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RESEARCH ARTICLE

Dysfunction of the retromer complex system contributes to amyloid and tau pathology in a stem cell model of Down syndrome

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Abstract

Introduction: Retromer complex proteins are decreased in Down syndrome (DS) brains and correlate inversely with brain amyloidosis. However, whether retromer dysfunction contributes to the amyloid beta (Aβ) and tau neuropathology of DS remains unknown.

Methods: Human trisomic induced Pluripotent Stem Cells (iPSCs) and isogenic controls were differentiated into forebrain neurons, and changes in retromer proteins, tau phosphorylated epitopes, and Aβ levels were assessed in euploid and trisomic neurons using western blot and enzyme-linked immunosorbent assay (ELISA). Genetic overexpression and pharmacological retromer stabilization were used to determine the functional role of the retromer complex system in modulating amyloid and tau pathology.

Results: Trisomic neurons developed age-dependent retromer core protein deficiency associated with accumulation of Aβ peptides and phosphorylated tau isoforms. Enhancing retromer function through overexpression or pharmacological retromer stabilization reduced amyloid and tau pathology in trisomic neurons. However, the effect was greater using a pharmacological approach, suggesting that targeting the complex stability may be more effective in addressing this neuropathology in DS.

Discussion: Our results demonstrate that the retromer complex is directly involved in the development of the neuropathologic phenotype in DS, and that pharmacological stabilization of the complex should be considered as a novel therapeutic tool in people with DS.

KEYWORDS
Alzheimert's disease, Aβ, Down syndrome, retromer, tau

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1 | INTRODUCTION

Down syndrome (DS) is a congenital condition that occurs in about 1 in 800 births worldwide and results from the presence of extra genetic material originating from human chromosome 21 (HSA21). Advances in medical care have led to a dramatically increased life expectancy for people with DS. However, with the growing population of aged DS patients has emerged an increased incidence of co-occurring age-related disorders, including Alzheimer's disease (AD).[1] The AD-like clinical phenotype is observed in the vast majority, up to 88%, of people with DS aged 65 and older and is the leading cause of death within this population.[2–5] Although the onset of clinical symptoms varies, virtually all people with DS develop the classical pathological hallmarks of AD—amyloid beta (Aβ) plaques and tau neurofibrillary tangles—by 40 years of age.[6] The high prevalence of AD in the DS population is known to be influenced at least in part by an increased gene dose of the amyloid precursor protein (APP), which resides on HSA21 and is cleaved to form the pathogenic species Aβ peptides; however, numerous studies have demonstrated that additional triplicated genes as well as factors outside of HSA21 can also modify AD-DS risk.[1,7]

The retromer complex is a highly conserved endosomal sorting system that regulates the retrieval and recycling of cargoes from early endosomes and, consequently, is a key regulator of intracellular protein trafficking. The central unit of the retromer complex is the cargo recognition core composed of the vacuolar protein sorting (VPS) 35, VPS26, and VPS29. Through its transient association with a tubulation module and VPS10 cargo adaptors, the recognition core directs cargoes from the early endosomes to the cell surface, trans-Golgi, or into the degradation pathway.[8] Loss of function of the retromer complex secondary to deficiencies of one of more of its core components is reported in the brains of AD patients,[9] and complementary functional studies have demonstrated the role of the retromer system in influencing amyloid, tau, and synaptic pathology in models of AD.[10–12] We recently reported that retromer cargo recognition core proteins are decreased in the cortices and hippocampi of people with DS.[13] Furthermore, in the same people, retromer protein levels correlated negatively with levels of Aβ and phosphorylated tau,[13,14] consistent with the concept that retromer dysfunction can promote pathogenic cleavage of APP in early endosomes while also influencing degradation of pathological tau proteins.

In the current study, we further explore the relationship between retromer dysfunction and the AD-DS pathologic phenotype using human trisomic induced pluripotent stem cell (iPSC)-derived neurons and isogenic euploid controls. After first establishing that trisomic neurons develop retromer recognition core protein deficiency similar to people with DS, we utilized both genetic and pharmacological approaches to examine the functional role of the retromer system in the development of amyloid and tau pathology in this stem cell model of DS. Herein we report that restoring retromer complex function, using both VPS35 overexpression and a stabilizing pharmacological chaperone, reduces the AD-like phenotype of trisomic neurons.

RESEARCH IN CONTEXT

1. **Systematic Review**: A literature review conducted using PubMed revealed that people with Down syndrome (DS) have decreased expression of the retromer complex proteins that inversely correlates with levels of amyloid beta (Aβ) and phosphorylated tau. However, the functional role of the retromer system in the development of this type of neuropathology in DS remains unclear.
2. **Interpretation**: Our findings demonstrate that dysfunction of the retromer system is responsible for the pathological accumulation of Aβ and phosphorylated tau in a stem cell model of DS, since its genetic overexpression or pharmacological stabilization prevents both pathologies. These results provide mechanistic support to previous work demonstrating that modulation of the retromer complex system is a viable target in preclinical models of brain amyloidosis.
3. **Future Directions**: This article highlights the therapeutic potential of targeting the retromer complex system to address amyloid and tau pathology in people with DS. Future studies are needed to evaluate the safety and effectiveness of pharmacological stabilization of the retromer system in additional preclinical models of DS.

