# ACCELERATING CELLULAR SENESCENCE IN THE BRAIN OF SIV-INFECTED YOUNG RHESUS MACAQUES

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## BACKGROUND

HIV infection plays a role in accelerating aging. Limited studies have found cellular senescence can occur in some tissues in HIV-infected individuals. However, it is unclear whether HIV infection can accelerate senescence in the brain partially due to challenges of access to human brain tissues. Here we used the SIV infected rhesus macaque model to determine whether SIV contributes to aging of the brain.

## METHODS

**Animal information.** Four groups of rhesus macaques were studied, which included SIVmac251-infected young (Mean 6.65 ± SD 0.94 years) and old aged animals (Mean 20.26 ± SD 3.91 years), and SIV-naïve age-matched animals for comparison.

**Basic Information of animals on this study**

| Animal # | Sex | Age (Years) | Group |
|---|---|---|---|
| H972 | FEMALE | 18.58 | Old SIV+ |
| I774 | FEMALE | 18.45 | Old SIV+ |
| FB04 | MALE | 17.86 | Old SIV+ |
| L618 | MALE | 17.6 | Old SIV+ |
| DT55 | FEMALE | 17.45 | Old SIV+ |
| P427 | MALE | 17.01 | Old SIV+ |
| N444 | MALE | 26.14 | Old SIV- |
| I161 | MALE | 22.22 | Old SIV- |
| FA98 | MALE | 19.26 | Old SIV- |
| M083 | FEMALE | 28.06 | Old SIV- |
| JH29 | FEMALE | 5.71 | Young SIV+ |
| IF44 | FEMALE | 6.42 | Young SIV+ |
| GD41 | MALE | 7.43 | Young SIV+ |
| IF29 | FEMALE | 6.94 | Young SIV+ |
| GB40 | MALE | 6.6 | Young SIV+ |
| GJ23 | MALE | 5.27 | Young SIV+ |
| JM95 | FEMALE | 7.35 | Young SIV- |
| JR95 | MALE | 6.03 | Young SIV- |
| KC32 | MALE | 5.16 | Young SIV- |
| IL53 | MALE | 7.89 | Young SIV- |
| IJ61 | MALE | 7.62 | Young SIV- |
| IM10 | FEMALE | 7.41 | Young SIV- |

**Brain tissue collection.** Brain frontal lobes were collected and formalin-fixed paraffin-embedded (FFPE).

**Lipofuscin measurement.** The lipofuscin area were determined by the overlapped autofluorscent signals in both fluorescent channels (423 nm and 555 nm excitation wavelength). The relative fold change were calculated by ratio of lipofuscin area in the Regions of Interest (ROI) then normalized by Young SIV- group. Image data quantification analysis was performed by HALO software.

**RT-qPCR.** RNA was extracted from FFPE brain frontal cortex using the Qiagen RNeasy FFPE Kit. cDNA was then prepared with the Promega Reverse Transcription System and amplified using the Thermofisher TaqMan Gene Expression Assays. The data was used for comparison of gene expression levels between groups. p16, p21, Cyclin D1 (CCND1), and Caveolin 1 (CAV1) were used as biomarkers of brain cellular senescence.

**Statistical analysis:** Unpaired t-test were used to compare lipofuscin levels and cellular senescence gene expression in frontal cortex between groups. The Welch's correlation was used when groups' variances are unequal. GraphPad Prism 7.0 statistical software was used to analyze data and statistical results were set two-sided at $p < 0.05$ as significant.

**SIV infection** contributes to accelerating **brain cellular senescence** in **young** but not in old rhesus macaques.

## RESULTS

**Lipofuscin significantly increased in SIV-infected young animals.**

In healthy SIV-naïve groups, a significantly higher amount of lipofuscin was observed in old animals than young animals. However, interestingly, this age-dependent discrepancy disappeared between groups of young and old animals with SIV infection, although both groups had higher levels of lipofuscin than young uninfected group. Moreover, the increase of lipofuscin was significantly higher in SIV-infected young animals than those age-matched animals without SIV infection, this was not observed between the older groups of animals with or without SIV infection.

