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Alzheimer Aβ Peptide Induces Chromosome Mis-Segregation and Aneuploidy, Including Trisomy 21: Requirement for Tau and APP

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Both sporadic and familial Alzheimer's disease (AD) patients exhibit increased chromosome aneuploidy, particularly trisomy 21, in neurons and other cells. Significantly, trisomy 21/Down syndrome patients develop early onset AD pathology. We investigated the mechanism underlying mosaic chromosome aneuploidy in AD and report that FAD mutations in the Alzheimer Amyloid Precursor Protein gene, *APP*, induce chromosome mis-segregation and aneuploidy in transgenic mice and in transfected cells. Furthermore, adding synthetic Aβ peptide, the pathogenic product of APP, to cultured cells causes rapid and robust chromosome mis-segregation leading to aneuploid, including trisomy 21, daughters, which is prevented by LiCl addition or Ca^{2+} chelation and is replicated in *tau* KO cells, implicating GSK-3β, calpain, and Tau-dependent microtubule transport in the aneugenic activity of Aβ. Furthermore, APP KO cells are resistant to the aneugenic activity of Aβ, as they have been shown previously to be resistant to Aβ-induced tau phosphorylation and cell toxicity. These results indicate that Aβ-induced microtubule dysfunction leads to aneuploid neurons and may thereby contribute to the pathogenesis of AD.

INTRODUCTION

Developing early diagnoses and successful treatments for Alzheimer's disease (AD) will be greatly aided by a clear understanding of all steps in the pathogenic pathway that leads to amyloid deposition, neurofibrillary tangle formation, inflammation, and neurodegeneration in the brain. Although most AD is "sporadic," a large proportion is at least partly "familial" in that patients develop the disease by inheriting a mutant gene or a risk-enhancing genetic polymorphism. Autosomal dominant mutations, accounting for ~5% of AD, have been described in three genes, and their analysis has provided especially important insights into the AD pathogenic pathway (Glenner and Wong, 1984; Hardy and Selkoe, 2002). One of these genes encodes the amyloid precursor protein (APP) from which the key amyloid component, the Aβ peptide, is derived by proteolysis. Although mutations in the *APP* gene itself account for <1% of AD, they provided the proof that APP and Aβ are central to the disease process.

Most autosomal dominantly inherited familial Alzheimer's disease (FAD) is caused by mutations in two presenilin genes, most commonly PS-1. The PS proteins must therefore also occupy a key place in the AD pathogenic pathway together with APP and the Aβ peptide. The role of the presenilins in AD pathology was clarified when they were found to form the enzymatic core of the γ-secretase complex that cleaves APP in its transmembrane region and generates the C-terminus of the Aβ peptide (Wolfe, 2003).

Several lines of evidence indicate that both sporadic and familiar AD patients, including those carrying *APP* and *PS* mutations, are abnormal in one or more aspects of the cell cycle (for reviews, see Obrenovich *et al.*, 2003; Potter, 2004, 2008). For example, Down syndrome (DS) patients, who carry three copies of chromosome 21 in all of their cells due to meiotic chromosome mis-segregation in one of (usually) their mother's germ cells, invariably develop AD-like pathology by the age 30–40 (Olson and Shaw, 1969; Glenner and Wong 1984; Epstein, 1990). This and other findings led us to propose that over a lifetime, defective mitoses lead to the accumulation of aneuploid cells throughout the body, including the brain. When such chromosome mis-segregation generates trisomy 21 cells, the extra copy of the *APP* gene on chromosome 21 contributes to the development of Alzheimer neuropathology and dementia (Potter, 1991). The microtubule (MT) disfunction likely responsible for the aneuploidy in AD patients could also affect other aspects of cell physiology, especially in neurons.

The chromosome mis-segregation/MT disfunction hypothesis of AD makes several easily-testable predictions (Potter, 1991). For example, AD patients should be mosaic for trisomy 21, and, indeed, we found trisomy 21 and other aneuploid cells in primary skin fibroblast cultures from patients with both the familial (early age of onset) and sporadic (late age of onset) forms of the disease (Potter *et al.*, 1995; Geller and Potter, 1999). Trisomy 21 cells have also been observed among peripheral blood lymphocytes, buccal cells, and brain neurons from sporadic AD patients and among lymphocytes of mothers who, at a young age, gave birth to a DS child and are prone themselves to develop AD later in life (Schupf *et al.*, 1994; Migliore *et al.*, 1999, 2006; Yang *et al.*,

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2001; Mosch *et al.*, 2007; Thomas and Fenech, 2008; Iourov *et al.*, 2009). Conversely, between 1 and 10% trisomy 21 mosaicism has also been found in individuals with otherwise unexplained AD-like dementia in middle age, indicating that even small numbers of trisomy 21 cells can lead to cognitive deficits (Rowe *et al.*, 1989; Shapiro *et al.*, 1989; Puri *et al.*, 1994; Ringman *et al.*, 2008).

Advanced AD patients also develop tetraploid neurons (Yang *et al.*, 2001; Mosch *et al.*, 2007), which may indicate entry into an incomplete cell cycle (Vincent *et al.*, 1996; Obrenovich *et al.*, 2003; Varvel *et al.*, 2008). Through the elegant use of several techniques, Arendt and colleagues found increases in both aneuploid and tetraploid neurons in AD brain, with the ~30% aneuploid cells (between 2n and 4n) being >10 times more common than tetraploid neurons (Mosch *et al.*, 2007).

Another prediction of the chromosome mis-segregation/MT disfunction hypothesis for AD is that the very genes that, when mutant, cause familial AD should encode proteins that are involved in the cell cycle and chromosome segregation. Indeed, a polymorphism in the PS-1 gene is associated with both an increased risk of developing AD and of having a Down syndrome child (Wragg *et al.*, 1996; Higuchi *et al.*, 1996; Petersen *et al.*, 2000; Lucarelli *et al.*, 2004). Furthermore, immunocytochemical and FRET results have shown that endogenous PS-1 and APP and some of their interacting proteins reside in cell structures involved in mitosis, such as the nuclear membrane, centrosomes, or kinetochores (Zimmermann *et al.*, 1988; Li *et al.*, 1997; Honda *et al.*, 2000; Johnsingh *et al.*, 2000; Kimura *et al.*, 2001; Tezapsidis *et al.*, 2003; Zitnik *et al.*, 2006; Nizzari *et al.*, 2007) and become hyperphosphorylated during mitosis (Pope *et al.*, 1994; Suzuki *et al.*, 1994; Preuss *et al.*, 1995).

