

Research Report

Increased exploratory activity of APP23 mice in a novel environment is reversed by siRNA

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ARTICLE INFO

Article history: Accepted 11 September 2008 Available online 18 September 2008

Keywords: Amyloid precursor protein (APP) Alzheimer's disease (AD) Short interfering RNA (siRNA) Knockdown APP23 mouse Locomotor behaviour

ABSTRACT

Genetic abnormalities in *amyloid precursor protein* (APP) are associated with Down's syndrome and familial Alzheimer's disease where hallmark plaques contain A^β peptides derived from APP. Both APP and its derivatives are implicated in neurodegenerative processes and may play important physiological and pathophysiological roles in synaptic function. Here, we show that young APP23 transgenic mice overexpressing human APP with the Swedish double mutation display altered novelty seeking behavior before the age of plaque onset. Using short interfering RNA (siRNA) targeted against APP, we investigate the direct contribution of APP and its derivatives to this behavioral deficit. After validating siRNAs targeting human APP in vitro, siRNAs were infused directly into the brain of APP23 mice for 2 weeks. Behavioral analysis shows that infusion of siRNA targeted against APP completely reverses increased exploratory activity in APP23 mice. Collectively, these data suggest that excessive APP and/or its derivatives, causes a hyperactive phenotype in APP23 mice when placed in a novel environment, which is fully reversible and not linked to plaque deposits.

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1. Introduction

Alzheimer's Disease (AD) is characterized by progressive cognitive decline and histopathological hallmarks including neurofibrillary tangles and plaques composed of the proteolytic derivatives of Amyloid Precursor Protein (APP), Amyloid β (A β) peptides (Selkoe, 2001). It is important to note that APP may also play a physiological role in synaptic plasticity and hippocampal-dependent learning and memory (Senechal et al., 2006). Based on the link between mutations in the gene *app* and familial early onset AD (Tanzi and Bertram, 2005), a variety of transgenic mouse lines overexpressing different forms of human APP (hAPP) have been created as models of AD and studied behaviorally (Kobayashi and Chen, 2005). One of these transgenic mice, referred to as APP23, overexpresses human APP751 with the Swedish double mutation (K670N/M671L) under the control of a neuron-specific Thy-1 promoter, which induces a strong neuronal expression especially in the

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Abbreviations: APP, Amyloid precursor protein; AD, Alzheimer's disease; siRNA, short interfering RNA; CHO, Chinese hamster ovary; YFP, Yellow fluorescent protein

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hippocampus and cerebral cortex (Sturchler-Pierrat et al., 1997).

APP23 mice develop amyloid plaques in the neocortex and hippocampus starting at the age of 6 months, and plaque load increases with age (Sturchler-Pierrat et al., 1997). These plaques are accompanied by additional AD-like histopathological features including microglial reactivity (Stalder et al., 1999), dystrophic neurites and hyperphosphorylated tau (Sturchler-Pierrat et al., 1997; Sturchler-Pierrat and Staufenbiel, 2000). In addition to the parenchyma, amyloid deposition also occurs in vessel walls leading to cerebral amyloid angiopathy (Calhoun et al., 1999; Winkler et al., 2001). APP23 animals exhibit modest neuronal loss in the CA1 subregion of the hippocampus (Calhoun et al., 1998), a reduction of total cholinergic fiber length (Boncristiano et al., 2002) and decreased activities of acetylcholinesterase and choline acetyltransferase in basal forebrain nuclei (Van Dam et al., 2005). These animals also display progressive age-related behavioral and learning impairments in passive avoidance and Morris water maze (Van Dam et al., 2003; Kelly et al., 2003; Lalonde et al., 2002; Dumont et al., 2004). In addition, APP23 transgenic mice

show overnight cage hyperactivity and altered exploration and activity levels in open field tests (Van Dam et al., 2003). Consistent with spatial memory deficits, APP23 mice are impaired in the acquisition phase of the Morris water maze task (Lalonde et al., 2002; Dumont et al., 2004). Thus, APP23 mice exhibit neuropathological and behavioral AD-like deficits which progress with age (Selkoe, 2001).

