

1 Genome sequencing for early-onset dementia: high diagnostic yield and frequent observation of
2 multiple contributory alleles

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4 J. Nicholas Cochran, Ph.D.^{1,5}, Emily C. McKinley, B.S.², Meagan Cochran, M.S.¹, Michelle D. Amaral,
5 Ph.D.¹, Bryan A. Moyers, Ph.D.¹, Brittany N. Lasseigne, Ph.D.¹, David E. Gray, M.S.¹, James M.J.
6 Lawlor, M.S.¹, Jeremy W. Prokop, Ph.D.^{1,3}, Ethan G. Geier, Ph.D.⁴, James M. Holt, Ph.D.¹, Michelle L.
7 Thompson, Ph.D.¹, J. Scott Newberry, B.S.¹, Jennifer S. Yokoyama, Ph.D.⁴, Elizabeth A. Worthey,
8 Ph.D.¹, David S. Geldmacher, M.D.², Marissa Natelson Love, M.D.², Gregory M. Cooper, Ph.D.¹,
9 Richard M. Myers, Ph.D.^{1,*}, Erik D. Roberson, M.D., Ph.D.^{2,*}

10
11 ¹HudsonAlpha Institute for Biotechnology, 601 Genome Way, Huntsville AL 35806, USA
12 ²Alzheimer's Disease Center, Department of Neurology, University of Alabama at Birmingham, 1720
13 7th Avenue South, Room 640, Birmingham AL 35294, USA
14 ³Department of Pediatrics and Human Development, Michigan State University, East Lansing, MI, USA
15 ⁴Department of Neurology, Memory and Aging Center, University of California San Francisco, 675
16 Nelson Rising Lane, Suite 190, San Francisco CA 94158, USA
17 ⁵Correspondence: ncochran@hudsonalpha.org, 256-327-5220
18 *Richard M. Myers and Erik D. Roberson contributed equally to this study.

19
20 Running title: Utility of early-onset dementia genome sequencing

21 **ABSTRACT**

22 We assessed the utility of genome sequencing for early-onset dementia. Participants were selected
23 from a memory disorders clinic. Genome sequencing was performed along with *C9orf72* repeat
24 expansion testing. All returned sequencing results were Sanger validated clinically. Prior clinical
25 diagnoses included Alzheimer's disease, frontotemporal dementia, and unspecified dementia. The
26 mean age-of-onset was 54 (41–76). 50% of patients had a strong family history, 37.5% had some, and
27 12.5% had no known family history. Nine of 32 patients (28%) had a variant defined as pathogenic or
28 likely pathogenic (P/LP) by American College of Medical Genetics standards, including variants in *APP*,
29 *C9orf72*, *CSF1R*, and *MAPT*. Nine patients (including three with P/LP variants) harbored established
30 risk alleles with moderate penetrance (odds ratios of about 2–5) in *ABCA7*, *AKAP9*, *GBA*, *PLD3*,
31 *SORL1*, and *TREM2*. All six patients harboring these moderate penetrance variants but not P/LP
32 variants also had one or two *APOE* ϵ 4 alleles. One patient had two *APOE* ϵ 4 alleles with no other
33 established contributors. In total, 16 patients (50%) harbored one or more genetic variants likely to
34 explain symptoms. We identified variants of uncertain significance (VUSs) in *ABI3*, *ADAM10*, *ARSA*,
35 *GRID2IP*, *MME*, *NOTCH3*, *PLCD1*, *PSEN1*, *TM2D3*, *TNK1*, *TTC3*, and *VPS13C*, also often along with
36 other variants. In summary, genome sequencing for early-onset dementia demonstrated high utility,
37 with particular advantages where targeted testing may fail such as atypical variant-disease associations
38 or presence of multiple moderate impact alleles. One or more established contributory alleles is often
39 present in early-onset dementia, supporting an oligogenic model.

40 INTRODUCTION

41 Genomic technologies are increasingly being used in clinical settings, but clinical large-scale
42 sequencing for adult-onset neurological conditions has not been heavily applied. Possible reasons
43 include the use of disease-specific gene panels and uncertain genetic yield, despite promising signals
44 for yield using comprehensive approaches (Blauwendraat et al. 2018). We sought to assess the
45 diagnostic yield with genome sequencing and *C9orf72* expansion testing in cases of early-onset
46 dementia.

