Neurobiology of Aging 84 (2019) 26–32

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

# A pleiotropic role for exosomes loaded with the amyloid β precursor protein carboxyl-terminal fragments in the brain of Down syndrome patients

Rocío Pérez-González [a,b,1,*], Sébastien A. Gauthier [a,1], Ajay Sharma [a], Chelsea Miller [a], Monika Pawlik [a], Gurjinder Kaur [a], Yohan Kim [a,b], Efrat Levy [a,b,c,d]

[a] Center for Dementia Research, Nathan S. Kline Institute for Psychiatric Research, Orangeburg, NY, USA
[b] Department of Psychiatry, NYU Langone Health, New York, NY, USA
[c] Department of Biochemistry & Molecular Pharmacology, NYU Langone Health, New York, NY, USA
[d] Neuroscience Institute, NYU Langone Health, New York, NY, USA

ARTICLE INFO

*Article history:*
Received 25 February 2019
Received in revised form 25 June 2019
Accepted 26 July 2019
Available online 1 August 2019

*Keywords:*
Extracellular vesicles
Exosomes
Brain
Down syndrome
APP
APP-CTFs
Aging

ABSTRACT

Down syndrome (DS) is characterized by cognitive deficits throughout the life span and with the development of aging-dependent Alzheimer's type neuropathology, which is related to the triplication of the amyloid β precursor protein (APP) gene. A dysfunctional endosomal system in neurons is an early characteristic of DS and APP metabolites accumulate in endosomes in DS neurons. We have previously shown enhanced release of exosomes in the brain of DS patients and the mouse model of DS Ts[Rb(12.17$^{16}$)]2Cje (Ts2), and by DS fibroblasts, as compared with diploid controls. Here, we demonstrate that exosome-enriched extracellular vesicles (hereafter called EVs) isolated from DS and Ts2 brains, and from the culture media of human DS fibroblasts are enriched in APP carboxyl-terminal fragments (APP-CTFs) as compared with diploid controls. Moreover, APP-CTFs levels increase in an age-dependent manner in EVs isolated from the brain of Ts2 mice. The release of APP-CTFs–enriched exosomes may have a pathogenic role by transporting APP-CTFs into naïve neurons and propagating these neurotoxic metabolites, which are also a source of amyloid β, throughout the brain, but also provides a benefit to DS neurons by shedding APP-CTFs accumulated intracellularly.



## 1. Introduction

DS is a genetic disease caused by trisomy of human chromosome 21. After the fourth decade of life, DS is linked to a neurodegenerative affliction that resembles Alzheimer's disease (AD) (Wisniewski et al., 1985), dependent on the triplication of the *APP* gene (Rovelet-Lecrux et al., 2006; Sleegers et al., 2006). A dysfunctional endosomal pathway and abnormally numerous and enlarged early endosomes are found within vulnerable AD and DS neurons early in life (Cataldo et al., 1997, 2000). APP overexpression leads to higher levels of APP-CTFs in the brain of DS patients and mouse models of the disease, including the trisomic mouse model Ts[Rb(12.17$^{16}$)]2Cje (hereafter Ts2) (Jiang et al., 2016; Kaur et al., 2014; Villar et al., 2005). APP overexpression and mainly high levels of APP-CTFs are involved in the endosomal pathology in DS (Cataldo et al., 2000, 2004, 2008; Jiang et al., 2016). APP is an integral membrane protein with a large extracellular amino terminus and a short cytoplasmic carboxyl terminus and it is cleaved by α- and β-secretases to generate APP-αCTF and APP-βCTF, respectively. In Ts2 mice, partial β-secretase (BACE1) genetic reduction prevents AD-related pathological features, including endosomal abnormalities and neurodegeneration (Jiang et al., 2016). These findings emphasize the critical role of APP in the development of these abnormalities as previously suggested (Cataldo et al., 2003; Salehi et al., 2006).

Full-length APP (flAPP), APP-CTFs, and APP cleaving secretases are trafficked through the endosomal pathway and loaded into intraluminal vesicles in the late-endosomal multivesicular bodies (MVBs) that will be either directed to lysosomes for degradation or secreted as exosomes into the extracellular space upon fusion of MVBs with the plasma membrane (Guix et al., 2017; Laulagnier et al., 2018; Miranda et al., 2018; Perez-Gonzalez et al., 2012; Rajendran et al., 2006; Sharples et al., 2008; Vella et al., 2008;

* Corresponding author at: Biomedical Research Institute Sant Pau (IIB-Sant Pau), Carrer Sant Quintí 77-79, 08041 Barcelona, Spain. Tel.: (+34) 93 553 7671; fax: (+34) 93 553 7872.
*E-mail address:* rperezgo@santpau.cat (R. Pérez-González).
[1] Both authors contributed equally to this study.

0197-4580/$ – see front matter © 2019 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.neurobiolaging.2019.07.016

Vingtdeux et al., 2007). Thus, exosomes have an endosomal origin unlike other types of extracellular vesicles (reviewed in van Niel et al., 2018). We have hypothesized that endosomal material can be released by MVBs into the extracellular space via exosomes to relieve neurons of accumulated endosomal contents when the endosomal pathway function is compromised (Levy, 2017). Supporting this, we found that partially blocking exosome release exacerbates the endosomal pathology in DS fibroblasts and that exosome secretion is enhanced in the brains of DS patients and Ts2 mice and by DS fibroblasts (Gauthier et al., 2017).

Given the higher levels of APP expression in the brain of DS patients, we investigated the content of flAPP and APP-CTFs in exosome-enriched extracellular vesicles (hereafter called EVs) isolated from the brains of DS patients and the Ts2 mouse model of the disease, and from the conditioned media of primary DS fibroblasts compared with diploid (2N) controls. Our data show higher levels of flAPP and APP-CTFs in both DS brains and EVs compared with 2N controls. No age-dependent changes were observed in the level of flAPP in brain homogenates or in EVs of Ts2 mice. Similarly, we did not detect changes in the levels of APP-CTFs in Ts2 brain homogenates. However, the levels of both APP-αCTF and APP-βCTF increased with age in EVs isolated from the brain of Ts2 mice. Thus, EVs may have a protective role by removing APP-CTFs that accumulate with age in neuronal endosomes in the brains of DS patients.

