**The *PSEN1*, p.E318G variant increases the risk of Alzheimer's disease in *APOE*-ε4 carriers**

**Authors and Affiliations**

Benitez BA1§, Karch CM1, Cai Y1, Jin SC1, Cooper B1, Carrell D1, Bertelsen S1, Lori Chibnik3,4,5, Julie A. Schneider6, David A. Bennett6 Alzheimer’s Disease Neuroimaging Initiative (ADNI)†, Genetic and Environmental Risk for Alzheimer's Disease Consortium (GERAD1)γ, Fagan AM6,8,Hotlzman D6,8, Morris JC6,8, Goate AM1,6,7,8, Cruchaga C1,8§\*,

1. Department of Psychiatry, Washington University, St. Louis, MO, USA. 2. Program in Translational NeuroPsychiatric Genomics, Institute for the Neurosciences Department of Neurology, Brigham and Women's Hospital, Boston, MA 02115, 3. Harvard Medical School, Boston, MA 02115, 4. Program in Medical and Population Genetics, Broad Institute of Harvard University and M.I.T., Cambridge, MA 02142. 5. Rush Alzheimer’s Disease Center and Department of Neurological Sciences, Rush University Medical Center, Chicago, IL 60062. 6. Department of Neurology, Washington University, St. Louis, MO, USA. 7. Department of Genetics, Washington University, St. Louis, MO, USA 8. Hope Center Program on Protein Aggregation and Neurodegeneration, Washington University St. Louis, MO, USA

§These authors contributed equally to this work.

\*To whom correspondence should be addressed at: Department of Psychiatry, Washington University School of Medicine, 660 South Euclid Avenue B8134, St. Louis, MO 63110. E-mail: [cruchagac@psychiatry.wustl.edu](mailto:cruchagac@psychiatry.wustl.edu), tel. 314-286-0546, fax. 314-747-2983

†Data used in preparation of this article were obtained from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (adni.loni.ucla.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: <http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf>

γ Data used in the preparation of this article were obtained from the Genetic and Environmental Risk for Alzheimer’s disease (GERAD1) Consortium. As such, the investigators within the GERAD1 consortia contributed to the design and implementation of GERAD1 and/or provided data but did not participate in analysis or writing of this report. A full list of GERAD1 investigators can be included in either supplementary content or acknowledgements.

**Genetic and Environmental Risk for Alzheimer’s disease (GERAD1) Consortium Author List**

Denise Harold1, Rebecca Sims1, Amy Gerrish1, Jade Chapman1, Valentina Moskvina1, Richard Abraham1, Paul Hollingworth1, Marian Hamshere1, Jaspreet Singh Pahwa1, Kimberley Dowzell1, Amy Williams1, Nicola Jones1, Charlene Thomas1, Alexandra Stretton1, Angharad Morgan1, Kate Williams1, Simon Lovestone2, John Powell2, Petroula Proitsi2, Michelle K Lupton2, Carol Brayne3, David C. Rubinsztein4, Michael Gill5, Brian Lawlor5, Aoibhinn Lynch5, Kevin Morgan6, Kristelle Brown6, Peter Passmore7, David Craig7, Bernadette McGuinness7, Janet A Johnston7, Stephen Todd7, Clive Holmes8, David Mann9, A. David Smith10, Seth Love11, Patrick G. Kehoe11, John Hardy12, Rita Guerreiro13,33, Andrew Singleton13, Simon Mead14, Nick Fox15, Martin Rossor15, John Collinge14, Wolfgang Maier16, Frank Jessen16, Reiner Heun16, Britta Schürmann16,17, Alfredo Ramirez16, Christine Herold34, André Lacour34, Dmitriy Drichel34, Hendrik van den Bussche18, Isabella Heuser19, Johannes Kornhuber20, Jens Wiltfang21, Martin Dichgans22,23, Lutz Frölich24, Harald Hampel25, Michael Hüll26, Dan Rujescu27,Alison Goate28, John S.K. Kauwe29, Carlos Cruchaga28, Petra Nowotny28, John C. Morris28, Kevin Mayo28, Gill Livingston30, Nicholas J. Bass30, Hugh Gurling30, Andrew McQuillin30, Rhian Gwilliam31, Panagiotis Deloukas31, Markus M. Nöthen32, Peter Holmans1, Michael O’Donovan1, Michael J.Owen1, Julie Williams1.

**Affiliations**

1 Medical Research Council (MRC) Centre for Neuropsychiatric Genetics and Genomics, Neurosciences and Mental Health Research Institute, Department of Psychological Medicine and Neurology, School of Medicine, Cardiff University, Cardiff, UK.