Previously, we used transgenic and knockin mice and transfected cells in culture to test directly the effect of the PS-1 gene on the cell cycle. All assays, tissues, and cells yielded the same results and allowed the conclusion that overexpression or mutation of PS-1 leads to chromosome mis-segregation and aneuploidy, including trisomy 21 (Boeras *et al.*, 2008; Potter, 2008). Analysis of the PS-1–transfected cells by immunocytochemistry revealed numerous abnormalities in the mitotic spindle apparatii, including improper MT arrays and lagging chromosomes. Finally, dominant negative mutant forms of presenilin failed to induce chromosome mis-segregation, showing that presenilin/γ-secretase is directly involved in the mutant PS-1–induced cell cycle and chromosome segregation defects.

During the course of these studies, we noted that the percentage of cells with abnormal chromosome complements that arose in the *PS-1*–transfected cultures was often higher than the measured transfection efficiency. This fact strongly suggested that the *PS-1* effect on chromosome mis-segregation was not restricted to the PS-1–expressing cells, but also extended to adjacent, nontransfected cells (i.e., was non-cell autonomous) and thus might be induced by a secreted molecule. Coupling this observation to the fact that γ-secretase activity was essential for the *PS-1*–induced chromosome mis-segregation and to the previous finding that patients carrying FAD mutations in *APP* also developed trisomy 21 mosaicism led us to hypothesize that secreted Aβ peptide itself might induce cell cycle defects including chromosome mis-segregation (Boeras *et al.*, 2008).

In this article we test whether 1) expression of an FAD mutant *APP* gene in transgenic mice or in transfected cells leads to chromosome mis-segregation, particularly in brain neurons, 2) exposing cells in culture to Aβ peptide itself leads to chromosome mis-segregation, 3) Ca^{2+} chelation or exposure to LiCl (two treatments that have been shown to obviate Aβ toxicity by inhibition of calpain and GSK-3β, respectively) prevent Aβ from inducing chromosome mis-segregation, and finally whether 4) knocking out the MT associated protein *Tau* involved in both mitosis and AD also causes chromosome mis-segregation.

MATERIALS AND METHODS

Mice

Transgenic mice expressing human *APP* with the V717F *APP* mutation (19–21 months) or knocked out for APP (3 months) and their nontransgenic littermates were used. *Tau*+/− mice and *Tau*−/− mice and their nontransgenic *Tau*+/+ controls were 6–7 mo old (Jackson Labs). All mice used in this study had C57BL/6 background strain.

Primary Cells

Mouse primary splenocytes were prepared for metaphase chromosome analysis and fluorescence in situ hybridization (FISH; Boeras *et al.*, 2008). Mouse brains were harvested, the meninges and cerebella were removed, and cells were obtained by triturating brain pieces in ice-cold 1× PBS (Cellgro, Manassas, VA) ~40 times with fire-polished Pasteur pipettes of different pore sizes. The cell suspension was fixed in cold 3:1 anhydrous methanol:acetic acid fixative and kept on ice for at least 30 min. Fixed brain cells were stored at −20°C before any downstream assay was performed.

Cell Line

The hTERT-HME1 cell line is a primary human mammary epithelial cell line that permanently expresses the telomerase reverse transcriptase (Clontech, Palo Alto, CA) and has a stable karyotype (Jiang *et al.*, 1999). Cells were maintained in supplemented mammary epithelium basal medium (MEBM, Lonza, Hopkinton, MA) and passaged every 2–3 d according to the supplier's recommendations. All experiments were conducted with the hTERT cells passages 3–6.

Plasmids

Plasmids constructed by inserting an FAD combination mutant NL-*APP* K595N/M596L (Swedish) and V642I (London) *APP* gene cDNA into the pcDNA3.1 expression vector or FAD mutant V717I into pAG3 vector were gifts of Dr. Chad Dickey (University of South Florida, Tampa) and Todd Golde (Mayo Clinic, Jacksonville). NucleoBond Plasmid Purification kit (BD Biosciences, San Diego, CA) was used for nucleic acid purification.

Transient Transfections

One day before the transfection, the hTERT cells (1–1.5 × 10^5 cells/2 ml) were plated in a six-well plate and grown in supplemented MEBM. A FuGene6 (Roche Applied Science, Indianapolis, IN)-DNA complex was prepared according to the manufacturer's recommendations using a ratio of Fugene 6 to DNA of 3 μL:1 μg and was applied to the cells. At 48 h after transfection, cells were either harvested immediately for FISH or treated with 37 ng/ml colcemid for 6–7 h before the harvest, collected, fixed, and scored for aneuploidy in metaphase chromosome spreads.

Peptides and Salts

Synthetic Aβ1-40, Aβ1-42, and Aβ42-1 from either BioSource International (Camarillo, CA) or American Peptide Company (Sunnyvale, CA) were resuspended in sterile HPLC water at room temperature, aliquoted, and stored at −80°C before use. Other control peptides were custom made by either Sigma Genosys (St. Louis, MO) or Bio-Synthesis (Lewisville, TX), and were designed by random scrambling of the Aβ12-28 or Aβ1-42 sequence (NH2-VHHQKLVFFAEDVGSNK-OH and NH2-ADFVGSVINIGKLELKMVGQVGVHGIAEVHFDYSFADHEARG-OH), respectively. Similarly, LiCl (Fisher Scientific, Pittsburgh, PA) and BAPTA (Invitrogen, Carlsbad, CA) salts were resuspended in HPLC water.

