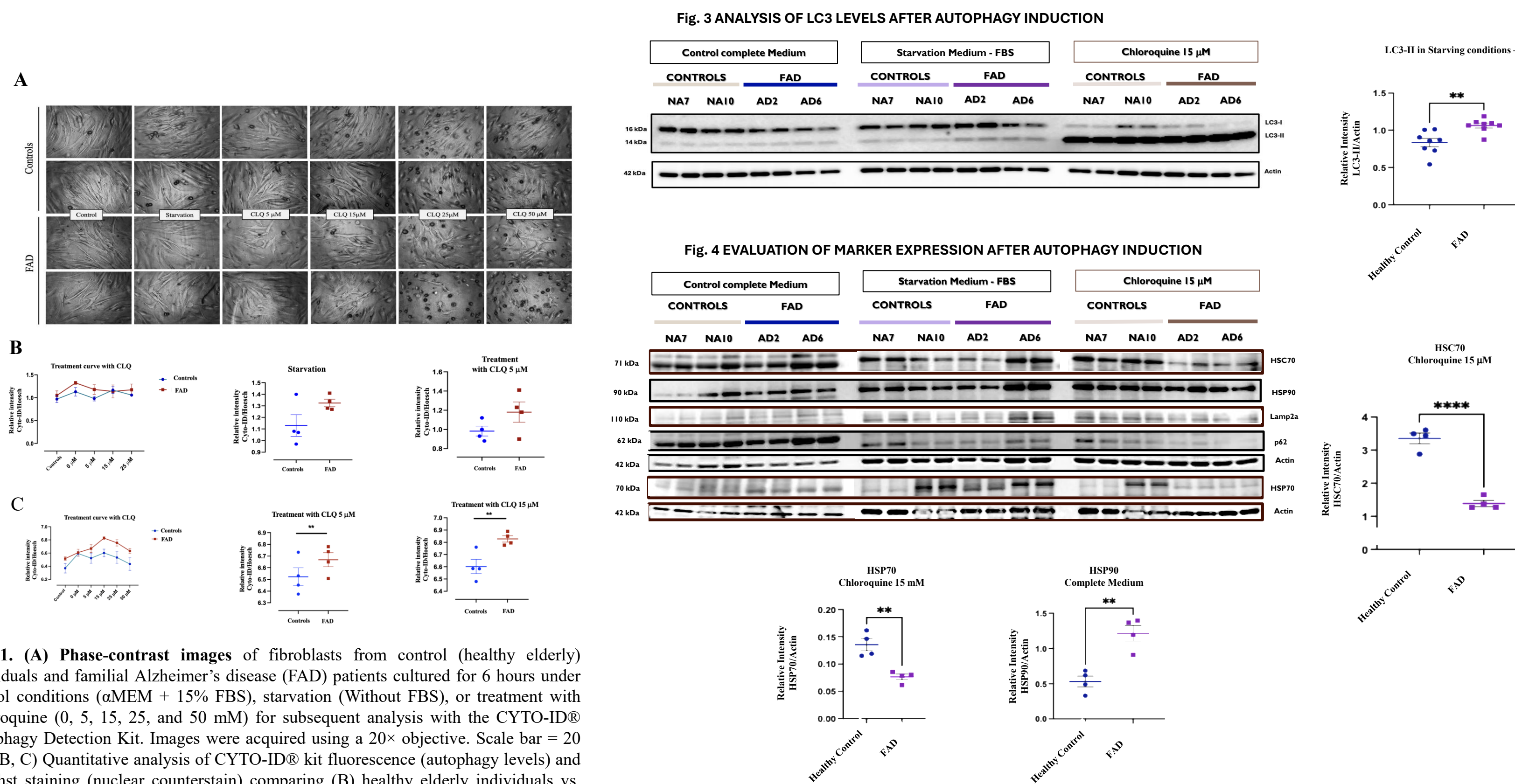


Fig. 1. (A) Phase-contrast images of fibroblasts from control (healthy elderly) individuals and familial Alzheimer's disease (FAD) patients cultured for 6 hours under control conditions (aMEM + 15% FBS), starvation (Without FBS), or treatment with Chloroquine (0, 5, 15, 25, and 50 mM) for subsequent analysis with the CYTO-ID[®] Autophagy Detection Kit. Images were acquired using a 20 $\times$ objective. Scale bar = 20 μ m. (B, C) Quantitative analysis of CYTO-ID[®] kit fluorescence (autophagy levels) and Hoechst staining (nuclear counterstain) comparing (B) healthy elderly individuals vs. FAD patients and (C) healthy young individuals vs. FAD patients.