2 | METHODS

2.1 | Human iPSC culture

Trisomic and isogenic euploid control iPSC lines generated from a person with mosaic DS were purchased from WiCell Stem cell bank (Madison, WI, USA) and have been characterized previously.[15] Human iPSC lines were maintained using a feeder-free culture system in mTesR plus medium (Stemcell Technologies, Vancouver, CA, USA) on Matrigel Growth Factor Reduced Basement Membrane Matrix (#354230, Corning, Corning, NY, USA), and differentiated into neurons using the StemDiff SMADi Neural Induction, Forebrain Neuron Differentiation, and Forebrain Neuron Maturation kits (Stemcell Technologies) according to the manufacturer's instructions. Neuronal differentiation was considered complete following 7 days of forebrain neuron differentiation and confirmation of neuronal cell type. (Detailed methods are available in the supplemental materials.)

2.2 | Immunocytochemistry

Cells plated on coated chamber slides were fixed, permeabilized, and probed for primary and Alexa-fluor conjugated secondary antibodies. Antibodies are included in Table 1. Detailed methods are available in the supplemental materials.

TABLE 1 Antibodies used in the study

Antigen	Catalog number	Dilutions (Immunoblot)	Immunocytochemistry (ICC)
VPS35	Abcam ab10099	1:200	
VPS26	Abcam ab23892	1:250	
VPS29	Abcam ab236796	1:250	
APP	Abcam ab32136	1:100	
GAPDH	Cell Signaling Technologies 2118	1:500	
HT7	Invitrogen MN1000	1:200	
AT270	Invitrogen MN1050	1:150	
PHF1	Gift from Dr. Peter Davies	1:100	
Vimentin	Abcam ab92547		1:200
Pax6	Santa Cruz sc-81649		1:100
Oct4	Abcam ab134218		1:100
DCX	Abcam ab18723		1:200
Tuj1	Abcam ab18207		1:200
MAP2	Abcam ab5492		1:500
Alexa Fluor-Rb	Abcam ab150061, ab175692, ab150063		1:300
Alexa Fluor-G	Abcam ab150129, ab175704		1:300
Alexa Fluor-M	Abcam ab150111, ab175700		1:300
Alexa Fluor-Ch	Abcam ab64507, ab150075		1:300

2.3 | Genetic overexpression of VPS35

For genetic overexpression experiments, purified AAV1 vectors expressing human VPS35 under the CAG promoter were purchased from Vector Biolabs.

2.4 | Treatment with TPT-172

Trisomic iPSC-derived neurons were grown for 23 days post-differentiation and then incubated with the pharmacological chaperone TPT-172 (Benjamin Blass, Temple University) diluted in culture media at a total concentration of 25 μM for 48 hours prior to harvest. The TPT-172 concentration was chosen based on previous studies[26] and an initial screening demonstrating high potency without toxicity for the 25 μM concentration under our experimental conditions.

2.5 | Immunoblot analysis

Cell lysates were extracted using radioimmunoprecipitation assay (RIPA) buffer and subjected to immunoblot analysis as previously described.[13,16] Antibodies are listed in Table 1. (Detailed methods are available in the supplemental materials.)

2.6 | qRT-PCR

RNA was extracted with the miRNeasy kit using the manufacturer's instructions (Qiagen, Germantown, MD, USA). One microgram of total RNA was used to synthesize cDNA using the RT2 First Strand kit (Qiagen). Quantitative reverse transcription polymerase chain reaction (RT-qPCR) reaction was prepared using TaqMan Universal PCR master mix II and commercially available TaqMan primers and amplified using the QuantStudio3 Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control.

2.7 | Enzyme-linked immunosorbent assay (ELISA)

Culture media was assayed for Aβ1-40 after 48-hour incubation using Human Aβ 1-40 sandwich enzyme-linked immunosorbent assay (ELISA) kits (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.8 | Lactate dehydrogenase assay

Lactate dehydrogenase (LDH) was measured in culture media following AAV-VPS35 infection or TPT-172 treatment using a colorimetric assay kit according to the manufacturer's instructions (Abcam, ab65393).

2.9 | Cycloheximide chase assay

N2A cells were pre-treated with 0 or 25 μM TPT-172 diluted in pH-stabilized media for 24 hours. Cycloheximide (Millipore-Sigma, Burlington, MA, USA) was added to cells at a concentration of 100 μg/mL and cells were harvested at 0, 4, and 8 hours post-cycloheximide for time-course analysis of retromer stability via immunoblot.

2.10 | Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). For comparison of two groups, the unpaired two-tailed student *t*-test was used. Comparisons between more than two groups were made using a two-way analysis of variance (ANOVA) and Tukey post hoc test when two independent variables were present. All in vitro experiments were performed 3 to 5 times in duplicate. Statistical significance was set at $P < .05$. GraphPad Prism for Mac version 9.00 was used for the above data analyses.

3 | RESULTS

Trisomic and isogenic euploid control iPSCs derived from a person with mosaic DS[15] were differentiated into forebrain neurons for use in the following study. During neural induction, differentiation markers were examined to confirm cell type. At 6 days post-neural induction, cells expressed the pluripotency marker OCT4 and neural ectoderm lineage markers Pax6 and Vimentin. Cells were positive for the neural progenitor marker DCX by day 18, indicating successful neural induction. Neural progenitors were then differentiated into forebrain neurons and assessed for the neuronal markers Tuj1 and Map2 (Figure 1).

Following neuronal differentiation, cells were grown in culture and assessed at 5, 15, and 25 days post-neuronal differentiation for changes in AD-like neuropathology markers. At 5 days post-differentiation, Aβ40 production and levels of total tau (HT7) did not differ between euploid and trisomic cells (Figure 2A,B,G). Phosphorylated tau isoforms at epitopes Thr181 and Ser396/Ser404, recognized by the antibodies AT270 and PHF1, respectively, were not detectable in all samples, and thus were not included in the analysis at the 5-day post-differentiation timepoint. By 15 and 25 days post-differentiation, trisomic neurons accumulated significantly more phosphorylated tau than euploid neurons did as detected by the antibodies AT270 and PHF1 (Figure 2C–F). In trisomic neurons, Aβ40 production was elevated significantly compared to euploid controls by 15 days post-differentiation and increased further with the age of the culture, whereas euploid neurons did not display aging-related increases in Aβ40 levels (Figure 2G).

