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Brief communication

# Identification of *PSEN2* mutation p.N141I in Argentine pedigrees with early-onset familial Alzheimer's disease

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ABSTRACT

Presenilin 2 gene (*PSEN2*) mutations account for <5% of all early-onset familial Alzheimer's disease (EOFAD) cases and only 13 have strong evidence for pathogenicity. We aimed to investigate the presence of *PSEN2* mutation p.N141I and characterize the clinical phenotypes in 2 Argentine pedigrees (AR2 and AR3) with clinical symptoms of EOFAD. Detailed clinical assessments and genetic screening for *PSEN2* and *APOE* genes were carried out in 19 individuals of AR2 and AR3 families. The p.N141I mutation was identified in all affected subjects and was associated with prominent early onset, rapidly progressive dementia, neurologic, and behavioral symptoms. AR2 and AR3 families share the same Volga German ancestry as all the families reported presenting this mutation. To our knowledge, this is the first report of *PSEN2* mutation p.N141I in Argentina and even more, in South America. Our contribution increases the total number of described families carrying this mutation and help to improve the characterization of clinical phenotype in EOFAD associated to *PSEN2* mutations.



## 1. Introduction

Most cases of early-onset familial Alzheimer's disease (EOFAD) are in association to autosomal dominant mutations in 3 genes: the amyloid precursor protein (*APP*) and presenilin 1 and 2 (*PSEN1* and *PSEN2*), respectively (Cruts and Van Broeckhoven, 1998; Goate et al., 1991; Levy-Lahad et al., 1995; Rogaev et al., 1995; Sherrington et al., 1995). In particular, *PSEN2* mutations are the more rare ones, accounting for <5 % of all EOFAD cases. Although 185 mutations have been described in *PSEN1*, only 25 have been postulated in *PSEN2* (www.molgen.vib-ua.be/ADMutations; Cruts et al., 2012), among which 13 have strong evidence for pathogenicity (Canevelli et al., 2014; Jayadev et al., 2010). Clinical features associated to *PSEN2* mutations differ from those in *PSEN1* mainly in age-at-onset (AAO), which widely range from 39 to 89 years and can vary by >20 years within subjects in the same family (Canevelli et al., 2014; Jayadev et al., 2010). In addition, the limited number of families presenting mutations in *PSEN2* described to date strongly limits the genotype-phenotype correlations. Among *PSEN2* mutations, the p.N141I is the most frequently detected. Twelve families with the p.N141I mutation have been described so far, most of them from USA. Interestingly, all families share the same Volga German (VG) ancestry (Canevelli et al., 2014; Jayadev et al., 2010). This means that their ancestors moved from Germany to the southern Volga region of Russia in the 1760s, suggesting a founder effect for this mutation (Bird et al., 1988). In the present study, we describe the first families from Argentina with the *PSEN2* p.N141I mutation, being the first report in South America. These families share the VG ancestry with most PSEN2 p.N141I pedigrees previously reported, however, their ancestors immigrate to Argentina from the city of Saratov unlike families reported by Bird et al. (1988), which were from or near the villages of Walter and Frank. Affected individuals show a relatively early onset of disease characterized by mnesic disorders, and an evolution of a typical Alzheimer's disease (AD) with apathy as the most prominent psychiatric symptom.