Since APP23 mice do not exhibit amyloid plaques before the age of 6 months (Sturchler-Pierrat and Staufenbiel, 2000), yet show increasing levels of APP and A β peptides at the age of 3 months (Capetillo-Zarate et al., 2006), we examined early behavioral deficits that could be caused directly by over-expression of APP and/or its derivatives. In the present study, to assess further the direct contribution of APP overexpression to the early behavioral deficits displayed by APP23 mice, APP was acutely downregulated in the brains of APP23 transgenic mice using a previously described siRNA-infusion approach (Senechal et al., 2007; Thakker et al., 2004). After siRNAs targeting human APP (hAPP-siRNAs) were validated in vitro, they were continuously infused for 2 weeks into three-monthold APP23 mice. Here we report the effects of APP knockdown by siRNA and confirm a direct role of APP/A β overexpression in

Si1 hAPP AUGAGUUUGGCAAACAUCGAT ++ TTUACUCCAAAGGGUUUGUAGG Si2 hAPP UUCACUAAUCAUGUUGGCCAAAA Si3 hAPP UUGCUUCACUUUGUCGCCCUCTT + TTAAGGAACUGCAAGACGGGAG Si4 hAPP UUCCUUGGUACACUUCGCGCCCUCT ++ TTAAGGAACCGUAUUAGUAAGGACGGAG Si5 hAPP UUCCUUGGUACAUCCAAGGAGAT +++ TTAAGGAACCGUAUUAGUUAGGUCG Si5 hAPP UUCCUUGGUACCAAGACGGGAG ++++ TTAAGGAACCGUAUUCAUGCAAGGGG ++++ Si6 TTAAGGAACCGUAUUAGUUCAUGGCGC Si7 hAPP UUCCUUGGUACAAGCAAGUGGGG +++ Si8 hAPP UUCCUUCGUACCAAGCAAGUGGGG +++ Si8 hAPP UUCCUUCGUACCAAGCUCAAGUACGGGAG +++ TTAACGUAAAGCCAAGUUUCAUGGUCGC Si8 hAPP UUCCUUGGUAAAGCAAGUGGGG +++ Si9 hAPP UUCCUUAGACCUUGGUCAAGAACUGGGG +++ Si10 hAPP UUCCUUAGGACUCAGUGGG +++ Si10 hAPP UUCCUUAGGACUCAGUGGG +++ Si11 hAPP UUCCGUAACGUAGGUGCGG +++ Si12 hAPP AUUCGUAAGGACUCAGGAACC Si12 hAPP AUUCGUAAGGACUAGGACC + SI12 hAPP AUCAGCUUGGGCACUAGGACC ++ TTAAGGAAUGAGCAAGUUGTT ++ SI12 hAPP AUCAGCUUAGGACCUAGGACC ++ TTAAGGAAUGAGCAAGUUCTT ++ MM5 hAPP mismatch UUCUGGGCACUACGGCGGGG STFAGGAAGUUGACUAGGGUCGC SIYFP YFP VIP UUGAAGUUCACUUGACUGGCG STAGGAAGUUGACUAAGGUUCACCUUGAGUGGG STFAGGCAGUUGACUUGACUGGGG STFAGGCAGUUGACUUGGUCGC SIYFP YFP UUGAAGUUCACUUGACUUGACUGGCGGCGCGCGCGCGCGC	Table 1 – Sequence of the siRNAs used in the human APP silencing study				