In Vitro Incubation of the Primary Cells and Cell Lines with Aβ Peptides, BAPTA, and LiCl

For each experiment, a fresh aliquot of the various Aβ peptides, BAPTA, or LiCl was used and thawed on ice to avoid repeated thaw-freeze cycles and possible changes in the structure of the molecules. Primary mouse splenocytes were stimulated to divide in concanavalin A (Con-A) containing media for 44 h in either 100-mm dishes (7.5–8 × 10^5 cells/10 ml growth media) or in six-well plates (4–5 × 10^5 cells/3 ml growth media). The cells were treated with Aβ peptide for 44–48 h and prepared according to established cytogenetic procedures described below. In coincubation experiments, the Tau+/+ splenocytes were pretreated

with either 1 μM of BAPTA for 3 min before the peptides were added or with 2.5 mM of LiCl for the last 7 h of Aβ peptide incubation.

Similarly, 24 h before Aβ treatment, a low passage of the hTERT-HME1 cells was seeded in either six-well dish (1–1.5 × 10^5 cells/well) or in a 100-mm culture dish (4–5 × 10^5 cells/55 cm^2). For the last 10–12 h of peptide exposure, the cells were treated with 33 ng/ml colcemid, harvested, fixed, and scored for aneuploidy by either karyotype analysis or FISH.

Metaphase Chromosome Analysis

After colcemid treatment, cells were harvested according to standard cytogenetic methods as described (Boeras *et al.*, 2008). Genus 2.81 software (Applied Imaging, San Jose, CA) and the Metafer 3.31 Slide Scanning System (MetaSystems, Altslussheim, Germany) with Isis 5.2 (ver. 2007; MetaSystems) was used for metaphase spreads capture and chromosome analysis. At least 80 mouse splenocyte metaphases and 45 hTERT-HME1 cells metaphase spreads were analyzed per each sample.

FISH

A bacterial artificial chromosome (BAC) containing a mouse chromosome 16–specific sequence (a gift from Dr. Bruce Lamb at Case Western Reserve University) was labeled by nick translation (Abbot, Vysis, Downer's Grove, IL) as described elsewhere (Kulnane *et al.*, 2002; Boeras *et al.*, 2008) with modification. Specifically, 1 mM of either Spectrum Green dUTP (Abbot, Vysis) or Spectrum Orange dUTP (Enzo Life Science, Bedford, MA) was used to label 1 μg of BAC DNA. The preincubated (37°C over night) BAC probe was used for FISH of mouse primary cells. Labeled BAC probe and mouse brain cells dehydrated in ethanol solutions (70, 80, and 90%) were codenatured at 74°C for 4 min and hybridized at 37°C for 22 h in the HyBrite (Vysis) hybridization chamber followed by additional 20–22 h of hybridization at 38°C. Excess probe was removed by three consecutive washes in 0.4× SSC at 37°C for 4 min, 2× SSC/0.1% NP40 at room temperature for 3 min, and 4× SSC/0.1% NP40 for 2 min. DAPI II (Abbot, Vysis) or Vectashield (Vector Laboratories, Burlingame, CA) counterstain was used to stain nuclear DNA. Mouse spleen cells were subject to similar in situ protocol except they required shorter hybridization time and less thorough washing.

Interphase FISH of hTERT-HME1 cells was performed using the LSI TEL/AML1 ES Dual Color Translocation Probe (Abbot, Vysis). Hybridizations were done according to Vysis recommendations for LSI probes in the HyBrite hybridization chamber (Vysis) and counterstained with DAPI II.

Image Acquisition and Analysis

Hybridization signals were scored according to Vysis guidelines using either a Nikon Eclipse E1000 microscope (Melville, NY) with a 4912 CCIR high-performance Cohu CCD camera (San Diego, CA) and Genus 2.81 software for image processing (Applied Imaging, San Jose, CA) or a Zeiss Imager.M1 Axio microscope with a CV-M4+CL high-resolution camera (Thornwood, NY). In interphase cells, only bright and compact signals were counted separately for each probe using DAPI, FITC, and TRITC Nikon filter cubes with a Nikon Eclipse E1000 fluorescence microscope and Genus 2.81 software or under 49 DAPI, 38 HE Green Fluorescent, and 43HE Red Zeiss filters with a Zeiss Imager.M1 Axio fluorescence microscope. The Abbott/Vysis (http://www.abbottmolecular.com/DualColor Enumeration_36752.aspx) guidelines were followed meticulously. Particularly important is that, as required, closely adjacent double spots, double spots linked by a light fluorescent thread, and defused signals were counted as only single spots (i.e., one chromosome) and *not* two spots (i.e., two chromosomes).

Antibodies

For immunocytochemistry and immunofluorescence we used anti-α-tubulin (Sigma, clone B-5-1-2), 1:500; Ms X Neuronal Nuclei (NeuN) AlexaFluor 488 (Millipore, Bedford, MA), 1:100; and Alexa Flour 488 rabbit α-mouse IgG (Invitrogen, Molecular Probes, Eugene, OR), 1:1000.

Immunocytochemistry after FISH

Immediately after hybridization, brain cells were incubated in 1× PBS for 10 min. The slides were blocked in 10% goat serum/0.1% Triton X-100 1× PBS solution for 1 h. Conjugated Ms X Neuronal Nuclei AlexaFluor 488 (Millipore) was diluted 1:100 in 1% BSA/0.1% Triton X-100 1× PBS and applied to the slides overnight to stain for neurons. After final washes, coverslips were mounted onto slides with DAPI II (Abbot, Vysis).

Statistical Analysis

Paired Student's *t* test was used to compare the aneuploidy induced by different peptide and inhibitor treatments and plasmid transfections in multiple experiments and to compare the levels of aneuploidy in mouse primary cell line with and without a human FAD transgene. At least 10–12 mice were analyzed for each graph and five to seven transfections/treatments of each plasmid/peptide were scored for aneuploidy.

RESULTS

Expression of FAD Mutant APP *Causes Chromosome Mis-Segregation in Transgenic Mice*

To understand the mechanism by which chromosome aneuploidy arises in AD, we asked whether the expression of FAD-mutant APP disrupts the cell cycle and causes chromosome mis-segregation. First, metaphase chromosome analysis was used to compare the chromosome complement of FAD-APP–expressing transgenic mice (PDAPP) and age-matched normal mice. Primary splenocytes were chosen for this analysis because they can be induced to divide in culture, a requirement for metaphase analysis, and the transgene promoter is active in the spleen. The dividing cells were arrested at metaphase by colcemid treatment and the chromosomes stained and counted. The data showed twice the level of aneuploidy (i.e., 25% of cells with both less or more than the normal mouse complement of 40 chromosomes) in the FAD-APP animals compared with nontransgenic animals (Figure 1A). There was no increase in the number of tetraploid/polyploid cells (not shown).

