SUPPLEMENTAL MATERIAL

Immunohistochemistry:

5μm frozen sections formalin post-fixed were blocked in PBS/BSA 5% for 15 minutes and stained for 90 minutes at room temperature using anti-oligomer (A11) antibody that recognizes a generic, sequence independent epitope specific to prefibrillar amyloid oligomers (Biosource, AHB0052, 1:400). The antigen/protein complexes were visualized using fluorochrome-conjugated secondary antibody (Invitrogen, 1:1000, 45 minutes at RT) and counterstained with DAPI (invitrogen, 1:1000). The slides were observed on a Zeiss confocal microscope. 8μm frozen sections were also fixed with buffered paraformaldehyde and pretreated with formic acid (according to the manufacturer guidelines) before exposing to anti-Aß antibody (Covance 6E10). The antigen/protein complexes were also visualized using fluorochrome-conjugated secondary antibody and counterstained with DAPI.

Immunoblotting:

The protein concentration of tissue lysate preparations were measured using the Bradford method [15]. Immunoblotting was performed under reducing condition on a gradient gel using standard techniques. For immunoreactions, the blots were incubated with antibodies for anti-PS1 N-terminal. GADPH was used for normalization against total proteins (Zymed, 39-8600). The blots were then incubated in a solution containing HRP conjugated IgG (DAKO anti-mouse IgG, p0161, anti-rabbit IgG, 0449, 1:2000).

Electron microscopy:

Human ventricular tissue fixed with 2% glutarhaldehyde was dehydrated, infiltrated with EPON-812 resin and embedded in capsules. The enclosed tissue was cut on a Reichter Ultracut E ultramicrotome in super thin sections. The sections was collected onto formvar-coated slot grid, post stained with uranyl acetate and lead citrate and viewed in a transmission electron microscope (JEM 1011 transmission electron microscope with digital acquisition of images) at 80 kV.

Immunogold staining for electron microscopy (EM) using anti-oligomer antibodies:

PLP fixed human heart samples were dehydrated through a graded series of ethanol and embedded in lR with resin, medium grade (EMsciences, Hatfield, PA) at 50°C overnight. Thin sections were cut on a Reichert Ultracut E ultra microtome and collected on fomvar-coated gold grids. For the immunostaining the sections were blocked with 5% goat serum then incubated for 1 hr at room temperature with anti-oligomer (A11) antibody (Biosurce AHB0052). The antibodies were labeled with IgG gold, 10 nM at the final concentration of 1:20 in DAKO diluent for 1 hr at room temperature. The grids were rinsed and stained on drops of 2% aqueous uranyl acetate for 5 minutes, rinsed and dried. The sections were examined at 80 kV in a JEOL 1011 TEM equipped with an ATM digital camera.

PCR and sequencing

Heart tissue samples for DNA analysis were obtained from iDCM patients. Lymphoblast cells lines were obtained from AD patients and non-AD controls. Genomic DNA was extracted using QUIAamp mini DNA isolation kits (Qiagen). The entire coding and 5' promoter regions of *PSEN1* and *PSEN2* gene were amplified from DNA. Primers were designed on the basis of the annotated genomic sequence across each exon, including

regions of at least 20–40 bp of flanking intervening sequence. PCR was performed in 30-µl reaction volumes using DNA Engine (PTC-200) from MJ Research (now Bio-Rad). PCR products were directly sequenced on an ABI 48-capillary Prism 3730 DNA analyzers (PE Applied Biosystems). Sequence electropherograms were compared with gene sequence from GenBank (http://www.ncbi.nlm.nih.gov/, NCBI Build 36.1), the UCSC Human Genome Database (http://genome.ucsc.edu, The March 2006 Human Reference Sequence) and control samples. The allele frequency of detected variants in *PSEN1* and *PSEN2* gene in AD patients was screened by single-nucleotide–polymorphism (SNP) genotypes with the use of the fluorescence polarization–detected single-base extension method on a Criterion Analyst AD high-throughput fluorescence detection system (Molecular Devices). SNP data from DCM patients were also compared with allele frequency and heterozygosity for Caucasian controls from the NCBI SNP database (the CEPH collection, Utah residents with ancestry from northern and western Europe).

Relative luciferase activity measurements:

The promoter region spanning the 72672641-72673058 (UCSC Genome Database) was PCR amplified from the DCM patient with *PSEN1* -92C/delC variant and the promoter region spanning the 72673065-72673530 was PCR amplified from the DCM patient with PSEN1 –21G>A variant. The PCR fragments were confirmed by sequence analyses and cloned into the pGL2 promoter-less Basic Luciferase Reporter Vector (Promega). Four pGL2 constructs were established containing *PSEN1*-92C, -92delC and *PSEN1* -21G and -21A, respectively. Human H4 naïve neuroglioma cells were cultured in Dulbecco's

modified Eagle's medium (high glucose) containing 9% heat-inactivated fetal calf serum, 100 units/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine. For transient transfection, H4 cells were seeded in 24-well tissue culture plate at $8X10^4$ cells/well and co-transfected each appropriate construct together with constitutive Renilla construct (Promega) for controlling transfection efficiency using Lipofectamine-2000 according to manufacturer's instructions (Invitrogen). Firefly and Renilla luciferase activities were quantified sequentially using the Dual Luciferase Assay System (Promega) in a Turner TD20-20 Luminometer. The promoter transcriptional activity was expressed as the ratio of the Firefly luciferase activity of the promoter construct to the constitutive Renilla activity and normalized using baseline Firefly luciferase activity from pGL2 promoter-less Basic Luciferase Reporter Vector alone. Results were presented as percentage change (mean±standard deviation) of variant constructs (*PSEN1*-92delC and -21A) in comparison with wild-type control constructs (*PSEN1*-92C or -21G), arbitrarily designated as 100%. Each experiment was repeated 4 times and experiment results were averaged. Statistical analysis was performed using Wilcoxon Two Sample Test.