TTUACUCAAAGCGUUUGUAGG\$12hAPPUUCACUAAUCAUGUUGCCUAAAA\$13hAPPUUGCUUGACAGUCGCUCTT\$14TTAAGGAACCAAGACGAG\$14HAPPUUCCUUGACAUAUGUACAGGGAG\$15hAPPUUCCUUGGAAUAUGUCAGGGAT\$14TTAAGGAACCAUAGUUACGUC\$15hAPPUUCCUUGGCAAGACGGAGAT\$16TTAAGGAACCCAUAGUUACGUCC\$17MAPPUUCCUUGGCAAAGCAAGCCGGAT\$18hAPPUUCCUUGCCAAAGUACCACCGGAGGAT\$19hAPPUUCCUUUGCCAAAGCAACCAGUGAGGAC\$19hAPPUUGCACCUUUGUUUGAACAAGUAGCGC\$10hAPPUUCGCUAAACAACUUGG\$11hAPPUUCCGUAACAGCAGUUGCGGAAC\$12TTAAGGACUCAUGGACCAGUUGCUGGAAC\$13hAPPUUCCGUAACUGAUCGUGGAAC\$14TTAAGGACUCAGUAUGAGCACCAGUUCUACG\$15hAPPUUCCGUAACUGAUCCUUGGAT\$16TTAAGGACUGAGUCAGUACAAC\$17HAPPUUCCGUAACUGAUCAGGAAC\$18hAPPUUCCGUAACUGAUCCUUGGAT\$19hAPPUUCCGUAACUGAUCCUUGGAT\$11hAPPUUCCGUAACUGAUCCUUGGAT\$12hAPPUUCCGUAACUGAUCCUUGGAAC\$132hAPPUUCCGUAACUGAUCCUUGGAACC\$14TTTAAGGACCUUAAGCAUUGAGCAAGUUCTT\$15hAPPUUCCUGGGACUAGGCAUAGGAAC\$16hAPPUUCCUGGGACUAGGCAGGAAT\$17HAPPUUCCUGGGACUAGGCAGGAAT\$18hAPPUUCCUGGGACUAGGCAGUACGCGUCC\$19MAPUUCCGUAACUCGUUCAAG\$10MAPUUCCUGG	siRNA name	Gene targeted	Sequence (5'–3')	sRNA efficiency	
Si2 hAPP UUCACUAUCAUGUUGGCCdAdA ++ TTAAGUGAUUAGUACAACCCG TTAAGUGAUUAGUACAACCCG ++ Si3 hAPP UUGCUUGACGUUCUGCCUCTT + Si4 hAPP UUCCUUGGAACUCCAAGCGGAG ++ Si5 hAPP UUCCUUGGCAUUACAUCCAGGGAT +++ Si5 hAPP UUCCUUGGCAAUACUGCAGGGAT +++ Si6 hAPP UUCCUUUCGCAAAGUACCAGCGGGGGAT +++ Si7 hAPP UUCCUUUCGUCAAAGUACCAGCGGGGGG - Si8 hAPP UUCGUUUUCGUCAAAGACCGCGAG - Si9 hAPP UUCGUUUUUUUUUUUUUUUUUAACAACCGCAA +++ Si10 hAPP UUCGUUUUCGUCAGAUCAUUUCUGGT ++ Si11 hAPP UUCCGUAACUGAUCCUUGGGT +++ Si12 hAPP UUCCGUAACUGAUCCUUGGGT +++ Si12 hAPP UUCCGUAACUGAUCCUUGGGT +++ Si12 hAPP UUCCGUCGAAGUUCAUCGUUCAG +++ Si12 hAPP MUCCGUCGCAGUUAGGAACCC +++ MM5 hAPP MUCCGUCGCAGUUCAGGAACC +++ Si12 hAPP M	Si1	hAPP	AUGAGUUUCGCAAACAUCCdAT	++	