Having established that, compared with euploid controls, trisomic iPSC-derived neurons develop age-related increases in Aβ40 and phosphorylated tau, we next examined the retromer recognition core protein levels to determine whether this model displayed retromer deficiency as we reported previously in the brains of DS patients.[13] To this end, we evaluated protein levels of the VPS35, VPS26, and VPS29, and the retromer cargo protein, APP, at 5, 15, and 25 days post-differentiation. APP was elevated in trisomic neurons at all timepoints. Retromer proteins did not differ between euploid and trisomic neurons at the 5-day timepoint, but at 15 days VPS29 was decreased ($P = .056$), and by 25 days post-differentiation all three retromer core proteins were decreased significantly in trisomic neurons compared to euploid controls (Figure 3A–F). To determine whether the reductions in retromer core proteins were due to transcriptional changes, we examined mRNA levels of VPS35, VPS26, VPS29, and APP (Figure 3G). Retromer mRNA levels did not differ between euploid and trisomic neurons, but APP mRNA was significantly increased in trisomic neurons, consistent with its genetic locus on HSA21.

After establishing trisomic iPSC-derived neurons as a representative model of AD-DS–like neuropathology and retromer deficiency, we aimed to examine the effects of restoring retromer core protein levels on these pathology output measures. At 10 days post-differentiation trisomic neurons were transduced with AAV1 vectors expressing human VPS35 (AAV-VPS35) and maintained in culture until 25 days post-differentiation. To determine transduction efficiency, we assessed retromer core protein and message in transduced trisomic neurons compared to euploid and trisomic controls (Figure 4A–C). We found that AAV-VPS35 transduction significantly increased VPS35 and VPS29 protein levels, and VPS35 mRNA compared to trisomic control neurons, indicating successful overexpression of VPS35 following AAV-VPS35 transduction. Next we investigated the effect of restoration of VPS35 and VPS29 protein levels on amyloid and tau pathology. As expected, Aβ 1-40 peptide levels were significantly elevated in trisomic neurons compared to euploid controls. However, compared to trisomic cell controls VPS35 overexpression significantly reduced Aβ1-40 levels in the supernatant (Figure 4D). To assess tau pathology, we examined the levels of total soluble tau (HT7) and the phospho-tau epitopes recognized by the antibodies AT270 and PHF1. VPS35 overexpression significantly reduced the phospho-tau epitope recognized by PHF1 to euploid levels; however, but it did not significantly affect levels of total tau or the other phosphorylated tau isoform (Figure 4E,F). Because an empty vector AAV control was not used in the current study, potential toxicity from AAV transduction was assessed by measuring LDH released by transduced and control cells. Under these experimental conditions we found that cell viability was not affected, as the levels of LDH in the conditioned media did not differ between AAV-treated and control cells (Figure S1).

To confirm these findings and expand upon our approach to restore retromer function in trisomic neurons, we utilized a small molecule pharmacological chaperone, TPT-172, to stabilize the retromer complex. Previous reports demonstrate that TPT-172 can restore retromer complex function by protecting it from denaturation and increasing stability of the VPS35-VPS29 binding domain, which is the weakest link of the VPS35-VPS26-VPS29 heterotrimer.[17] Trisomic neurons were grown in culture for 23days post-differentiation, then incubated with 25μM TPT-172 or vehicle in culture media for 48 hours prior to assessment. First, we assessed whether the chaperone treatment affected steady-state levels of the retromer recognition core proteins.