47 Patients were selected from the Memory Disorders Clinic at the University of Alabama at
48 Birmingham (UAB). Inclusion criteria were clinician-diagnosed early-onset dementia. When possible,
49 unaffected parents were included as participants to allow filtering for *de novo* variants in patients
50 without a family history (a fruitful approach in pediatric genetic disorders (Vissers et al. 2010; Bowling et
51 al. 2017) and amyotrophic lateral sclerosis (ALS) (Chesi et al. 2013; Steinberg et al. 2015a)). In
52 addition, unaffected siblings past the age of onset of the patient were enrolled as participants when
53 possible for variant filtering and segregation.

54 Before starting analysis, we set criteria for return of results to patients. First, we used the
55 American College of Medical Genetics (ACMG) criteria for pathogenicity (Richards et al. 2015) to
56 identify highly penetrant causal variation. For moderately penetrant variants, we set criteria to return: (i)
57 *APOE* $\epsilon 4$ status for early-onset Alzheimer's disease (EOAD), (ii) any variant with a disease-associated
58 odds ratio greater than two in multiple reports as an "established risk variant," or (iii) one strong report
59 with a disease-associated odds ratio greater than two with replication included in the study design as a
60 "likely risk variant."

61

62 RESULTS

63 Clinical presentation and family history

64 Prior clinical diagnoses for patients included EOAD, frontotemporal dementia (FTD), and other
65 unspecified dementias. 21 patients were female and 11 were male. 28 self-reported Caucasian and
66 four self-reported African American, all reported non-Hispanic ethnicity. The mean age of onset was 54

67 (range 41–76). 10 patients had ages of onset in their 40's, 17 in their 50's, 4 in their 60's, and 1 in their
68 70's.

69 In addition to enrolling patients, we also enrolled reportedly unaffected family members for
70 variant filtering and segregation analyses. 31 unaffected relatives were enrolled, 29 of which had
71 genome sequencing (2 were only checked for variants by Sanger). Only two families had complete trios
72 (mother, father, and proband) to allow for searching for *de novo* variants, of which none of interest were
73 identified. In total, 20 unaffected siblings, 9 unaffected parents, and 2 unaffected cousins were enrolled.

74 A strong family history of dementia was reported for 50% of patients (16/32), while 37.5%
75 (12/32) had some family history, and 12.5% (4/32) had no reported family history. Our definition of
76 family history is based on a modification of a four point scoring system first put forward by Jill Goldman
77 (Goldman et al. 2005) where we modified the score as follows: (1) At least three people in two
78 generations affected with EOAD, FTLN, ALS, CBD, or PSP with one person being a first-degree
79 relative of the other two, (1.5) Same as (1) but with LOAD instead of EOAD, (2) At least three relatives
80 with dementia or ALS but where criteria for autosomal dominant inheritance were not met, (3) A single
81 affected first or second degree family member with early-onset dementia or ALS, (3.5) A single affected
82 first or second degree family member with late-onset dementia or ALS, (4) No contributory family
83 history or unknown family history. We considered a score of 1 or 1.5 as strong family history, a score of
84 2, 3, or 3.5 as some family history, and a score of 4 as no reported family history. All family history
85 information is listed alongside phenotype and variant information in **Supplemental Table 1**.

86 To protect patient information, more detailed diagnoses and phenotype information beyond that
87 provided here and listed in **Supplemental Table 1** are only provided in the controlled access dataset,
88 NIAGADS project NG00082, to qualified researchers approved for access.

89

90 **Genomic analyses**

91 Nine of 32 (28%) patients had a highly penetrant variant relevant to their clinical diagnosis
92 (ACMG P/LP (Richards et al. 2015)), while seven (22%) had multiple moderately penetrant risk alleles
93 (**Figure 1**). Individual cases are discussed next, with variants identified summarized by **Table 1** and
94 listed alongside phenotype information in **Supplemental Table 1**.

95

96 ***Pathogenic or Likely Pathogenic Diagnoses***

97 Variants were first evaluated using ACMG criteria for pathogenicity, and all P/LP variants were
98 returned to patients (Richards et al. 2015). We provide a summary below, with detail on the ACMG
99 evidence codes for variants provided in the Supplemental ACMG Pathogenicity Evidence Details.

100

101 *APP Pathogenic Variant (V717F) in Two Siblings*

102 Two siblings with ages of onset in the mid-to-late 40s and a family history of EOAD suggestive
103 of dominant inheritance harbored a pathogenic variant in *APP* (NM_000484.3, c.2149G>T, V717F), a
104 well-established pathogenic variant (see Supplemental ACMG Pathogenicity Evidence Details). This
105 variant is an example of one that would have been identified on commonly-used panels for genetic
106 testing for EOAD.