Si3 hAPP UUGCUUGCAGCGUCUCCCUCTT + Si4 hAPP UUCCUUGGUAUCAAUGCAAGGAGGT ++ Si5 hAPP UUCCUUGGUAUCAAUGCAGGAGT ++ Si5 hAPP UUCCUUGGUAUCAAUGCAGGGAGT +++ Si5 hAPP UUCCUUGGUAUCAAUGCAGGGAGT +++ Si6 hAPP UUCCUUUGGUUCAAUGCAGGGGGG +++ Si7 hAPP UUCCUUUGGUUCAAGGUCC - Si7 hAPP UUCCUUUGGUUCAAGCAGCGGGGG - Si8 hAPP UUCCUUUGUUUGGUCAAGCUCAGUACUUGGGC +++ Si9 hAPP UUCCAUAUCCUUGGUCAGUCAUGGGGGG ++ Si10 hAPP UUUCCUUGUUUGGUCAUGGUCAUGGGGGG ++ Si11 hAPP UUUCCUUAGUUCAUGGGT ++ Si12 hAPP UUUCCGUAACUGAUCCUUGGGT ++ Si12 hAPP UUUCCGUUAGGGAACACC - Si14 hAPP UUCCGUUAGGGCACUAGUACCUUGGGT ++ Si12 hAPP UUCCGUUAGGGCACUAGGACC - Si14 hAPP UUCCGUUAGGCACUAAGGACC - MM5 hAPP UUCCGU			TTUACUCAAAGCGUUUGUAGG		
Si3 hAPP UUGCUUGACGUUCUGCCUCTT + TTAACGAACUGCAAGACGGAG TTAACGAACUGCAUGCAGGGAG ++ Si4 hAPP UUCCUUGGCAUACUUACGUC ++ Si5 hAPP UUCUUGGCAUACUGACGGCACU +++ Si6 hAPP UUCCUUGGCAUACUGACGGGGAG +++ Si6 hAPP UUCCUUUGACGUUCAUGGUCC +++ Si7 hAPP UUCGUUUCAUGGUCAAGAUAGUGAGG - Si8 hAPP UUGCAUUUCAUGUUUGACCACGA ++ Si8 hAPP UUUCGUUUGACCAUGUACGAUA ++ Si9 hAPP UUUCGUUUGGACCAUUGUAGG ++ Si10 hAPP UUUCCUUAGGAUCAUUGAGAC ++ Si11 hAPP UUUCCGUAACUGAUCCUUGGGT ++ Si12 hAPP UUUCCGUAACUGAUCCUUGGAT ++ Si12 hAPP UUUCCGUAACUGAUCGUUCAGAACC ++ Si12 hAPP UUUCGGCACUUGACUCUUGGTT ++ MM5 hAPP mismatch UUUCGGCACUUAGGCACGUACGCAGGAAT - MM6 hAPP mismatch UUCCACUCAACGUAACGCAGGGGGGG - SiYFP YFP UUGAAGUUCACCUUGAGGAGGGCGGGGGGGGGGGGGGGG	Si2	hAPP	UUCACUAAUCAUGUUGGCCdAdA	++	
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Si6 hAPP UCACAUCAAAGUACCAGCGdGdG +++ Si7 hAPP UUCGUUUCAUGGUCAAAGUAGCAGCGC +++ Si8 hAPP UUGCACUUUGUUUGAUCGUCAAGUUUCUAC + Si8 hAPP UUGCACUUUGUUUGAUCGUCAGGAGA + Si9 hAPP UUGCACCUUUGUUUGAUCAUGGUCAG ++ Si9 hAPP UUGCACCUUUGUUGAGUCAUGAGACAACUAGG ++ Si10 hAPP UUUCCGUAACUGAUCCUUGGGT ++ Si11 hAPP UUCCGUAACUGAUCCUUGGGT ++ Si12 hAPP UUCCGUAACUGAUCCUUGGACC ++ Si12 hAPP UUCUGGGCACUUAGGCACC + Si12 hAPP MUCAGCUUUAGGCAGGACC + Control siRNAs UUCUGGGCACUUAGGCACUCAGGGAAT - MM5 hAPP mismatch UUCUGGGCACUAACGUGCGUCC - MM6 hAPP mismatch UCCACCUCAACGUAACAGCGGGGAT - TTAGGGAGUUGCAUUGCAUUGCGCC iTTAGUGGAGUUGCAUUGCAUUGCGCG - -			TTAAGGAACCAUAGUUACGUC		