## 2. Materials and methods

### 2.1. Mice

A breeding colony of Ts[Rb(12.17$^{16}$)]2Cje (Ts2) mice (Villar et al., 2005) was maintained on B6EiC3SnF1/J background. Trisomic mice and age-matched 2N controls were studied at 3, 8, 12, and 24 months of age. Both females and males were used for all analyses. All animal procedures were performed following the National Institutes of Health guidelines with approval from the Institutional Animal Care and Use Committee at the Nathan S. Kline Institute for Psychiatric Research.

### 2.2. Human brain tissues

Samples of Brodmann Area 9 of human DS and age-matched 2N control subjects were kindly provided by Dr. Jerzy Wegiel, Director, Brain Bank for Developmental Disabilities and Aging, Institute for Basis Research in Developmental Disabilities, Staten Island, New York (details of the human samples in the study of Gauthier et al., 2017).

### 2.3. EV isolation from brain tissues

EVs were isolated from frozen cortical human brain samples and from right murine hemibrains (without the cerebella and the olfactory bulbs). In each experiment, EVs were simultaneously isolated from a brain of either a DS patient or a Ts2 mouse and from an age-matched 2N control. Brain EVs were isolated and purified as we have previously described (Perez-Gonzalez et al., 2012).

### 2.4. Western blot analyses

Brain homogenates (10 μg protein) and EV proteins (15 μL of the lysate corresponding to 25% of the EV lysate total volume) were separated by 4%–20% Tris-HCl electrophoresis gels (Criterion precast gel, Bio-Rad, Hercules, CA, USA) except when 16.5% Tris-Tricine electrophoresis gels were used for the resolution of the APP-αCTF and APP-βCTF bands. The proteins were transferred onto PVDF membranes (Immobilon, EMD Millipore, Billerica, MA, USA). Membranes were incubated with antibodies to CD63 (1:250, Santa Cruz Biotechnology, Dallas, TX, USA), Alix (1:1000, Millipore), TSG101 (1:1000, GeneTex, Irvine, CA, USA), APP and APP-CTFs (C1/6.1 [Mathews et al., 2002], 1:1000), BACE1 (1:1000, Rockland Immunochemicals Inc, Limerick, PA, USA), ADAM10 (1:1000, Millipore), and Nicastrin (1:1000, Millipore). Protein bands were quantified using ImageJ (NIH, Bethesda, MD). All data are shown as the trisomic to 2N ratio.

### 2.5. Cell culture

Skin fibroblasts from a DS patient (Cat#AG06922, Coriell Institute for Medical Research), and an age-matched 2N control subject (#AG07095, Coriell Institute for Medical Research) were grown in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific), 100 units/mL of penicillin and 100 μg/mL streptomycin (Thermo Fisher Scientific), 2 mM GlutaMAX (Thermo Fisher Scientific) at 5% $CO_2$ at 37 °C in humidified air. Experiments were performed on DS and 2N cells with passage numbers from P8 to P14.

### 2.6. EV isolation from fibroblast-conditioned media

EVs were isolated from conditioned media of DS and 2N controls fibroblasts. Cell culture media were replaced with DMEM supplemented with 10% fetal bovine serum that was depleted of EVs by ultracentrifugation at 100,000g for 16 hours, 100 units/mL of penicillin and 100 μg/mL streptomycin (Thermo Fisher Scientific), and 2 mM GlutaMAX (Thermo Fisher Scientific). The conditioned media were collected after 48 hours and EVs were isolated as previously described (Thery et al., 2006). Briefly, the medium was centrifuged at 300g for 10 minutes. The supernatant was sequentially centrifuged at 2,000g for 10 minutes, at 10,000g for 30 minutes, and finally at 100,000g to pellet the EVs. The EVs were resuspended in cold PBS and then centrifuged at 100,000g. The washed EV pellet was resuspended in PBS and lysed in 2X radioimmune precipitation assay buffer (2% Triton-X, 2% sodium deoxycholate, 0.2% SDS, 300 mM NaCl, 100 mM Tris–HCl pH 7.4, and 1 mM EDTA in double distilled water).

### 2.7. Statistical analyses

Data are presented as mean and SEM. Unpaired, two-tailed, Student's t-test statistical analyses were used to compare differences in brain or EV levels of flAPP and APP-CTFs between DS patients and control subjects, Ts2 mice and age-matched 2N controls, and to compare the relative amount of APP-CTFs to flAPP signal intensity between brain and EVs in DS and Ts2 as well as in 2N controls. Age-dependent differences between flAPP and total APP-CTFs levels in Ts2 brains or Ts2 EVs were assessed by one-way ANOVA followed by Tukey post hoc multiple comparison tests. The nonparametric Kruskal-Wallis test was used to compare the levels of APP-αCTFs or APP-βCTFs with age.

## 3. Results

### 3.1. EVs transport flAPP, APP metabolites, and enzymes involved in the metabolism of flAPP

Western blot analysis of the content of EVs isolated from DS brain samples, fractionated on a sucrose gradient, showed that these vesicles float at densities higher than 1.07 g/mL and lower than 1.17 g/mL corresponding to fractions b, c, and d, and that they cargo the exosomal markers Alix and CD63 (Fig. 1). The proteins

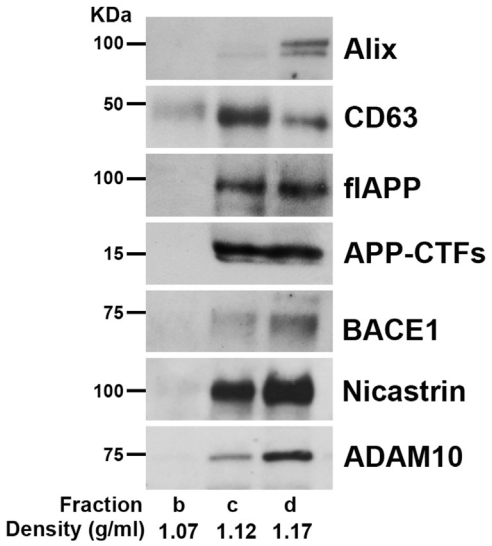


**Fig. 1.** Brain EVs transport flAPP and APP metabolites, and the α-, β-, and γ-secretases. Western blot analysis of human DS brain EVs showed that the sucrose gradient fractions containing the exosomal markers Alix and CD63 also contain flAPP and APP-CTFs, and the enzymes involved in the metabolism of flAPP including α-secretase (ADAM10), β-secretase (BACE1), and a subunit of γ-secretase (Nicastrin). Abbreviations: APP, amyloid β precursor protein; flAPP, full-length APP; APP-CTFs, APP carboxyl-terminal fragments.

flAPP and their metabolites APP-CTFs, as well as α-secretase (ADAM10), BACE1, and the γ-secretase subunit Nicastrin were also identified by western blot analysis in the same fractions that were immunoreactive to the exosomal markers (Fig. 1).