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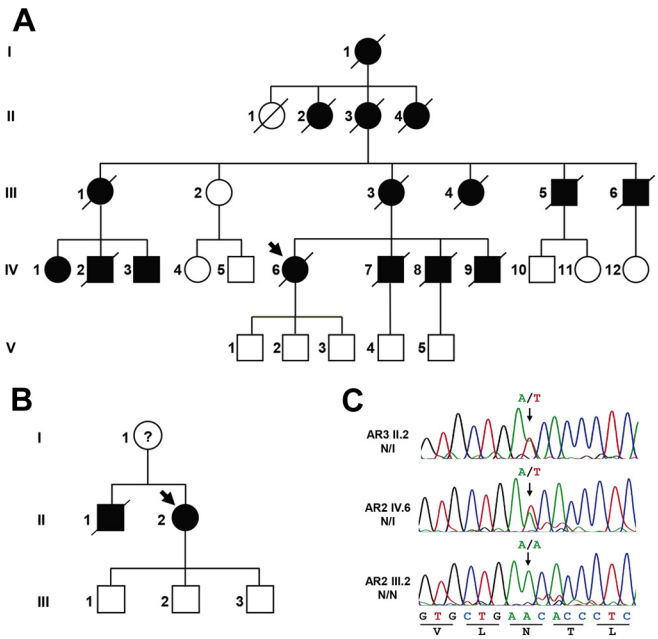


**Fig. 1.** Pedigrees of Argentine families with *PSEN2* mutation. (A) Family AR2 and (B) family AR3. Probands are marked by arrow, black symbols denote affected subjects, white symbols denote unaffected members, square denotes man and circle denotes women. Crossed symbols denote deceased subjects. (C) Sequencing chromatograms showing the p.N141I (c141 A > T) mutation.

This report provide experimental evidence that increases the total number of families carrying p.N141I mutation in *PSEN2* and reinforce the utility of the genetic screening to improve the characterization of the clinical phenotype to get insights into the biological basis of EOFAD associated to *PSEN2* mutations.

## 2. Methods

This study enrolled 2 Argentine pedigrees (AR1 and AR2). Patients with cognitive impairments, and individuals without obvious clinical dysfunction from 2 families, were clinically evaluated by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable AD (McKhann et al., 1984). AAO was determined by memory impairment and severe behavioral changes (Canevelli et al., 2014; Jayadev et al., 2010). All subjects were recruited from the outpatient Neurology Department of Instituto de Investigaciones Médicas A. Lanari. The study was approved by the institutional ethical committee and carried out with the family members' informed consent. All patients were subjected to clinical, neurological, and neuropsychological examinations and blood tests to discard reversible causes of dementia. Genetic results were informed to all participating subjects. Pedigrees of the 2 families with EOFAD are shown in Fig. 1. The sequencing methods are listed in Supplementary Materials. The APOE status was investigated as previously described (Hixson and Vernier, 1990).

## 3. Results

The clinical phenotype and genotype of 19 individuals of AR2 and AR3 families are summarized in Table 1. Formal neuropsychological testing was usually carried out 1–4 years after onset of symptoms. All affected individuals showed disorders in episodic, anterograde, and declarative memories. Verbal fluency, spatial perception, and executive function were also impaired, especially as measured by the Rey Auditory-Verbal Learning Test, Trail Making Test, and the Stroop test (Mitrushina et al., 1999). A psychiatric symptom frequently observed was apathy (Table 1). Sequencing analysis of exon 5 of *PSEN2* revealed a heterozygous AAC to ATC missense mutation at codon 141, which is predicted to result in an Asn > Ile substitution, in all affected subjects analyzed. In AR2 pedigree, the index case (IV.6) showed the p.*N141I* mutation as well as other 5 affected (IV.1, IV.2, IV.3, IV.8, and IV.9) and 4 unaffected (IV.10, V.1, V.4, and V.5) subjects. p.*N141I* mutation was absent in member III.2, a nondemented 95-year-old woman, as well as in members IV.11, IV.12, V.2, and V.3 (Fig. 1A). In AR3 pedigree, this mutation was observed in member II.2 (index case, and the only affected subject genetically characterized in this report), and in 2 of her 3 unaffected sons (III.2, III.3) (Fig. 1B).

The p.N141I mutation generates an *NdeII* restriction site that was used to confirm the AAC to ATC codon change by restriction fragment length polymorphism analysis (Supplementary Fig. 1). A 100% agreement between both methods was observed in all samples analyzed. These results demonstrate the cosegregation of the mutant allele with EOFAD in these Argentine kindred.