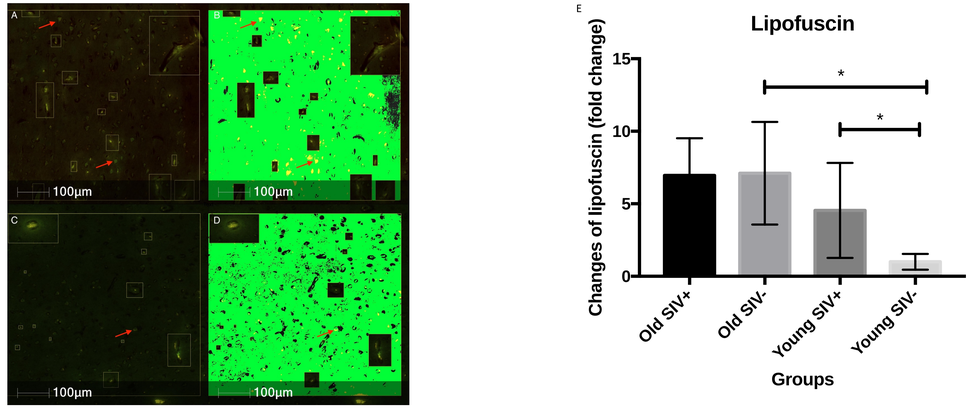


Fig 1. Frontal cortex lipofuscin quantification. Fluorescent microscopy for the detection of lipofuscin via autofluorescence (A-D). Frontal cortex of SIV infected (A&B) and naïve (C&D) young rhesus macaques. SIV-infected young rhesus macaques have higher numbers of cells that contain lipofuscin (A). In naïve young rhesus macaques, lipofuscin was rarely observed (C). Overlays of the HALO analysis results illustrated the autofluorescent area detected (yellow) in SIV-infected and naïve young rhesus macaques (pane B and D, respectively). Arrow: example of lipofuscin. Dotted box: nonspecific autofluorscent area that was excluded from analysis. The relative fold change of lipofuscin in different groups (E). Plot: mean with SD, *: *P<0.05*.

**Brain cellular senescence related genes highly expressed in SIV-infected young animals.**

CAV1 gene expression was significantly increased in the SIV-infected young animals. CCND1 was significantly higher in uninfected older animals than uninfected young animals, but SIV infection of young animals reduced this difference to insignificant. In the young groups, SIV-infected animals had a higher expression levels of p21, CCND1, and CAV1 than uninfected age-matched animals.

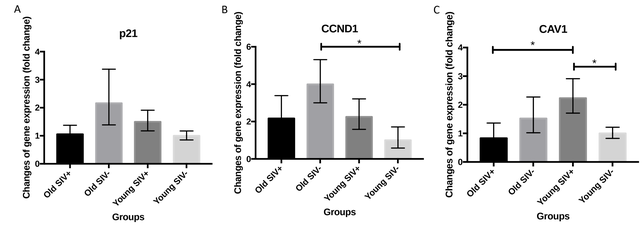


Fig 2. Relative gene expression levels. p21 gene expression level (A). CCND1 gene expression level (B). CAV1 gene expression level (C). Plot: mean with SD, *: *P<0.05*.

## SUMMARY and CONCLUSIONS

- SIV infection contributed to accelerating brain cellular senescence in young rhesus macaques.
- This brain cellular senescence changes were not obvious in old animals.
- p16 gene expression was undetectable in all brain frontal cortex samples.
- Lipofuscin is an ideal marker for cellular senescence measurement in FFPE brain tissue.
- Given that senescent cells in the brain contribute to the cognitive decline and neurodegeneration, our findings indicate that they play an important role in the acceleration of brain aging in young hosts and potentially contribute to the development of HIV-associated neurocognitive disorders.

## ADDITIONAL KEY INFORMATION

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