### 3.2. Brain EVs are enriched with APP-CTFs

We first analyzed by western blot the levels of flAPP and APP-CTFs in brain homogenates of DS patients (Fig. 2A) and 12-month-old (MO) Ts2 mice (Fig. 2B) compared with the brain of age-matched 2N controls. flAPP expression levels were 3.75- and 1.56-fold higher in the brain of DS patients (Fig. 2C) and Ts2 mice (Fig. 2D), respectively, compared with the flAPP expression measured in age-matched 2N controls, consistent with previous reports (Jiang et al., 2016; Salehi et al., 2006). APP triplication consequently increases 4 and 1.65 times the levels of APP-CTFs in the brain of DS patients (Fig. 2C) and Ts2 mice (Fig. 2D), respectively. These data support studies showing higher APP metabolite levels in cultured neuronal cells derived from DS fetal brain tissue (Busciglio et al., 2002), human DS fibroblast cultures (Cataldo et al., 2008), and brain samples of DS patients (Cenini et al., 2012). Consistent with the levels in brain homogenates, flAPP and APP-CTFs loading levels were also higher in EVs isolated from the brain of DS human brains (3.42 times for flAPP and 1.79 times for APP-CTFs, Fig. 2E and F), and 12-MO Ts2 mice (1.61 times for flAPP and 1.93 times for APP-CTFs, Fig. 2G and H) compared with 2N controls after normalization of the intensity of the bands to total EV protein content in fractions b, c, and d. Normalization of the intensity of the flAPP and APP-CTFs bands in EVs to total protein content in the brain homogenate revealed higher secretion levels of flAPP and APP-CTFs in EVs isolated from the brain of DS patients (4.34 times for flAPP and 2.31 times for APP-CTFs, Fig. 2E and I) and 12-MO Ts2 mice (1.96 times for flAPP and 2.28 times for APP-CTFs, Fig. 2G and J) compared with 2N controls. These results indicate the release of higher flAPP and APP-CTFs levels into the extracellular space of the brain of DS patients and Ts2 mice as compared with controls, impacted by a synergy of the increased level of APP expression in the DS and Ts2 brain and the enhanced EV release in the DS and Ts2 brain that we previously reported (Gauthier et al., 2017).

We next calculated the relative amount of APP-CTFs to flAPP signal intensity on the same western blot with the C1/6.1 antibody that targets the carboxyl terminal amino acids of flAPP and identifies both flAPP and APP-CTFs. We show that brain EVs have higher APP-CTFs to flAPP levels ratio than brain homogenates in both human (5.73 vs 0.28 in 2N, and 2.52 vs 0.85 in DS, Fig. 2K) and mice (3.66 vs 0.73 in 2N, and 2.15 vs 0.80 in Ts2, Fig. 2L) regardless of the genotype. These results are consistent with our previous finding of APP-CTFs enrichment in brain EVs of the mouse model of β amyloidosis Tg2576 and their littermate control mice (Perez-Gonzalez et al., 2012).

### 3.3. EVs secreted by fibroblasts are enriched with APP-CTFs

We corroborated the results obtained in the DS brain, in an in vitro model by using human skin fibroblasts from a DS patient and a 2N control individual. Western blot analysis of cell lysates and EVs isolated from the conditioned media of DS and 2N fibroblasts with antibodies to the exosomal markers Alix, TSG101, and the tetraspanin CD63 confirmed the presence of exosomes in the isolated EVs (Fig. 3A). The higher levels of Alix and TSG101 are due to higher EV release by DS cells compared with 2N controls due to enhanced CD63 expression (Gauthier et al., 2017). DS fibroblasts express higher levels of flAPP (Fig. 3B), contain higher levels of APP-CTFs (Fig. 3B), and secrete EVs containing higher levels of flAPP and APP-CTFs compared with 2N control fibroblasts (Fig. 3C). Similar to brain EVs, EVs secreted by 2N and DS fibroblasts into the culture media were enriched with APP-CTFs as shown by a higher APP-CTFs to flAPP ratio than cell lysates (Fig. 3D).

### 3.4. Age-dependent increase in APP-CTFs loading levels of EVs in the brain of Ts2 mice

To determine the effect of aging on the content of flAPP and APP-CTFs, we analyzed by western blot brain homogenates and brain EVs of Ts2 mice at 3, 8, 12, and 24 months of age. We quantified the levels of flAPP and APP-CTFs per EV by normalizing the intensity of the bands to the total amount of EV proteins. Thus, the changes in the level of EV secretion with age were not taken into account in the analysis. The levels of flAPP were 1.25, 1.38, 1.56, and 1.57 times higher in 3-, 8-, 12-, and 24-MO Ts2 mice, respectively, compared with littermate controls and no significant changes were detected with age (Fig. 4A). Similarly, APP-CTFs levels were 1.78, 1.75, 1.65, and 1.63 times higher in the brain homogenate of 3-, 8-, 12-, and 24-MO Ts2 mice respectively, compared with 2N controls with no significant changes with age (Fig. 4B). However, in brain EVs, while the level of flAPP was not significantly affected by age (Fig. 4C), the content of APP-CTFs was roughly 50% higher in brain EVs of older (12 and 24 MO) compared with younger (3 and 8 MO) Ts2 mice (Fig. 4D). Resolution of the 2 APP-CTFs bands by western blot analysis (Fig. 4E) and separate analysis of the levels of APP-αCTFs (Fig. 4F) and APP-βCTFs (Fig. 4G) revealed that the levels of both metabolites increased with age in Ts2 brain EVs.