Affected members of both families (AR2: III.1, III.3, IV.1, IV.2, IV.3, IV.6, IV.7, IV.8, and IV.9, and AR3: II.1, and II.2) show a mean ± standard deviation (SD) of AAO of 52.7 ± 3.2 years with a range of 50–58 years. The mean ± SD of age at death of all individuals analyzed (AR2: III.1, III.3, III.5, III.6, IV.2, IV.6, IV.7, IV.8, and IV.9 and AR3: II.1) was 60.7 ± 3.6 years with a range of 55–65 years. Finally, the mean ± SD of disease duration in members of these families (AR2: III.1, III.3, IV.2, IV.6, IV.7, IV.8, and IV.9, and AR3: II.1) was 7.9 ± 3.1 years with a range of 3–13 years. The penetrance of the mutation in these families is 100 %, as no individual ˃50 years harboring the mutation is unaffected.

Interestingly, affected subjects of AR2 family had either the ε3/ε3 or ε2/ε3 genotypes, whereas all subjects analyzed in the AR3 family had ε4/ε4 genotype (Table 1). Because there is only 1 affected subject characterized in AR3 family, it is not possible to determine the impact of ε4 allele in the AAO or progression of the disease. However, AAO and disease duration for AR2 ε2/ε3 members was 54 ± 3.2 years and 5.7 ± 2.3 years, respectively.

## 4. Discussion

We have previously described a South American pedigree from Argentina (AR1) with EOFAD (mean AAO 38.9 ±3.9 years) due to *PSEN1* p.M146L mutation (Morelli et al., 1998). Here, by clinical and genetic approaches we described the first families with a *PSEN2* p.N141I mutation borne and living in Argentina. With the exception of a German patient with the PSEN2 p.N141I mutation without VG ancestry (Nikisch et al., 2008), most of the subjects carrying this mutation have VG ancestry as AR2 and AR3 families. The ancestors of VG families immigrated from south and center of Germany to Russia in the 1760s and subsequently to the United States, Canada, Brazil, and Argentina. Several VG colonies settled in Argentina since 1878, and nowadays there are about 2.000.000 descendants of VG in this country (http://www.aadav.org.ar), suggesting that additional cases could be reported in the region.

It is unclear why mutations in *PSEN2* are rarely identified worldwide, and 1 possibility may be the difficulty to be recognized because of the relatively later AAO in most cases. The individuals of the VG families reported so far (n = 84), showed a mean of AAO of 53.7 ± 7.8 years, ranging from 39 to 75 years with a mean of disease duration of 10.6 years, similar to that observed for late-onset sporadic AD (Canevelli et al., 2014; Jayadev et al., 2010). The observed AAO and duration of the disease in the AR2 and AR3 pedigrees are in accordance with these observations. It was proposed that the wide range of AAO observed in *PSEN2* p.N141I carriers could be due to the presence of other modifying factors including the *APOE* genotype. It

