Supplementary Tables

Supplementary Table S1. Clinical features of patients in Family A.

Patient ID	III:1	III:3	III:5
Sex	Female	Male	Male
Age at onset (years)	72	69	57
Age at first exam (years)	79	73	64
Disease duration (years)	10	13	10
Age of death	82	82	67
Cause of death	Pneumonia	Asphyxia caused by foreign body aspiration	Unknown
Clinical features			
Initial symptoms	episodic memory loss	episodic memory loss	episodic memory loss
Orientation dysfunction	Yes	Yes	Yes
Language problems	Yes	Yes	Yes
Changes in personality and behavior	Social withdrawal/distrust in others/Irritability and aggressiveness/Changes in sleeping habits	Social withdrawal/distrust in others/Irritability and aggressiveness/Changes in sleeping habits	Distrust in others/Irritability and aggressiveness/Changes in sleeping habits
Executive disfunction	Yes	Yes	Yes
Neurological examination			
Muscle strength	Normal	Normal	Normal
Muscle tone	Normal	Normal	Normal
Tremor	-	-	-
Tendon reflexe	++	++	++
Babinski’s sign	-	-	-

Neuropsychological tests			
MMSE (first evaluation)	17/30	19/30	6/30
MoCA (first evaluation)	10/30	12/30	Can't finish
Other examinations			
Blood pressure	Normal	Normal	Normal
Thyroid hormones	N/A	Normal	Normal
VitB12	N/A	Normal	Normal
TPHA and RPR test	N/A	-	-
Brain MRI	N/A	Atrophy in widespread cortex and hippocampus	Lacunar stroke and whole brain atrophy
FDG-PET	N/A	Hypometabolic activity in temporoparietal, frontal and occipital cortices	N/A
PiB-PET	N/A	Increased 11C-Pittsburgh compound B (PiB) accumulation	N/A
Autospy	N/A	Senile Plaque（+）; Neurofibrillary Tangle（+）	N/A

TPHA:a passive particle agglutination assay for the qualitative and semi-quantitative detection of IgG and IgM antibodies to Treponema pallidum.

RPR:rapid plasma regain test；

N/A: not available

Supplementary Table S2. Clinical features of controls in Family A.

ID	Year of Birth	Age of last examination	Muscle strength	Muscle tone	Tremor	Tendon reflexe	Babinski's sign	Years of education	MMSE	MoCA
III:2	1930	83	N	N	-	-	-	< 6 years	22	17
III:4	1942	70	N	N	-	-	-	< 6 years	22	18
III:6	1952	61	N	N	-	-	-	< 6 years	24	20
III:7	1939	73	N	N	-	-	-	< 6 years	21	19
III:8	1942	70	N	N	-	-	-	< 6 years	22	17
III:9	1944	68	N	N	-	-	-	< 6 years	25	18
III:10	1946	66	N	N	-	-	-	< 6 years	24	21
III:11	1948	64	N	N	-	-	-	9 years	26	24
III:12	1940	72	N	N	-	-	-	< 6 years	21	19
III:13	1942	70	N	N	-	-	-	< 6 years	22	18
III:14	1958	61	N	N	-	-	-	9 years	30	27

N=normal; "-"= absent

MMSE: Chineses version of Mini-Mental State Examination. The cut-off points were 16/17 for illiterate individuals, 19/20 for individuals with 1-6 years of education, and 23/24 for individuals with 7 or more years of education. MoCA: Montreal Cognitive Assessent Beijing Version. The cut-off scores for AD screening were 13 for individuals with no more than 6 years of education, 15 for individuals with 7 to 12 years of education, and 16 for individuals with more than 12 years of education.

Supplementary Table S3. Filtering of the candidate genes for Family A by whole-exome sequencing.