## 4. Discussion

We have previously reported that EVs isolated from human and mouse brains contain flAPP and the APP metabolites APP-CTFs (Perez-Gonzalez et al., 2012), as was shown previously for EVs isolated from the media cultured by neuronal cells in vitro (Laulagnier et al., 2018; Vingtdeux et al., 2007). Moreover, we found that brain EVs, regardless of the genotype, are enriched with APP-

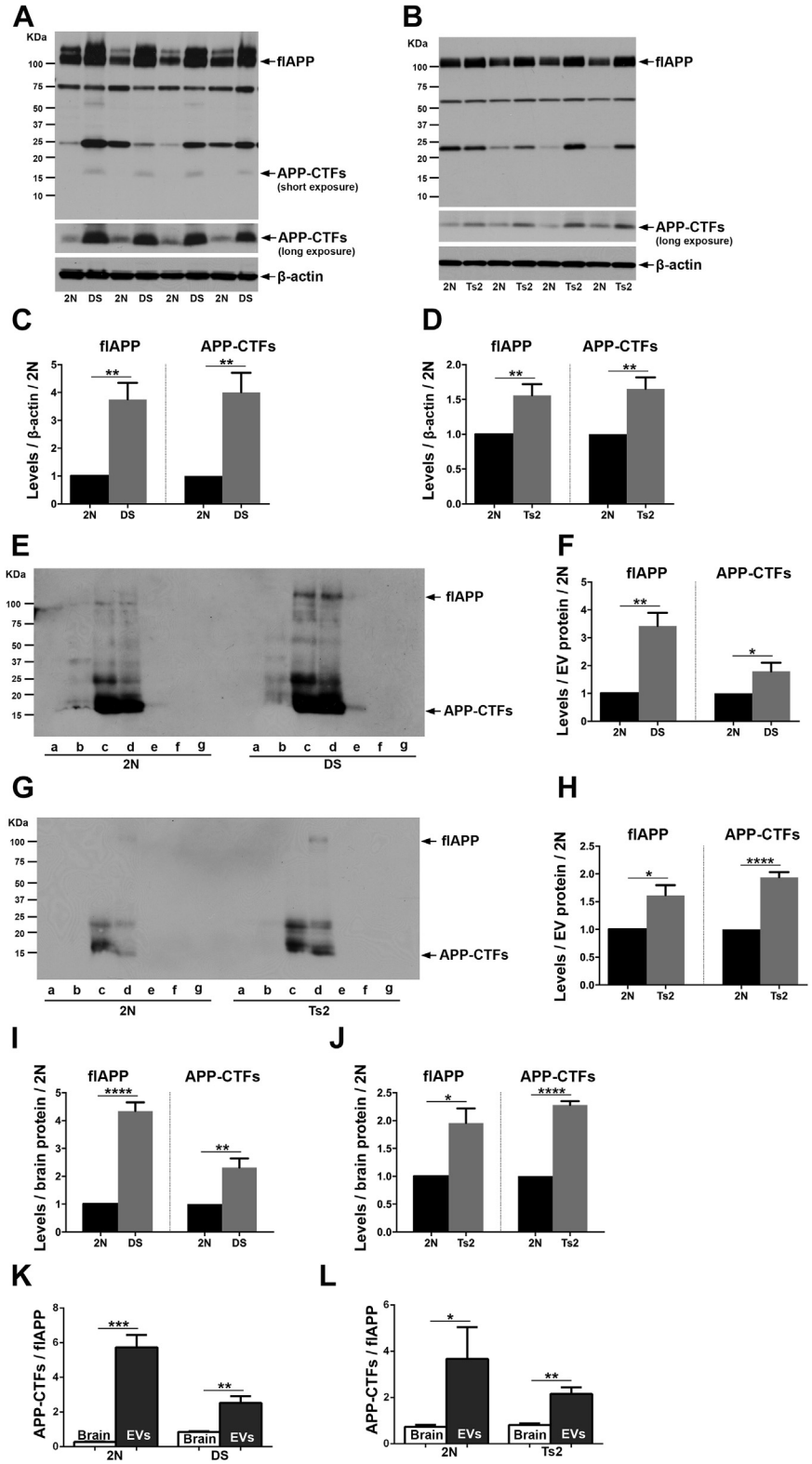


**Fig. 2.** Brain EVs are enriched with APP-CTFs. Western blot analysis with the C1/6.1 antibody of brain homogenates of human DS patients and age-matched 2N controls (A) and 12-MO Ts2 and littermate 2N mice (B) revealed higher levels of flAPP and APP-CTFs in DS (C) and Ts2 (D) brains compared to 2N. Long exposure time of membranes was used to reveal APP-CTFs in brain homogenates. The flAPP and APP-CTFs band intensities in brain homogenates were normalized by β-actin. Western blot analysis with C1/6.1 of EVs isolated from the brain extracellular space of DS patients (E) and Ts2 mice (G) and age-matched diploid controls showed higher loading of flAPP and APP-CTFs levels in the DS (F) and Ts2 (H) EVs compared with controls when the intensity of the APP and APP-CTFs bands was normalized to total EV protein content in fractions b, c, and d. Normalization of the intensity of the bands to total protein content in the brain homogenates shows higher secretion levels of flAPP and APP-CTFs in DS (I) and Ts2 (J) brains through EVs compared with 2N controls. The values are presented as the ratio of trisomic to 2N levels. Calculation of the APP-CTFs to flAPP signal intensity ratio on the same western blot shows APP-CTFs enrichment relative to flAPP in human (K) and murine (L) EVs compared with brain homogenates. Student's t-test, n = 5 (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$). Abbreviations: EV, exosome-enriched extracellular vesicle; flAPP, full-length APP; APP-CTFs, APP carboxyl-terminal fragments; DS, Down syndrome; 2N, diploid control.

