



Novel presenilin 1 variant (P117A) causing Alzheimer's disease in the fourth decade of life

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ABSTRACT

Over 160 rare genetic variants in presenilin 1 (*PSEN1*) are known to cause Alzheimer's disease (AD). In this study we screened a family with early-onset AD for mutations in *PSEN1* using direct DNA sequencing. We identified a novel *PSEN1* genetic variant which results in the substitution of a Proline with an Alanine at codon 117 (P117A). The P117A variant was present in all demented individuals and fifty percent of at risk individuals. This variant occurs at a site where three other disease-causing variants have been previously observed. *In vitro* functional studies demonstrate that the P117A variant results in an altered A β 42/total A β ratio consistent with an AD causing mutation. The P117A variant is a novel mutation in *PSEN1*, which causes early-onset AD in an autosomal dominant manner.

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The presenilins form the catalytic core of the γ -secretase complex, which is required to produce amyloid-beta (A β) from full length amyloid-beta precursor protein (APP) [9]. Over 160 rare genetic variants in *PSEN1* are known to cause Alzheimer's disease (AD) [1] (<http://www.molgen.ua.ac.be/ADMutations/>). With few exceptions [3] these variants result in onset of dementia before age 55 years, or early-onset AD (EOAD). Most of these variants occur in transmembrane regions of *PSEN1* and appear to alter the production and deposition of 42 amino acid A β fragments (A β 42) [10]. Thirteen variants, including P117L, P117R and P117S occur in the first hydrophilic loop of *PSEN1* [2,6,8,11]. In this study we sequenced *PSEN1* in a family with EOAD and identified a novel variant which segregates with AD.

The pedigree is shown in Fig. 1. Eight individuals over four generations of this family have developed dementia during the fourth decade of life. At age 35 the proband (IV-3) was identified in the local hospital of a small town in the state of Valle del Cauca, Colombia. The clinical evaluation included a comprehensive interview with the subject as well as collection of information from medical records and family members. The clinical diagnoses were made using the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria

for probable Alzheimer's disease along with a mini-mental state exam (MMSE). The routine evaluation of a patient with dementia included computed tomography (CT) head scan, and the following laboratory tests: complete blood count (CBC), standard chemistry, thyroid stimulant hormone (TSH), Venereal Disease Research Laboratory test (VDRL), Folic acid and Vitamin B12 levels. Information regarding the affected deceased family members was obtained through family members and review of hospital records.

We sequenced *PSEN1* in three AD cases and two at risk individuals. Primers were designed to target each exon of *PSEN1* and at least 50 bp of 3' and 5' flanking intronic sequence. Primers for sequencing were designed using consensus sequence from Ensembl (<http://www.ensembl.org/index.html>) and the software PRIMER3 [5] (primer sequences will be provided on request). Sequencing was performed using ABI Big Dye version 3.1 (Applied Biosystems, Foster City, CA). Sequence analysis was performed using Sequencher software (Gene Codes, Ann Arbor, MI).

To test the effects of the P117A mutation, we transfected P117A mutant *PSEN1* cDNA into HEK cells and measured secreted A β 40 and A β 42. The QuickChange II site-directed mutagenesis kit (Stratagene, Cedar Creek, TX) was used to introduce the P117A point mutation into *wtPSEN1* cDNA. The construct was confirmed by direct sequencing. HEK cells were transiently transfected with *APP Δ NL* and *wtPSEN1*, *P117A* or Δ E9 (a *PSEN1* familial AD variant which is known to have a large effect on APP processing [4]). The cDNA constructs for *wtPSEN1*, *APP Δ NL* and Δ E9 and methods for 40 amino acid A β (A β 40) and A β 42 measurement have been

