LETTER TO JMG

Molecular diagnosis of autosomal dominant early onset Alzheimer's disease: an update

G Raux, L Guyant-Maréchal, C Martin, J Bou, C Penet, A Brice, D Hannequin, T Frebourg, D Campion

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Background: Autosomal dominant early onset Alzheimer's disease (ADEOAD) is genetically heterogeneous. Mutations of the *amyloid precursor protein (APP)*, *presenilin 1 (PSEN1)*, and *presenilin 2 (PSEN2)* genes have been identified.
Objective: To further clarify the respective contribution of these genes to ADEOAD.
Methods: 31 novel families were investigated. They were ascertained using stringent criteria (the occurrence of probable or definite cases of Alzheimer's disease with onset before 60 years of age in three generations). All cases fulfilled the NINCDS-ADRDA criteria for probable or definite Alzheimer's disease. The entire coding regions of *PSEN1* and *PSEN2* genes and exons 16 and 17 of *APP* gene were sequenced from genomic DNA
Results: *PSEN1* mutations, including eight previously unreported mutations, were detected in 24 of the 31 families, and *APP* mutations were found in five families. In this sample, the mean ages of disease onset in *PSEN1* and *APP* mutation carriers were 41.7 and 51.2 years, respectively.
Conclusions: Combining these data with previously published data, yielding 65 ADEOAD families, 66% of the cases were attributable to *PSEN1* mutations and 16% to *APP* mutations, while 18% remained unexplained.

Ten years after the discovery of the *presenilin 1 (PSEN1)* gene,[1] which is involved in autosomal dominant early onset Alzheimer's disease (ADEOAD), there is an increasing requirement for presymptomatic testing in affected families. ADEOAD is genetically heterogeneous and mutations of the *amyloid precursor protein (APP)* and *presenilin 2 (PSEN2)* have also been identified in ADEOAD patients. However, there is no consensus about the relative contribution of *PSEN1* mutations to ADEOAD. Accurate determination of the relative contribution of *PSEN1*, *PSEN2*, and *APP* mutations to ADEOAD is important to ensure efficient genetic counselling in affected families.

In a previous report, mutational analysis of these three genes in 34 ADEOAD families led us to conclude that 71% of cases in these families were attributable to mutations within the *PSEN1* and *APP* genes.[2] However, other groups have recently reported lower estimates on smaller samples.[3–5] To further clarify the respective contribution of these genes to ADEOAD, we analysed 31 novel families ascertained using the stringent criteria we have previously defined (that is, the occurrence of probable or definite Alzheimer's disease cases with onset before 60 years of age in three generations).[2] All cases fulfilled the NINCDS-ADRDA criteria for probable or definite Alzheimer's disease. Clinical and molecular investigations were carried out after informed consent of participating family members was obtained.

The entire coding regions of *PSEN1* and *PSEN2* were sequenced, either from reverse transcriptase polymerase chain reaction (RT-PCR), undertaken on mRNA extracted from lymphoblastoid cell lines as previously described,[6] or from genomic DNA, and exons 16 and 17 of *APP* were sequenced from genomic DNA.[1] *APOE* genotypes were also determined. The results are summarised in table 1. *PSEN1* mutations, including eight previously unreported mutations, were detected in 24 of the 31 families, and *APP* mutations were found in five. In this sample, the mean ages of Alzheimer's disease onset in *PSEN1* and *APP* mutation carriers were 41.7 and 51.2 years, respectively. We detected no *PSEN2* mutations in the two remaining families without mutation within *PSEN1* or *APP*. In these families without detectable mutations within *PSEN1, PSEN2*, or *APP* exons 16 and 17, we completed the analysis by sequencing exons 1–15 of *APP* and exons 1–5, 7, and 9–14 of *MAPT* (*microtubule associated protein tau*) from genomic DNA, as the clinical presentation of Alzheimer's disease and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) may overlap. This complementary analysis revealed no additional mutations.

Among the eight novel *PSEN1* mutations, four affected residues that had already been shown to be mutated in ADEOAD patients. The four other novel *PSEN1* mutations concerned residues that are conserved between *PSEN1* and *PSEN2* genes and which are also conserved in evolution. For two of these eight novel *PSEN1* mutations, cosegregation with the disease could clearly be demonstrated in two families with multiple affected living members. Three *PSEN1* mutations (P264L, E280G, and F386S) were associated with an atypical presentation including spastic paraparesis. We had previously reported the occurrence of the P264L mutation in another family with spastic paraparesis,[7] but the novel F386S mutation appears to be also associated with this phenotype. The E318G variation, which had been described either as a non-pathogenic polymorphism[8 9] or as a mutation with reduced penetrance,[10 11] was present in three families. In these three families, DNA was only available from the proband, thus precluding any cosegregation analysis. In family ALZ-231, in which the Q120N mutation cosegregates with the disease, one patient also bore the R35G substitution already described by Rogaeva *et al*.[12] Absence of cosegregation of the R35G substitution with the disease within this family shows that this substitution is a non-causative rare variant. Finally, 78% of the affected subjects had an *APOE* 3-3 genotype and none had the *APOE* 4-4 genotype.

