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Supplementary Data

Measurement of Altered AβPP Isoform Expression in Frontal Cortex of Patients with Alzheimer's Disease by Absolute Quantification Real-Time PCR

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AβPP 695 Forward
AAAAACTGAATGATGACGAGGAGATGAGGATGGTGATGAGGTAGAGGAAGAGGCTGAGGAACCCTACGAAGAAGCCAC
AGAGAGAACCACCAGCATTGCCACCACCACCACCACCACCACAGAGTCTGTGGAAGAGGTGGTTCGAGTTCCTACAACA
GCAGCCAGTACCCCTGATGCCGTTGACAAGTATCTCGAGACACCTGGGGATGAGAATGAACATGCCCATTTCCAGAAAG
CCAAAGAGAGGCTTGAGGCCAAGCACCGAGAGAAA

AβPP 695 Reverse
CTTGGCTTCTGGAATGGGCATGTTCATTCTCATCCCCAGGTGTCTCGAGATACTTGTCAACGGCATCAGGGGTACTGGCT
GCTGTTGTAGGAACTCGAACCACCTCTTCCACAGACTCTGTGGTGGTGGTGGTGGTGGTGGCAATGCTGGTGGTTCTCT
CTGTGGCTTCTTCGTAGGGTTCCTCAGCCTCTTCCTCTACCTCATCACCATCCTCATCGTCCTCGTCATCATCGGCTTCTT
CTTCTTCCACCTCAGCCACTTCTTCCTACTAAAA

AβPP 751 Forward
TTAGGTGATGAANGGGAGTGTGCCCCATTCTTTTACGGCGGATGTGGCGGCAACCGGAACAACTTTGACACAGAAGAGT
ACTGCATGGCCGTGTGTGGCAGCGCCATTCCTACAACAGCAGCCAGTACCCCTGATGCCGTTGACAAGTATCTCGAGAC
ACCTGGGGATGAGAATGAACATGCCCATTTCCAGAAAGCCAAAGAGAGGCTTGAGGCCAAGCACCGAGAGAA

AβPP 751 Reverse
CTTTGCATTCTGGAATGGGCATGTTCATTCTCATCCCCGGTGTCTCGAGATACTTGTCAACGGCATCAGGGGTACTGGCT
GCTGTTGTAGGAATGGCGCTGCCACACACGGCCATGCAGTACTCTTCTGTGTCAAAGTTGTTCCGGTTGCCGCCACATC
CGCCGTAAAAGAATGGGGCACACTTCCCTTCAGTCACATCAAAGTACCAGCGGGAGATCATTGCTCGGCAAAA

AβPP 770 Forward
GGCGGTCGAACATGATCTCCCGCTGGTACTTTGATGTGACTGAAGGGAAGTGTGCCCCATTCTTTTACGGCGGATGTGG
CGGCAACCGGAACAACTTTGACACAGAAGAGTACTGCATGGCCGTGTGTGGCAGCGCCATGTCCCAAAGTTTACTCAAG
ACTACCCAGGAACCTCTTGCCCGAGATCCAAAA

AβPP 770 Reverse
GGCATGGATAACTTTGGGACTGGCGCTGCCACACACGGCCATGCAGTACTCTTCTGTGTCAAAGTTGTTCCGGTTGCCG
CCACATCCGCCGTAAAAGAATGGGGCACACTTCCCTTCAGTCACATCAAAGTACCAGCGGGAGATCATTGCTCGGCACG
GCCCCGTCTCGGCTTGTTCAGAGCACACAAAA