FISH allows aneuploidy for particular chromosomes to be assessed at all phases of the cell cycle. A BAC carrying a 300-kb fragment of mouse chromosome 16 (Kulnane *et al.*, 2002) was labeled with spectrum green dUTP (Abbott) by nick translation and used as a hybridization probe to tag chromosome 16 in transgenic and normal mouse splenocytes (Figure 1B). Expression of the mutant *APP* transgene induced a many-fold increase in both trisomy and monosomy 16 (Figure 1, C and D).

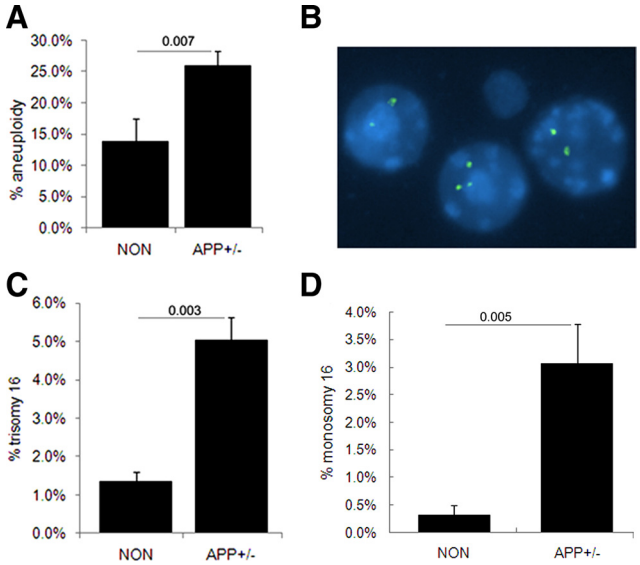


Figure 1. Chromosome aneuploidy induced in transgenic mouse splenocytes carrying an AD mutant *APP* transgene (V717F). (A) Karyotype analysis of nontransgenic (NON) and transgenic *APP*+/− mice showed significantly higher levels of aneuploidy in the cells harboring a mutant *APP* transgene, but no increase in tetraploidy/polyploidy. In situ DNA FISH with a BAC plasmid containing mouse chromosome 16 (B) showed significantly higher levels of trisomy 16 (C) and monosomy 16 (D) in *APP*+/− splenocytes compared with cells from NON transgenic controls.

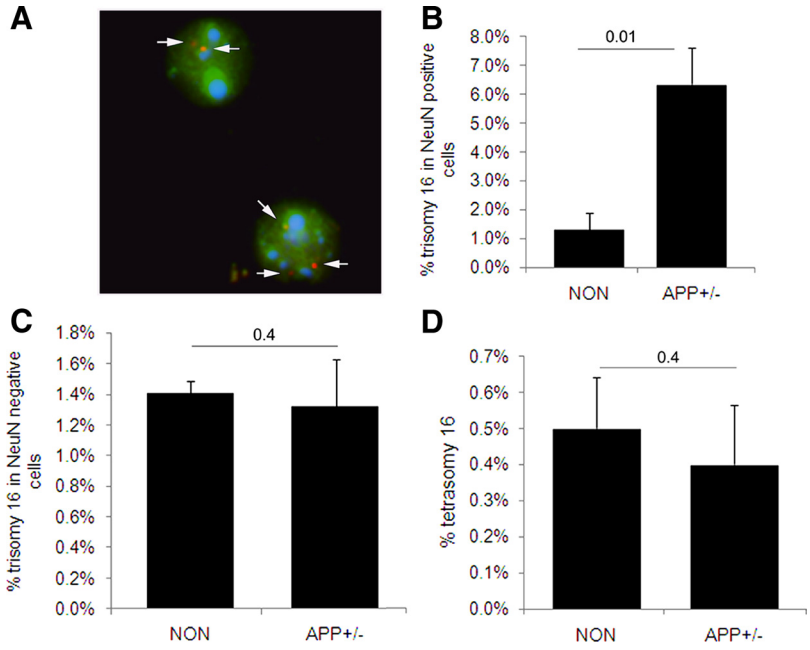


Figure 2. Trisomy 16 induced in neurons of mice carrying a mutant *APP* transgene. Quantitative FISH analysis of resuspended cells from the whole brain of FAD mutant V717F mice costained with the NeuN antibody (A) revealed significantly higher levels of trisomy 16 compared with nontransgenic animals. Most of the aneuploidy was due to trisomy 16 neurons (B and C). No significant increase in tetrasomy 16 was detected (D).

Chromosome Aneuploidy in Neurons from FAD-APP Transgenic Mice

The effect of an FAD mutation (V717F) in the *APP* gene on the chromosome complement of brain cells was examined using DNA FISH, which can be used to count chromosomes in both the nondividing neurons and cycling cells such as glia. Whole brains from *PS-1* transgenic and nontransgenic mice were processed to yield suspensions of mixed primary cells. The cells were fixed to slides and hybridized with the mouse chromosome 16 BAC probe labeled with Orange-dUTP. The hybridization efficiency was ~90%, with most cells being disomic, i.e., exhibiting two signals (Figure 2A). By colabeling with Neu-N antibody, we found that ~6.5% of neurons in *APP* transgenic mice were trisomic for chromosome 16 compared with 1.5% of neurons in nontrangenic mice brain (Figure 2B). There was no increase in aneuploidy in nonneuronal cells (Neu-N negative; Figure 2C). These robust results with one chromosome probe indicate that even more tgAPP neurons are likely to be aneuploid, just as 5% trisomy 16 among spleen cells correlated to 25% total aneuploidy in metaphase spreads (which cannot be generated for postmitotic neurons).

