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# Partial BACE1 Reduction in a Down Syndrome Mouse Model blocks Alzheimer-related Endosomal Anomalies and Cholinergic Neurodegeneration: Role of APP-CTF

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## Abstract

β-amyloid precursor protein (APP) and amyloid beta peptide (Aβ) are strongly implicated in Alzheimer's disease (AD) pathogenesis, although recent evidence has linked APP-βCTF generated by BACE1 (β-APP cleaving enzyme 1) to the development of endocytic abnormalities and cholinergic neurodegeneration in early AD. We show that partial BACE1 genetic reduction prevents these AD-related pathological features in the Ts2 mouse model of Down syndrome (DS). Partially reducing BACE1 by deleting one BACE1 allele blocked development of age-related endosome enlargement in the medial septal nucleus (MSN), cerebral cortex, and hippocampus and loss of choline acetyltransferase (ChAT)-positive MSN neurons. BACE1 reduction normalized APP-βCTF elevation but did not alter Aβ40 and Aβ42 peptide levels in brain, supporting a critical role *in vivo* for APP-βCTF in the development of these abnormalities. While ameliorative effects of BACE1 inhibition on β-amyloidosis and synaptic proteins levels have been previously noted in AD mouse models, our results highlight the additional potential value of BACE1 modulation in therapeutic targeting of endocytic dysfunction and cholinergic neurodegeneration in DS and AD.

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**Keywords**

trisomic mice; BACE1; APP-βCTF; Alzheimer's disease; endosomes; basal forebrain cholinergic neurons

## 1. Introduction

Endosomes are sites of highly active APP processing and genes that influence endocytosis are over-represented as AD risk factors (Israel, et al., 2012; Nixon, 2013). Moreover, abnormalities of neuronal endocytosis, characterized by swelling of rab5-positive early endosomes and upregulated expression of rab5 and other endocytosis-related genes, are the earliest disease-specific response of neurons in AD so far reported (Cataldo, et al., 1997). In Down syndrome (DS, Trisomy 21), a cause of early-onset AD has been linked to an extra copy of APP, and these changes appear progressively, beginning before birth (Cataldo, et al., 2000). Similar endosomal changes in fibroblasts derived from DS individuals and in neurons of the Ts65Dn mouse model of DS have been shown to represent the pathological acceleration of endocytosis rate (Cataldo, et al., 2008), which is dependent on APP triplication, rab5 activation (Cataldo, et al., 2003), and possibly additional triplicated genes on human chromosome 21 (HSA21) (Cossec, et al., 2012). Aberrant signaling from abnormal endosomes (Laifenfeld, et al., 2007; Salehi, et al., 2006) disrupts neurotrophin signaling leading to degenerative changes in cholinergic neurons in DS and AD mouse models (Choi, et al., 2013; Salehi, et al., 2006). In DS fibroblasts and neuronal APP models of AD, rab5-mediated endosomal dysfunction is driven by an elevated level of APP-βCTF, the product of APP cleavage by β-site APP cleaving enzyme 1 (BACE1) (Jiang, et al., 2010). APP-βCTF elevation is found in both AD and DS human brains (Kim, et al., 2015; Pera, et al., 2013). APP-βCTF has also been shown recently to pathologically activate rab5 by recruiting APPL1 (adaptor protein containing pleckstrin homology domain, phosphotyrosine binding (PTB) domain, and leucine zipper motif), an adaptor protein unrelated to APP, to early endosomes where it binds both APP-βCTF and GTP-rab5 and stabilizes this activated GTP form of rab5 on the endosome. Pathological rab5 activation enlarges endosomes, which slows their axonal transport in an APPL1-dependent manner (Kim, et al., 2015). These findings and others (Choi, et al., 2013; Salehi, et al., 2006) have increasingly implicated APP-βCTF in the pathogenesis of AD and DS, but so far there is no *in vivo* validation. Partial BACE1 reduction in a model without β-amyloid deposition enabled the possibility of reducing APP-βCTF while minimally changing Aβ levels, as previously reported, (McConlogue, et al., 2007; Nishitomi, et al., 2006) to investigate its possible therapeutic effects. Genetic reduction of BACE1 has been shown to reduce amyloid burden, delay the onset of basal forebrain cholinergic neurons (BFCN) neurodegeneration and improve cognitive function in several mouse models of β-amyloidosis (McConlogue, et al., 2007; Ohno, et al., 2007; Singer, et al., 2005), although its impact on other key features of AD pathology, including endosomal pathology and BFCN neurodegeneration has not been previously examined in a DS mouse model.

BACE1 inhibitors are currently undergoing clinical trials as a therapy for AD (http://clinicaltrials.gov/ct2/results?term=BI1181181) and have potential advantages over other

anti-amyloid strategies by modulating a broader array of APP metabolites, including APP-βCTF and the corresponding soluble amino-terminal fragment (sAPPβ). Here, we tested the therapeutic effects of BACE1 inhibition in a trisomic mouse line (Ts2) by deleting one BACE1 allele (Ts2.BACE1$^{+/-}$) to lower BACE1 expression while avoiding undesirable effects of complete BACE1 deletion on synaptic function, neuroplasticity, and behavior (Kobayashi, et al., 2008; Laird, et al., 2005). We used the Ts[Rb(12.17$^{16}$)]2Cje (Ts2) DS model (Villar, et al., 2005) because the commonly used Ts65Dn mouse model is challenging to breed (Moore, et al., 2010). By contrast, the Ts2 mouse, which expresses the same complement of trisomic genes as Ts65Dn and displays the same overt DS phenotype, yields male mice that are fertile and female mice that have higher trisomy transmission rates, resulting in a ∼3-fold higher viable offspring compared to Ts65Dn mice. We demonstrate that Ts2 and Ts65Dn mice exhibit indistinguishable age-dependent endosomal and cholinergic phenotypes as well as similar elevated APP-βCTF levels. Collectively, our findings on BACE1 reduction in Ts2 mice highlight the likely importance of lowering APP-βCTF levels to attain maximum therapeutic effects against an APP-related target in AD and DS.