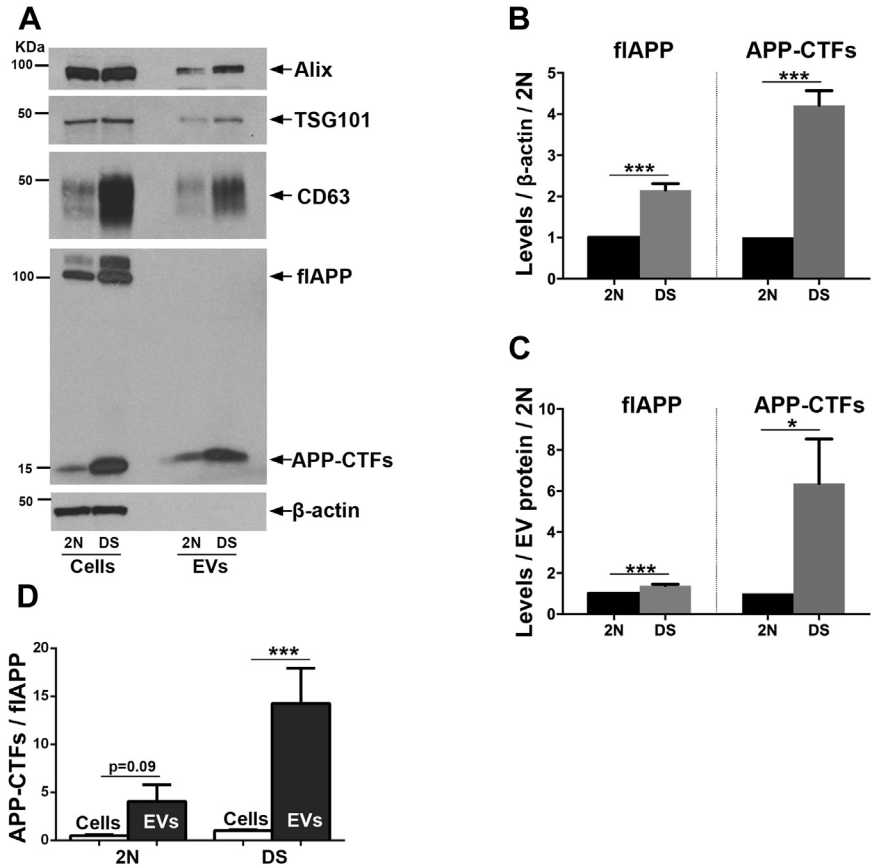


**Fig. 3.** EVs secreted by fibroblasts are enriched with APP-CTFs. Western blot analysis of cell lysates and EV proteins show the presence of the exosomal markers Alix, TSG101 and CD63, and flAPP and APP-CTFs (A). Of note, flAPP in EVs was only detected after longer exposure times (not shown). Quantification of the APP bands show higher level of flAPP and APP-CTFs in cell lysates (B) and EVs (C) of DS fibroblasts compared to 2N and a higher ratio of APP-CTFs to flAPP in EVs secreted by human 2N and DS fibroblasts compared to 2N and DS cell lysates, respectively (D). The flAPP and APP-CTFs band intensities in cell lysates and EVs were normalized by β-actin and EV protein, respectively. Student's t-test, n = 4 independent experiments (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Abbreviations: EV, exosome-enriched extracellular vesicle; flAPP, full-length APP; APP-CTFs, APP carboxyl-terminal fragments; DS, Down syndrome; 2N, diploid control.

CTFs compared with brain homogenates (Perez-Gonzalez et al., 2012). Here we analyzed the content of flAPP and APP-CTFs in EVs secreted in the brain of DS patients and in in vivo and in vitro models of the disease. In accordance with our previous work, we found that all the EVs studied were enriched with APP-CTFs. Consistent with higher levels of APP expression, EVs released into the brain extracellular space of DS patients and Ts2 mice, as well as EVs secreted into the conditioned media by DS fibroblasts, contained higher levels of APP-CTFs compared with 2N controls.

EV release potentially plays pleiotropic roles. The transport of APP-βCTF, a neurotoxic metabolite that is also a source of amyloid β (Deyts et al., 2012; Flammang et al., 2012; Oules et al., 2012; Tamayev et al., 2012) throughout the brain may result in the spread of the disease, suggested as a general mechanism of propagation of pathology in neurodegenerative disorders (Howitt and Hill, 2016; Xiao et al., 2017). Given that we found enhanced accumulation of APP-CTFs, including APP-βCTFs, in EVs at older ages in the brain of Ts2 mice, in addition to the higher EV release compared with 2N, the propagation of the neurotoxic material may be a mechanism for the age-dependent progression of the pathology in DS. Alternatively, we previously suggested that endosomal abnormalities affect the exosome secretory pathway in DS as a protective mechanism and explored the molecular mechanism underlying changes in exosome secretion in vivo in the brain of DS patients and the Ts2 murine model, and in human DS fibroblasts grown in vitro. We found higher levels of EV secretion and identified an increase in EV levels in the brain extracellular space of the DS mouse model Ts2 as compared with 2N littermates at an older age than the age at which endosomal pathology appear (Gauthier et al., 2017). Here we report that in addition to the age-dependent increase in EV release, these EVs are enriched with APP-CTFs compared with 2N controls. Therefore, the combination of the trisomy-induced age-dependent higher level of EV release (Gauthier et al., 2017), with each EV containing higher amount of APP-CTFs with age results in large amounts of this metabolite transported by EVs into the extracellular space as the mice age. These results support the hypothesis that in neurodegenerative disorders with endosomal-lysosomal dysfunction, such as DS, exosome secretion serves as a disposal mechanism of toxic material that is abnormally accumulated in dysfunctional endosomal compartments. In support of the neuroprotective role of EV release to mitigate the accrued neurotoxic threat associated with cellular accumulation, it was reported that exosome uptake by microglia is more efficient than by neurons (Fitzner et al., 2011; Yuyama et al., 2012). Moreover, in vitro studies have shown that after internalization into HEK293 cells, exosomes shuttle within endocytic vesicles and are sorted into the lysosome as their final intracellular destination (Heusermann et al., 2016), suggesting that exosome release in DS may be a protective function leading to the degradation of toxic material in non-neuronal cells (Joshi et al., 2015).