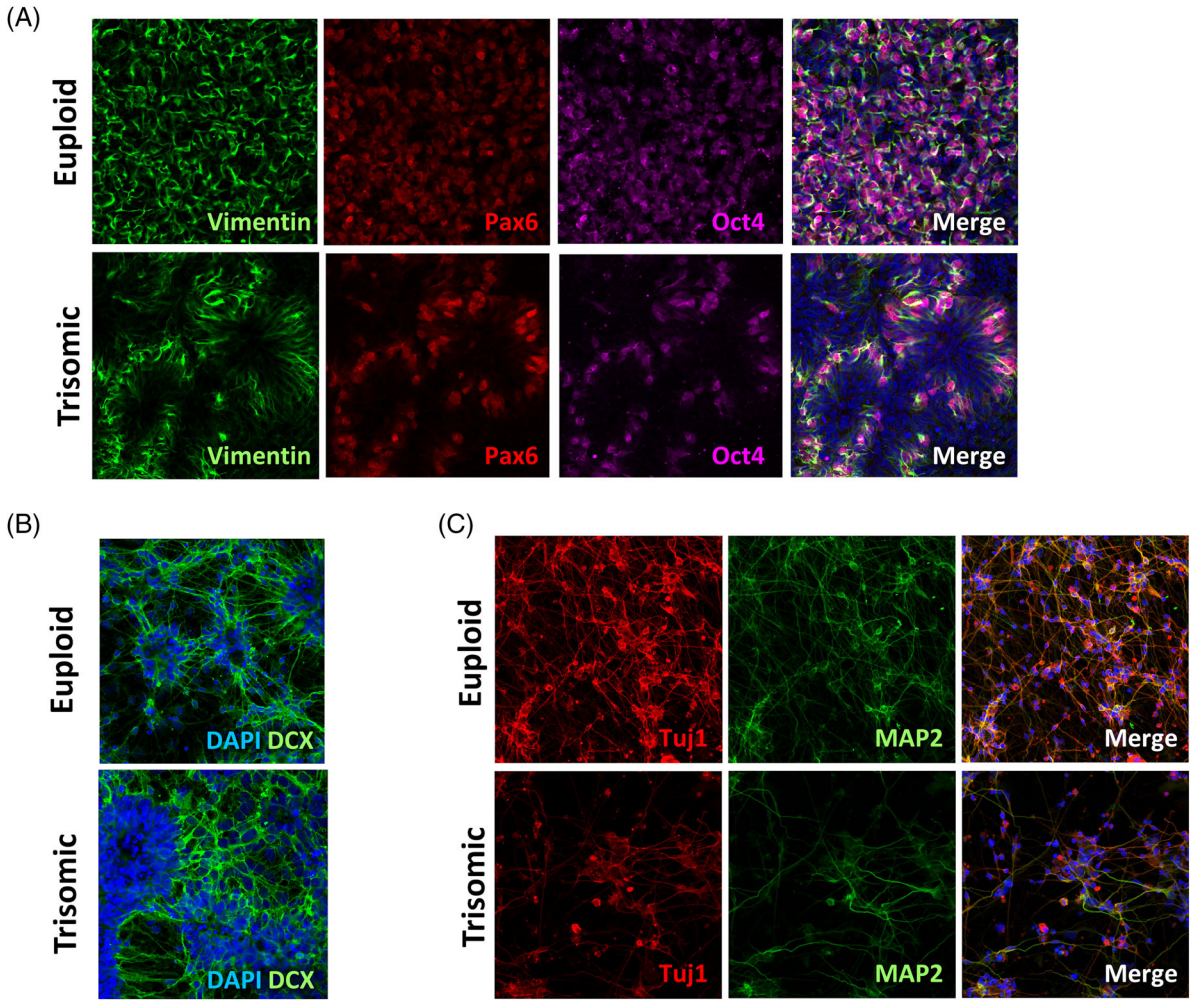

FIGURE 1 Characterization of iPSCs during neural induction and differentiation. (A) Fluorescence microscopy images of Vimentin (green), Pax6 (red), Oct4 (magenta), and DAPI (blue) in euploid and trisomic iPSCs at 6 days post-neural induction. (B) Fluorescence microscopy images of DCX (green) and DAPI (blue) in euploid and trisomic iPSCs at 18 days post neural induction. (C) Fluorescence microscopy images of Tuj1 (red), MAP2 (green), and DAPI (blue) in euploid and trisomic iPSCs following neuronal differentiation

Incubation with TPT-172 did not result in any significant changes of VPS35, VPS26, and VPS29 protein levels compared to trisomic control cells. In addition, we found that cell viability was not affected because the levels of LDH in the conditioned media did not differ between TPT-treated and control cells (Figure S1).

Despite lack of an apparent effect on the retromer core proteins, we hypothesized that the drug could be working primarily through its effects on complex stability and promoting restoration of its function, so we examined the effects on Aβ and tau pathology in our cell model. Treatment with TPT-172 reduced the amount of Aβ1-40 in the supernatant of trisomic neurons to levels indistinguishable from the supernatant of euploid control neurons (Figure 5C). We next evaluated whether the same treatment affected tau pathology in trisomic neurons. Total levels of soluble tau (HT7) were unchanged by TPT-172 treatment; however, phospho-tau epitopes recognized by the antibodies AT270 and PHF1 were both significantly reduced in trisomic neurons and restored to euploid control levels (Figure 5E,F).

To confirm that the effect of TPT-172 treatment was secondary to the stabilization of the retromer core protein against degradation, we performed an analysis of protein stability by examining VPS35, VPS26, and VPS29 degradation rates using the cycloheximide chase assay (Figure S2). N2A cells were pretreated with 0 or 25 μM TPT-172 for 24 hours, and then treated with cycloheximide. Cells were harvested at 0, 4, and 8 hours post-cycloheximide treatment, and VPS35, VPS26, and VPS29 protein levels were measured using western blot. We observed that cells exhibited a significant decrease in VPS35 and VPS29 protein levels after cycloheximide treatment in the absence of TPT-172. However, in cells pretreated with TPT-172, all three retromer core proteins were unchanged (Figure S2).

4 | DISCUSSION

In addition to the aberrant accumulation of intracellular proteins, defects in endocytic trafficking pathways are a common feature shared