## 2. Materials and Methods

### 2.1 Mice

Mouse experimentation and animal care were approved by the Institutional Animal Care and Use Committee (IACUC) of Nathan S. Kline Institute. Ts65Dn, Ts2, and wild type breeding partners (C57BL/6JEi × C3H/HeSnJ) were obtained from Jackson Labs (Bar Harbor, ME). Heterozygous BACE1 knockout mice (BACE1$^{+/-}$) were kindly provided by Dr. Joseph D. Buxbaum (Pastorino, et al., 2004). Male and female mice were used.

**Antibodies, Immunocytochemistry, Western Blot Analysis and Aβ ELISA—**At the appropriate ages, mice were transcardially perfused with 4% paraformaldehyde, and single label immunohistochemistry was performed on 40 μm thick vibratome sections (Cataldo, et al., 2003), using commercial antibodies against rab5b (Santa Cruz Biotechnology Inc., CA; 1:50), and choline acetyltransferase (Chat, Millipore, MA; 1:250), and visualized with diaminobenzidine (DAB, Sigma, MO) after incubation with biotinylated secondary antibodies (Vector Laboratories, CA; 1:500) and peroxidase using a Vectastain ABC kit (Vector). Rudy4, our in house antibody against cathepsin D was used as positive control (rabbit polycolonal antibody, 1:5000), and secondary antibody only was used as negative control. Double immunofluorescence labeling was used to identify co-localization of ChAT and rab5 as described previously (Choi, et al., 2013). For protein analyses, mouse hemibrains and cortex were homogenized (Schmidt, et al., 2005a) and Western blot analyses were performed with antibodies against APP (c1/6.1; 1:1000) (Choi, et al., 2009; Schmidt, et al., 2005a; Schmidt, et al., 2005b), BACE1 (Sigma;1:500), sAPPβ (IBL; 1:100), sAPPα (M3.2; 1:1000) (Choi, et al., 2009; Schmidt, et al., 2005a; Schmidt, et al., 2005b), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Sigma; 1:2000). All the secondary antibodies for Western blot analyses were used according to the manufacturer's recommendations (Jackson ImmunoResearch Laboratories, PA). All the Western blot X-ray films were scanned using a Canon 9000F MarkII scanner and quantified using ImageJ

(imagej.nih.gov/ij) with GAPDH as an internal control. Mouse brain Aβ40 and Aβ42 were determined by sandwich ELISA (Choi, et al., 2009; Schmidt, et al., 2005a; Schmidt, et al., 2005b).

### 2.2 Morphometric Analysis

Morphometric analyses were performed as previously reported (Jiang, et al., 2010). Brain tissue sections from Ts65Dn, Ts2, and littermates from the cross of Ts2 and BACE1$^{+/-}$ mouse lines were immunolabeled with rab5 antibody in tandem under identical experimental conditions and images were obtained at 100× magnification (0.1049 μm/pixel, AxioVision 4.5 software; Carl Zeiss, Inc., NY). ImageJ (imagej.nih.gov/ij) was used to obtain numbers and sizes of rab5-positive endosomes. A Student's t-test was used for statistical analyses.

### 2.3 Stereological counting of MSN-ChAT cells

The numbers of ChAT-immunoreactive BFCNs in the MSN were determined using the optical fractionator method (West, et al., 1991) using ImageJ software as previously described (Smiley, et al., 2012). The MSN was sampled dorsal to the ventral edge of the anterior commissure, in every third consecutive section rostral to the commissure. Sections were sampled with a grid of optical dissector counting sites. At each site, a 40× oil-immersion objective was used to collect a z-stack of seven 2-micron spaced images. A 160 × 116 micron counting box was drawn onto each z-stack, with an upper guard zone of 2 microns (1 slice), counting box of 4 microns, and lower guard zone made of the remaining z-slices. Cell counts were corrected for z-axis shrinkage in each brain. Section thickness was 11 +/- 1.2 microns (mean +/- S.D.) and did not differ between treatment groups (t-test p-values > 0.5). On average, 329 +/- 93 cells were counted per MSN, and the coefficient of error was 0.06 +/- 0.01 (mean +/- S.D.).

## 3. Results

### 3.1 Higher transmission frequency of trisomy in Ts2 males and females compared to Ts65Dnmice

In agreement with the initial description of the Ts2 mouse (Villar, et al., 2005), female Ts2 mice transmitted trisomy more frequently (35.32± 5.12 %; n=59 from 9 litters), than female Ts65Dn mice (24.22± 2.63%, p=0.048; n=94 from 13 litters,), while average litter sizes were comparable (6.56 ± 0.41 Ts2; 7.23 ± 0.39 Ts65Dn; p=0.263). Notably, Ts2 males were equally as fertile as Ts2 females, producing average litters of 9 pups with a 34.30± 4.03% transmission frequency (p=0.041 comparing to Ts65Dn females, n=69 from 8 litters), whereas male Ts65Dn mice are sub-fertile (Davisson, et al., 1993). Given the higher transmission of trisomy in female Ts2 mice and equivalent transmission in fertile males, the production of Ts2 mice is ∼3 times higher than in the Ts65Dn line. We used approximately equal numbers of male and female Ts2 and Ts65Dn mice in the following studies.