Si6 hAPP UCACAUCAAAGUACCAGCGGGGG ++++ TTAGUGUAGUUUCAUGGUCGC Si7 hAPP UUCGUUUCGGUCAAAGAUGGGC - TTAAGCAAAGCCAGUUUCUAC Si8 hAPP UUCCAUCUUUGUUUGAACCGCAAAACUUGG Si9 hAPP UUCAUAUCCUGAGUCAUGUACGGAACAAACUUGG ++ Si10 hAPP UUUCCGUAACUGAGUCAUGAUCAUGGAACA Si11 hAPP UUUCCGUAACUGAUCCUUGGT + Si11 hAPP UUUCCGUAACUGAUCCUUGGT ++ Si12 hAPP AUAAGCAUUGACUAGGAACC Si12 hAPP MISMATCH UUCUGGUAACUGAUCCUUGGT ++ Si12 hAPP MISMATCH UUCUGGUAACUGAUCCUUGAGT +- Si12 hAPP MISMATCH UUCUGGCACUUAAGGACUCAUGGAACC Si12 hAPP MISMATCH UUCUGGCACUUAAGGCAUGAGCAUGAGCUCTT + Si12 hAPP MISMATCH UUCUGGCACUUAAGGCAUGAGCAUGAGCAUGACUCAUGAUCCUUGAGT +- Si12 hAPP MISMATCH UUCUGGCACUUAAGGAACC Si12 hAPP MISMATCH UUCUGGCACUUAAGGCAUGAGCAUGAGCUCTT +- SI14 SI14 SAPP SISMACH SUCUCUGGCACUUCAAGGAACC SAGUUCTT SSI14 SAPP SISMAS SAGUGAAAUCCGUUCAAGGAACC SAGUUCTT SSI14 SAPP SISMACH SUCUCGGCACUUAAGGCAUGGCAUGACUGAUGACUGAGGAACC SAGUUCTT SSI14 SAPP SISMACH SUCUCGGCACUUAAGGCAUGGCGUCC SAGUGAAGUGAAAUCCGUUCAAGGAACU SAGUGAAAUCCGUUCAAGGAAAUCCGUUCAAGG SAGUGCAUGACUGAGGAAAUCCGUUCAAGG SAGUGACUGAAAUCCGUUCAAGG SAGUGCAUGAAUCGUUCAAGG SAGUGCAUGAAUCCGUUCAAGG SAGUUCTT SSI5 SAPP SISMACH SUCCACCUCAACGUAACAGCGGAGAG SAGUGCAUGCCGUCC SAGUGAAUCCGUUCAAGG SAGUGCAUGCCGUCC SAGU SAGUUGAAUGCCUUCAAGG SAGU SAGUGAAUCGCUUCAAGG SAGU SAGUGAAUCGCUUCAAGG SAGU SAGUUGAAUGCCUUCAAGG SAGU SAGUGAAUCCGUUCAAGG SAGU SAGUUGAAUGCCUUCAAGG SAGU SAGUUGAAUGCCUUCAAGG SAGU SAGUUGAAUGCCUUCAAGG SAGU SAGUUGAAUGCCUUCAAGGAGU SAGUGAAUCGCUUCAAGGGAGG SAGU SAGUUGAAUGCCUUCAAGGAGU SAGUUGAAUUGCCC SAGU SAGUUGAAUUGCCUUAAGGAGUUGCAUUGCCG SAGU SAGUUGAAUUGCUUCGC SAGU SAGUUGAAUUGCAUUGCCG SAGUUGAAUUGCUUGAGUUGAAUUGCUUGCG SAGUUGAAUUGCUUGAGUUGAAUUGCUUGAGG SAGUUGAAUUGCUUGAGUUGAAUUGCAUUGUCGC SAGU SAGUUGAAUUGAUUGACGUUGAAUUGCUUGAGUUGAAUUGAUUG	Si5	hAPP	UUCUUGGCAAUACUGCAGGdAT	+++	
Si7 hAPP UUCGUUUCAUGGUCGC - Si8 hAPP UUGCACCUUUGUUUCAACGUCGA + Si9 hAPP UUGCAUCGUGAAAAGUUGGUCA + Si10 hAPP UUCCAUAUCCUGAGUAACUGGUCA + Si11 hAPP UUCCGUAACUGAUGACCUUGGGT + Si12 hAPP UUCCGUAACUGAUGACCAGUUCTT ++ Si12 hAPP UUCCGGCACUUAGGAACCAGUUCTT ++ Si12 hAPP UUCUGGGCACUAAGGAACCAGUUCTT + Si12 hAPP UUCUGGGCACUUAGGAACC + MM5 hAPP mismatch UUCUGGGCACUUAGCGCAGGAAT - MM6 hAPP mismatch UCACCUCAACGUAACGAGCGGGGAG - TTAAGUGGAAUUGCAUUGCAUUGCACCUUGAGCAGGUAG - - - SiYYYP YFP UUGAAGUUCACCUUGAUGCACGGGGAGG -			TTAAGAACCGUUAUGACGUCC		