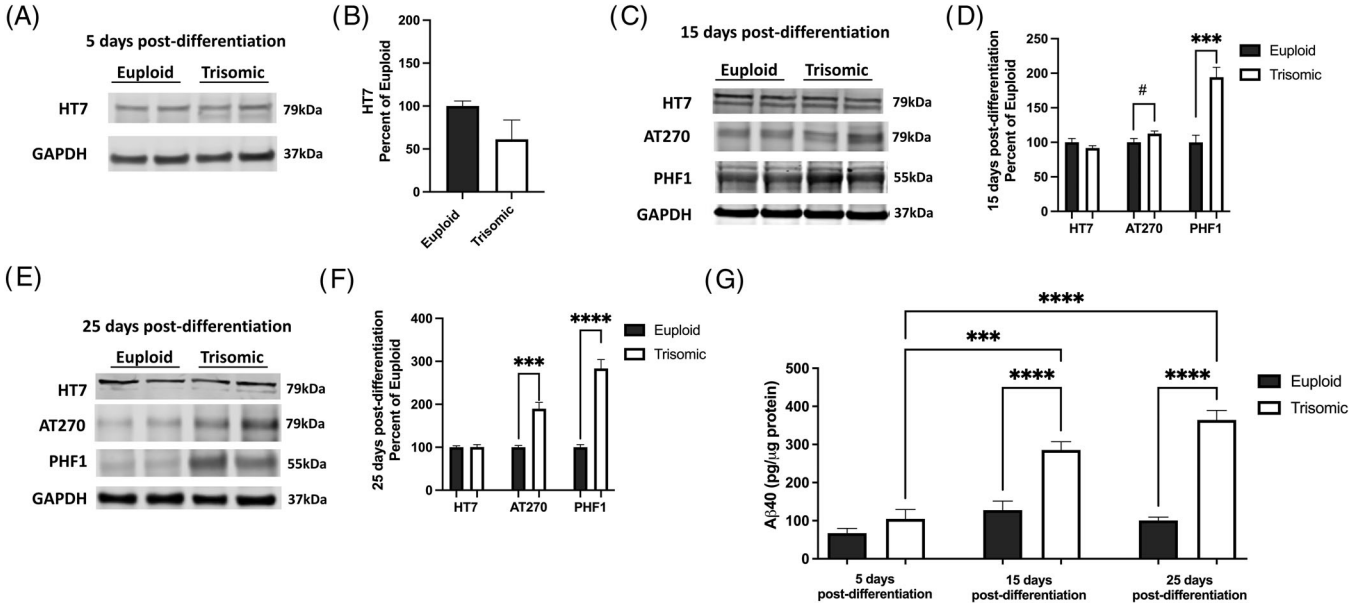

FIGURE 2 Trisomic neurons display age-dependent elevations in Aβ40 and phosphorylated tau. (A) Representative immunoblot of total soluble tau (HT7) in euploid and trisomic neurons at 5 days post-differentiation. (B) Densitometry analysis of immunoblots shown in panel A ($n = 3$, in duplicate). (C) Representative immunoblots of total soluble tau (HT7), and phosphorylated tau epitopes (AT270 and PHF1) in euploid and trisomic neurons at 15 days post-differentiation. (D) Densitometry analysis of immunoblots shown in panel C ($n = 3$, in duplicate). (E) Representative immunoblots of total soluble tau (HT7) and phosphorylated tau epitopes (AT270 and PHF1) in euploid and trisomic neurons at 25 days post-differentiation. (F) Densitometry analysis of immunoblots shown in panel E ($n = 3$, in duplicate). (G) Aβ1-40 levels detected in the culture media from euploid and trisomic neurons at 5, 15, and 25 days post-differentiation (day 5: $n = 5$, day 15: $n = 10$, day 25: $n = 6$). Comparisons between groups were made using the Student t-test. Values represent mean ± standard error of the mean, #$P < .10$, *$P < .05$, **$P < .01$, ***$P < .001$, and ****$P < .0001$

among multiple neurodegenerative diseases.[9,18–20] Within the context of AD, endocytic pathway abnormalities have been associated with deficiencies in core components of the retromer complex, a multi-protein endosomal sorting system. The retromer complex acts as a "master conductor" of endocytic trafficking by sorting cargoes from early endosomes toward the cell surface, trans-Golgi, or to the lysosome for degradation.[21] Through its actions within these three pathways, the retromer can influence numerous aspects of neuronal physiology and health including excitability, signaling, survival, lysosomal degradation, and autophagy.[8,22–25] Conversely, retromer complex dysfunction promotes neurodegeneration by increasing the production of Aβ and reducing pathological tau degradation.[17,26–28]

In the present study we showed that human DS neurons manifest an age-dependent retromer complex dysfunction associated with progressive accumulation of Aβ peptides and phosphorylated tau isoforms, and that restoring the complex function, either through overexpression of VPS35 or pharmacological stabilization of the cargo recognition core proteins, results in an amelioration of this AD-like phenotype. We reported previously that retromer cargo recognition core proteins are significantly decreased in the cortices and hippocampi of people with DS, and that their levels correlate inversely with the amyloidotic phenotype commonly found in DS brains.[13] To further dissect the relationship between retromer dysfunction and AD-DS pathogenesis, in the current study we investigate whether this dysfunction is involved directly in modulating the development of the AD-DS pathologic phenotype using a stem cell model of DS. To this end, we examined the retromer core complex system in human trisomic and isogenic euploid control iPSC-derived forebrain neurons. Initially, we characterized changes in the retromer cargo recognition core proteins in this model and found that compared with euploid controls, the trisomic neurons develop an age-dependent retromer core protein deficiency. It is important to note that although the reduction we found was statistically significant, compared with the reduction we observed in DS post-mortem brain tissues the percentage in the iPSC is smaller. Although it is always very difficult to compare data obtained in vitro versus in vivo, we believe that the difference could be because tissues contain multiple cell types, which can influence the results. Furthermore, our previous work examined the retromer system in brain tissues from over 60 patients, whereas in the current study we examine cells derived from only one donor. This neuronal model does develop reductions in retromer proteins; however, it is likely that there is variability in this phenotype between individual DS donors.