### 3.2 Ts2 mice display endosomal phenotypes similar to those in Ts65Dn mice

Ts2 mice, like Ts65Dn mice, demonstrate increased rab5 immunolabeling in MSN (Fig. 1 B, C, n=6), cingulate cortex (CCX) (Fig.1 H, I) and hippocampus (not shown) compared to their normal disomic controls (2N, Fig. 1 A and G, respectively; n=6). Also, Ts2 mice, like

their Ts65Dn counterparts, show enlarged rab5 early endosomes in the MSN (Fig. 1E, F; n=6), CCX (Fig. 1H and I, insets).

### 3.3 Ts2 and Ts65Dn mice display a similar degenerative BFCN phenotype, APP and APP-CTFexpression profile

Previous studies of Ts65Dn mice have shown that BFCNs are selectively vulnerable and undergo age-related neurodegenerative changes and loss of their cholinergic phenotype, which starts at 6 months and is robust by 9 months of age (Davisson and Costa, 1999; Davisson, et al., 1990; Granholm, et al., 2000; Holtzman, et al., 1996; Lockrow, et al 2009). ChAT immunohistochemistry indicated that Ts2 mice undergo a similar loss of cholinergic neurons within the MSN at 9 months of age (Fig. 2B, E; n=6) as Ts65Dn (Fig. 2C, F; n=6), compared with their 2N littermates (Fig. 2A, D; n=6). As also seen in Ts65Dn mice (Choi, et al., 2009), levels of full-length APP and APP-CTFs, identified by c1/6.1 antibody (Choi, et al., 2009; Schmidt, et al., 2005a; Schmidt, et al., 2005b), are significantly elevated in Ts2 mouse brain cortex homogenates at 8-9 months of age compared to their 2N littermates, with APP-βCTF more than doubled that of 2N (Fig. 3A, B; n=3-4 for each genotype; p=0.006 for APPfl, p=0.004 for APP-βCTF).

### 3.4 Age-related neuronal endosomal pathology in Ts2 mice precedes cholinergic neur odegenerative changes

Consistent with published reports using Ts65Dn mice (Granholm, et al., 2000; Hunter, et al., 2004), cholinergic loss is not evident in Ts2 mice at 4 months of age (Fig. 4A); BFCN numbers in MSN of Ts2 mice were not significantly decreased (Fig. 4B, n=4 for 2N and Ts2, p=0.54). Qualitative analysis of endosomal pathology showed enlarged rab5-positive endosomes within the MSN (Fig. 4C-F) and CCX (Fig. 4G, H) in Ts2 mice at 4 months of age, and the enlargement is comparable to that seen in age-matched Ts65Dn mice (Cataldo, et al., 2003). Semi-quantitative morphometric analysis of cortical neurons in Ts2 mice and their 2N littermates confirmed that numbers of the larger rab5-positive early endosomes are disproportionately increased in Ts2 mice starting at 4 months of age (p=0.003, size 0.51-1.4μm; p=0.0008, size 1.4-7.0μm), and this pathological morphology persists throughout adulthood (Fig. 4I; n=4 for each age group). The increased endosome size and number in Ts2 mice also results in a higher cross-sectional area occupied by rab5 positive endosomes per cell (Fig. 4J, p=1.176E-08, 5.825E-06, 6.803E-08 for at 4, 9 and 16 month old of age).

### 3.5 APP-CTFs, but not Aβ are reduced by BACE1 dosage reduction in Ts2 mice

To examine the therapeutic effects of lowering APP-βCTF levels in Ts2 mice, we partially reduced BACE1 expression by crossing the trisomic mice with $BACE1^{+/-}$ mice to yield a $Ts2.BACE1^{+/-}$line. Brain levels of BACE1 by western blot analysis were comparably reduced in $2N.BACE1^{+/-}$ (p=1.046E-04 compared to 2N) and $Ts2.BACE1^{+/-}$mice (p=9.245E-05 compared to Ts2). [(Fig. 5A, B), n=4 (2 male and 2 female) for each genotype], BACE1 reduction also decreased brain levels of APP-βCTF (p=0.0119) and sAPPβ (p=9.245E-05) in $Ts2.BACE1^{+/-}$ (Fig. 5A, B). Because APP-βCTF can be cleaved by APP α-secretase to generate APP-αCTF (Gouras, et al., 1998; Haass, et al., 1992; Jiang, et al., 2010; Kaether, et al., 2006), BACE1 reduction also lowered APP-αCTF levels

(p=0.00271), as expected, although to a lesser extent than the drop in APP-βCTF levels. By contrast, Aβ40 and Aβ42 levels measured by sandwich ELISA, remained unchanged with BACE1 reduction [Fig. 5C, n=6 (3 male and 3 female) for each genotype], as previously observed with partial BACE1 reduction (Nishitomi, et al., 2006).