2 King's College London, Institute of Psychiatry, Department of Neuroscience, De Crespigny Park, Denmark Hill, London.

3 Institute of Public Health, University of Cambridge, Cambridge, UK.

4 Cambridge Institute for Medical Research, University of Cambridge, Cambridge, UK.

5 Mercer's Institute for Research on Aging, St. James Hospital and Trinity College, Dublin, Ireland.

6 Institute of Genetics, Queen's Medical Centre, University of Nottingham, UK.

7 Ageing Group, Centre for Public Health, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, UK.

8 Division of Clinical Neurosciences, School of Medicine, University of Southampton, Southampton, UK.

9 Clinical Neuroscience Research Group, Greater Manchester Neurosciences Centre, University of Manchester, Salford, UK.

10 Oxford Project to Investigate Memory and Ageing (OPTIMA), University of Oxford, Level 4, John Radcliffe Hospital, Oxford, UK.

11 University of Bristol Institute of Clinical Neurosciences, School of Clinical Sciences, Frenchay Hospital, Bristol, UK

12 Department of Molecular Neuroscience and Reta Lilla Weston Laboratories, Institute of Neurology, UCL, London, UK.

13 Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, Maryland, United States of America

14 MRC Prion Unit, Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK.

15 Dementia Research Centre, Department of Neurodegenerative Diseases, University College London, Institute of Neurology, London, UK.

16 Department of Psychiatry, University of Bonn, Sigmund-Freud-Straβe 25, 53105 Bonn, Germany.

17 Institute for Molecular Psychiatry, University of Bonn, Bonn, Germany

18 Institute of Primary Medical Care, University Medical Center Hamburg-Eppendorf, Germany.

19 Department of Psychiatry, Charité Berlin, Germany.

20 Department of Psychiatry, University of Erlangen, Nürnberg, Germany.

21 LVR-Hospital Essen, Department of Psychiatry and Psychotherapy, University Duisburg-Essen, Germany.

22 Institute for Stroke and Dementia Reserach, Klinikum der Universität München, Marchioninistr. 15, 81377, Munich, Germany.

23 Department of Neurology, Klinikum der Universität München, Marchioninistr. 15, 81377, Munich, Germany.

24 Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Germany.

25 Department of Psychiatry, Psychosomatic Medicine and Psychotherapy, Goethe University, Frankfurt, Germany

26 Centre for Geriatric Medicine and Section of Gerontopsychiatry and Neuropsychology, Medical School, University of Freiburg, Germany.

27 Alzheimer Memorial Center and Geriatric Psychiatry Branch, Department of Psychiatry, Ludwig-Maximilian University, Munich, Germany

28 Departments of Psychiatry, Neurology and Genetics, Washington University School of Medicine, St Louis, MO 63110, US.

29 Department of Biology, Brigham Young University, Provo, UT, 84602, USA.

30 Department of Mental Health Sciences, University College London, UK.

31 The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK.

32 Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany

33 Department of Molecular Neuroscience, Institute of Neurology, University College London, Queen Square, London WC1N 3BG, UK

34 Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Bonn

**Information about the known variants:**

**Known pathogenic variant**

One known pathogenic mutation *PSEN1* p.A426P (rs63751223) was found. Rs63751223 was identified in five members of a family with early onset autosomal dominant AD [[1](#_ENREF_1),[2](#_ENREF_2)]. Interestingly, we found this variant in a non-demented (CDR=0) individual (56 years old) with low CSF tau levels (189.52 pg/ml, WU series) but relatively lower levels of CSF Aβ42 levels (653.69 pg/ml). No family history of dementia has been documented to date.