Although it is challenging to put side by side and compare the results obtained in 25-day-old cells with a 40- to 50-year-old organ, we found that DS neurons were able to model key features of the AD neuropathological phenotype. It is notable that in association with the reduction of the recognition core proteins, we observed a progressive accumulation of Aβ peptides and phosphorylated tau. Intriguingly, we found that changes in these two pathological markers were altered a bit earlier (day 15) than the significant changes in the retromer proteins,

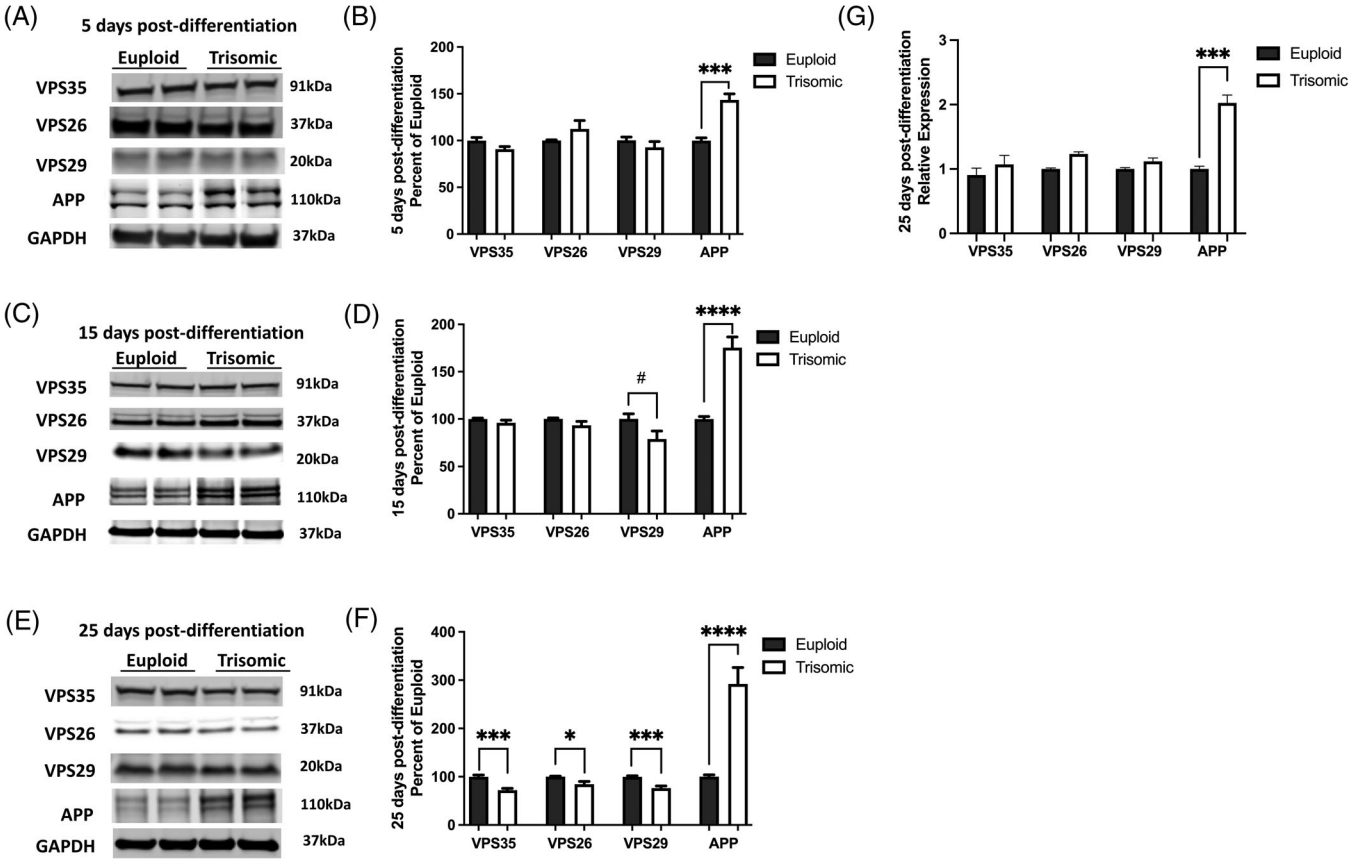

FIGURE 3 Retromer cargo recognition core proteins are decreased in trisomic neurons by 25 days post-differentiation. (A) Representative immunoblots of VPS35, VPS26, VPS29, and APP in euploid and trisomic neurons at 5 days post-differentiation. (B) Densitometry analysis of immunoblots shown in panel A ($n = 3$, in duplicate). (C) Representative immunoblots of VPS35, VPS26, VPS29, and APP in euploid and trisomic neurons at 15 days post-differentiation. (D) Densitometry analysis of immunoblots shown in panel C ($n = 4$, in duplicate). (E) Representative immunoblots of VPS35, VPS26, VPS29, and APP in euploid and trisomic neurons at 25 days post-differentiation. (F) Densitometry analysis of immunoblots shown in panel E ($n = 3$, in duplicate for VPS35, VPS26, and VPS29; $n = 4$, in duplicate for APP). (G) Relative expression of VPS35, VPS26, VPS29, and APP message in euploid and trisomic neurons at 25 days post-differentiation ($n = 3$, in duplicate). Comparisons between groups were made using the Student *t*-test. Values represent mean ± standard error of the mean, #$P < .10$, *$P < .05$, **$P < .01$, ***$P < .001$, and ****$P < .0001$

suggesting that a reduction in function of the complex may antecede the alterations in their steady state levels. In this sense, our model of trisomic iPSC-derived neurons should be considered relevant, since it recapitulated the retromer recognition core deficiency and the AD-DS phenotype that we reported previously in brain tissues from people with DS.[13–15]

Given that retromer core proteins were decreased in trisomic neurons, to assess for its functional role in the development of the AD-DS phenotype of these cells we decided to examine the effects of genetic overexpression of VPS35. Because this sub-unit is considered the backbone of the recognition core, over-expressing or stabilizing VPS35 is proposed to affect the function of the entire trimer retromer cargo recognition core.[11,17] Under our experimental conditions, we found that VPS35 overexpression rescued reductions in VPS29 levels but failed to increase VPS26 protein levels, suggesting that retromer deficiency in DS is unable to be completely resolved by increased expression of VPS35 alone. To this point we observed that the effects of VPS35 overexpression on the AD-like phenotype were also somewhat incomplete. Thus we found a reduction in only one specific epitope of phosphorylated tau and a decrease in Aβ1-40 levels, which although significant did not reach the levels of the euploid controls.