### 3.6 Partial reduction of BACE1 prevents neuronal endosomal pathology in Ts2 mice at 8-9months

BACE1 reduction diminished rab5-endosomal volume in Ts2 mice to that of 2N littermates (Fig. 6A; n=6-8 for each genotype, approximately equal numbers of male and female mice), as reflected by both decreased numbers of enlarged endosomes (Fig. 6B; p=0.015, 0.0051, 0.035 for the three size ranges in a comparison of Ts2.BACE1$^{+/-}$ with Ts2.BACE1$^{+/+}$ in MSN; p=0.107, 0.0038, 0.030 for the three size ranges in a comparison of Ts2.BACE1$^{+/-}$ with Ts2.BACE1$^{+/+}$ in HC) and total cross-sectional areas of rab5-endosomes (Fig. 6C) in MSN (p=8.258E-05 in a comparison of Ts2.BACE1$^{+/-}$ to Ts2.BACE1$^{+/+}$) and hippocampal neurons (p=0.00539, comparing Ts2.BACE1$^{+/-}$ to Ts2.BACE1$^{+/+}$) (Fig. 6D, E). BACE1 reduction in wild-type mice (2N.BACE1$^{+/-}$) did not alter any of these measured endocytic parameters (Fig. 6B-E).

### 3.7 Partial reduction of BACE1 rescues ChAT-immunoreactive BFCNs in the MSN of Ts2 mice

To investigate the effect of partial BACE1 reduction on cholinergic dysfunction, we first confirmed by double immunolabeling that rab5-positive endosomes are enlarged in ChAT-containing neurons in the MSN of 9 month old Ts2 mice, including in those neurons with reduced ChAT signal (Fig. 7A, B). DAB staining with ChAT also confirmed that BFCN numbers are significantly lowered in MSN of Ts2 mice (Fig. 7D). By comparison, partial deletion of BACE1 prevented the loss of ChAT- positive MSN neurons in Ts2.BACE1$^{+/-}$ mice (Fig. 7E). Further stereological analysis showed that partial reduction of BACE1 preserved ChAT positive neurons in the MSN in Ts2 mice at a number comparable to that in 2N mice [Fig. 7F; n=6-7 (approximately equal numbers of male and female mice) for each genotype; p=0.00088 for Ts2.BACE1$^{+/-}$ versus Ts2.BACE1$^{+/+}$].

## 4. Discussion

The Ts2 mouse, a model of DS generated by a spontaneous Robertsonian fusion, was identified and first characterized by Villar and colleagues (Villar, et al., 2005), who noted advantages of Ts2 over Ts65Dn mice in both the transmission of the triplicated distal segment of mouse chromosome 16 (MMU16) and the male fertility, two features that significantly hinder breeding of Ts65Dn mice for large scale investigations (Davisson, et al., 1993) Our data extend these findings by showing even higher rates of male fertility (averaging 9 pups per litter) comparable to those of females (averaging 7 pups per litter), resulting in nearly a 3-fold overall increase in breeding productivity in Ts2 mice over Ts65Dn mice, and yielding both substantial time and space savings as well as improving breeding success when crossed with other mouse lines. These features and a pathological phenotype comparable to Ts65Dn establish the Ts2 mouse as a suitable, if not superior, alternative to the widely used Ts65Dn model.

Beyond the trisomic and phenotypic similarities previously reported (Villar, et al., 2005), we show that Ts2 mice develop a similar regional pattern of APP-dependent adult-onset anomalies of neuronal endosomes as seen in Ts65Dn mice (Cataldo, et al., 2003), which appear at a comparable age and precede a similar loss of cholinergic phenotype in MSN BFCNs beginning between 6 and 8 months of age (Davisson and Costa, 1999; Davisson, et al., 1990; Granholm, et al., 2000; Holtzman, et al., 1996). The evidence of endosomal pathology prior to the onset of substantial BFCN loss in Ts2 mice indicates that early endosomal alterations are an early manifestation of pathology and precede BFCN neuron loss. Ts2 and Ts65Dn mice also show similar hippocampal glutamatergic neuronal deficits (Kaur, et al., 2014) and similar MRI anomalies (Chen, et al., 2009).

Our demonstration that BACE1 reduction blocks development of the AD-related phenotype in Ts2 mice adds *in vivo* support to previous evidence that APP-βCTF elevation, in the absence of a change in APP levels, is necessary and sufficient to induce the endosome phenotype in DS fibroblasts (Jiang, et al., 2010) and primary neuron culture models (Kim, et al., 2015). BACE1 cleaves APP to generate APP-βCTF (Sinha, et al., 1999), which is located mainly in early endosomal compartments (Walter, et al., 2001). Elevated APP-βCTF signaling in endosomes initiates the pathological activation of rab5 in endosomes (Jiang, et al., 2010). Rab5 activation upregulates endocytosis and endosomal fusion that promotes endosomal enlargement and further endolysosomal dysfunction and altered endosomal signaling associated with reduced neuronal survival (Cataldo, et al., 2008). Retrograde signaling by rab5-immunoreactive endosomes also mediates retrograde nerve growth factor-derived trophic support of BFCNs (Deinhardt, et al., 2006), which is dysfunctional in aged Ts65Dn mice (Salehi, et al., 2006; Seo and Isacson, 2005). APP-FL and APP-CTF are proposed to be key factors in restoring the BFCN number in MSN of Ts65Dn mice, when the copy number of *App* is lowered to normal disomic levels (Salehi, et al., 2006). An association between elevated APP-βCTF levels, abnormal endosome morphology, and BFCN degenerative changes has also been seen in mouse models of familial AD due to APP mutations (APP23 mice) (Choi, et al., 2013). Two mouse models were used in their study: APP23 (Swedish K670N/M671L double mutation) and APPLd2 (London V717I mutation), while both showed increasing overall Aβ production/levels, the Swedish mutation in the APP23 mice also promoted higher levels of βCTF. APP23 mice showed both endosomal and cholinergic abnormalities, indicating the relevance of the βCTF.