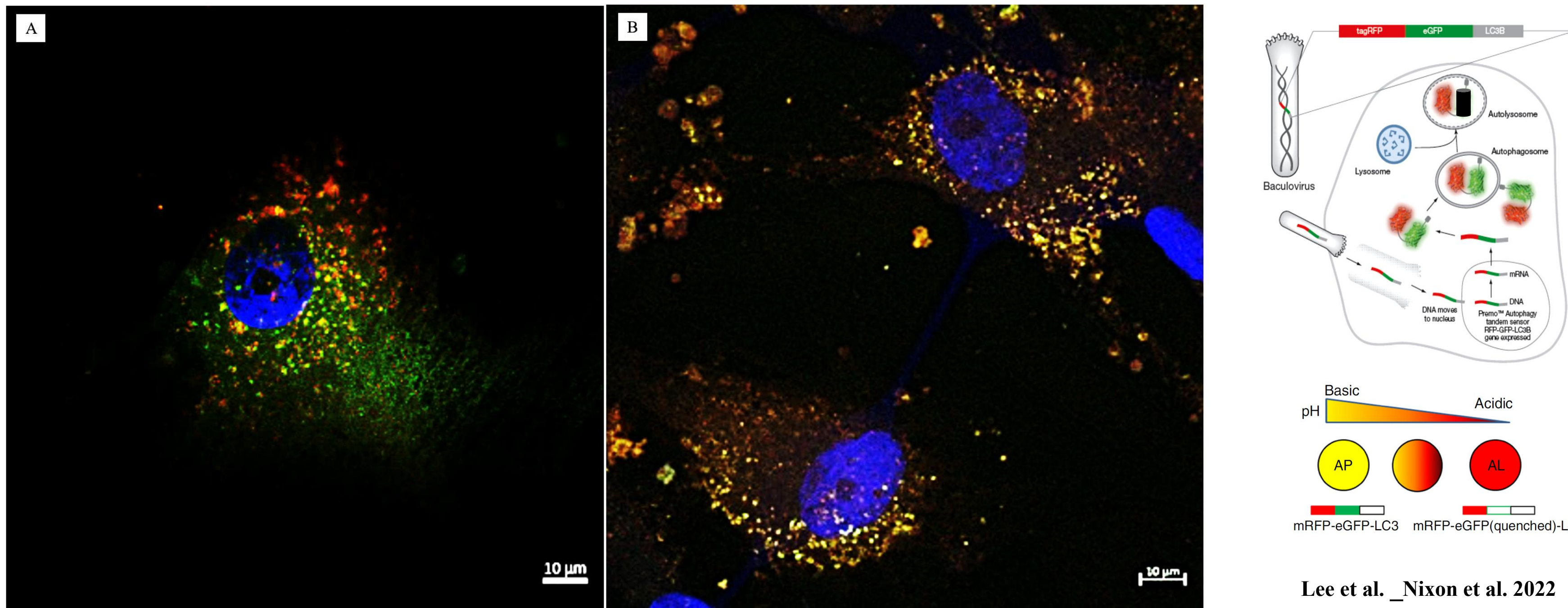


Fig. 2 PremoTM Autophagy Tandem Sensor assay results. representative Confocal microscopy images (63 $\times$ magnification) of fibroblasts from (A) a control non-affected individual (NA4) and (B) a FAD patient (AD2) after 6 hours of treatment with 5 mM Chloroquine (CLQ) and 45 viral particles per cell (PPC) from the PremoTM Autophagy Tandem Sensor Kit.

CONCLUSION

-CMA markers (LAMP2A and HSC70) were downregulated in FAD patients versus controls. Macroautophagy markers (LC3-II and p62) exhibited increased levels under autophagy induction, suggesting dysregulation of both CMA and macroautophagy pathways.

-Autophagic activity and flux were impaired in FAD fibroblasts compared to healthy controls.

-The Cyto-ID[®] assay revealed higher basal autophagy activity in FAD fibroblasts under the tested conditions.