Considering that VPS35 overexpression yielded only some improvement of AD-like pathology in trisomic neurons, we decided to supplement our overexpression studies with a slightly different approach, a pharmacological one designed to target the whole retromer recognition core complex. The small molecule pharmacological chaperone TPT-172 is known to stabilize the VPS35-VPS29 interface, the weakest interaction of the trimeric retromer core, and consequently protects retromer core proteins from denaturation and increases their half-life.[17] Stabilization of the retromer complex using TPT-172 has been shown also to increase retromer core protein levels and reduce production of Aβ in both in vivo and in vitro models of AD.[12,17,29] Contrary to previous studies using TPT-172, under our experimental conditions we did not observe any significant changes in the steady-state levels of retromer core proteins. However, upon examination of AD pathology outputs, we observed a dramatic reduction of Aβ1-40 levels and

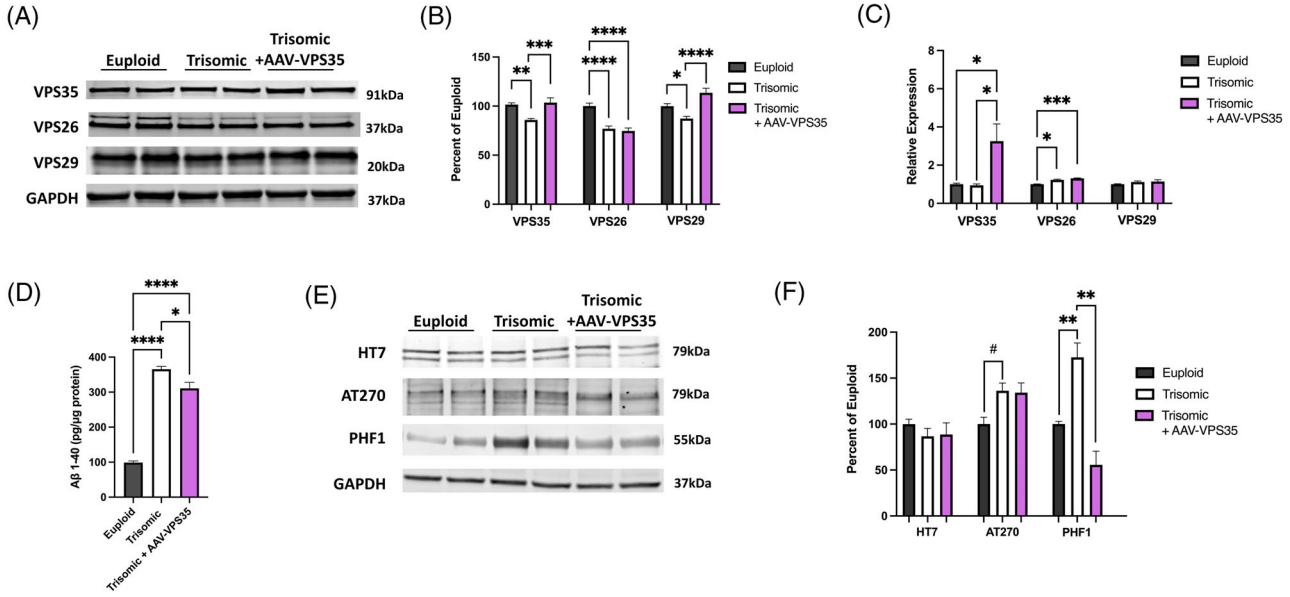

FIGURE 4 VPS35 overexpression reduces Aβ1-40 and phosphorylated tau in trisomic neurons. (A) Representative immunoblots of VPS35, VPS26, and VPS29 in euploid, trisomic, and trisomic neurons overexpressing VPS35 (trisomic + AAV-VPS35) at 25 days post-differentiation. (B) Densitometry analysis of immunoblots in panel A ($n = 5$, in duplicate for VPS35; $n = 4$, in duplicate for VPS26 and VPS29). (C) Relative expression of VPS35, VPS26, and VPS29 message in euploid, trisomic, and trisomic neurons overexpressing VPS35 (trisomic + AAV-VPS35) at 25 days post-differentiation ($n = 3$, in duplicate). (D) Levels of Aβ1-40 detected in the culture media from euploid, trisomic, and trisomic neurons overexpressing VPS35 (trisomic + AAV-VPS35) ($n = 4$). (E) Representative immunoblots of HT7, AT270, and PHF1, in euploid, trisomic, and trisomic neurons overexpressing VPS35 (trisomic + AAV-VPS35). (F) Densitometry analysis of immunoblots shown in panel E ($n = 3$, in duplicate). Comparisons between groups were using a main-effects model two-way ANOVA. Values represent mean ± standard error of the mean, #$P < .10$, *$P < .05$, **$P < .01$, ***$P < .001$, and ****$P < .0001$