Si7 hAPP UUCGUUUCGGUCAAGAUGdGdC - TTAAGCAAAGCCAGUUUCUAC + TTAACGUGGAAACAACUUGG + TTAACGUGGAAACAACUUGG + TTAACGUGGAAACAAACUUGG + TTAACGUGGAAACAAACUUGG + TTAAGGUAUGGACUAGUACG + TTAAGGUAUGGACUAGUACG + TTAAGGAUUGACUAGGACCAGUAC + TTAAGGCAUUGACUAGGAACCAGUAC + TTAAGGCAUUGACUAGGAACC + TTAAGGCAUUGACUAGGAACC + TTAAGGCAUUGACUAGGAACC + TTAAGGCAUUGACUAGGAACC + TTAAGGCAUUGACUAGGAACC + Si12 hAPP UUCCGUAACUGAUCCUUGGTT + MM5 hAPP mismatch UUCUGGGCACUACGGCAGGdAT - TTAAGACCCGUGAUGCCUUCAAG - MM6 hAPP mismatch UCACCUCAACGUACGCAGGGAGG - SiYFP YFP UUGAAGUUCACCUUGAUCCUUGAUGCCUGC -	Si6	hAPP	UCACAUCAAAGUACCAGCGdGdG	+++	
Si8 hAPP UUGCACCUUUGUUUGAACCdCdA + Si9 hAPP UUCAUAUCCUGAGUCAUGUdCdG ++ TTAAGGUAUAGGACUCAUGUdCdG ++ ++ Si10 hAPP UUCCQUAACUGAUCCUUGGGT ++ Si11 hAPP UUCCGUAACUGAUCCUUGGGT ++ Si11 hAPP UUCCGUAACUGAUCCUUGGGT ++ Si12 hAPP UUCCGUAACUGAUUCCUUGGACC ++ Si12 hAPP UUCCGUAACUCGUUCAAG ++ MM5 hAPP mismatch UUCUGGGCACUACGGCAGGAAT - MM6 hAPP mismatch UUCCUGAACUGAUGCAUUGACAGGUAGGGAGGGGGGGGGG			TTAGUGUAGUUUCAUGGUCGC		
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TTAACGUGGAAACAAACUUGGSi9hAPPUUCAUAUCCUGAGUCAUGUdCdG++Si10hAPPUUUCCGUAACUGAUCCUUGdGT+Si11hAPPUUCCGUAACUGAUCCUUGGTT++Si12hAPPUUCCGUAACUGAUCCUUGGTT++Si12hAPPAUCAGCUUUAGGCAAGUUCTT+Si2hAPPAUCAGCUUUAGGCAAGUUCTT+Si2hAPPAUCAGCUUUAGGCAAGUUCTT+Si2hAPP mismatchUUCUGGGCACUACGGCAGGdAT-Control siRNAsMM5hAPP mismatchUUCUGGGCACUACGGCAGCGUCCMM6hAPP mismatchUCACCUCAACGUAACAGCGGGGGGGGGGGGGG-SiYFPYFPUUGAAGUUCACCUUGAUGCCGUCC-			TTAAGCAAAGCCAGUUUCUAC		
Si9 hAPP UUCAUAUCCUGAGUCAUGUdCdG ++ TTAAGUAUAGGACUCAGUACA Si10 hAPP UUUCCGUAACUGAUCCUUGGT + TTAAAGGCAUUGACUAGGAAC Si11 hAPP UUCCGUAACUGAUCCUUGGTT ++ Si22 hAPP APP AUCAGCUUUAGGAACC Control siRNAS MM5 hAPP mismatch UUCUGGGCACUACGGCAGGAT - TTAAGACCCGUGAUGCCGUCC MM6 hAPP mismatch UCACCUCAACGUACAGGAGGG - TTAGUGCGAGUUGCAUUGUCGC SiYFP YFP UUGAAGUUCACCUUGAUGCCGUCC -	Si8	hAPP	UUGCACCUUUGUUUGAACCdCdA	+	