Small numbers of tetraploid neurons have been reported in AD humans and mice and interpreted as being due to adult neurons reentering the cell cycle and completing DNA replication, but failing to complete cell division (Yang *et al.*, 2001, 2006; Obrenovich *et al.*, 2003; Mosch *et al.*, 2007; Zhu *et al.*, 2008). We observed no increase in tetrasomy 16 in disaggregated cells from *APP* mice brains compared with nontransgenic brains (Figure 2D); see also Iourov *et al.* (2009), who also report finding human AD cortical neurons with trisomy 21 but very few neuronal or nonneuronal cells showing tetrasomy or polyploidy.

These and previous results show that expression of FAD mutant forms of either *APP* or *PS-1* in transgenic mouse models of AD disrupts normal mitosis, leading to chromosome mis-segregation and the development of aneuploid cells in both the brain and the periphery. The presence of aneuploid neurons in both *APP* and *PS-1* transgenic mice indicates that both classes of FAD-causing mutations cause cell cycle and chromosome mis-segregation defects in neuronal precursor cells.

Expression of FAD Mutant APP Causes Chromosome Mis-Segregation and Aneuploidy in Transfected Cells

To confirm that the aneuploidy observed in FAD-*APP* transgenic mice was caused directly by the expression of the mutated gene, we asked whether mutant *APP* could also induce aneuploidy after transient expression in mammalian cells in culture. To assure a low background level of cell cycle defects such as aneuploidy, we used as a transfection recipient the hTERT-HME1 cell line, a (karyotypically stable) primary human cell line transfected with a telomerase reverse transcriptase gene to confer immortality (Jiang *et al.*, 1999; Morales *et al.*, 1999).

Parallel cultures of hTERT cells were transiently transfected with plasmids expressing a human *APP* gene carrying both the NL-*APP* K595N/M596L (Swedish) and V642I (London) mutations or the V717I *APP* mutation. The empty vectors served as controls. FISH with a double-labeled probe showed that expression of mutant *APP* induced chromosome mis-segregation and the development of aneuploidy for both chromosome 21 and 12 (Figure 3, A–C), but did not induce an increase in tetraploid cells (Figure 3D). Together, these results show that the aneugenic activity of mutant APP expression likely affects all chromosomes randomly and therefore probably alters an essential aspect of normal mitosis. Mutant *APP* did not induce aborted cell cycles in which DNA replication occurs without a subsequent cell division in these short-term experiments.

AD-causing Peptides Induce Chromosome Mis-Segregation and Aneuploidy

Because overexpression or mutation in either *PS-1* or *APP* cause cell cycle defects and chromosome mis-segregation, we hypothesized that the product of APP cleavage by PS-1—the Aβ peptide itself—might be the effector molecule responsible for disrupting the mitotic spindle (Boeras *et al.*, 2008). We tested

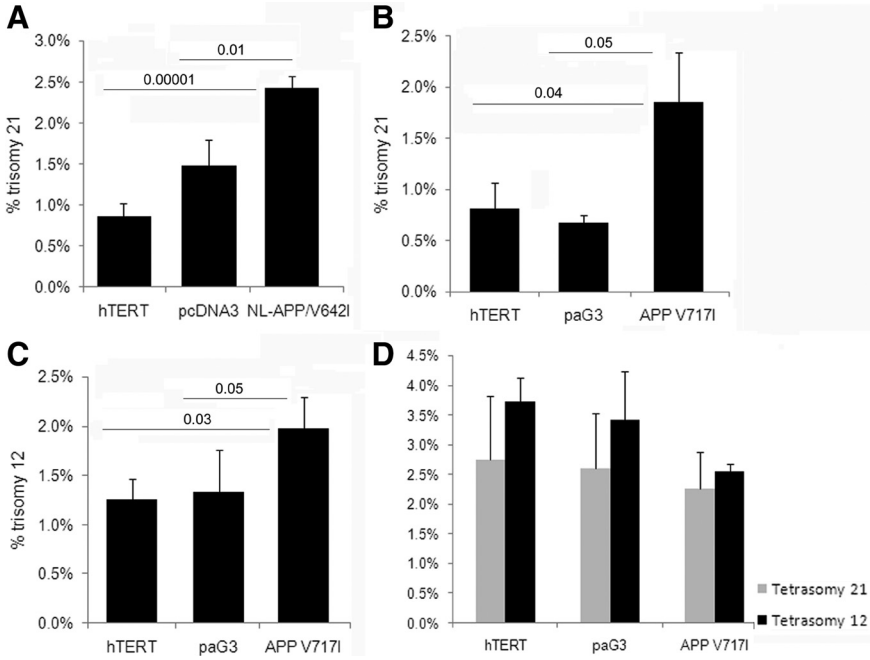
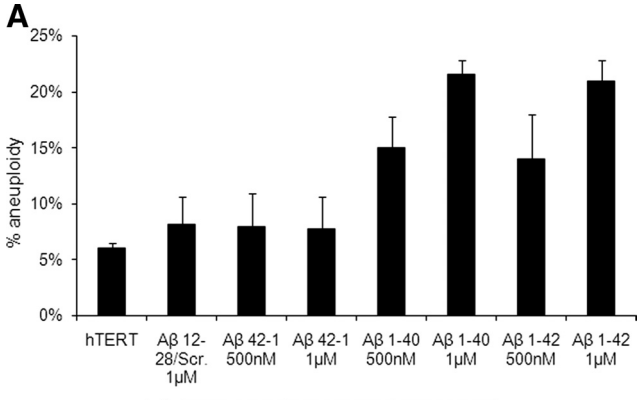


Figure 3. Chromosome aneuploidy induced in cells expressing either NL-APP K595N/M596L and V642I or V717F mutant *APP*. hTERT cells were transfected with expression vectors for the mutant human APP genes with the empty vectors pcDNA3 or pAG3 serving as control. The transfected cells were analyzed 48 h later. Expression of *APP* caused many cells to become aneuploid, as indicated by trisomy 21 and trisomy 12 (A–C). *APP* expression failed to induce a significant increase in tetrasomy for chromosome 16 (D).

this hypothesis by directly exposing cells in culture to Aβ and various control peptides for 48 h and counting chromosomes by karyotype analysis and by FISH. Both Aβ1-40 and Aβ1-42 induced significant chromosome mis-segregation and aneuploidy in cultured hTERT cells within 48 h whereas the Aβ 42-1 reverse peptide, the Aβ1-42 scrambled peptide and the Aβ12-28 scrambled peptide had no significant effect on untreated cells (Figure 4A,B), a result confirmed by FISH analysis of chromosomes 21 and 12 (Figure 5, A–C).