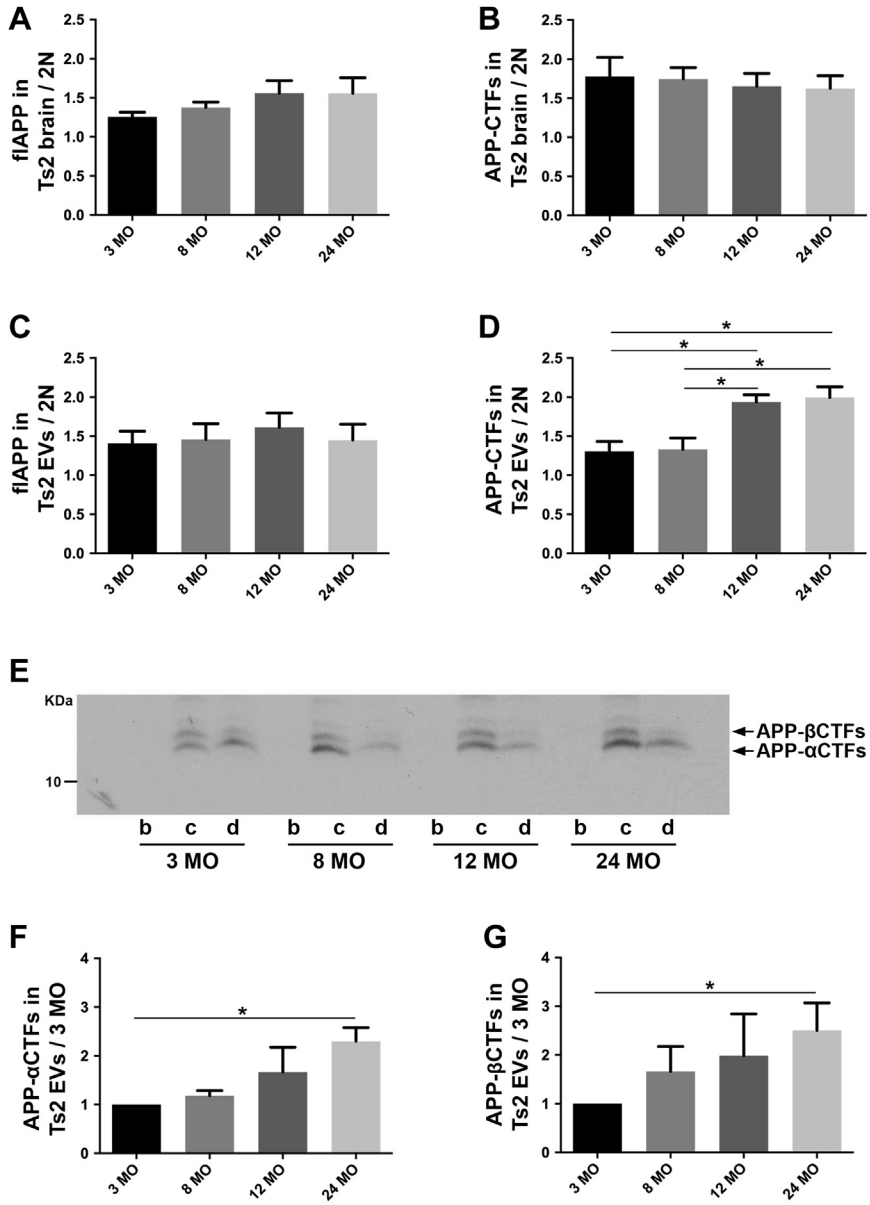


**Fig. 4.** Content of APP-CTFs in brain EVs of Ts2 mice increases with age. The relative levels of flAPP (A) and APP-CTFs (B) in brain homogenates of Ts2 to 2N mice did not show significant changes with age (3, 8, 12, and 24 MO). Although the EV loading content of flAPP was not affected by age (C), the amount of APP-CTFs in Ts2 EVs relative to 2N EVs increased with age (D). The values in EVs were normalized to total EV protein in fractions b, c, and d. One-way ANOVA followed by Tukey post hoc multiple comparison tests, n = 4 (*, $p < 0.05$). Western blot analysis with the C1/6.1 antibody of Ts2 EV proteins separated by 16.5% Tris-Tricine gels were used to separate between the bands of APP-αCTFs and APP-βCTFs (E). Quantification of the bands revealed an increase of both APP-αCTFs (F) and APP-βCTFs (G) with age. Kruskal-Wallis test followed by Dunn's multiple comparison test, n = 4 (*, $p < 0.05$). Abbreviations: EVs, exosome-enriched extracellular vesicles; flAPP, full-length APP; APP-CTFs, APP carboxyl-terminal fragments.

## 5. Conclusions

We report here that EVs are loaded with the APP metabolites APP-αCTF and APP-βCTF and that this loading is exacerbated in DS. Moreover, we show an age-dependent increase in the level of APP-CTFs in each EV in the brain of Ts2 mice that combined with the age-dependent higher levels of EV secretion results in removal of large amounts of the toxic metabolite with age. Although APP-CTFs loading of EVs may play a pathogenic role by transporting these metabolites into naïve neurons and propagating pathology throughout the brain, we suggest that the release of APP-CTFs–loaded EVs alleviates the endosomal-lysosomal pathology that characterizes neurodegenerative disorders such as DS. Given that endosomal abnormality causes neuron degeneration (Nixon, 2004), mitigation of this pathology by enhanced exosome release might be protective and a target for therapeutic intervention.

## Disclosure

The authors report no conflicts of interest related with this work.

## Acknowledgements

This work was supported by the Alzheimer's Association (NIRG-14-316622) and the National Institute on Aging (AG017617 and AG056732).

Authors' contributions: E.L. conceived the ideas. R.P.G. and S.A.G. designed, performed, and analyzed experiments. A.S. performed brain EV isolation. Y.K, G.K., A.S., and C.M. conducted western blot

analysis. M.P. maintained the Ts2 mouse colony. R.P.G., S.A.G., and E.L. wrote the manuscript.

## References


Busciglio, J., Pelsman, A., Wong, C., Pigino, G., Yuan, M., Mori, H., Yankner, B.A., 2002. Altered metabolism of the amyloid beta precursor protein is associated with mitochondrial dysfunction in Down's syndrome. Neuron 33, 677–688.