Filter	III:1	III:3	III:5	III:2
Status	Patient	Patient	Patient	Control
Total number of variants	108081	113406	112478	107568
Number of NS/SS/Indel[1]	13889	15280	15088	15070
Number of rare NS/SS/Indel variants[2]	2262	2261	2103	2073
Shared NS/SS/Indel variants in 3 cases	54 variants			
Number of variants presented in cases but not in unaffected[3]	20 variants			
Number of variants after removing non-dementia associated genes[4]	16 SNV			
Number of variants after removing variants not segregated with disease status[5]	3 SNV			

1. NS/SS/Indel, non-synonymous/splice acceptor and donor site/insertions or deletions variants;
2. Based on the hypothesis that the mutation underlying Family A with dementia should not be common in the general population, we removed the NS/SS/Indel variants with MAF **>** 0.5% in the Genome Aggregation Database (gnomAD), ExAc Database, the 1000 Genome Project, the eight previously exome-sequenced HapMap samples ('HapMap 8'), the BGI in-house exome variant dataset, and the PVFD database (Population variation frequency database), which are based on the Chinese population. BGI In-house database: sequence data from 1000 controls from a research on diabetes in BGI-Shenzhen.
3. In this step, NS/SS/Indel variants identified in the control WES data were excluded from the gene list.
4. In this step, we removed 4 known disease-causing genes because the clinical symptoms associated with them did not match the phenotype of family A.
5. In this step, we removed 13 variants because they are not co-segregated with disease status.

Supplementary Table S4. Variants of known non-dementia associated pathogenic genes.

Gene	Position[a]	RefSeq	Mutation type	cDNA change	Protein change	Phenotype	Inheritance	OMIM
CUBN	16877124	NM_001081.3	hete	c.10251T>A	N3417K	Megaloblastic anemia-1	AR	602997
CEP57	95561049	NM_001243776.1	hete	c.958A>G	S320G	Mosaic variegated aneuploidy syndrome 2	AR	607951
TBP	170871055	NM_003194.4	hete	c.231_264del	Q77fs	Spinocerebellar ataxia 17	AD	600075
FLT4	180057281	NM_002020.4	hete	c.457G>A	A153T	Lymphatic malformation-1	AD	136352

Supplementary Table S5. Identification of the candidate variants for Family A by exome sequencing.

Gene	Chr	Position[a]	RefSeq	cDNA change	Protein change	Mutation type	REVE score	SIFT	Polyphen2	Mutation Taster
ECE-2	**chr3**	**183995194**	**NM_001100120.1**	**c.556C>T**	**R186C**	**hete**	**0.89**	**D**	**D**	**D**
YEATS2	chr3	183470023	NM_018023.4	c.1132G>A	D378N	hete	0.114	D	P	N
APEH	chr3	49718618	NM_001640.3	c.1384C>T	H462Y	hete	0.082	D	B	N

[a.] According to Human Feb. 2009 (GRCh37/hg19) Assembly;

RefSeq, NCBI reference sequences;

PolyPhen-2 (Polymorphism Phenotyping v2; http://genetics.bwh.harvard.edu/pph/);

SIFT (Sorting Intolerant from Tolerant; http://sift.jcvi.org/);

MutationTaster (http://www.mutationtaster.org/);

ReVe, a pathogenicity-computation method combining both REVEL and VEST3.

In this table, genes are ranked according Reve score.

Supplementary Table S6. Damaging variants of ECE-2 gene predicted by ReVe in the Chinese cohort.

Refseq	Location	Variant	Protein change	AD cases(n)	Controls (n)	ReVe value
ECE-2	183995194	C556T	R186C	1	0	0.89
NM_001100120.1	183995814	C718A	P240T	1	0	0.952
	184001651	A1033G	M345V	1	0	0.833
	184002848	A1241T	D414V	1	0	0.815
	184007304	G1592A	R531H	1	0	0.779
	184007449	G1632T	M544I	2	0	0.876
	184008118	C1765G	Q589E	1	0	0.76
	184008386	C1835A	P612Q	1	0	0.931
	184008858	G2003A	R668Q	2	0	0.882
	184009220	T2252C	F751S	2	1	0.97
Carriers/non-carriers (n/n)				13/741	1/545	
Frequency (%)				1.75%	0.18%	
SKAT-O			p=0.01; odds ratio=9.71, 95% CI=1.27-74.48			

Variants with ReVe value over 0.7 are classified as damaging variants.