B

Control	Aβ40	Aβ42
Aβ12-28-Scr.	0.005	0.005
Aβ 42-1	0.005	0.002
Untreated	0.000001	0.0000002

Figure 4. High levels of total aneuploidy induced in hTERT cells by either Aβ40 or Aβ42. In the series of seven experiments, metaphase karyotypes of peptide-treated hTERT cells were examined after 48 h exposure to Aβ and control peptides (A). Significant levels of aneuploidy were induced by 1 μM Aβ40 and Aβ42 compared with Aβ12-18-Scrambled peptide and Aβ42-1 reverse peptide (B).

Mechanism of Aβ-induced Chromosome Mis-Segregation: Roles for Tau and APP

To investigate the mechanism by which Aβ, and by implication mutant APP and PS, cause cells to undergo chromosome mis-segregation and yield aneuploid daughters, we assumed that its aneugenic function was related to the peptide's other toxic activities, especially those related to MT-based transport, which is, of course, required for proper chromosome segregation. The data of Figure 6, A and B, show that pretreating normal splenocytes with BAPTA, which chelates extracellular Ca^{2+} and thus inhibits, for instance, calpain activation, or with LiCl, which inhibits GSK-3β, prevents Aβ from inducing chromosome mis-segregation. BAPTA and LiCl have previously been shown to inhibit Aβ toxicity (Lee *et al.*, 2000; Takashima 2006).

Numerous studies, both in vitro and in vivo, have shown that Aβ (and FAD mutant *PS-1* and *APP*) induces increased phosphorylation of the MT-associated protein Tau, the main component of the intraneuronal paired helical filaments and neurofibrillary tangles of AD (Lee 1996; Pigino *et al.*, 2001; Small and Duff, 2008). Indeed, the toxicity of Aβ depends on the presence of Tau (Rapoport *et al.*, 2002; Roberson *et al.*, 2007). Interestingly, activated calpain cleaves Tau, inactivating it, and yielding a toxic fragment, and GSK-3β phosphorylates Tau at AD-PHF-relevant sites and is itself involved in chromosome segregation (Lee 1996; Wakefield *et al.*, 2003; Park and Ferreira, 2005). We therefore investigated the role of Tau in chromosome segregation. Spenocytes were prepared from normal, *Tau*+/−, and full *Tau*−/− knockout mice, allowed to grow for 48 h in the presence and absence of Aβ, and analyzed for chromosome aneuploidy. As shown in Figure 7, A and B, knocking out one, or even more effective, both copies of *Tau* led to increased aneuploidy. Addition of Aβ only induced a clear increase in chromosome mis-segregation in normal cells, indicating that the aneugenic effect of Aβ requires and disrupts normal, Tau-stabilized, MT function.

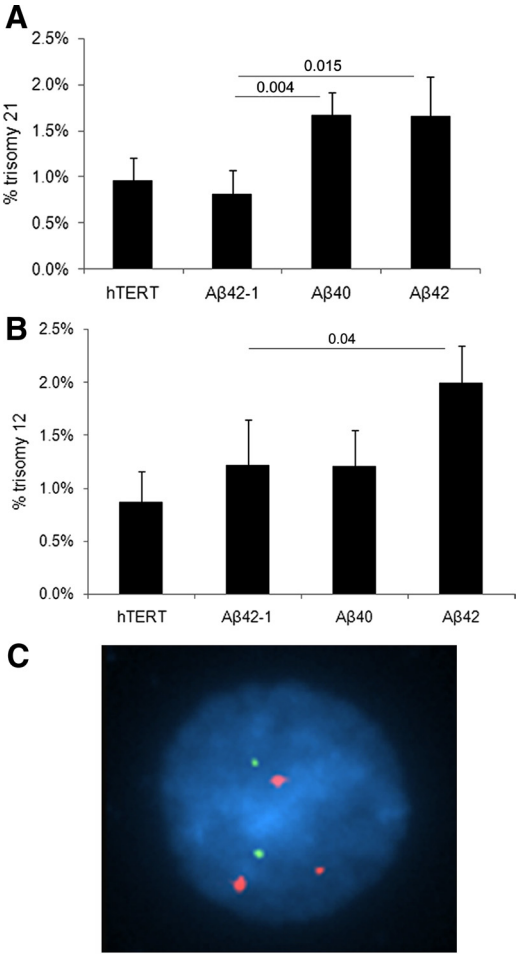


Figure 5. Specific chromosome aneuploidy, including trisomy 21 and trisomy 12 induced by exposure of hTERT cells to Aβ peptide. Quantitative FISH analysis with a dual color probe detecting both chromosome 21 (SpectrumOrange) and 12 (SpectrumGreen) revealed induction of trisomy 21 and 12 (A–C), but no significant increase of either tetrasomy 21 or 12 (data not shown). Comparing these results with the data of Figure 3 indicates that Aβ peptides exert a general disruptive effect on chromosome segregation during mitosis that includes but is not restricted to chromosome 21.