-Autophagolysosome formation was reduced or absent in FAD cells, as evidenced by PremoTM Autophagy Tandem Sensor (lack of red vesicles).

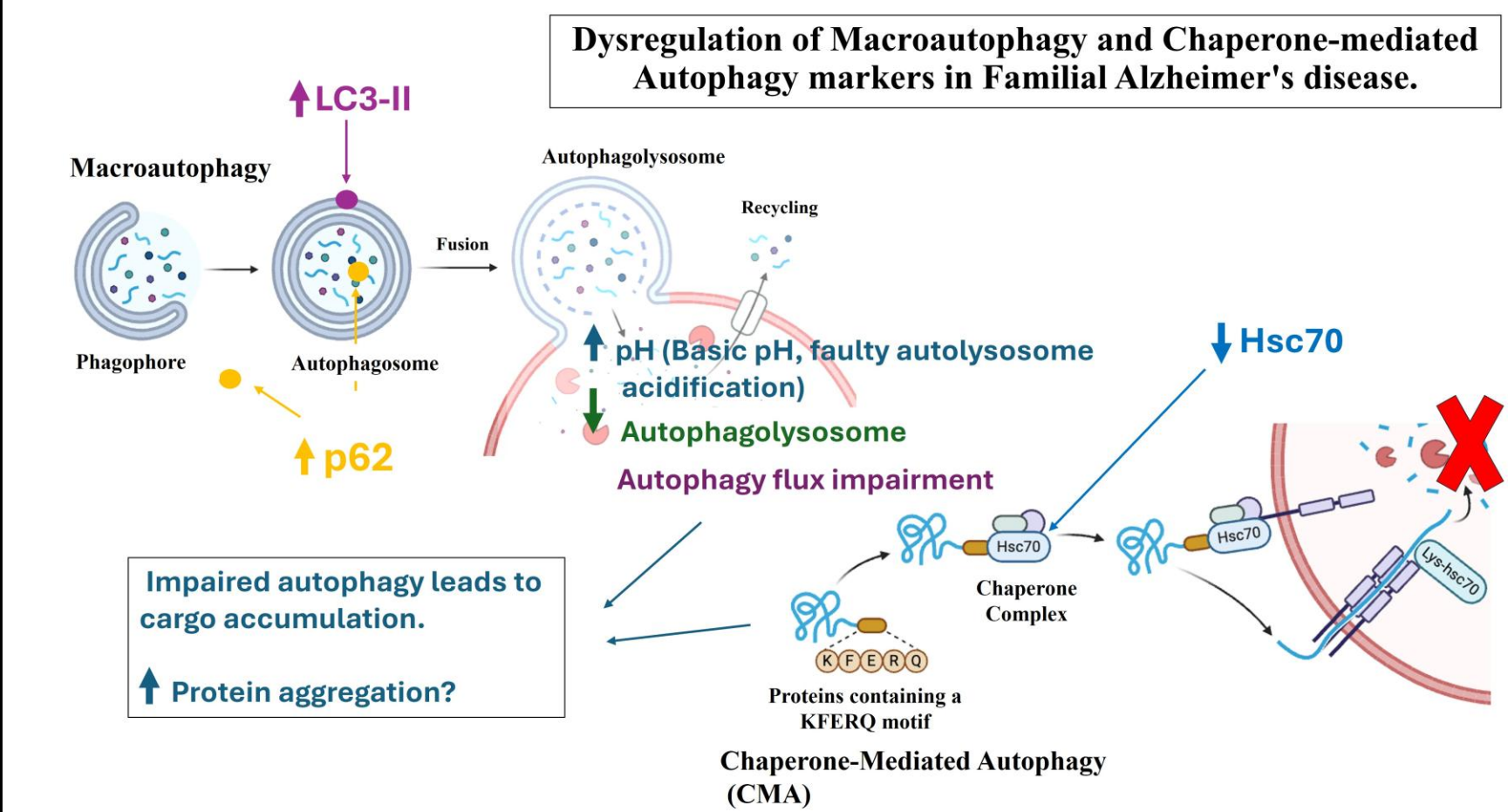
-Potential applications of these findings in peripheral cells include:

Early diagnosis of FAD

Identification of novel biomarkers

Drug screening

Disease modeling



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