Cataldo, A.M., Barnett, J.L., Pieroni, C., Nixon, R.A., 1997. Increased neuronal endocytosis and protease delivery to early endosomes in sporadic Alzheimer's disease: neuropathologic evidence for a mechanism of increased beta-amyloidogenesis. J. Neurosci. 17, 6142–6151.

Cataldo, A.M., Peterhoff, C.M., Troncoso, J.C., Gomez-Isla, T., Hyman, B.T., Nixon, R.A., 2000. Endocytic pathway abnormalities precede amyloid beta deposition in sporadic Alzheimer's disease and Down syndrome: differential effects of APOE genotype and presenilin mutations. Am. J. Pathol. 157, 277–286.

Cataldo, A.M., Petanceska, S., Peterhoff, C.M., Terio, N.B., Epstein, C.J., Villar, A., Carlson, E.J., Staufenbiel, M., Nixon, R.A., 2003. App gene dosage modulates endosomal abnormalities of Alzheimer's disease in a segmental trisomy 16 mouse model of down syndrome. J. Neurosci. 23, 6788–6792.

Cataldo, A.M., Petanceska, S., Terio, N.B., Peterhoff, C.M., Durham, R., Mercken, M., Mehta, P.D., Buxbaum, J., Haroutunian, V., Nixon, R.A., 2004. Abeta localization in abnormal endosomes: association with earliest Abeta elevations in AD and Down syndrome. Neurobiol. Aging 25, 1263–1272.

Cataldo, A.M., Mathews, P.M., Boiteau, A.B., Hassinger, L.C., Peterhoff, C.M., Jiang, Y., Mullaney, K., Neve, R.L., Gruenberg, J., Nixon, R.A., 2008. Down syndrome fibroblast model of Alzheimer-related endosome pathology: accelerated endocytosis promotes late endocytic defects. Am. J. Pathol. 173, 370–384.

Cenini, G., Dowling, A.L., Beckett, T.L., Barone, E., Mancuso, C., Murphy, M.P., Levine 3rd, H., Lott, I.T., Schmitt, F.A., Butterfield, D.A., Head, E., 2012. Association between frontal cortex oxidative damage and beta-amyloid as a function of age in Down syndrome. Biochim. Biophys. Acta 1822, 130–138.

Deyts, C., Vetrivel, K.S., Das, S., Shepherd, Y.M., Dupre, D.J., Thinakaran, G., Parent, A.T., 2012. Novel GalphaS-protein signaling associated with membrane-tethered amyloid precursor protein intracellular domain. J. Neurosci. 32, 1714–1729.

Fitzner, D., Schnaars, M., van Rossum, D., Krishnamoorthy, G., Dibaj, P., Bakhti, M., Regen, T., Hanisch, U.K., Simons, M., 2011. Selective transfer of exosomes from oligodendrocytes to microglia by macropinocytosis. J. Cell Sci. 124 (Pt 3), 447–458.

Flammang, B., Pardossi-Piquard, R., Sevalle, J., Debayle, D., Dabert-Gay, A.S., Thevenet, A., Lauritzen, I., Checler, F., 2012. Evidence that the amyloid-beta protein precursor intracellular domain, AICD, derives from beta-secretase-generated C-terminal fragment. J. Alzheimers Dis. 30, 145–153.

Gauthier, S.A., Perez-Gonzalez, R., Sharma, A., Huang, F.K., Alldred, M.J., Pawlik, M., Kaur, G., Ginsberg, S.D., Neubert, T.A., Levy, E., 2017. Enhanced exosome secretion in Down syndrome brain - a protective mechanism to alleviate neuronal endosomal abnormalities. Acta Neuropathol. Commun. 5, 65.

Guix, F.X., Sannerud, R., Berditchevski, F., Arranz, A.M., Horre, K., Snellinx, A., Thathiah, A., Saido, T., Saito, T., Rajesh, S., Overduin, M., Kumar-Singh, S., Radaelli, E., Corthout, N., Colombelli, J., Tosi, S., Munck, S., Salas, I.H., Annaert, W., De Strooper, B., 2017. Tetraspanin 6: a pivotal protein of the multiple vesicular body determining exosome release and lysosomal degradation of amyloid precursor protein fragments. Mol. Neurodegener. 12, 25.

Heusermann, W., Hean, J., Trojer, D., Steib, E., von Bueren, S., Graff-Meyer, A., Genoud, C., Martin, K., Pizzato, N., Voshol, J., Morrissey, D.V., Andaloussi, S.E., Wood, M.J., Meisner-Kober, N.C., 2016. Exosomes surf on filopodia to enter cells at endocytic hot spots, traffic within endosomes, and are targeted to the ER. J. Cell Biol. 213, 173–184.

Howitt, J., Hill, A.F., 2016. Exosomes in the pathology of neurodegenerative diseases. J. Biol. Chem. 291, 26589–26597.

Jiang, Y., Rigoglioso, A., Peterhoff, C.M., Pawlik, M., Sato, Y., Bleiwas, C., Stavrides, P., Smiley, J.F., Ginsberg, S.D., Mathews, P.M., Levy, E., Nixon, R.A., 2016. Partial BACE1 reduction in a Down syndrome mouse model blocks Alzheimer-related endosomal anomalies and cholinergic neurodegeneration: role of APP-CTF. Neurobiol. Aging 39, 90–98.

Joshi, P., Benussi, L., Furlan, R., Ghidoni, R., Verderio, C., 2015. Extracellular vesicles in Alzheimer's disease: friends or foes? Focus on abeta-vesicle interaction. Int. J. Mol. Sci. 16, 4800–4813.

Kaur, G., Sharma, A., Xu, W., Gerum, S., Alldred, M.J., Subbanna, S., Basavarajappa, B.S., Pawlik, M., Ohno, M., Ginsberg, S.D., Wilson, D.A., Guilfoyle, D.N., Levy, E., 2014. Glutamatergic transmission aberration: a major cause of behavioral deficits in a murine model of Down's syndrome. J. Neurosci. 34, 5099–5106.