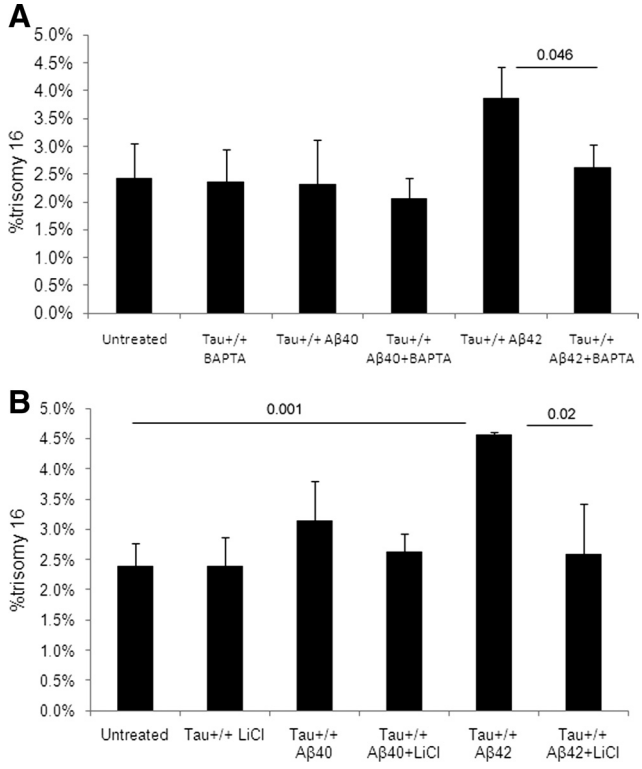


Figure 6. Pretreating normal splenocytes for 3 min with 1 mM BAPTA, a chelator of extracellular Ca^{2+}, showed a reduction in Aβ 1-42–induced trisomy 16 (A). Similarly, the coincubation with 1 μM of Aβ 1-42 for 48 h and 2.5 mM of LiCl, a GSK-3β inhibitor, for the last 7 h decreased trisomy 16 (B).

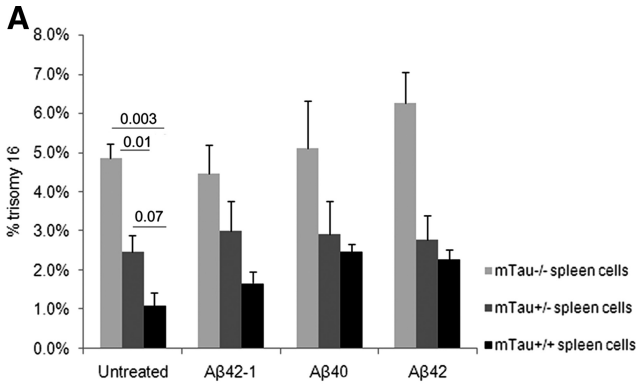
Finally, Aβ-induced cellular toxicity has been shown to require the presence of full-length APP as a receptor on the surface of the target cell, perhaps to aid in the uptake of exogenous Aβ or to undergo induced endocytosis and processing to generate intracellular Aβ (Lorenzo *et al.*, 2000; Shaked *et al.*, 2006; Sola Vigo *et al.*, 2009). We have tested whether the effect of Aβ on chromosome segregation in treated cells similarly requires interaction with endogenous APP. Addition of Aβ 40 or 42 to *APP*$-/-$ spleen cells fails to induce chromosome mis-segregation and aneuploidy over background, (Figure 8). This result further indicates that Aβ-induced chromosome mis-segregation is part of, and likely contributes to, the peptide's toxic action.

DISCUSSION

The preponderance of the data—from pathology, from genetics, from biochemistry, from cell biology, and from the mouse models designed to mimic the human disease—point to the Aβ peptide as playing a central role in the pathogenic pathway to AD (Hardy and Selkoe, 2002). For example, mutations in *APP* or *PS* affect either the sequence of Aβ, or the cleavage of the APP protein, so as to generate forms of Aβ that, under the essential catalytic influence of inflammatory proteins, specifically apolipoprotein E and antichymotrypsin, are more prone to first oligomerize and then polymerize and aggregate into the toxic amyloid deposits of AD (Wolfe, 2003; Potter *et al.*, 2001). Both in vitro and in vivo studies have shown that oligomerized and/or polymerized Aβ is toxic to neurons (Yankner *et al.*, 1989; Ma *et al.*, 1994, 1996; Wisniewski *et al.*, 1994; Kayed *et al.*, 2003; Chromy *et al.*, 2003; Townsend *et al.*, 2006).

The importance of Aβ is also supported by the fact that DS patients, whose cells carry three copies of chromosome 21 (and of the *APP* gene) in all of their cells due to chromosome mis-segregation during meiosis produce increased levels of Aβ peptide and invariably develop AD pathology at an early age (Olson and Shaw 1969; Epstein, 1990, Potter, 1991, 2008). Even mosaic DS individuals, with only a small proportion of trisomy 21 cells, develop early dementia. Furthermore, FAD can be caused by a duplication of one *APP* gene on one chromosome, confirming that a mere 50% overproduction of APP/Aβ is sufficient to cause very early AD in the context of an otherwise normal human brain (Sleegers *et al.*, 2006; Rovelet-Lecrux *et al.*, 2006).

The data of this article, together with our earlier results, support the hypothesis that a potentially important step in the pathogenic pathway by which Aβ overproduction leads



B

p value	mTau-/-	mTau+/-	mTau+/+
AβR/Aβ42	0.06	0.3	0.06
AβR/Aβ40	0.3	0.4	0.008
Untreated/ Aβ42	0.04	0.35	0.009
Untreated/ Aβ40	0.4	0.33	0.01

Figure 7. Knocking out *Tau* replicates/replaces ability of Aβ to induce chromosome mis-segregation. Spleen cells from normal (WT) and *Tau*+/− and *Tau*−/− mice were cultured ± Aβ1-42 and Aβ1-40 for 48 h, and the resulting chromosome aneuploidy was assessed (A and B). *Tau*+/− and −/− cells displayed a higher-than-normal inherent level of aneuploidy, consistent with the requirement for Tau in the MT function in the mitotic spindle. Aβ's ability to induce chromosome mis-segregation was greatly attenuated (i.e., replaced) in *Tau*+/− and *Tau*−/− cells.

to AD is Aβ's interference with the cytoskeleton, leading to chromosome mis-segregation during mitosis (Potter, 1991, 2008; see also Rassoulzadegan *et al.*, 1998). More specifically, the Aβ product of the PS γ-secretase enzyme acting on APP, at least in part by inactivating Tau, may contribute to AD by inducing the mis-segregation of chromosomes and the development of aneuploid, including trisomy 21, cells. Knocking out the *Tau* gene also causes chromosome mis-segregation and aneuploidy.