107

108 *C9orf72 Expansion Carriers*

109 Testing for a pathogenic G₄C₂ hexanucleotide expansion at the *C9orf72* locus associated with
110 ALS and FTD was ordered for 30 of 32 patients (with two excluded for technical reasons, see
111 Methods). GeneDx conducted a repeat-primed PCR test with 95% sensitivity and 98% specificity
112 (Akimoto et al. 2014) to detect *C9orf72* expansions. As a technical aside, *C9orf72* expansions were not
113 detectable using ExpansionHunter (Dolzhenko et al. 2017) or STRetch (Dashnow et al. 2018) in
114 genome sequencing libraries prepared with PCR amplification assessed here. ExpansionHunter
115 detects *C9orf72* expansions in PCR-free genome preparations (Dolzhenko et al. 2017), so PCR-free
116 genome preparations or secondary testing (such as testing conducted by GeneDx for here) is
117 necessary for detection of *C9orf72* expansions (and would also be necessary for other repeat
118 expansions). Three patients with FTD (one patient also had ALS signs) with ages-of-onset in the 40s
119 and 50s harbored a pathogenic expansion in *C9orf72* (see Supplemental ACMG Pathogenicity
120 Evidence Details).

121 Some studies have suggested that additional contributing alleles could lower age of onset
122 and/or alter clinical presentation for *C9orf72* expansion carriers (van Blitterswijk et al. 2012; van

123 Blitterswijk et al. 2014; Pottier et al. 2015; Giannoccaro et al. 2017; Farhan et al. 2018). Consistent with
124 this, all three *C9orf72* expansion carriers harbored other possibly contributory variants.

125 One carrier had three additional variants that may be contributory: an “established risk” stop
126 gained variant in *ABCA7* (NM_019112.3, c.5035G>T, p.E1679*), one *APOE* ϵ 4 allele, and a VUS in
127 *PSEN1* (NM_000021.3, c.103C>T, p.R35W) (see Supplemental ACMG Pathogenicity Evidence
128 Details). These variants may have contributed to the patient’s family history of multiple
129 neurodegenerative diseases including ALS and EOAD.

130 Another carrier had a different “established risk” variant in *ABCA7* (NM_019112.3,
131 c.2126_2132delAGCAGGG, p.E709Afs*86) (see Supplemental ACMG Pathogenicity Evidence Details),
132 along with memory symptoms and a family history of AD, consistent with a possible contributory role of
133 *ABCA7*.

134 The third carrier had two VUS in *ARSA*, associated with recessive metachromatic
135 leukodystrophy (discussed further in Supplemental ACMG Pathogenicity Evidence Details).

136

137 *MAPT* R406W Pathogenic Variant in Three Alzheimer’s Disease Patients

138 Three patients with EOAD (one patient also exhibited FTD signs) with ages-of-onset in the mid
139 50s to early 60s harbored a pathogenic variant in *MAPT* (NM_005910.5, c.1216C>T, p.R406W).
140 Although *MAPT* pathogenic variants are typically associated with FTD (Cruts et al. 2012), this variant
141 has been reported in patients with clinically diagnosed Alzheimer’s disease (AD) in multiple studies
142 (see Supplemental ACMG Pathogenicity Evidence Details). This variant would not have been detected
143 on many AD-specific panels, which often test for only *APP*, *PSEN1*, and *PSEN2*.

144 All three of these patients exhibited a possible contribution from another allele, just as in
145 *C9orf72* expansion carriers. One patient had a loss-of-function “established risk” variant in *ABCA7*
146 (NM_019112.3, c.2126_2132delAGCAGGG, p.E709Afs*86). Another patient had a VUS in *APP*
147 (NM_000484.3, c.1090C>T, p.L364F). The third patient had a loss-of-function splice variant in *GRID2IP*
148 (NM_001145118.1, c.429+2T>G), which, while not yet firmly associated with EOAD and thus not yet
149 returnable, was implicated in a recent large sequencing study (Raghavan et al. 2018).

150 The presence of this rare variant in three individuals enrolled at the same clinic suggests they
151 may share a common ancestor. However, none of these individuals are aware of any extended family
152 members participating in the study. Furthermore, the patients are not detectably related by software
153 used for routine checks of close familial relationships (KING).

154

155 *CSF1R R900K in an FTD Patient*

156 A patient presenting with behavioral variant FTD (bvFTD) harbored a likely pathogenic variant in
157 *CSF1R* (NM_005211.3, c.2699G>A, p.R900K) (see Supplemental ACMG Pathogenicity Evidence
158 Details). Patients with variants in *CSF1R* can present with bvFTD, but the underlying pathology of
159 pathogenic *CSF1R* variants is leukoencephalopathy (Rademakers et al. 2011; Stabile et al. 2016).
160 Consistent with this, this patient had white matter abnormalities on MRI with frontal-predominant
161 confluent white matter hyperintensity (**Figure 2A**) and global atrophy (**Figure 2B–D**). This variant would
162 not have been detected on typical panels testing for FTD.