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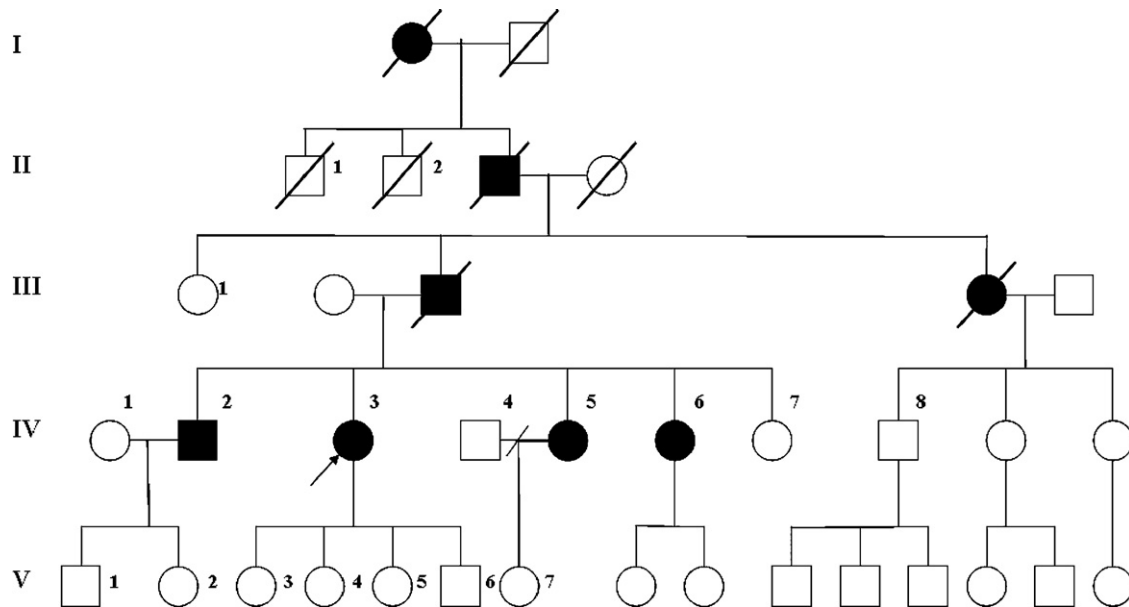


Fig. 1. Pedigree. Open symbols denote unaffected individuals; closed symbols denote affected individuals; diagonal lines denote deceased individuals. The index case is denoted with an arrow.

described previously [7]. Total A β (A β 42 + A β 40) and A β 42/total A β ratio were compared between the *wtPSEN1* and *P117A* variant cells using a *t*-test.

We have identified a pedigree with a four-generation history of early-onset dementia of the Alzheimer type. Eight individuals in this pedigree have been diagnosed with a dementing disorder. The transmission of AD in this family is consistent with autosomal dominant inheritance (Fig. 1). The proband (IV-3) visited the local hospital and was evaluated by Dr. Henao-Martinez.

The proband (IV-3) developed progressive memory impairment with gradual onset at 32 years of age. She visited the hospital repeatedly (even several times a day), often forgetting the information she had provided previously. She is a single mother and experienced difficulties in caring for her children; preparing baby bottles several times and misplacing documents or personal objects frequently. This difficulty led to the need for a neighbor's assistance. Finally, local government authorities took custody of her children because she was unable to provide them with appropriate care. Her speech was characterized by confabulation and repetitive phrases. At age 35 she exhibited significant cognitive impairment affecting more than two cognitive areas, with an MMSE score of 10/30, showing severe memory dysfunction, acalculia, agraphia, impairment in visuospatial tasks and some degree of ideational apraxia. The CT of her head showed diffuse cerebral atrophy with all laboratory measurements in the dementia workup within normal limits.

The proband's brother developed symptoms at 33 years of age. He was married with two children and worked as an auto mechanic. He started to forget and misplace objects frequently. He became unable to perform his duties satisfactorily and was released from his job. Later, he attempted to assist another auto mechanic without success. He required his wife's assistance in several daily activities and wandered frequently. At the time of the evaluation he presented with marked cognitive deficits, including disorientation, memory dysfunction, acalculia, agraphia and ideational apraxia, with an MMSE score of 5/30. The physical exam and laboratory workup was unremarkable but the CT showed diffuse cerebral atrophy.

Subject IV-5 was also a single mother with one child. She started to develop symptoms at the age of 33 years. She required

her daughter's assistance for common tasks at home. At 36 years old she exhibited repetitive speech with marked forgetfulness. She worked as a housekeeper, but gradually became incapable of performing her job satisfactorily. When examined by a physician the patient presented with acalculia, agraphia and memory impairment (MMSE of 15/30), with an otherwise normal dementia workup.