The therapeutic effects of partial BACE1 inhibition in our DS model are consistent with those in β-amyloidosis models (Ohno, et al., 2007; Singer, et al., 2005). In our study, levels of APP-βCTF, but not Aβ, were lowered by BACE1 reduction, which prevented endosomal and BFCN abnormalities. Notably, Aβ levels remain unchanged in non-transgenic mice with only one copy of BACE1 (Nishitomi, et al., 2006). Moreover, partial BACE1 reduction in the PDAPP β-amyloidosis mouse does not significantly change levels of Aβ at ages prior to the onset of Aβ deposition (McConlogue, et al., 2007). These findings suggest that γ-cleavage of APP-βCTF may not be rate-limiting unless levels of APP-βCTF are significantly elevated by over-expression or increased APP-βCTF formation. In this regard, in mice over-expressing APPswe (Swedish APP mutation), partial BACE1 deletion did reduce Aβ levels (Laird, et al., 2005; Luo and Gallwitz, 2003); however, APPswe is 60 times more efficient

BACE1 substrate than wild-type APP (Barman and Prabhakar, 2014; Cai, et al., 2001; Tomasselli, et al., 2003). The reduction of APP-αCTF in Ts2.BACE1+/- we observed is consistent with the reported existence of an alternative non-amyloidogenic pathway for APP-βCTF that is cleaved by α-secretase to form APP-αCTF (Gouras, et al., 1998; Haass, et al., 1992). Although, APP-αCTF decreased in Ts2.BACE1$^{+/-}$ as expected, our previous studies in fibroblasts from individuals with DS have shown the endosomal abnormalities to be dependent on APP-βCTF levels and not on changes in APP-αCTF (Jiang, et al., 2010; Kim, et al., 2015).

Notably, in large clinical trials, treatments with APP γ-secretase inhibitors, which lowered Aβ but are predicted to raise APP-βCTF levels, worsened the neurological status of subjects (Imbimbo, 2008). Inhibition of γ-secretase also deteriorated the memory deficits in a genetically congruous mouse model of Danish dementia (Tamayev and D'Adamio, 2012), and induced defects of BDNF axonal trafficking and signaling in normal rat cortical neurons (Weissmiller, et al., 2015). APP-βCTF levels and BACE1 activity are elevated in the brain in familial AD (FAD) (Pera, et al., 2013) and in sporadic AD (Kim, et al., 2015) and APP-βCTF has various pathologic and neurotoxic properties that are independent of Aβ (Devi and Ohno, 2011; Jiang, et al., 2010). The present results add to a growing body of evidence that, independently of its conversion to Aβ, APP-βCTF has neurotoxic properties that are pathogenic in AD (Devi and Ohno, 2011; Kim, et al., 2015; McPhie, et al., 2001; Tamayev, et al., 2012). In fact, elevated BACE1 expression has been reported to increase APP β-cleavage and cause neurodegeneration even though Aβ levels are lowered (Lee, et al., 2005). Moreover, it was recently shown in neurons generated from induced pluripotent stem cells of FAD patients that APP metabolism regulates tau protein levels and that the effect seems to be mediated by APP-βCTF levels, rather than by Aβ (Moore, et al., 2015). Collectively, these studies strongly suggest that APP-βCTF may contribute significantly to APP pathogenicity in AD and that the outcomes of treatments with BACE1 inhibitors, which are currently in clinical trials, should be evaluated in light of their potential effects on APP-βCTF as well as Aβ. The protection afforded by BACE1 inhibitors over an increased range of putatively pathogenic APP metabolites, while possibly improving the balance of pro-survival metabolites of APP, may prove to be advantages of this therapeutic strategy over approaches specifically targeting only Aβ.

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**Dedication**: We dedicate this work to the memory of Dr. Anne Cataldo, our colleague and friend, who was a pioneer in describing neuronal endosomal pathology in the brain of Alzheimer's disease and Down syndrome patients. Dr. Anne Cataldo passed away on April 13, 2009.

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## Highlights

- Genetic BACE1 reduction blocks AD-related endosomal pathology in Ts2 mice.
- Genetic BACE1 reduction ameliorates cholinergic neurodegeneration in Ts2 mice.
- APP-CTFs, but not Aβ levels, are reduced by genetic BACE1 knockdown in Ts2 mice.
- Ts2 have similar endosomal & BFCN pathology to Ts65Dn mice but are easier to breed.