163

164 ***High Impact Risk Alleles***

165 One unique aspect of this study is that we returned to patients moderately penetrant risk
166 variants that meet criteria we have described. Intriguingly, rare variants meeting these criteria were
167 observed only along with one or two *APOE* ϵ 4 alleles, the most common moderately penetrant risk
168 allele for AD (see Supplemental ACMG Pathogenicity Evidence Details). In all cases, *APOE* ϵ 4 alleles
169 were returned as “established risk variants.” The presence of one *APOE* ϵ 4 allele was returned as likely
170 only a small contributor to symptoms, while presence of two *APOE* ϵ 4 alleles or one or two *APOE* ϵ 4
171 alleles in combination with a rare moderately penetrant risk variant was returned with language
172 indicating that such a combination of variants is likely to explain a large portion of the genetic
173 contribution to symptoms (but with the caveat that family members should not be presymptomatically
174 tested given incomplete penetrance). We continue with detail on some cases falling into this category.

175

176 *A case with APOE ε4 Homozygosity, PLD3 V232M, APP D248N, and ABI3 V97E*

177 In a patient with EOAD whose symptoms began in the late 40s with enrolled unaffected parents,
178 we observed an example of how EOAD may occur from a combination of inherited alleles from each
179 parent, consistent with previous observations that EOAD can appear recessive in nature (Wingo et al.
180 2012). The patient had two *APOE* ε4 alleles (returned as “established risk,”) a *PLD3* variant
181 (NM_012268.3, c.694G>A, p.V232M) (returned as “likely risk,”) an *APP* variant (NM_000484.3,
182 c.742G>A, p.D248N) (returned as a VUS), and a private variant in *ABI3* (NM_016428.2, c.290T>A,
183 p.V97E) (not returned but predicted damaging by PolyPhen-2 (Adzhubei et al. 2010) and SIFT (Ng and
184 Henikoff 2003), with a CADD score (Kircher et al. 2014) of 33) (see Supplemental ACMG Pathogenicity
185 Evidence Details). The *ABI3* variant was not returned to the patient because of insufficient evidence to
186 consider the variant as a returnable VUS or risk variant, but is highlighted because a different coding
187 variant in *ABI3* (NM_012268.3, c.1124T>C, p.S209F) (Sims et al. 2017) was associated with AD in a
188 rigorous case-control study with an odds ratio of 1.4, yet is not predicted to be as damaging
189 (CADD=13.5) and is relatively common in population databases (allele frequency of 0.6%). Therefore,
190 we speculate that perhaps the variant we observed could have an effect of similar or greater magnitude
191 given its higher predicted deleteriousness and absence from population databases. One of the *APOE*
192 ε4 alleles and the variants in *PLD3* and *APP* was inherited from a parent with neurologic symptoms but
193 not EOAD. The other parent harbored an *APOE* ε4 allele and the *ABI3* variant and did not have
194 neurologic symptoms. This case serves as an example of how EOAD may arise with either no family
195 history or limited family history of late-onset disease.

196

197 *A case with APOE ε4 Heterozygosity and SORL1 M105T*

198 An individual with EOAD with onset in the mid 50s and a strong family history of AD had one
199 *APOE* ε4 allele and a variant in *SORL1* (NM_003105.5, c.314T>C, p.M105T). While *SORL1* variants
200 are not completely penetrant, loss-of-function variants in *SORL1* confer one of the highest levels of risk
201 for AD outside of dominant pathogenic variants and *APOE*. Loss-of-function *SORL1* variant carriers in
202 cases from a recent study (Raghavan et al. 2018) are present at an odds ratio of about four compared
203 to population databases, a likely underestimate given that some individuals in population databases

204 may develop AD. Indeed, a recent meta-analysis suggests the odds ratio for loss-of-function *SORL1*
205 variants could be as high as 12.3 for all AD and 27.5 for EOAD (Campion et al. 2019).