A transversion from C to G, resulting in a change from Proline (CCA) to Alanine (GCA) at codon 117 (P117A) was found in each of the demented subjects and fifty percent of at risk individuals (Figs. 1 and 2). Codon 117 is located in the first hydrophilic loop of the PSEN1 protein [6] and is the site of three other disease-causing variants [8,11]. No variants that segregate with disease were found elsewhere in the *PSEN1* gene in members of this family.

The A β 42/total A β ratio in media from cells transfected with *P117A* was significantly higher than those with *wtPSEN1* ($P=2.79 \times 10^{-7}$; Fig. 3). Total A β levels from *P117A* were not significantly different from *wtPSEN1*.

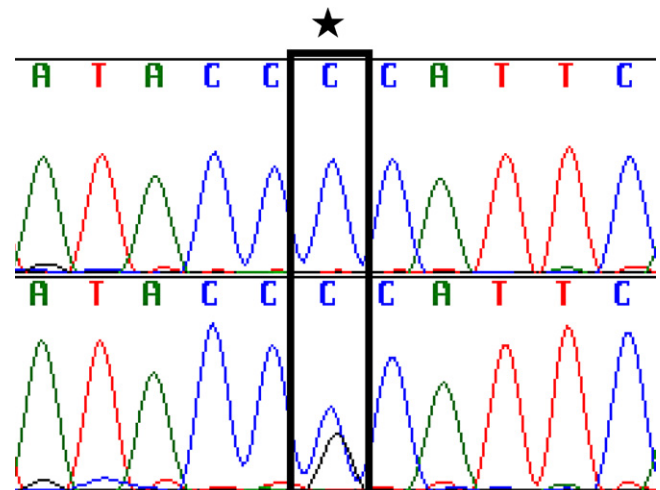


Fig. 2. Chromatogram of a portion of *PSEN1* exon 5 sequence. This displays a base pair change (P117A; bottom) compared to the family control (top).

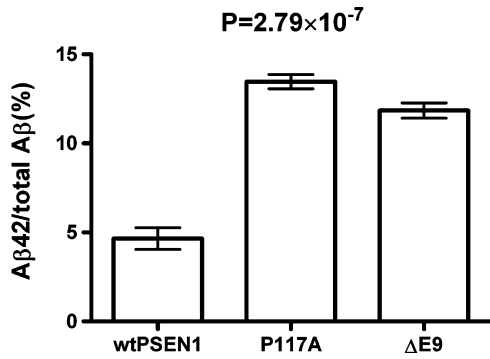


Fig. 3. Ratio of Aβ42 to total Aβ (Aβ42 + Aβ40) in the media of wild type *PSEN1* versus P117A HEK cells. The y-axis represents Aβ42 as a percentage of total Aβ levels. The x-axis shows the levels for cells with wild type *PSEN1* (wt*PSEN1*), cells with the *PSEN1* P117A variant (P117A), and cells with the *PSEN1* mutation delta E9 (ΔE9). *P*-value is for a t-test contrasting wt*PSEN1* and P117A.

The P117A variant segregates with disease in this Columbian kindred with an autosomal dominant pattern of early-onset AD. Three disease-causing variants at codon 117 of *PSEN1* have been described previously. The P117L mutation was described in a single family with onset between 24 and 34 years [8]. The P117R variant was identified in a female patient, who has had complaints of memory loss beginning at 36 years of age [11]. The P117S mutation was observed in a family with AAO ranging from 29 to 33 years [2].

AAO for the P117A variant is similar to that of the previously reported variants at codon 117, with mean AAO at 32.7 years. Clinical features of individuals with these four variants are quite similar; cognitive impairment in memory, learning and visuospatial tasks and rapid disease progression were observed with all four variants. Variants at this codon consistently cause a very severe early-onset form of the disease even for *PSEN1* mutations.

HEK cells carrying the P117A variant showed significantly higher Aβ42/total Aβ ratio than wild type cells. This finding is clearly consistent with the observation of increased Aβ42/total Aβ ratio in several other known *PSEN1* AD mutations, including P117L [8] and P177S [2] and suggests that this mutation causes AD via a similar mechanism. These findings reiterate the central role of *PSEN1* in AD and add to the list of known disease-causing variants in *PSEN1*.