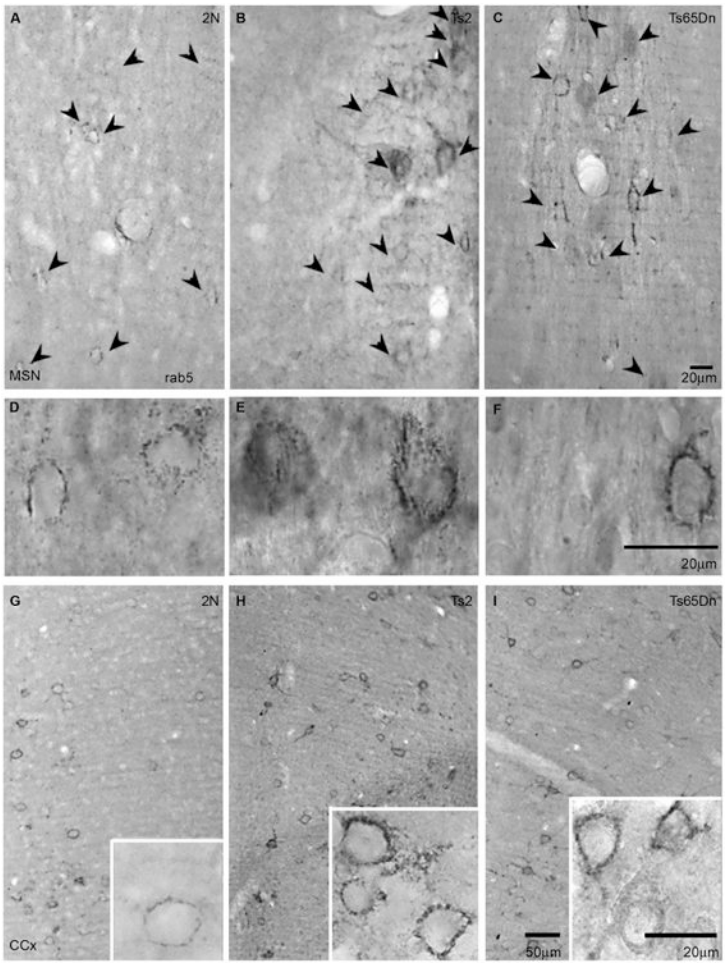


**Figure 1. Ts2 mice display endosomal abnormalities similar to Ts65Dn mice**

Representative neurons labeled with anti-rab5 antibody in the MSN (A-F) and cingulate cortex (G-I) of 9 month old 2N (A, D, G), Ts2 (B, E, H) and Ts65 (C, F, I) mice. rab5-positive early endosomes are enlarged in Ts2 mice (E, H inset) compared to 2N (D, G inset), and resemble those seen in Ts65Dn mice (F, I inset).

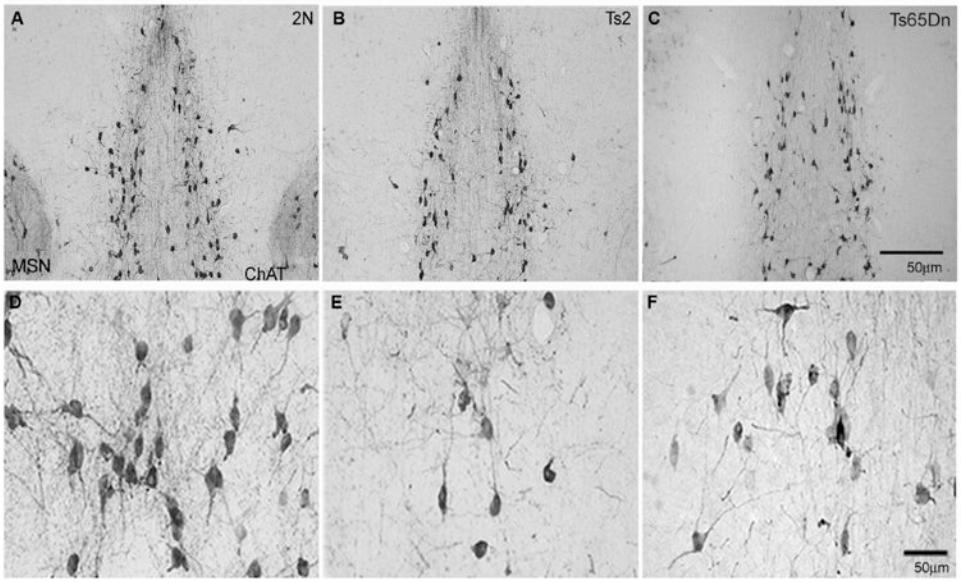


**Figure 2. Ts2 mice have a degenerative BFCN phenotype similar to Ts65Dn mice**

Morphometric analyses of the MSN reveals decreased numbers of ChAT-immunoreactive BFCNs at 9 months of age in representative images of Ts2 mice (B, E) and Ts65Dn mice (C, F) compared to 2N mice (A, D).

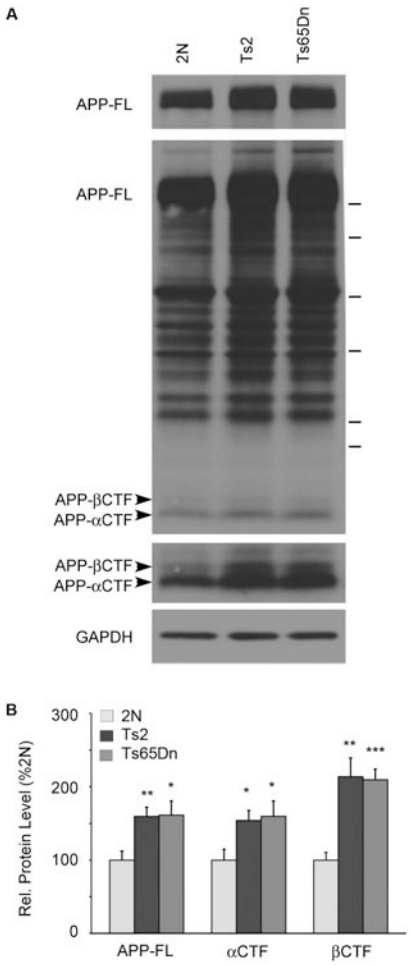


**Figure 3. Upregulation of APP holoprotein, APP-αCTF, and APP-βCTF in Ts2 and Ts65Dn mice**

Representative Western blots (A) and quantitation (B) of full-length APP (APP-FL), APP-αCTF, and APP-βCTF of mouse brain cortex homogenates at 8-9 months of age (n=4 for each genotype). GAPDH is shown as a loading control. Statistical significance was determined by Student's t-test (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$). Graphs show mean ± SEM.

