

Supplementary Material

A Novel Heterozygous Missense Variant in the CIAO1 Gene in a Family with Alzheimer's Disease: The Val67Ile Variant Promotes the Interaction of CIAO1 and Amyloid- β Protein Precursor

MATERIALS AND METHODS

Expression constructs

The coding regions of human *APP* and *CIAO1* cDNAs were amplified using polymerase chain reaction, and then subcloned into the *NheI* and *SgfI* sites of the pFN21 (Promega) and pUY-3 \times Flag (home-made) vectors, respectively. *In vitro* mutagenesis of the CIAO1 (V67I)-expressing plasmid was performed by PCR-based methods. We verified the complete nucleotide sequences of the expression plasmids.

Cell culture, transfection, and membrane fractionation

HEK293T cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% newborn calf serum and a non-essential amino acids solution (Thermo Fisher Scientific). DNA transfection was performed by the polyethyleneimine-based method [1] using PEI MAX reagents (Polysciences). Briefly, plasmid DNA (2 μ g) was complexed with 10 μ g of PEI MAX reagent in 0.2 ml of OPTI-MEM (Thermo Fisher) for 20 min at room temperature, and then added to a HEK293T cell culture in a 35 mm diameter dish. After 24 h of transfection, cells were harvested in lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA) supplemented with

cOmplete™ protease inhibitor cocktail (Roche), and then lysed with a short pulse (1 s) of sonication. Cell debris and nuclei were removed by centrifugation at $1,000 \times g$ for 10 min. The postnuclear supernatant was centrifuged at $100,000 \times g$ for 1 h to separate the membrane (pellet) and cytosol (supernatant) fractions.

Immunoprecipitation and western blotting

The membrane fraction was solubilized in the lysis buffer containing 1% Triton X-100, and then subjected to immunoprecipitation. Protein samples were mixed with anti-DDDDK antibody-coupled magnetic beads (MBL) for 1 h, and then the immunoprecipitates were washed extensively with the lysis buffer containing 1% Triton X-100. Proteins were eluted from the beads in the Laemmli sample buffer and then subjected to SDS-PAGE. The separated proteins were transferred to PVDF membranes (Millipore). Anti-DDDDK-tag (MBL) and anti-APP (Cell Signaling Technology, rabbit monoclonal antibody, E8B3O) antibodies were used for western blotting. The antibody-targeted proteins were detected using Immobilon Forte chemiluminescence HRP substrate (Millipore) and imaged using an ImageQuant LAS 4000 system (Cytiva).

Gene dosage study using comparative genomic hybridization (CGH) arrays

The DNA sample of the proband was compared with the universal control DNA sample of NA10851 (Male). Array CGH (aCGH) was designed in a 2x400K format using the Agilent SureDesign website (<https://earray.chem.agilent.com/suredesign/>, AMADID# 021529). CGH data

were extracted from scanned images (TIFF files) using Feature Extraction software (v11.0.1.1) (Agilent Technologies). We defined gains and losses over a continuous three probes and as a linear log₂ ratio average of ≥ 0.25 or ≤ -0.25 , respectively.

Whole-exome sequencing (WES) study

We carried out WES of genomic DNA from the proband. The genomic DNA was isolated from peripheral blood leukocytes. Exome capture was performed with a SureSelect Human All Exon V6+UTR (89Mb) Kit (Agilent Technologies, Santa Clara, CA, USA). Paired-end sequencing was carried out on a HiSeq2500 (Illumina, San Diego, CA, USA) using a HiSeq SBS Kit V4 (Illumina), which generated 100-bp reads. The average and minimum sequencing depths were 125 \times and 20 \times , respectively. The reference databases utilized included hg38 (GRCh38) (<http://genome.ucsc.edu>), HGMD (<https://portal.biobase-international.com>), GnomAD (<http://gnomad.broadinstitute.org>), and dbSNP (<https://www.ncbi.nlm.nih.gov/snp/>). Genes known to be associated with or as risk factors for the conditions of Alzheimer's dementia, frontotemporal dementia, parkinsonism, and amyotrophic lateral sclerosis are summarized below:

DCTN1 PRKN FLNC ABCA7 MAPT NPC1 LRRK2 TGM6 ATXN2 STH APP GRN PSEN1 PSEN2 APOE CR1 BIN1 SORL1 CHMP2B PSENEN TREM2 SETX SIGMAR1 EWSR1 SPG11 GAK OGG1 GRIN2B MS4A7 TREML2 TREML4 MS4A1 VCP CHCHD10 CD33 TARDBP PRNP IL6 OPTN TBK1 THBS2 MKL2 DAAM2 PLEKHG5 CLECL1 CTNNA1 CD163L1 AKAP9 GALR3 MIEF1 INPP5D EPHA1 UNC5C NME8 PLCD1 UNC13C CD2AP CLU CHRN2B CHRNA4 EPHA5 CDH2 EPHA6 CRMP1 PIN1 FUS C9orf72 PCDH11X NAMPT CPE CSF1R UBQLN2 SERPINI1 ADAM17 ERMP1 VLDLR SRCAP APH1A HFE NLGN3 MS4A6A CALHM1 TFCEP2 CDKN1A CREB1 AGER TNF ABCG2 A2M AR IL6R CYP46A1 NOS1 MAPK8IP1 RTN3 MME CTSD CFH LPL CHAT ABCA1 GSTM3 SERPINA3 IL1B NCSTN BLMH PPP2R2B SLC19A1 BDNF ATP7B PICALM CST3 GSK3B ADRB2 TP53 CASP8 OTC MS4A6E TF IL1A TYROBP CTSF VPS35 MARK4 SLC30A3 MEOX2 FPR2 KLK1 SQSTM1 PLD3 GSTT1 XBPI ABI3 LRP2 CAV1 BACE1 CYP19A1 CTNNA3 MPO ADAM10 PRKCA TTC3 TCOF1 MLKL AMBN MTHFR

AXIN1 TNK1 TM2D3 TRIML2 TOMM40 TRAK2 MYH13 PGBD1 GALP ACAN LRP6 LRPAP1 TFAM IP6K3 NOTCH3 TSHZ3
CASP7 PLCG2 ESR2 COMT OLR1 ARMS2 ADAM9 ECE1 ARC DRD1 FGF1 VEGFA DRD3 CCR1 CETP HTR2C LRP1 DIO2
ESR1 CCL11 APOC1 MIR146A ADRA2B PNMT APBB3 LAMA1 DAPK1 PLAU NGF IDE HMGCR NGFR VDR NTRK1 NRG1
TLR2 PNP PCK1 GBA ZNF224 IL18 PGRN TDP hmRNPA1 hmRNPA2 hmRNPA2B1 UBQLN SNCA UCHL1 PINK1 ATP13A2
GIGYF2 HTRA PLA2G6 FBXO7 RAB29 EIF4G1 DNAJC6 SYNJI DNAJC13 CHCHD2 ABCB1 SCN9A MAOB SLC6A4 CYP2D6
NQO2 PITX3 HMOX2 XRCCI FMRI DRD4 CHRNB3 HNMT HTRA2 HSPA1A POLG ADH1C XRCC3 CPXM1 RAD51B
ZFYVE26 RIC3 GLUD2 SLURP1 RUNDC3A COL12A1 EPPK1 ANKRD13A PAPD4 PMEL VPS53 SLC5A9 KCNV2 FBXL17 LCT
SLC52A1 MKSI PEPD PTEN MGA ACMSD VAPB PACRG RAB39B MTX1 HLA-DRA SLC6A3 NDUFV2 ERBB2 GCHI COQ2
HMOX1 SLC18A2 VPS13C PODXL SLC39A14 CP SLC30A10 SPR ATP6AP2 TNK2 GSTP1 TYR SLC2A1 PRRT2 CACNA1A
CLCN1 CLCN2 PIGT TERC PNKD CLEC7A IL10 ATG12 SNPD1 PTK2B TBP SIRT1 OVOS2 MS4A6ATV1 TMEM230 HSPA9
SLC41A1 SCARB2 EEF1D PLXNA4 LAMP2 NOD2 ATG7 FTH1 SNCAIP ARSBTV2 ATG5 GPATCH2L PTPRH UHRF1BP1L
PARL TNR TNK2TV1 NPC2 APTXBSN PUS1 SHC2 ZNF231 COQ10D1 NACP MLASA1 CLN11 SHCB MSA1 SCK CXCL8 ICAM-
1 EIF4EBP1 SLC1A4 CAV2 COX5B FGFR2 LAMA4 LTBP1 MAP1A PDGFC PSMB5 SPOCK TNFAIP TNFRSF21 HBB ABCA8
ACT RAB7L1 ADH7 UCHL-1 DM2 DBH USP24 AAOPD TH DDC ADORA1 DRD2 GLIS1 LINGO1 MCCC1 NR4A2 PDE8B
PTRHD1 RIT2 PANK2 PARK7 PSAP NOS3 CYLD TIA1 CCNF

REFERENCE

- [1] Longo PA, Kavran JM, Kim MS, Leahy DJ (2013) Transient mammalian cell transfection with polyethylenimine (PEI). *Methods Enzymol* **529**, 227-240.