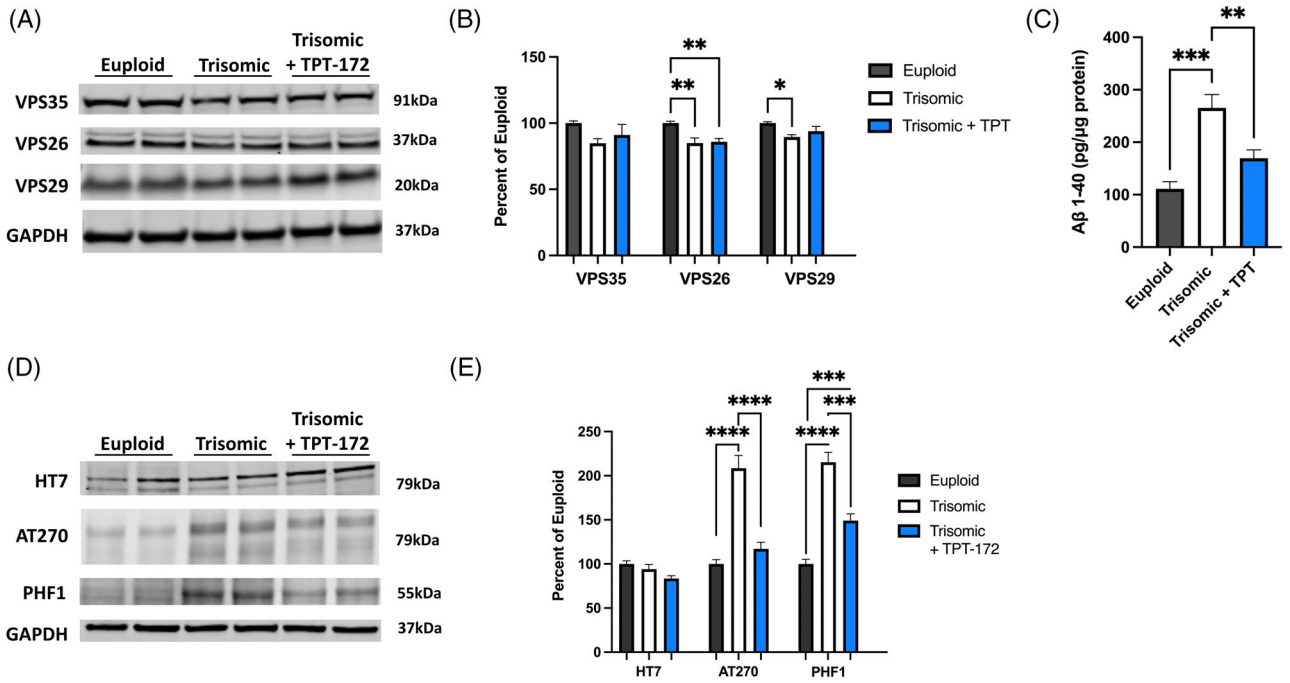

FIGURE 5 Pharmacological stabilization of the retromer complex reduces Aβ1-40 and phosphorylated tau in trisomic neurons. (A) Representative immunoblots of VPS35, VPS26, and VPS29 in trisomic neurons treated with 25 μM TPT-172, and trisomic and euploid control neurons. (B) Densitometry analysis of immunoblots in panel A ($n = 4$, in duplicate). (C) Levels of Aβ1-40 detected in the culture media from trisomic neurons treated with 25 μM TPT-172, and trisomic and euploid control neurons ($n = 5$). (D) Representative immunoblots of HT7, AT270, and PHF1. (E) Densitometry analysis from panel D ($n = 3$, in duplicate). Comparisons between groups were using a main-effects model two-way ANOVA. Values represent mean ± standard error of the mean, #$P < .10$, *$P < .05$, **$P < .01$, ***$P < .001$, and ****$P < .0001$

tau protein phosphorylated epitopes, which were indistinguishable for the ones found for the euploid control cells. Taken together these findings support the concept that the mechanism of action of the molecule is the restoration of retromer function via its actions on the recognition core complex stability. Overall, our findings are in agreement with a previous report in which iPSC-derived neurons from AD patients were incubated with the same drug,[29] although in that study the authors described some degree of variability in the effect of the drug on the stabilization of VPS35 because they used iPSC derived from different donors; in our study we used cells obtained from one donor only. To this end, we recognize that because we have used iPSC-derived neurons from only one donor, our results may not accurately reflect the status of the retromer complex system in a generalized manner (for further discussion see *Study Limitations and Future Directions* in the supplemental materials).

Nonetheless, in both our genetic and pharmacological studies we show that the retromer complex system can directly modulate the development of amyloid and tau neuropathology in trisomic neurons. However, comparison of our two approaches may provide new insight into the neurobiology of the retromer complex dysfunction in DS. Previous studies have focused on increasing specifically VPS35 when addressing retromer complex dysfunction; yet this approach is incomplete in resolving retromer dysfunction and the associated AD-like pathological measures in DS neurons. Instead, stabilization of the retromer recognition core complex using the pharmacological chaperone, TPT-172, yields a more robust reduction of AD-like pathology measures in trisomic neurons. We propose that genetic components of HSA21 cause an inherent instability of the retromer complex that is not overcome by VPS35 overexpression alone, and that targeting recognition core complex stability as a whole is more effective at improving and restoring retromer function and reducing neuropathology.

In conclusion, our findings provide mechanistic support for the role of retromer complex system dysfunction as an upstream driver for both amyloid and tau pathology during the development of the AD phenotype in people with DS. Given that modulation of the retromer system can independently target multiple pathways implicated in the AD-like pathologic phenotype, pharmacological stabilization of the retromer recognition core has unique potential as a preventive disease-modifying therapeutic for the DS patients.

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CONFLICT OF INTEREST

The authors have no conflicting financial interests to disclose. Author disclosures are available in the supporting information.

AUTHOR CONTRIBUTIONS

Mary Elizabeth Curtis and Domenico Praticò designed the study; Mary Elizabeth Curtis and Tiffany Smith performed the experiments and collected the data; Mary Elizabeth Curtis analyzed the data and prepared the figures; Benjamin E. Blass synthesized the TPT-172; Mary Elizabeth Curtis performed the statistical analyses; Mary Elizabeth Curtis and Domenico Praticò drafted the manuscript; all authors have seen and approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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