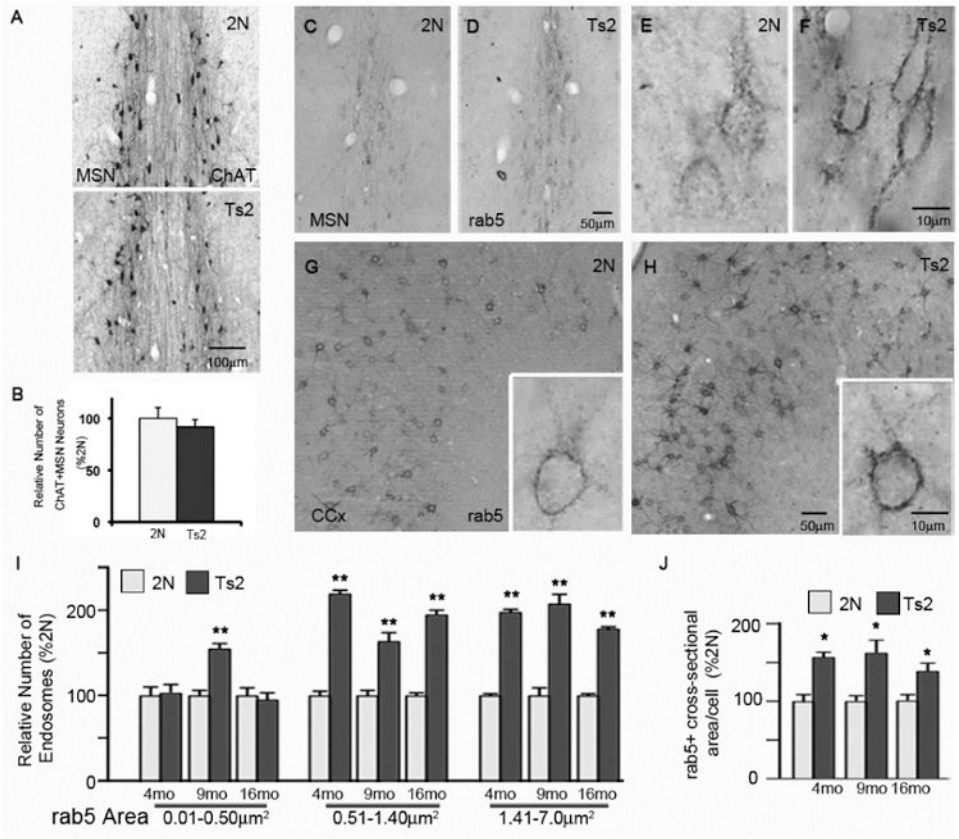


**Figure 4. Ts2 mice develop neuronal endosomal pathology preceding cholinergic neurodegenerative changes**

Quantitative analysis revealed that ChAT-positive MSN numbers remain unchanged in 4 month old Ts2 mice compared to 2N mice (Fig. A, B). Representative neurons labeled with anti-rab5 antibody are shown in the MSN (C-F) and cingulate cortex (G, H) 4 month old 2N and Ts2 mice. Rab5-positive early endosomes are enlarged in Ts2 mice (F, H inset) compared to 2N (E, G inset). Increased rab5-positive endosome numbers and sizes are seen in mice from 4 months old to 16 months of age (I, J, n=9-10, for each genotype and age group) (*, $p<0.05$, **, $p<0.01$). Graphs show mean ± SEM.