**Novel coding variants**

The novel variants are distributed among all the genes: A*PP* p.A741S and p.V287G, GRN p.C247Y, *MAPT* p.T263P, *PSEN1* p.V63G, and *PSEN2*, p.G270S, p.A346S, p.T347P and p.T369S (Table 2 and 3). Interestingly, the variants *APP* p.V287G, *PSEN1* p.V63G, and *PSEN2*, p.T347P and p.T369S were found in cognitively normal individuals with high Aβ42 CSF levels. On the other hand, the variant GRN p.C247Y was found in a 77 year-old individual with a CDR=0 but with low CSF Aβ42 levels (75 pg/ml, ADNI series) and high CSF ptau levels (35 pg/ml), which is considered biomarker criteria for AD [[3](#_ENREF_3)]. This variant is located in a highly conserved nucleotide (GERP=4.81); the residue p.C247 is conserved among all granulins and is very close related to a known pathogenic variant (p.P248L) reported in individuals with frontotemporal dementia (FTD) [[2](#_ENREF_2),[4](#_ENREF_4)].The variant *MAPT,* p.T263P was found in a cognitively normal individual (last CDR=0, 74 years old) with low CSF Aβ42 levels (406 pg/ml, WU series). This variant is located in exon 9 in a highly conserved nucleotide (GERP=5.41) and is predicted to be damaging for MAPT protein. Additionally, it falls within the first microtubule-binding domain of tau and is surrounded by known pathogenic mutations causing taupathies [[2](#_ENREF_2)]. The variant *PSEN2*, p.G270S was found in a demented individual (90 years old, CDR=0.5, MMSE= 24) with high CSF Aβ42 levels (247 pg/ml, ADNI series) and low CSF levels of ptau (19 pg/ml).

**Known low frequency variants**

We also identified four previously recognized high-risk variants for LOAD (*APOE*, p.L46P; *MAPT*, p.A152T; *PSEN2*, p.R62H and p.R71W) [[2](#_ENREF_2),[5](#_ENREF_5),[6](#_ENREF_6),[7](#_ENREF_7)]. *PSEN2* p.R71W was identified in one AD case (CDR=1, 82 years old) within the bottom 1% of CSF Aβ42 levels (81 pg/ml, ADNI series) and the top 18% of the CSF tau levels (141 pg/ml). *PSEN2* p.R71W exhibited a higher frequency in clinical cases than in controls (p=0.03, OR=10.3, 95%CI=1.1-96.2). However, it did not reach statistical significance in individuals with Aβ deposition (p=0.27, OR=3.4, 95%CI=0.38-30.7) (Table 3). *MAPT* p.A152T was found in one AD case (CDR=1, 84 years old) at the bottom 8% of CSF Aβ42 levels (140.3 pg/ml, ADNI series). It also occurred more frequently in clinical cases (MAF=0.006) than in controls (MAF=0.001), however, it did not achieve statistical significance in our dataset, possibly for the sample size (p=0.13, OR=3.9, 95%CI=0.64-23.3). *PSEN2* p.R62H was found in one AD case (CDR=0.5, 80 year old) within the bottom 9% of the CSF Aβ42 levels (273.41 pg/ml in the WU series) and *APOE* p.L46P was initially found in one AD case (CDR=1, 78 years old) with CSF Aβ42 levels within the bottom 7% (99.2 pg/ml, ADNI series). *PSEN2* p.R62H and *APOE* p.L46P carriers showed similar frequency among cases and controls (Table 3).

Seven variants that have been recently reported in public databases with no clear roles in human diseases to date were also found (*APOE*, p.E37K; *GRN*, p.C231W; *MAPT*, p.G107S, p.S318L, p.V224G; *PSEN2*, p.E317G and p.V300G) (Table 2). The variants *APOE* p.E37K, *MAPT* p.G107S and *PSEN2* p.V300G were found in cognitively normal individuals with high Aβ42 CSF levels (Table 3). In fact, *APOE* p.E37K was found in an individual (78 years old) within the top 1% of Aβ42 CSF levels (1211.68 pg/ml, WU series, *APOE* ε3/ ε3 genotype).

Six variants previously reported in families with AD or FTD but classified as non-pathogenic were also identified (*GRN*, p.R433W, p.P458L, p.R19W; *MAPT*, p.Q230R; *PSEN1*, p.R35Q and p.E318G) [[2](#_ENREF_2)]. The variant *GRN* p.P458L was found in a 37-year old individual with cognitive deficit (CDR=0.5), low Aβ42 CSF levels (241.6 pg/ml, WU series) and high tau CSF levels (924.09 pg/ml), which meets biomarker criteria for AD [[8](#_ENREF_8)]. *PSEN1* p.R35Q was found in a demented individual (Caucasian, 77 years old and CDR=0.5) who exhibits high Aβ42 CSF levels (221 pg/ml, ADNI series) and low CSF ptau levels (20 pg/ml), which suggest a non-AD type of dementia.

1. Poorkaj P, Sharma V, Anderson L, Nemens E, Alonso ME, et al. (1998) Missense mutations in the chromosome 14 familial Alzheimer's disease presenilin 1 gene. Hum Mutat 11: 216-221.

2. Cruts M, Theuns J, Van Broeckhoven C (2012) Locus-specific mutation databases for neurodegenerative brain diseases. Hum Mutat 33: 1340-1344.

3. Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, et al. (2009) Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol 65: 403-413.

4. Guerreiro RJ, Washecka N, Hardy J, Singleton A (2010) A thorough assessment of benign genetic variability in GRN and MAPT. Hum Mutat 31: E1126-1140.

5. Scacchi R, Gambina G, Ferrari G, Corbo RM (2003) Screening of two mutations at exon 3 of the apolipoprotein E gene (sites 28 and 42) in a sample of patients with sporadic late-onset Alzheimer's disease. Neurobiol Aging 24: 339-343.

6. Cruchaga C, Haller G, Chakraverty S, Mayo K, Vallania FL, et al. (2012) Rare variants in APP, PSEN1 and PSEN2 increase risk for AD in late-onset Alzheimer's disease families. PLoS One 7: e31039.

7. Coppola G, Chinnathambi S, Lee JJ, Dombroski BA, Baker MC, et al. (2012) Evidence for a role of the rare p.A152T variant in MAPT in increasing the risk for FTD-spectrum and Alzheimer's diseases. Hum Mol Genet 21: 3500-3512.

8. Fagan AM, Head D, Shah AR, Marcus D, Mintun M, et al. (2009) Decreased cerebrospinal fluid Abeta(42) correlates with brain atrophy in cognitively normal elderly. Ann Neurol 65: 176-183.

|  |  |  |
| --- | --- | --- |
| **Table S1: Biomarkers and covariates in the different series** | | |
| **Pool1** | **vs Pool 2** | **vs Additional Set** |
| **Age** | ns | ns |
|
|
| **Gender** | ns | ns |
|
|
| **Apoe e4+** | ns | ns |
|
|
| **CDR = 0** | ns | ns |
|
|
| **Aß42** | <0.0001, \* | <0.0001 δ |
|
|
| **Tau** | <0.0001 | <0.01 |
|
|
| **Ptau181** | <0.0001 | <0.01 |
|
|

|  |
| --- |
| ANOVA was used to identify the important differences for each endophenotype, age and each set. The p-value shown. Fisher exact test was used for gender, apoe e4 + and CDR analysis, separate by series. ADNI pool1 vs pool2 p=0.01, δ ADNI pools vs additional data set is not significant |

**Table S2. Summary of exon coverage per gene.**

|  |  |  |
| --- | --- | --- |
| **GENE** | **Exon** | **Mean Coverage** |
| **p53 positive control** |  | 335.9582 |
| **pCMV6-XL5 negative control** |  | 111.6862 |
| **APOE** | 1 | 196.4079 |
|  | 2 | 107.3219 |
|  | 3 | 82.1116 |
|  | 4 | 52.93052 |
| **GRN** | 1 | 158.054 |
|  | 2\_4 | 175.2848 |
|  | 5\_7 | 143.983 |
|  | 8\_1 | 205.4358 |
|  | 11\_13 | 207.851 |
| **PSEN1** | 1 | 221.6 |
|  | 2\_3 | 77.77825 |
|  | 4 | 80.86913 |
|  | 5 | 95.76458 |
|  | 6 | 31.50099 |
|  | 7 | 54.82842 |
|  | 8 | 92.2601 |
|  | 9 | 85.52308 |
|  | 10 | 56.08312 |
|  | 11 | 64.90789 |
|  | 12 | 125.9575 |
| **PSEN2** | 1\_2 | 16.47196 |
|  | 3 | 123.2815 |
|  | 4 | 140.2585 |
|  | 5 | 111.291 |
|  | 6 | 118.9234 |
|  | 7\_8 | 112.3041 |
|  | 9 | 104.7415 |
|  | 10\_11 | 141.1574 |
|  | 12 | 26.11573 |
|  | 13 | 74.70342 |

**Table S2. Summary of exon coverage per gene**

|  |  |  |
| --- | --- | --- |
| **GENE** | **Exon** | **Mean Coverage** |
| **APP** | 1 | 27.54009 |
|  | 2 | 197.2113 |
|  | 3 | 145.7021 |
|  | 4 | 94.60729 |
|  | 5 | 59.49297 |
|  | 6 | 130.5826 |
|  | 7 | 154.9414 |
|  | 8 | 98.93752 |
|  | 9 | 123.3836 |
|  | 10 | 62.32779 |
|  | 11 | 130.3605 |
|  | 12 | 124.6781 |
|  | 13 | 105.8592 |
|  | 14 | 97.83711 |
|  | 15 | 184.1279 |
|  | 16 | 118.9949 |
|  | 17 | 80.41112 |
|  | 18 | 64.74846 |
| **MAPT** | 1 | 61.95862 |
|  | 2 | 53.77114 |
|  | 3 | 81.24887 |
|  | 4 | 92.15595 |
|  | 5 | 62.5825 |
|  | 5 | 45.68207 |
|  | 7 | 47.82681 |
|  | 8 | 83.24144 |
|  | 9 | 79.76721 |
|  | 10 | 108.4224 |
|  | 11 | 96.46175 |
|  | 12 | 177.2816 |
|  | 13 | 137.7167 |
|  | 14 | 74.16285 |