Abbreviations: ADEOAD, autosomal dominant early onset Alzheimer's disease; NINCDS-ADRDA, National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association

Table 1 Detection of *PSEN1* and *APP* mutations among 29 families with autosomal dominant early onset Alzheimer's disease

Family	AOO (years)	Affected subjects (n)	*APP*	*PSEN1*
ALZ 184	53 to 58	3	WT	c.315T→A; F105I*
ALZ 157	30 to 33	4	WT	c.347C→A; T116N
ANG 008	40 to 47	4	WT	c.347C→T; T116I*
ALZ 231	43 to 48	4	WT	c.360A→C; Q120N†
ROU 001	34 to 35	3	WT	c.428T→C; I143W
ALZ 175	45 to 50	5	WT	c.428T→A; I143N*
ALZ 148	34 to 40	5	WT	c.459C→G; L153V†
ALZ 180	39 to 44	4	WT	c.459C→G L153V†
ALZ 156	36 to 42	4	WT	c.529T→C; F177L†
ALZ 219	32 to 34	5	WT	c.617G→A; G206N†
KRI 001	30 to 35	15	WT	c.616G→A; G206S†*
ALZ 178	37 to 45	5	WT	c.640C→T; H214Y‡
ALZ 163	37 to 46	5	WT	c.698T→C; M233T
ALZ.202	38 to 40	3	WT	c.698T→C; M233T
ALZ 183	47 to 51	4	WT	c.791C→T; P264L†
ALZ 150	43 to 47	4	WT	c.839A→G; E280G
ALZ 179	52 to 58	3	WT	c.953A→G; E318G
LEF 063	45 to 51	3	WT	c.953A→G; E318G
KER 061	49 to 59	3	WT	c.953A→G; E318G
POI 060	37 to 58	5	WT	c.1157T→C; F386S†‡
ALZ 174	42 to 45	3	WT	c.1171G→T; V391F‡
ALZ 154	39 to 47	5	WT	c.1174C→G; L392V†
ALZ 147	41 to 48	6	WT	c.1174C→G; L392V†
ALZ 161	38 to 42	3	WT	c.1271T→A; L424H‡
ALZ 191	35 to 41	3	c.2084C→T; T714I	WT
ALZ 196	50 to 60	3	c.2092G→A; V717I	WT
ALZ 166	42 to 56	4	c.2092G→A; V717I	WT
ALZ 170	45 to 57	6	c.2092G→A; V717I	WT
ALZ 221	40 to 54	3	c.2092G→A; V717I	WT

*Novel mutations at codons previously reported mutant in Alzheimer's disease.
†Cosegregation with Alzheimer's disease demonstrated.
‡Novel mutations at codons which had not previously been reported mutant in Alzheimer's disease.
AOO, age of onset (range in years); *APP*, Genbank accession number X06989; *PSEN1*, Genbank accession number L42110; WT, wild type.

When these data are combined with those that we previously published,[2] and taking into account that the diagnosis of Alzheimer's disease was not confirmed by neuropathological examination in two previously negative families, we conclude that among 65 ADEOAD families, 66% are attributable to *PSEN1* mutations (62% if the E318G variation is considered as a non-pathogenic polymorphism) and 16% to *APP* mutations, while 18% remain unexplained. These results, which are in accordance with those published by Janssen *et al*,[13] should be helpful for geneticists undertaking molecular diagnosis or genetic counselling for ADEOAD families.

ELECTRONIC DATABASE INFORMATION

The URL for data presented herein as follows:

Alzheimer Disease Mutations Database: http://www.molgen.ua.ac.be/ADMutations/

Genbank home page: http://www.ncbi.nlm.nih.gov/Genbank/

Authors' affiliations

G Raux, J Bou, D Campion, Inserm U 614, IFRMP, Faculty of Medicine, Rouen, France
L Guyant-Maréchal, C Martin, T Frebourg, Department of Genetics, Rouen University Hospital, France
C Penet, A Brice, Inserm U 679, Salpêtrière Hospital, Paris, France
D Hannequin, Department of Neurology, Rouen University Hospital

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Additional data including pedigree structures are available from the corresponding author upon request.

Correspondence to: Dr D Campion, Inserm U614, Faculty of Medicine, IFRMP, 22 Boulevard Gambetta, 76183 Rouen, France; dominique.campion@univ-rouen.fr

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