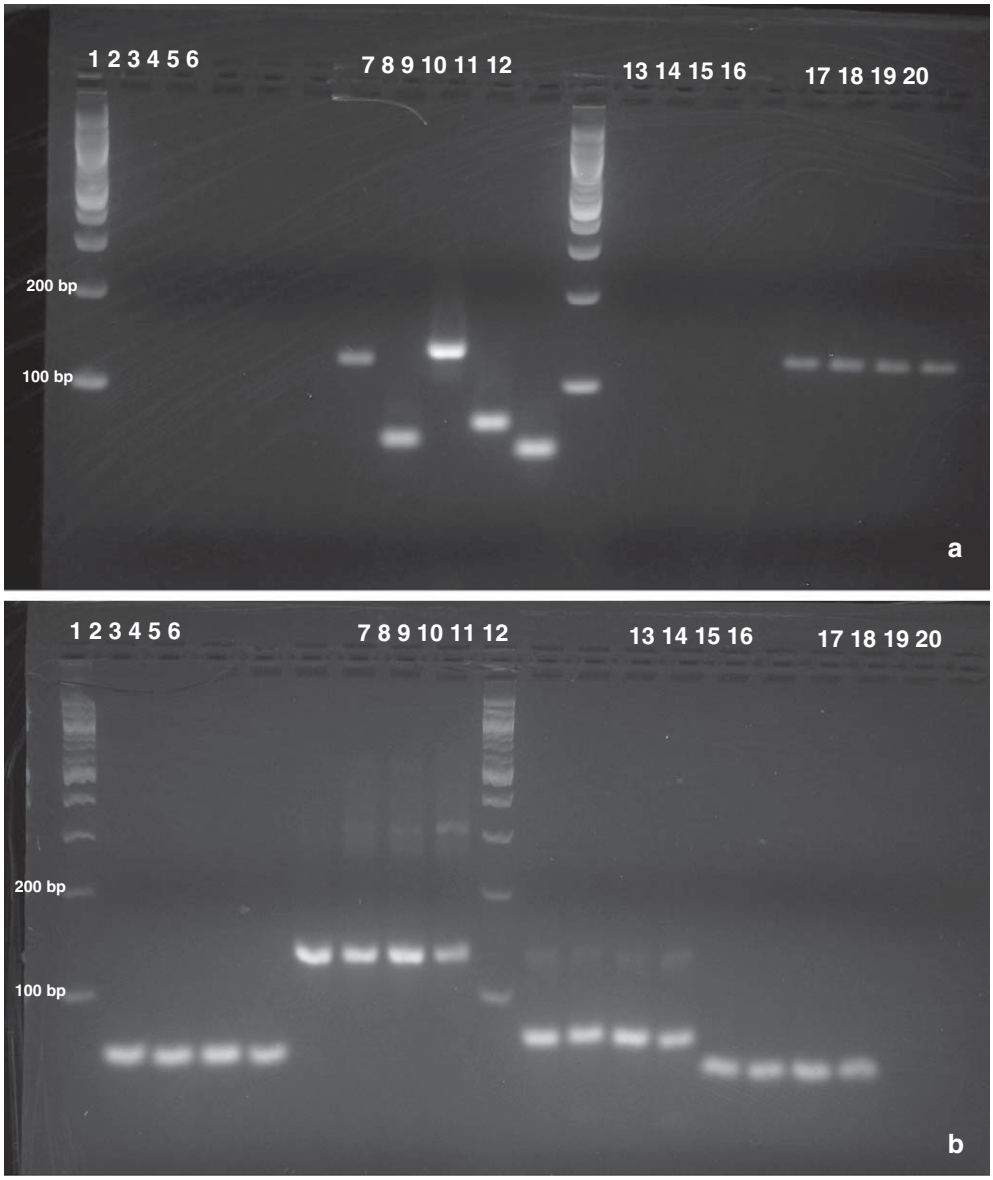
AβPP-common Forward
TCATCCGACGACATGGCCCCTGGAAACTACATCACCGCTCTGCAGGCTGTTCCTCCTCGGCCTCGTCACGTGTTCAATAT
GCTAAAGAAGTATGTCCGCGCAGAACAGAAGGACAGACAGCACACCCTAAAGCATTTCGAGCATGTGCGCATGGTGGAT
CCCAAGAAAGCCGCTCAGATCCGGTCCCAGGTTATGACACACCTCCGTGTGATTTATGAGCGCATGAATCAGTCTCTCAA
AA

AβPP-common Reverse
GGAGAGGTGTGTCTAACCTGGGGACCGGATCTGACGGTTTCTTGGGACCCCATGCGCACATGCTCGAAATGCTTTAGG
GTGTGCTGTCTGTCCTTCTGTTCTGCGCGGACATACTTCTTTACATATTGAACACGTGACGAGGCCGAGGAGGAAAGCCT
GCAGAGCGGTGATGTATTCTCCAGGGCCAGGCGGCGGCGGTCATTGAGCATGGCTTCCACTCTGGCCTGA