TTAAGUAUAGGACUCAGUACA TTAAGUAUAGGACUCAGUACA Si10 hAPP UUUCCGUAACUGAUCCUUGGT + TTAAAGGCAUUGACUAGGAAC TTAAAGGCAUUGACUAGGAAC ++ Si11 hAPP UUCCGUAACUGAUCCUUGGTT ++ Si12 hAPP AUCAGCUUUAGGCAAGUUCTT + Control siRNAs TTAAGACCCGUGAUGCCGUCC + MM5 hAPP mismatch UUCUGGGCACUACGCAGGAGT - MM6 hAPP mismatch UCACCUCAACGUAACAGCGGGGGG - siYFP YFP UUGAAGUUCACCUUGAUGCCGUCG -			TTAACGUGGAAACAAACUUGG		
Si10 hAPP UUUCCGUAACUGAUCCUUGGT + TTAAAGGCAUUGACUAGGAAC Si11 hAPP UUCCGUAACUGAUCCUUGGTT ++ TTAAGGCAUUGACUAGGAACC Si22 hAPP AVCAGCUUUAGGCAAGUUCTT + TTUAGUCGAAAUCCGUUCAAG Control siRNAS MM5 hAPP mismatch UUCUGGGCACUACGCCAGGAT - TTAAGACCCGUGAUGCCGUCC MM6 hAPP mismatch UCACCUCAACGUAACAGCGdGdG - TTAGUGGAGUUGCAUUGUCGC siYFP YFP UUGAAGUUCACCUUGAUGCdCdG -	Si9	hAPP	UUCAUAUCCUGAGUCAUGUdCdG	++	
Si11 hAPP UUCCGUAACUGAUCCUUGGTT ++ Si12 hAPP AUCAGCUUUAGGAACC + Si12 hAPP AUCAGCUUUAGGCAAGUUCTT + Control siRNAs TTAAGGCCAUUGACUACGGAGCU + MM5 hAPP mismatch UUCUGGGCACUACCGCAGGdAT - MM6 hAPP mismatch UCACCUCAACGUACGGCGGGdGG - siYFP YFP UUGAAGUUCACCUUGAUGCCGUCG -			TTAAGUAUAGGACUCAGUACA		
Si11 hAPP UUCCGUAACUGAUCCUUGGTT ++ TTAAGGCAUUGACUAGGAACC Si12 hAPP APP AUCAGCUUUAGGCAAGUUCTT + TTUAGUCGAAAUCCGUUCAAG + Control siRNAs MM5 hAPP mismatch UUCUGGGCACUACGGCAGGAT - TTAAGACCCGUGAUGCCGUCC MM6 hAPP mismatch UCACCUCAACGUAACAGCGdGdG - TTAGUGGAGUUGCAUUGUCGC siYFP YFP UUGAAGUUCACCUUGAUGCdCdG -	Si10	hAPP	UUUCCGUAACUGAUCCUUGdGT	+	
Si12 hAPP AUCAGCUUUAGGAACCC + Si12 hAPP AUCAGCUUUAGGCAAGUUCTT + Control siRNAs TTUAGUCGAAAUCCGUUCAAG - MM5 hAPP mismatch UUCUGGGCACUACGGCAGGdAT - MM6 hAPP mismatch UCACCUCAACGUAACGCGGGdGdG - TTAGUGGAGUUGCAUUGUCGC TTAGUGGAGUUGCAUUGUCGC - siYFP YFP UUGAAGUUCACCUUGAUGCdCdG -			TTAAAGGCAUUGACUAGGAAC		
Si12 hAPP AUCAGCUUUAGGAACCC + Si12 hAPP AUCAGCUUUAGGCAAGUUCTT + Control siRNAs TTUAGUCGAAAUCCGUUCAAG - MM5 hAPP mismatch UUCUGGGCACUACGGCAGGdAT - MM6 hAPP mismatch UCACCUCAACGUAACGCGGGdGdG - TTAGUGGAGUUGCAUUGUCGC TTAGUGGAGUUGCAUUGUCGC - siYFP YFP UUGAAGUUCACCUUGAUGCdCdG -	Si11	hAPP	UUCCGUAACUGAUCCUUGGTT	++	