206 For the *SORL1* variant identified here, we checked independent datasets for replication, and
207 observed one M105T carrier in one study (Sassi et al. 2016), three M105T carriers in Alzheimer's
208 Disease Sequencing Project (ADSP) exomes (Bis et al. 2018), and two M105T carriers in ADSP
209 genomes (one in an AD case and in one a mild cognitive impairment case) with no controls harboring
210 the variant in any of these datasets. No other carriers were identified in cases or controls in four other
211 studies (see Supplemental ACMG Pathogenicity Evidence Details). In addition to these four studies,
212 there is one record in ClinVar from GeneDx (RCV000489328.1), but it lacked a denominator of the
213 number of cases tested and thus was not considered in calculating the replication statistic. Taken
214 together, *SORL1* M105T is observed six times out of 13,390 AD cases compared to 11 of 189,196
215 individuals at a population level for a replication-only odds ratio of 7.7 ($p = 0.0005$ by Fisher's exact
216 test). This variant did not completely segregate with disease in four family members of our patient.
217 However, the age-of-onset range for similar variants in *SORL1* can be up to 24 years (Louwersheimer
218 et al. 2017), which is wider than the age differences between the family members we genotyped,
219 suggesting that this segregation analysis may not be completely informative. Considering all of the
220 evidence, we returned this variant to the patient as a VUS (it could also be considered a "likely risk
221 variant"). Modelling suggests M105T is a highly conserved residue (**Figure 3A**) where change to a
222 threonine may create a PLK1 kinase site that may disrupt function (**Figure 3B**) (discussed further in
223 Supplemental ACMG Pathogenicity Evidence Details).

224

225 *APOE ε4 with TREM2, AKAP9, and GBA Risk Variants*

226 In two cases with EOAD beginning in the late 40s, we observed a risk allele in *TREM2* and one
227 or two *APOE ε4* alleles. The first was *TREM2* (NM_018965.3, c.140G>A, p.R47H) (Guerreiro et al.
228 2013; Jonsson et al. 2013) with one *APOE ε4* allele. This *TREM2* variant was returned as an
229 "established risk variant." Second, we observed *TREM2* (NM_018965.3, c.259G>A, p.D87N) (Guerreiro
230 et al. 2013) (see Supplemental ACMG Pathogenicity Evidence Details) with two *APOE ε4* alleles. This
231 *TREM2* variant was returned as a "likely risk variant."

232 In an African American patient with features of both EOAD and FTD, we observed a variant in
233 *AKAP9* previously reported to increase risk in African Americans (NM_005751.4, c.7638A>G,
234 p.I2546M) (Logue et al. 2014). In this case, despite only being observed in one study with replication,
235 the specificity of this variant disease association to African American ethnicity and additional functional
236 data (Ikezu et al. 2018) provided enough evidence to return this as an “established risk variant.”

237 A patient with EOAD with onset in the mid 50s harbored *GBA* (NM_000157.3, c.1448T>C,
238 p.L483P [previous nomenclature, p.L444P]) and two *APOE* $\epsilon 4$ alleles, originally associated with Lewy
239 body disorders (Mata et al. 2008), but later also with mixed Dementia with Lewy Bodies and AD
240 (Tsuang et al. 2012; Nalls et al. 2013). Because of this and a recent association with accelerated
241 cognitive decline (Liu et al. 2016), we returned this as a “likely risk variant.”

242

243 *VPS13C* loss-of-function with *APOE* $\epsilon 4$

244 A patient with mixed symptoms of AD and FTD with onset in the late 60s harbored *VPS13C*
245 (NM_020821.2, c.10954C>T, p.R3652*) and two *APOE* $\epsilon 4$ alleles. A patient with EOAD with onset in
246 the late 40s had *VPS13C* (NM_020821.2, c.1988delC, p.T663Nfs*2), a variant in *PLCD1*
247 (NM_006225.3, c.631C>T, p.R211W), and one *APOE* $\epsilon 4$ allele. Only *APOE* $\epsilon 4$ was reported back to
248 these patients because of uncertain contribution of the other variants to the phenotype. Homozygous
249 loss of *VPS13C* is associated with early-onset Parkinson’s (Schormair et al. 2018). We do not know the
250 significance of the observation of one loss-of-function allele here, although unpublished studies have
251 reported an association between heterozygous loss-of-function in *VPS13C* and FTD (see Supplemental
252 ACMG Pathogenicity Evidence Details). *PLCD1* was proposed as a candidate gene for AD in one study
253 (Shimohama et al. 1998). Observing two loss-of-function variants in *VPS13C* in this small cohort leads
254 us to speculate that heterozygous loss-of-function variants in *VPS13C* may contribute to early-onset
255 dementia.