**Table 1**
Clinical characteristics of 2 novel Argentinean pedigrees with EOFAD

| Pedigree | Gender | Age (y) | *PSEN2* (p.N141I) | APOE genotype | AAO (y) | MMSE | GDS | CDR | FAST | Epilepsy | Apathy |
|---|---|---|---|---|---|---|---|---|---|---|---|
| AR2 | | | | | | | | | | | |
| III.1 | Female | 62[a] | NA | NA | 54 | NA | NA | NA | NA | No | NA |
| III.2 | Female | 95 | No | 3/3 | – | 28 | 2 | 0.5 | 2 | No | – |
| III.3 | Female | 60[a] | NA | NA | 50 | NA | NA | NA | NA | No | NA |
| III.5 | Male | 62[a] | NA | NA | NA | NA | NA | NA | NA | No | NA |
| III.6 | Male | 65[a] | NA | NA | NA | NA | NA | NA | NA | No | NA |
| IV.1 | Female | 65 | Yes | 3/3 | 58 | 4/30 | 6 | 3 | 6d | No | Yes |
| IV.2 | Male | 56 | Yes | 2/3 | 53 | NA | NA | NA | NA | No | Yes |
| IV.3 | Male | 63 | Yes | 2/3 | 55 | NA | NA | NA | NA | No | Yes |
| IV.4 | Female | 62 | ND | ND | – | NA | NA | NA | NA | No | – |
| IV.5 | Male | 57 | ND | ND | – | NA | NA | NA | NA | No | – |
| IV.6 | Female | 65[a] | Yes | 2/3 | 58 | 8/30 | 6 | 3 | 6c | No | Yes |
| IV.7 | Male | 55[a] | NA | NA | 50 | NA | NA | NA | NA | Yes | Yes |
| IV.8 | Male | 62 | Yes | 3/3 | 52 | 12/30 | 4 | 1 | 4 | No | Yes |
| IV.9 | Male | 57 | Yes | 2/3 | 50 | NA | NA | NA | NA | No | Yes |
| IV.10 | Male | 39 | Yes | 3/3 | – | NA | NA | NA | NA | No | – |
| IV.11 | Female | 36 | No | 2/3 | – | NA | NA | NA | NA | No | – |
| IV.12 | Female | 40 | No | 3/3 | – | NA | NA | NA | NA | No | – |
| V.1 | Male | 40 | Yes | 2/3 | – | 29/30 | NA | NA | NA | No | – |
| V.2 | Male | 36 | No | 2/3 | – | NA | NA | NA | NA | No | – |
| V.3 | Male | 33 | No | 3/3 | – | NA | NA | NA | NA | No | – |
| V.4 | Male | 30 | Yes | 3/3 | – | NA | NA | NA | NA | No | – |
| V.5 | Male | 24 | Yes | 3/3 | – | NA | NA | NA | NA | No | – |
| AR3 | | | | | | | | | | | |
| I.1 | Female | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| II.1 | Male | 63[a] | NA | NA | 50 | NA | NA | NA | NA | No | NA |
| II.2 | Female | 52 | Yes | 4/4 | 50 | 27 | 2 | 0.5 | 2 | No | Yes |
| III.1 | Male | 32 | No | 4/4 | – | NA | NA | NA | NA | No | – |
| III.2 | Male | 31 | Yes | 4/4 | – | NA | NA | NA | NA | No | – |
| III.3 | Male | 28 | Yes | 4/4 | – | NA | NA | NA | NA | No | – |

Key: AAO, age at onset; APOE, apolipoprotein E gene; AR, Argentine pedigree; CDR, Clinical Dementia Rating; EOFAD, early-onset familial Alzheimer's disease; FAST, Functional Assessment Staging Test; GDS, Global Deterioration Scale; MMSE, Mini-Mental State Examination; NA, not available; ND, not determined; –, not demented.
[a] Age at death.

is of note that in 9 VG families analyzed, the estimated contribution of APOE ε4 to total variance in AAO was significant but low (~2%), whereas no protective effect was observed for ε2 allele (Pastor et al., 2003; Wijsman et al., 2005). Although our results suggest no obvious effect of the *APOE* genotype on AAO in these Argentine pedigrees, the limited number of affected and genetically characterized members in our data set prevents rigorous conclusions. An interesting feature of our study is that when more individuals of both pedigrees will be analyzed, we would be able to compare the clinical features of *PSEN2* p.N141I mutation on *APOE* ε2 or ε4 carriers to shed light on the *APOE* contribution to resilience (a novel clinical concept that represent individuals' ability to remain cognitively intact) in EOFAD. By contrast, to experimental data that showed significant association between the *APOE* allele with resilience in sporadic AD (less resilience for ε4 and more resilience for ε2 carriers) (Negash et al., 2013), our preliminary data suggest that *APOE* genotype in subjects with VG mutation do not modify resilience for AD.