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References

- [1] K. Dillen, W. Annaert, A two decade contribution of molecular cell biology to the centennial of Alzheimer's disease: are we progressing toward therapy? *Int. Rev. Cytol.* 254 (2006) 215–300.
- [2] W.K. Dowjat, I. Kuchna, T. Wisniewski, J. Wegiel, A novel highly pathogenic Alzheimer presenilin-1 mutation in codon 117 (Pro117Ser): comparison of clinical, neuropathological and cell culture phenotypes of Pro117Leu and Pro117Ser mutations. *J. Alzheimers Dis.* 6 (2004) 31–43.
- [3] J.S. Kauwe, S. Jacquart, S. Chakraverty, J. Wang, K. Mayo, A.M. Fagan, D.M. Holtzman, J.C. Morris, A.M. Goate, Extreme cerebrospinal fluid amyloid beta levels identify family with late-onset Alzheimer's disease presenilin 1 mutation. *Ann. Neurol.* 61 (2007) 446–453.
- [4] N.D. Mehta, L.M. Refolo, C. Eckman, S. Sanders, D. Yager, J. Perez-Tur, S. Younkin, K. Duff, J. Hardy, M. Hutton, Increased Abeta42(43) from cell lines expressing presenilin 1 mutations. *Ann. Neurol.* 43 (1998) 256–258.
- [5] S. Rozen, H. Skaletsky, Primer3 on the WWW for general users and for biologist programmers, in: S. Krawetz, S. Misener (Eds.), *Bioinformatics Methods and Protocols: Methods in Molecular Biology*, Humana Press, Totowa, NJ, 2000, pp. 365–386.
- [6] D. Spasic, A. Tolia, K. Dillen, V. Baert, B. De Strooper, S. Vrijens, W. Annaert, Presenilin-1 maintains a nine-transmembrane topology throughout the secretory pathway. *J. Biol. Chem.* 281 (2006) 26569–26577.
- [7] J. Wang, D. Beher, A.C. Nyborg, M.S. Shearman, T.E. Golde, A. Goate, C-terminal PAL motif of presenilin and presenilin homologues required for normal active site conformation. *J. Neurochem.* 96 (2006) 218–227.
- [8] T. Wisniewski, W.K. Dowjat, J.D. Buxbaum, O. Khorkova, S. Efthimiopoulos, J. Kulczycki, W. Lojowska, J. Wegiel, H.M. Wisniewski, B. Frangione, A novel Polish presenilin-1 mutation (P117L) is associated with familial Alzheimer's disease and leads to death as early as the age of 28 years. *Neuroreport* 9 (1998) 217–221.
- [9] M.S. Wolfe, J. De Los Angeles, D.D. Miller, W. Xia, D.J. Selkoe, Are presenilins intramembrane-cleaving proteases? Implications for the molecular mechanism of Alzheimer's disease. *Biochemistry* 38 (1999) 11223–11230.
- [10] W. Xia, J. Zhang, D. Kholodenko, M. Citron, M.B. Podlisny, D.B. Teplow, C. Haass, P. Seubert, E.H. Koo, D.J. Selkoe, Enhanced production and oligomerization of the 42-residue amyloid beta-protein by Chinese hamster ovary cells stably expressing mutant presenilins. *J. Biol. Chem.* 272 (1997) 7977–7982.
- [11] C. Zekanowski, M. Styczynska, B. Peplonska, T. Gabryelewicz, D. Religa, J. Ilkowski, B. Kijanowska-Haladyna, S. Kotapka-Minc, S. Mikkelsen, A. Pfeiffer, A. Barczak, E. Luczywek, B. Wasiaik, M. Chodakowska-Zebrowska, K. Gustaw, J. Laczkowski, T. Sobow, J. Kuznicki, M. Barcikowska, Mutations in presenilin 1, presenilin 2 and amyloid precursor protein genes in patients with early-onset Alzheimer's disease in Poland. *Exp. Neurol.* 184 (2003) 991–996.