256

257 ***Variants of Uncertain Significance or Research Interest***

258 Five other patients harbored interesting – but speculative – VUSs or combinations of variants of
259 interest for future research. These include (1) a patient with possible CADASIL and a haplotype of

260 uncertain significance with two variants in *NOTCH3* (NM_000435.2, c.133G>C, p.D45H and
261 NM_000435.2, c.154G>A, p.G52R), (2) a patient with a VUS in *MAPT* (NM_005910.5, c.1174A>G,
262 p.I392V), (3) a patient with an *APOE* ϵ 4 allele and a variant in both *ADAM10* (NM_001110.3, c.359T>C,
263 p.I120T) and *TTC3* (NM_001001894.2, c.5557G>A, p.V1853M), (4) a patient with an *APOE* ϵ 4 allele,
264 and a variant in both *SORL1* (NM_003105.5, c.1247G>A, p.R416Q) and *MME* (NM_007289.2,
265 c.1241A>G, p.Y414C), and (5) a patient with variants in *TM2D3* (NM_078474.2, c.206C>T, p.P69L)
266 and *TNK1* (NM_001251902.1, c.393C>G, p.H131Q). Furthermore, one patient harbored a secondary
267 pathogenic variant in *KCNQ1* (NM_000218.2, c.1552C>T, R518*). We expand on these cases in the
268 Supplemental ACMG Pathogenicity Evidence Details.

269

270 **Quantitative Enrichment of Multiple Alleles**

271 Because we observed so many cases harboring multiple established alleles, we asked if this
272 effect was statistically enriched over a control population recruited from the same geographical area,
273 with controls reporting a family history of dementia excluded. We set criteria for qualifying variants as
274 follows: (1) *TREM2* or *GBA* missense or loss-of-function variants with CADD>20 and population
275 frequency <0.5% in both gnomAD (Lek et al. 2016) and TOPMed Bravo (NHLBI 2018), (2) *ABCA7*,
276 *SORL1*, *TBK1*, or *GRN* loss-of-function variants with CADD>20 and population frequency <0.5%, (3)
277 the specific *PLD3* and *AKAP9* variants observed here (since their associations are for single alleles),
278 (4) missense only variants with CADD>20 and population frequency <0.01% for *SORL1*, *CSF1R*, *APP*,
279 *PSEN1*, *PSEN2*, and *MAPT*, (5) expansion carriers in *C9orf72*, and (6) *APOE* ϵ 4 alleles. We recognize
280 that this may contain bias since these filtering criteria were selected after analysis of cases. However,
281 we attempted to mitigate this by selecting reasonable thresholds that would catch variants not identified
282 in this study but that would still have been considered if they had been identified. For example, we did
283 not observe any variants meeting these criteria in *TBK1* or *GRN* but included them here because of
284 their important role in disease. We also included *C9orf72* carriers without information on if any are
285 present in the control population, but this is a reasonable assumption (see Supplemental ACMG
286 Pathogenicity Evidence Details).

287 Variants meeting the criteria described are highly enriched in cases (**Figure 4A**). Intriguingly,
288 there is no enrichment of *APOE* ϵ 4 alleles in the absence of other qualifying alleles (**Figure 4B**). In
289 contrast, the presence of *APOE* ϵ 4 alleles in combination with another qualifying variant is highly
290 enriched in cases, regardless of whether Mendelian variants are included in the calculation (**Figure 4C**)
291 or excluded (**Figure 4D**). The odds ratios for *APOE* ϵ 4 alleles in combination with another qualifying
292 variant in cases without a Mendelian cause suggests that the presence of rare variants increases odds
293 ratios approximately multiplicatively over those typically reported for *APOE* ϵ 4 alone (typically reported:
294 ~2.5 for one *APOE* ϵ 4 allele, with a rare variant, 5.5; 10–15 for two *APOE* ϵ 4 alleles, with a rare variant,
295 39.1), see Supplemental ACMG Pathogenicity Evidence Details on *APOE*) (**Figure 4D**).

296

297 **DISCUSSION**

298 One key theme in this study was the frequent observation of multiple possible contributory
299 alleles. We even observed this in multiple cases with clear, highly penetrant, pathogenic variants
300 despite a small cohort size. The degree to which additional alleles contribute in dominant cases cannot
301 be assessed without larger cohorts to evaluate effects on age-of-onset or other variables. However,
302 given that other studies have made similar observations in ALS/FTD (van Blitterswijk et al. 2012; van
303 Blitterswijk et al. 2014; Pottier et al. 2015; Giannoccaro et al. 2017; Farhan et al. 2018), this
304 phenomenon clearly warrants further investigation.