Supplementary Figures

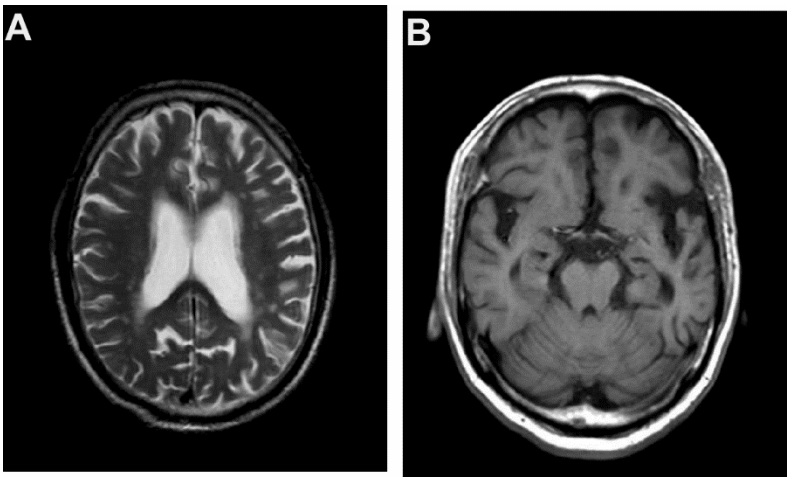

Figure S1. Structural magnetic resonance images of patient III:5 in family A. Axial T2-weighted MRI (A) and axial T1-weighted MRI (B) showed lacunar stroke and medial temporal lobe atrophy.

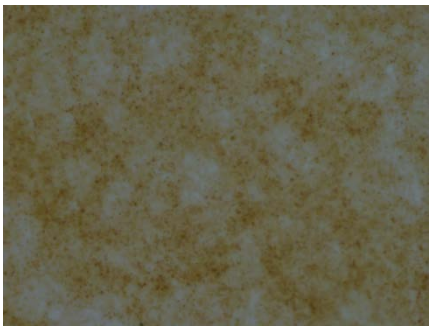

Figure S2. Immunohistochemical analysis of aSyn pathology. aSyn (LB509) immunostaining in the right temporal cortex region of patient III:3 in Family A.

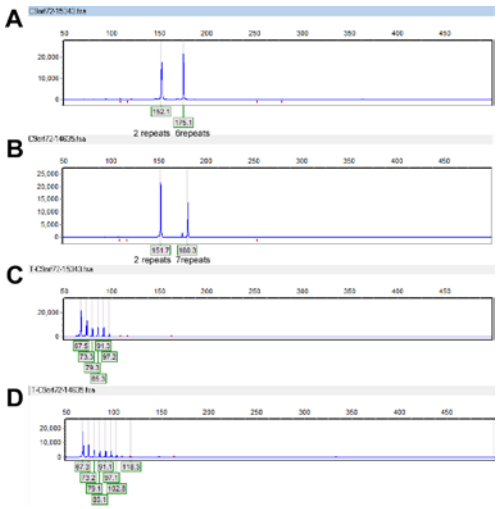

Figure S3. Detection of GGGGCC hexanucleotide expansion in noncoding region of C9ORF72 in Family A. Because pathogenic nucleotide repeat expansion cannot be detected by WES, we screened the presence of the GGGGCC hexanucleotide expansion in noncoding region of C9ORF72 of the proband (III:3, DNA NO. 15343) and patient III:5 (DNA NO. 14635) in Family A. Firstly, a two-step polymerase chain reaction protocol was adopted. In the first step, we used a polymerase chain reaction assay to detect the size of the expanded alleles. Briefly, DNA samples (50 ng/µl) were amplified using two primers (C9orf72-F: 5 FAM-CCTGTAGCAAGCTCTGGAACTC; C9orf72-R: 5 TCACTCACCCACTCGCCAC), and the primers ratio (1µl of 5µM C9orf72-F; 1µl of 5µM of C9orf72-R) were modified to improve the efficiency of the PCR. Other components of the PCR reaction included the following: 1µl of 50ng/µl of DNA samples, 0.15µl of KOD FX (Toyobo), 0.35µl of 100% DMSO, 0.4µl of 2nM dNTP, 4µl of 2xPCR buffer KOD FX(Toyobo), and 0.9µl ddH_2O. The total process was performed using a touchdown thermocycling program. The reaction conditions consisted of 95◦C for 5 min, 5 cycles of 94◦C for 30sec, 67◦C for 30sec, with a decrement of 1◦C per cycle, 72◦C for 3 min, followed by 30cycles of 94◦C for 30sec, 62◦C for 30sec, 72◦C for 30sec, 72◦C for 3min, the final temperature was then sustained at 4◦C. In the second step, we performed a classical FAM-fluorescent labeled PCR assay to accurately measure the repeat sizes. The fragment length analysis was performed on an ABI 3730×l DNA analyzer and was visualized by the GeneMarker software version 2.2.0. Secondly, repeat-primed PCR (RP-PCR) was performed to ascertain whether there were greater than 100 repeats present. In conclusion, pathogenic GGGGCC repeat expansion in C9orf72 were excluded from the two patients in Family A. **(A-B)** Capillary electrophoresis traces following flanking PCR, showing relatively balanced amplification of (**A**) 2 and 7 repeat alleles and (**B**) 2 and 6 repeat alleles. (**C-D**) The electropherograms of the polymerase chain reaction (PCR) products of RP-PCR reactions investigating the hexanucleotide repeat expansion in C9ORF72 of Patient III:3(**C**) and patient III:5(**D**).