Control siRNAs Control siRNAs MM5 hAPP mismatch UUCUGGGCACUACGGCAGGdAT - TTAAGACCCGUGAUGCCGUCC MM6 hAPP mismatch UCACCUCAACGUAACAGCGdGdG - TTAGUGGAGUUGCAUUGUCGC siYFP YFP UUGAAGUUCACCUUGAUGCdCdG -					
Control siRNAs Control siRNAs MM5 hAPP mismatch UUCUGGGCACUACGGCAGGdAT - TTAAGACCCGUGAUGCCGUCC MM6 hAPP mismatch UCACCUCAACGUAACAGCGdGdG - TTAGUGGAGUUGCAUUGUCGC siYFP YFP UUGAAGUUCACCUUGAUGCdCdG -	Si12	hAPP	AUCAGCUUUAGGCAAGUUCTT	+	
MM5 hAPP mismatch UUCUGGGCACUACGGCAGGdAT – TTAAGACCCGUGAUGCCGUCC MM6 hAPP mismatch UCACCUCAACGUAACAGCGdGdG – TTAGUGGAGUUGCAUUGUCGC siYFP YFP UUGAAGUUCACCUUGAUGCdCdG –					
MM5 hAPP mismatch UUCUGGGCACUACGGCAGGdAT – TTAAGACCCGUGAUGCCGUCC MM6 hAPP mismatch UCACCUCAACGUAACAGCGdGdG – TTAGUGGAGUUGCAUUGUCGC siYFP YFP UUGAAGUUCACCUUGAUGCdCdG –	Control siRNAs				
MM6 hAPP mismatch UCACCUCAACGUAACAGCGdGdG - TTAGUGGAGUUGCAUUGUCGC TTAGUGGAGUUGCAUUGUCGC - siYFP YFP UUGAAGUUCACCUUGAUGCdCdG -	MM5	hAPP mismatch	UUCUGGGCACUACGGCAGGdAT	_	
MM6 hAPP mismatch UCACCUCAACGUAACAGCGdGdG – TTAGUGGAGUUGCAUUGUCGC siYFP YFP UUGAAGUUCACCUUGAUGCdCdG –					
siYFP YFP UUGAAGUUCACCUUGAUGCdCdG –	MM6	hAPP mismatch		_	
siYFP YFP UUGAAGUUCACCUUGAUGCdCdG –					
	siYFP	YFP		_	
TTAACUUCAAGUGGAACUACG			TTAACUUCAAGUGGAACUACG		

Table indicating the siRNA sequences used in the study. Sequences of the antisense strand (upper sequence, 5'–3' orientation) and of the complementary sense strand (lower sequence, 3'–5' orientation) are indicated for each siRNA. Two-deoxyribonucleotide overhangs (dA, dG, dC or T) are placed at the 3' end of each siRNA strand. The abbreviation hAPP stands for human APP. Underlined bases indicate mismatch harbored by the control siRNAs as compared to match siRNA. The efficacy of APP knockdown in our co-transfection assay is indicated as follows: – no effect, + to +++ indicates increasing APP silencing efficiency.

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