305 In cases where a dominant pathogenic variant was not present, there was enrichment for
306 multiple established alleles contributing to disease risk. Every case with a moderately penetrant risk
307 variant established by case-control studies identified in this cohort also harbored one or two *APOE* ϵ 4
308 alleles, emphasizing the importance of *APOE* ϵ 4. Future efforts in analysis of large cohorts should
309 include analysis of level of risk when rare risk variants are present, for example by incorporation of
310 signal from rare variation in established risk genes into polygenic risk scores. Several groups have
311 begun developing polygenic risk scores for AD (Escott-Price et al. 2015; Desikan et al. 2017), but these
312 scores are based solely on common variation. This is, of course, a reasonable approach because it
313 maximizes reproducibility, as considering rare variants could lead to an over-trained model. However,
314 while rare variants are rare individually, aggregation approaches may provide replicable and meaningful

315 signals if incorporated for key genes where rare variants are now established to confer risk for AD, such
316 as *ABCA7*, *SORL1*, and *TREM2*. Similarly, while large FTD genetic studies are not as progressed as
317 those for AD, we can begin to consider genes where variation in a polygenic risk score may be
318 informative for FTD, such as *TBK1* (Cirulli et al. 2015), *MFSD8* (Geier et al. 2019), *DPP6*, *UNC13A*,
319 and *HLA-DQA2* (Pottier et al. 2019).

320 In Conclusion, this study demonstrates the high diagnostic and research utility of genome
321 sequencing in cases of early-onset dementia. Mendelian diagnostic yield in this population was 28%,
322 with an additional 22% of patients harboring risk-increasing variants that, in combination with *APOE* ϵ 4,
323 likely account for most of the genetic contribution to their symptoms. Genome sequencing is able to
324 identify relevant variation in conditions with high genetic heterogeneity, nonspecific phenotypes, or
325 established risk factors that do not follow a clear Mendelian pattern, and allowed for identification of
326 cryptic genotype-phenotype relationships that likely would have been missed by panel testing. In
327 addition to the research value of this study, it had value for patient care as well, for example by allowing
328 for referral of families to the Dominantly Inherited Alzheimer's Network and the Advancing Research &
329 Treatment for Frontotemporal Lobar Degeneration study. We conclude that application of more
330 comprehensive genetic testing (including genome sequencing, where appropriate) could aid in
331 evaluation of early-onset dementia cases currently and will continue to grow in utility for future use.

332

333 **METHODS**

334 *Genome sequencing*

335 Genome sequencing was performed at the HudsonAlpha Institute for Biotechnology on Illumina
336 HiSeq X or NovaSeq platforms using paired end 150 base pair reads. Mean depth was 34X with an
337 average of 91.5% of bases covered at 20X. Sequencing libraries were prepared by Covaris shearing,
338 end repair, adapter ligation, and PCR using standard protocols. Library concentrations were normalized
339 using KAPA qPCR prior to sequencing. All sequencing variants returned to patients were validated by
340 CAP/CLIA Sanger.

341

342 *Data processing and quality control*

343 Demuxed FASTQs were aligned with bwa-0.7.12 (Li and Durbin 2009) to hg19. BAMs were
344 sorted and duplicates were marked with Sambamba 0.5.4 (Tarasov et al. 2015). Indels were realigned,
345 bases were recalibrated, and gVCFs were generated with GATK 3.3 (McKenna et al. 2010). gVCFs
346 were batch called with GATK 3.8. KING 2.1.2 (Manichaikul et al. 2010) was used for sex checks on
347 VCFs, for validation of known familial relationships, and to check for unknown familial relationships
348 (none of which were identified).

349

350 *C9orf72 expansion testing*

351 Samples from 30 of 32 patients were tested for pathogenic *C9orf72* repeat expansion alleles by
352 GeneDx (Gaithersburg, MD). Two patients did not have sufficient material for testing, but both lacked
353 symptoms consistent with a *C9orf72* repeat expansion and also had another likely explanation of
354 symptoms: one had a pathogenic *APP* variant and another harbored both one *APOE* ϵ 4 allele and a
355 *TREM2* established risk allele).

356

357 *Genomic data analysis*

358 The HudsonAlpha-developed Codicem application ([http://envisiongenomics.com/codicem-](http://envisiongenomics.com/codicem-analysis-platform/)
359 [analysis-platform/](http://envisiongenomics.com/codicem-analysis-platform/)) was used to analyze and support interpretation of the variant data (described
360 elsewhere (Holt et al. 2019)). Although this software package was used for analysis, it would not be
361 necessary to use this package to reproduce this work. Simple filtering for population allele frequencies
362 (ie gnomAD (Lek et al. 2016) and TOPMed Bravo (NHLBI 2018)), *in silico* deleteriousness scores (ie
363 CADD (Kircher et al. 2014), PolyPhen-2 (Adzhubei et al. 2010), and SIFT (Ng and Henikoff 2003)), and
364 gene lists relevant to the phenotype of interest would recapitulate our findings using any suitable
365 software package, or even by a command line interface.