## 5. Conclusions

This is the first report of 2 Argentine EOFAD families with the missense *PSEN2* mutation (p.N141I) and VG ancestry. The clinical phenotype of subjects carrying the mutation is an early-onset dementia, with an evolution and duration of the disease similar to typical sporadic AD. This study constitutes a solid basis for future characterization of clinical phenotype in EOFAD associated to *PSEN2* mutations.

## Disclosure statement

The authors have no conflicts of interest to disclose.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2015.06.011.

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## Supplementary Materials

### *DNA purification and polymerase chain reaction*

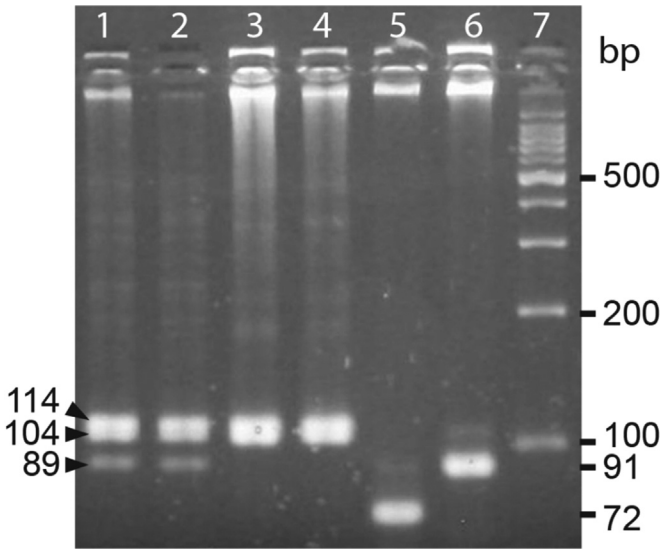
High molecular weight genomic DNA was extracted from peripheral blood using the salting out technique. *N141I* mutation was determined by restriction fragment length polymorphism and confirmed the results by DNA sequencing, based on the protocol described in Levy-Lahad et al.(1995). Briefly, exon 5 of *PSEN2* gene was polymerase chain reaction (PCR)-amplified using the following primers: FWint (5′-CATCAGCCCTTTGCCTTC-3′) and INT1R (5′-GTGGGGCAGACGGAGAGAA-3′). PCR was carried out in a total volume of 50 μL, containing 20 pmol of each primer, 50 pg of DNA, and 2.5 U of Taq DNA polymerase (Invitrogen). The reaction was done in a Gen Cycler (Bio-Rad) with an initial incubation at 95 °C for 3 minutes followed by 35 cycles for 1 minute at 94 °C, 1 minute at 60 °C, and 1 minute at 72 °C, with a final incubation for 10 minutes at 72 °C. Appropriate amounts of PCR products (218 bp) were subjected either to DNA sequencing or restriction fragment length polymorphism analysis.

### *Sequencing analysis*

PCR products were purified using spin columns (GenElute Gel Extraction Kit from Sigma) and sequenced forward and reverse with the same primers used to perform the PCR in an ABI3130 XL of 16 capillaries. Chromatograms were analyzed with ChromasPro version 1.33 (Fig. 1C).

### *Restriction fragment length polymorphism analysis*

Amplification products were digested overnight with *NdeII* (5 U in 25 μL) and restriction fragments resolved by electrophoresis in a 4% agarose gel. Fragments were visualized by ethidium bromide staining. The *N1411* mutation results in fragments of 114, 104, 89, and 15 bp, whereas in the absence of the mutation only the 114 and 104 bp fragments can be observed.




**Supplementary Fig. 1.** Detection of N141I by RFLP analysis. Representative fragments profile after digestion with *NdeII* and electrophoresed in 4% agarose gel. Lanes 1 and 2, carriers of N141I mutation showing the specific 89 bp fragment. Lanes 3 and 4, escapees without the 89 bp fragment. Lanes 5 and 6, PCR products of 91 and 72 bp, respectively, used as DNA marker. Lane 7, DNA ladder. Abbreviations: PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.