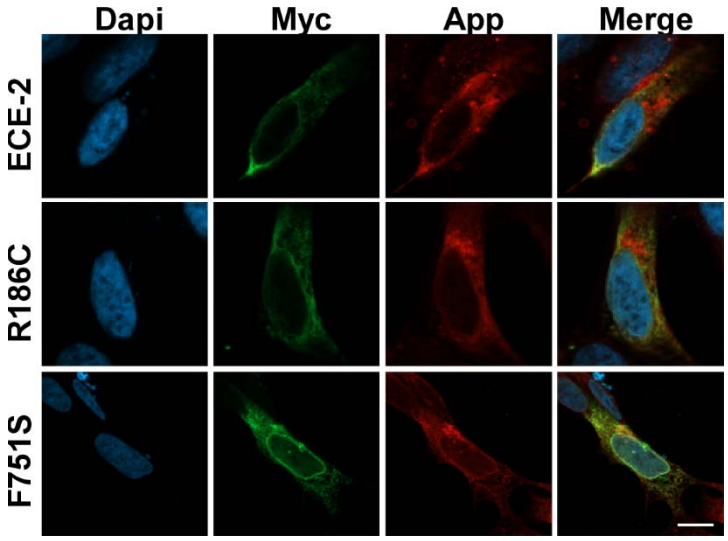

Figure S4. Co-localization of ECE-2 wildtype, ECE-2 R186C and ECE-2 F751S with APP. SH-SY5Y-APP cells were transfected with ECE-2 WT, ECE-2 R186C and ECE-2 F751S and immune-stained with the 9E10 antibody for ECE2 wildtype or mutants, and C20 antibody against the last 20 amino acids of APP C-terminus. Scale bar represents 10 μm.

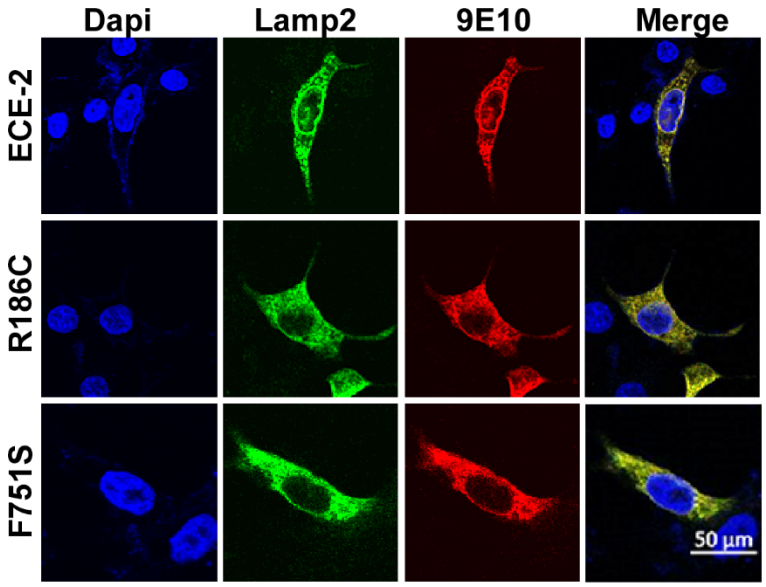

Figure S5. Intracellular distribution of ECE-2 WT, ECE-2 R186C and ECE-2 F751S. Cos7 cells were transfected with either pcDNA4/Myc-HisA-ECE-2-WT, pcDNA4/Myc-HisA-ECE-2-R186C or pcDNA4/Myc-HisA-ECE-2-F751S constructs and immuno-stained with anti-Myc antibody for ECE-2 wild type and mutant proteins and lamp-2 antibody for marker of lysosome. Scale bar represents 50 µm.