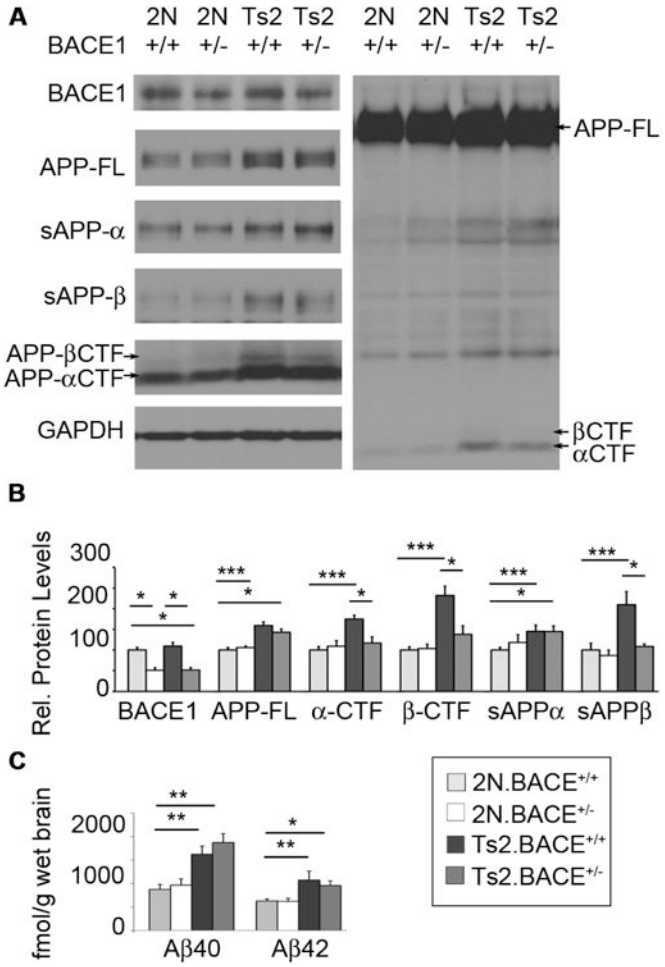


**Figure 5. Genetic reduction of BACE1 decreases APP-CTFs, but not Aβ levels in Ts2 mice**

Representative Western blots (A) and quantitation (B) reveals decreased expression of BACE1, APP-αCTF, APP-βCTF, and sAPPβ in Ts2.BACE$^{+/-}$ mouse brain homogenates (n=12 for each genotype) at 8-9 months of age. Elevated levels of Aβ40 and Aβ42 are not reduced by BACE1 reduction in Ts2 mice compared to 2N mice and 2N mice with BACE1 reduction (C; n=6 for each genotype) (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

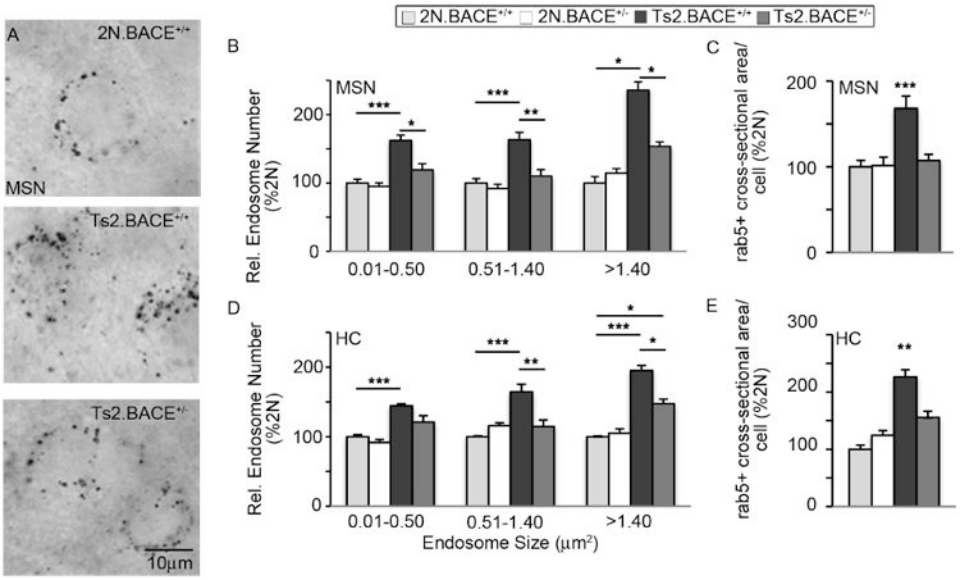


**Figure 6. Genetic reduction of BACE1 prevents neuronal endosomal pathology in Ts2 mice**

Representative neurons labeled with anti-rab5 antibody in the MSN (A) and quantitative analysis within the MSN (B, C) and hippocampal CA1 region (HC; D, E) of 9 month old mice (n=8-10 for each genotype). BACE1 genetic reduction in Ts2 mice shows endosome profiles similar to 2N mice, which are significantly reduced compared to Ts2 mice without BACE1 knockdown (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

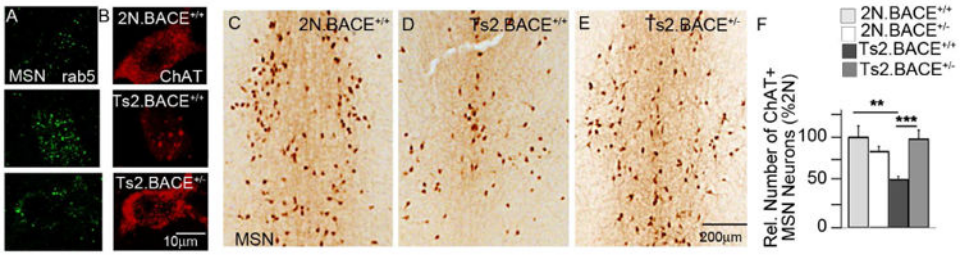


**Figure 7. BACE1 reduction rescues ChAT-immunoreactive BFCNs in the MSN of Ts2 mice**

Representative images show double immunofluorescence labeling of rab5-positive endosomes (A) and ChAT (B) in 2N, Ts2 mice and Ts2.BACE1$^{+/-}$ mice, with restoration of ChAT levels and normalization of rab5 expression by BACE1 partial deletion. DAB staining of ChAT-positive BFCNs in the MSN of 9 month old 2N (C), Ts2 (D) and Ts2.BACE$^{+/-}$ (E) mice was quantified by stereological analysis (F; n=6-7 for each genotype), which revealed rescue of phenotypic expression by BACE1 reduction in Ts2 mice (**, $p<0.01$; ***, $p<0.001$). Graphs show mean ± SEM.