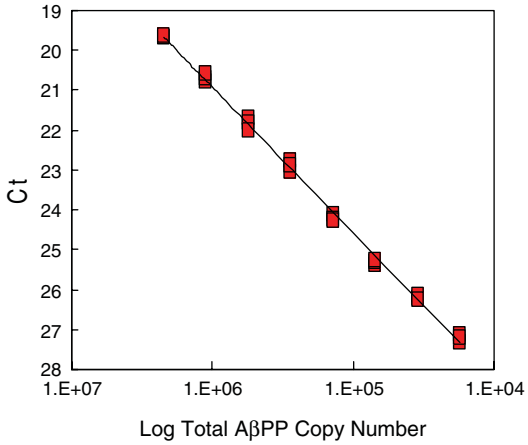
GAPDH Forward
CCCTTATTTGGTCGCAGCCGACCACTCGCTGAGACCATGGGGAAGGGAAGTCGGATCAACGGATTTGGTCGTATTGGG
CGCCTGGTCACCAGGGCTGCTTTTAACTCTGGTAAAGTGGATATTGTTGCCATCAATGACCCCTTCATTGACCTCAACTA
CATGGTTTACATGTTCCAATATGATTCCACCCATGGCAAATTCCATGGCACCGTCAAGGCTGAGAACGGGAAGCTTGTCA
TCAATGGAAATCCCATCACCATCTTCCAAAA

GAPDH Reverse
GTAGCTTTCCGNTTCCAGCCTTGACGGTGCCATGGAATTTGCCATGGGTGGAATCATATTGGAACATGTAAACCATGTAG
TTGAGGTCAATGAAGGGGTCATTGATGGCAACAATATCCACTTTACCAGAGTTAAAAGCAGCCCTGGTGACCAGGCGCC
CAATACGACCAAATCCGTTGACTCCGACCTTCACCTTCCCCATGGTGTCTCAGCGATGTGGCTCGGCTGGCGACGCAAA
AGAAGATGCGGCTGACTGTCGAACAGGAGGAGCAGAAAA

Supplementary Figure 1. Sequencing of AQ-PCR standards from each primer pair. Each sequence is shown 5′ to 3′ and all were the correct amplicon.



Supplementary Figure 2. Gel electrophoresis of AQ-PCR standards and brain samples before and after TaqMan amplification. a) 4% agarose gel with ethidium bromide under UV light: Lane 1) 100 bp DNA ladder. Lanes 2–6) Unamplified AQ-PCR standards: GAPDH, AβPPcm, AβPP695, AβPP751, AβPP770. Lanes 7–11) Post-TaqMan amplification of AQ-PCR standards (predicted amplicon size in bp): GAPDH (122), AβPPcm (66), AβPP695 (132), AβPP751 (75), AβPP700 (60). Lane 12) 100 bp DNA Ladder. Lanes 13–16) Unamplified brain tissue cDNA: ND CBL, ND SFG, AD CBL, AD SFG. Lanes 17–20) Post-GAPDH TaqMan amplification: ND CBL, ND SFG, AD CBL, AD SFG. b) 4% agarose gel with ethidium bromide under UV light: Lane 1) 100 bp DNA ladder. Lanes 2–5) Post-AβPPcm TaqMan amplification (predicted amplicon size 66 bp): ND CBL, ND SFG, AD CBL, AD SFG. Lanes 6–9) Post-AβPP695 TaqMan amplification (predicted amplicon size 132 bp): ND CBL, ND SFG, AD CBL, AD SFG. Lane 10) 100 bp DNA Ladder. Lanes 11–14) Post-AβPP751 TaqMan amplification (predicted amplicon size 75 bp): ND CBL, ND SFG, AD CBL, AD SFG. Lanes 15–18) Post-AβPP770 TaqMan amplification (predicted amplicon size 60 bp): ND CBL, ND SFG, AD CBL, AD SFG. Lanes 19–20) No template controls for TaqMan reactions.



Supplementary Figure 3. Representative absolute quantification PCR standard curve for total AβPP transcripts. $r^2 = 0.9970$ for the linear regression of log copy number versus threshold cycle (Ct). Each standard value was run in triplicate for each assay.