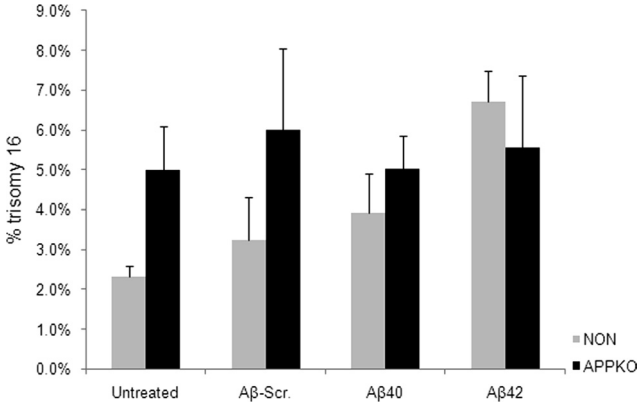


Figure 8. Knocking out APP in the target cells prevents Aβ from inducing further chromosome mis-segregation and aneuploidy. Spleen cells from *APP*−/− mice were cultured ± Aβ1-42, Aβ1-40, and scrambled Aβ1-42 for 48 h, and the resulting chromosome 16 aneuploidy was assessed. Aβ induced aneuploidy in the normal (NON) cells ($p = 0.01$), but failed ($p = 0.4$) to increase aneuploidy over background in the APPKO cells. Interestingly, like the *Tau*−/− cells examined in Figure 7, *APP*−/− cells also exhibited higher levels of aneuploidy compared with nontransgenic cells ($p < 0.05$).

The mechanism by which Aβ induces cell cycle abnormalities evidently involves activation of GSK-3β and the influx of Ca^{2+}, because blocking either of these two activities restores chromosome segregation to normal. In addition, Tau-stabilized MTs are a necessary substrate for the aneugenic activity of Aβ. These findings are linked, for Ca^{2+} is necessary for the Aβ-induced cleavage and inactivation of Tau and GSK-3β for Aβ-induced phosphorylation and inactivation of Tau and both for Aβ toxicity (Park and Ferreira, 2005; Takashima, 2006). Ca^{2+} is also necessary for activation of kinesin-like MT motors through Ca^{2+}-binding regulatory proteins (Vinogradova *et al.*, 2009; Wang and Schwartz, 2009). Indeed we consider it likely that Aβ directly damages the MT system such that Tau disconnects from MTs, becomes prone to cleavage, and phosphorylation, further destabilizing MTs.

Aβ's ability to inhibit MT function and cause chromosome mis-segregation will have major deleterious effects, particularly on neurons and on their precursors. Because neurogenesis occurs throughout life, especially following neuronal loss (Zhao *et al.*, 2008), Aβ-induced chromosome mis-segregation will yield aneuploid, defective, neuronal precursor cells and should thus inhibit the production of fully functional replacement neurons, as found in FAD-tg mice and AD patients. For instance the trisomy 21 cells that accumulate in AD patients overexpress APP and Aβ, which imbalance should promote the disease as it does in mosaic trisomy 21 or *APP*-duplication individuals who are born normal but later develop early AD pathology and dementia. Indeed, trisomy 21 neurons are prone to apoptosis, *PS-1* mutation or overexpression (and thus increased Aβ production) induces cell cycle defects in cultured cells, and mice harboring an FAD mutant human *APP* or *PS-1* gene have reduced neurogenesis and other cell cycle defects (Wolozin *et al.*, 1996; Busciglio and Yankner, 1997; Janicki and Monteiro, 1999; Chui *et al.*, 1999; Feng *et al.*, 2001; Wen *et al.*, 2004; Wang *et al.*, 2004; Chevallier *et al.*, 2005; Boeras *et al.*, 2008; Zhang *et al.*, 2007;Verret *et al.*, 2007; Boeras *et al.*, 2008; Varvel *et al.*, 2008). Any dividing precursor or mature cell, in culture or in vivo, which cannot undergo proper chromosome segregation will produce defective progeny prone to apoptosis.

Aβ damage to MT function will also lead to intracellular trafficking defects that will particularly affect mature neurons (see for example Stokin *et al.*, 2005; Lazarov *et al.*, 2007). Motor proteins needed for proper interaction of MTs with kinetochores or other cargo require Ca^{2+}, and stability of MTs in neurons is reduced by Ca^{2+}-dependent cleavage of Tau or by GSK-3β-dependent phosphorylation of Tau, both of which are induced by Aβ, as discussed above.

A direct connection between P-Tau and chromosome mis-segregation is reinforced by the recent finding that the P301L mutation in *Tau* that causes frontal temporal dementia (FTD) also causes Tau hyperphosphorylation, chromosome mis-segregation, and aneuploidy (Rossi *et al.*, 2008). Just as we showed for AD, the finding of FTD-related chromosome defects confirms the presence of Tau, MT disfunction, and chromosome mis-segregation on the causal path to neurodegeneration in these two related disorders. Interestingly, Pin1, which modulates Tau phosphorylation, also causes chromosome mis-segregation, aneuploidy, and oncogenic transformation in transfected cells (Suizu *et al.*, 2006).

The fact that both AD and FTD are associated with advancing age and that aging leads to increased aneuploidy even in normal neuronal precursors and other cells (Geller and Potter, 1999; Rehen *et al.*, 2001; Thomas and Fenech, 2008; Granic and Potter, unpublished data), further supports the hypothesis that slow development of such aneuploidy contributes to age-related neurodegenerative disease (Potter, 1991; Li *et al.*, 1997). Indeed two other common age-promoted diseases, cancer and cardiovascular disease, are also characterized and likely promoted by chromosome mis-segregation and aneuploidy (Duesberg, 1999; Potter *et al.*, 2008).

In sum, the data of this article and previous results show that the Aβ peptide found at increased levels in both sporadic and familial AD interferes with mitosis and chromosome segregation, thus leading to trisomy 21 mosaicism and other chromosome aneuploidy. The implication of the results is that MT disruption leading to cell cycle, chromosome mis-segregation, and other cytoskeletal defects in neuronal precursor cells may underlie many of the neurotoxic aspects of AD. The findings also suggests that novel approaches to diagnosis and treatment directed at detecting and preventing disruption of MT function and/or the development of chromosome aneuploidy with age may be successful against AD and possibly other age-associated disorders.

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