**Table S2b. SPLINTER raw ouput of different SNPs by pools**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Pool 1** |  |  |  |  |  |  |
| **Gene** | **Intronic** | **Missense** | **Coding-synonymous** | **Splicing** | **UTR** | **Near-gene** |
| **APOE** | 8 | 7 | 2 | 0 | 0 | 2 |
| **APP** | 62 | 2 | 2 | 0 | 18 | 0 |
| **GNR** | 13 | 3 | 2 | 0 | 4 | 0 |
| **MAPT** | 84 | 10 | 6 | 0 | 16 | 0 |
| **PSEN1** | 78 | 1 | 1 | 0 | 5 | 0 |
| **PSEN2** | 45 | 5 | 8 | 0 | 11 | 1 |
| **Total** | 290 | 28 | 21 | 0 | 54 | 3 |
|  |  |  |  |  |  |  |
| **Pool 2** |  |  |  |  |  |  |
| **Gene** | **Intronic** | **Missense** | **Coding-synonymous** | **Splicing** | **UTR** | **Near-gene** |
| **APOE** | 6 | 10 | 1 | 1 | 2 | 2 |
| **APP** | 56 | 1 | 1 | 0 | 4 | 0 |
| **GNR** | 8 | 3 | 1 | 2 | 2 | 1 |
| **MAPT** | 74 | 10 | 8 | 0 | 14 | 0 |
| **PSEN1** | 59 | 4 | 0 | 0 | 3 | 0 |
| **PSEN2** | 65 | 4 | 4 | 1 | 10 | 12 |
| **Total** | 268 | 32 | 15 | 4 | 35 | 15 |

SPLINTER software was used for call the SNPs. Here it is shown all the variants that passed the filter adjusting the sensitivity and specificity. Only variants with a predicted MAF less than 5 %, exonic missense and affecting the splicing, were selected to be validated.

**Table S3. Summary of sample Cerebrospinal Fluid (CSF) biomarker residual levels**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Sample** | **n** | **Residual\_Aβ42 Mean ± SD (range)** | **Residual\_Tau Mean ± SD (range)** | **Residual\_p-tau Mean ± SD (range)** |
| **Pool 1** | **WU-ADRC** | 70 | 0.26 ± 0.38 (-0.53-1.02) | -0.21 ± 0.46 (-1.1-0.64) | -0.20 ± 0.41 (-1.08-1.27) |
|  | **ADNI** | 28 | -0.15 ± 0.31 (-0.83-0.24) | -0.43 ± 0.42 (-1.41-0.52) | -0.15 ± 0.31 (-0.83-0.24) |
| **Pool 2** | **WU-ADRC** | 75 | -0.32 ± 0.42 (-1.06-0.68) | 0.25 ± 0.59 (-1.33-1.25) | 0.24 ± 0.42 (-1.06-0.68) |
|  | **ADNI** | 39 | 0.04 ± 0.32 (-0.5-0.57) | 0.32 ± 0.58 (-0.97-1.21) | 0.39 ± 0.68 (-1.2-1.23) |
| **Additional Set** | **WU-ADRC** | 340 | 0.01 ± 0.31 (-1.2-0.78) | -0.009 ± 0.41 (-1.02-1.45) | -0.01 ± 0.37 (-0.86-1.42) |
|  | **ADNI** | 192 | 0.01 ± 0.21 (-0.69-0.57) | -0.003 ± 0.36 (-0.98-1.35) | -0.01 ± 0.34 (-0.87-1.08) |
| **Total** | **WU-ADRC** | **475** | 0.0002 ± 0.38 (-1.2-1.02) | 0.0014 ± 0.48 (-1.3-1.45) | 0.0015 ± 0.42 (-1.14-1.42) |
|  | **ADNI** | **259** | -1.930e-005 ± 0.25 (-0.83-0.57) | 3.862e-006 ± 0.47 (-1.14-1.35) | 7.721e-006 ± 0.45 (-1.58-1.23) |