Laulagnier, K., Javalet, C., Hemming, F.J., Chivet, M., Lachenal, G., Blot, B., Chatellard, C., Sadoul, R., 2018. Amyloid precursor protein products concentrate in a subset of exosomes specifically endocytosed by neurons. Cell Mol. Life Sci. 75, 757–773.

Levy, E., 2017. Exosomes in the diseased brain: first insights from *in vivo* studies. In: Sarko, D.K., McKinney, C. (Eds.), Exosomes: Role in Cell Function, Neurodegeneration and Therapy, in Front. Neurosci. section neurodegeneration, 11, p. 142.

Mathews, P.M., Jiang, Y., Schmidt, S.D., Grbovic, O.M., Mercken, M., Nixon, R.A., 2002. Calpain activity regulates the cell surface distribution of amyloid precursor protein. Inhibition of calpains enhances endosomal generation of beta-cleaved C-terminal APP fragments. J. Biol. Chem. 277, 36415–36424.

Miranda, A.M., Lasiecka, C.M., Xu, Y., Neufeld, J., Shahriar, S., Simoes, S., Chan, R.B., Oliveira, T.G., Small, S.A., Di Paolo, G., 2018. Neuronal lysosomal dysfunction releases exosomes harboring APP C-terminal fragments and unique lipid signatures. Nat. Commun. 9 (1), 291.

Nixon, R.A., 2004. Niemann-Pick Type C disease and Alzheimer's disease: the APP-endosome connection fattens up. Am. J. Pathol. 164, 757–761.

Oules, B., Del Prete, D., Greco, B., Zhang, X., Lauritzen, I., Sevalle, J., Moreno, S., Paterlini-Brechot, P., Trebak, M., Checler, F., Benfenati, F., Chami, M., 2012. Ryanodine receptor blockade reduces amyloid-beta load and memory impairments in Tg2576 mouse model of Alzheimer disease. J. Neurosci. 32, 11820–11834.

Perez-Gonzalez, R., Gauthier, S.A., Kumar, A., Levy, E., 2012. The exosome secretory pathway transports amyloid precursor protein carboxyl-terminal fragments from the cell into the brain extracellular space. J. Biol. Chem. 287, 43108–43115.

Rajendran, L., Honsho, M., Zahn, T.R., Keller, P., Geiger, K.D., Verkade, P., Simons, K., 2006. Alzheimer's disease beta-amyloid peptides are released in association with exosomes. Proc. Natl. Acad. Sci. U. S. A. 103, 11172–11177.

Rovelet-Lecrux, A., Hannequin, D., Raux, G., Le Meur, N., Laquerriere, A., Vital, A., Dumanchin, C., Feuillette, S., Brice, A., Vercelletto, M., Dubas, F., Frebourg, T., Campion, D., 2006. APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. Nat. Genet. 38, 24–26.

Salehi, A., Delcroix, J.D., Belichenko, P.V., Zhan, K., Wu, C., Valletta, J.S., Takimoto-Kimura, R., Kleschevnikov, A.M., Sambamurti, K., Chung, P.P., Xia, W., Villar, A., Campbell, W.A., Kulnane, L.S., Nixon, R.A., Lamb, B.T., Epstein, C.J., Stokin, G.B., Goldstein, L.S., Mobley, W.C., 2006. Increased App expression in a mouse model of Down's syndrome disrupts NGF transport and causes cholinergic neuron degeneration. Neuron 51, 29–42.

Sharples, R.A., Vella, L.J., Nisbet, R.M., Naylor, R., Perez, K., Barnham, K.J., Masters, C.L., Hill, A.F., 2008. Inhibition of gamma-secretase causes increased secretion of amyloid precursor protein C-terminal fragments in association with exosomes. FASEB J. 22, 1469–1478.

Sleegers, K., Brouwers, N., Gijselinck, I., Theuns, J., Goossens, D., Wauters, J., Del-Favero, J., Cruts, M., van Duijn, C.M., Van Broeckhoven, C., 2006. APP duplication is sufficient to cause early onset Alzheimer's dementia with cerebral amyloid angiopathy. Brain 129 (Pt 11), 2977–2983.

Tamayev, R., Matsuda, S., Arancio, O., D'Adamio, L., 2012. beta- but not gamma-secretase proteolysis of APP causes synaptic and memory deficits in a mouse model of dementia. EMBO Mol. Med. 4, 171–179.

Thery, C., Amigorena, S., Raposo, G., Clayton, A., 2006. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr. Protoc. Cell Biol. 3.

van Niel, G., D'Angelo, G., Raposo, G., 2018. Shedding light on the cell biology of extracellular vesicles. Nat. Rev. Mol. Cell Biol 19, 213–228.

Vella, L.J., Sharples, R.A., Nisbet, R.M., Cappai, R., Hill, A.F., 2008. The role of exosomes in the processing of proteins associated with neurodegenerative diseases. Eur. Biophys. J. 37, 323–332.

Villar, A.J., Belichenko, P.V., Gillespie, A.M., Kozy, H.M., Mobley, W.C., Epstein, C.J., 2005. Identification and characterization of a new Down syndrome model, Ts [Rb(12.1716)]2Cje, resulting from a spontaneous Robertsonian fusion between T(171)65Dn and mouse chromosome 12. Mamm. Genome 16, 79–90.

Vingtdeux, V., Hamdane, M., Loyens, A., Gele, P., Drobeck, H., Begard, S., Galas, M.C., Delacourte, A., Beauvillain, J.C., Buee, L., Sergeant, N., 2007. Alkalizing drugs induce accumulation of amyloid precursor protein by-products in luminal vesicles of multivesicular bodies. J. Biol. Chem. 282, 18197–18205.

Wisniewski, K.E., Wisniewski, H.M., Wen, G.Y., 1985. Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. Ann. Neurol. 17, 278–282.

Xiao, T., Zhang, W., Jiao, B., Pan, C.Z., Liu, X., Shen, L., 2017. The role of exosomes in the pathogenesis of Alzheimer' disease. Transl Neurodegener 6, 3.

Yuyama, K., Sun, H., Mitsutake, S., Igarashi, Y., 2012. Sphingolipid-modulated exosome secretion promotes clearance of amyloid-beta by microglia. J. Biol. Chem. 287, 10977–10989.