366 In addition to searching for single nucleotide variants and small indels, we also searched for
367 large copy number variations using four callers (DELLY (Rausch et al. 2012), ERDS (Zhu et al. 2012),
368 CNVnator (Abyzov et al. 2011), and BIC-seq2 (Xi et al. 2016)), but did not identify any relevant to
369 patient phenotypes (including absence of *APP* duplications).

370

371 *SORL1 structural modeling*

372 SORL1 structural modeling and evolutionary conservation analysis was assessed using a
373 previously published sequence-to-structure-to-function workflow (Prokop et al. 2017).

374

375 *Statistics*

376 The exact conditional Cochran-Armitage trend test was calculated using the CATTexact 0.1.0
377 package and Fisher's exact test using fisher.test in R 3.4.1.

378

379 *Return of results*

380 Results meeting criteria for return were delivered to patients by clinicians in the UAB Memory
381 Disorders Clinic through letters written by a genetic counselor. Letters included information on the
382 variant, associated disease, recurrence risk, and management recommendations. Patients were given
383 the option to have a genetic counselor present for return of results via phone or videoconference or to
384 follow up with a genetic counselor after delivery of results. Primary results were provided only to
385 probands. Although a secondary result was identified in only one participant who was a patient, we did
386 also offer non-patient participants (family members) receipt of actionable secondary findings (ACMG
387 59™) if such a result had been identified. Family members of patients that received diagnostic results
388 were provided with information to seek out clinical genetic counseling and targeted testing for familial
389 variants if they desired.

390

391 **ADDITIONAL INFORMATION**

392 *Data Deposition and Access*

393 All data from participants enrolled as a part of this study, including more detailed phenotype
394 data for the cases described here, are available on the National Institute on Aging Genetics of
395 Alzheimer's Disease Data Storage (NIAGADS) site under project NG00082. Data from control subjects
396 not enrolled as a part of this study are available under dbGaP accession phs001089.v3.p1, which
397 contains data generated by the Clinical Sequencing Exploratory Research (CSER) Consortium

398 established by the NHGRI. Funding support for CSER was provided through cooperative agreements
399 with the NHGRI and NCI through grant numbers U01 HG007301 (Genomic Diagnosis in Children with
400 Developmental Delay). Information about CSER and the investigators and institutions who comprise the
401 CSER consortium can be found at <https://cser-consortium.org>.

402 ADNI (Alzheimer's Disease Neuroimaging Initiative, part of the ADSP genomes batch call) and ADSP
403 data are available at NIAGADS under projects NG00066 and NG00067 and on dbGap under accession
404 phs000572.v7.p4 (see Supplemental Extended Acknowledgements for full list of ADNI and ADSP
405 contributors and funding sources).

406

407 *Ethics Statement*

408 This study was approved by UAB IRB protocol X161202004, "Evaluation of Genomic Variants in
409 Patients with Neurologic Diseases." All participants described provided explicit written consent for
410 publication.

411

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419 correspondence indicating the absence from both cases and controls of the M105T variant in *SORL1* in
420 the dataset published in (Bellenguez et al. 2017).

421

422 *Authors' contributions*

423 JNC, GMC, RMM, and EDR designed the study. JNC and RMM secured funding. JNC and EDR
424 wrote the IRB protocol. ECM coordinated all aspects of patient interaction. JNC, MDA, BAM, and BNL
425 analyzed genomes with input from MEC, ECM, and EDR. MDA coordinated *C9orf72* testing. JNC,

426 DEG, JMJL, JWP, EGG, JMH, and JSN conducted other analyses. MEC wrote clinical letters and
427 provided genetic counseling. MLT provided phenotype information for controls. JSY accessed ADSP
428 and supervised EGG. EAW supervised JMH, JSN, and the software development team. EDR, DSG and
429 MNL recruited participants and returned results. GMC supervised DEG, JMJL, and MLT. JNC wrote the
430 manuscript, with edits by ECM, MEC, MDA, BAM, BNL, JWP, EGG, JMH, EAW, GMC, and EDR. All
431 authors approved the final manuscript.

432

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436

437 **SUPPLEMENTAL MATERIAL**

438 **ACMG Pathogenicity Evidence Details:**

439 ***APP (NM_000484.3, c.2149G>T, V717F).***

440 Two strong criteria, three moderate criteria, and one supporting criterion result in the ACMG-
441 recommended assertion of “pathogenic.”

- 442 • Strong segregation data (Murrell et al. 1991; Finckh et al. 2005) (ACMG criterion PP1S)
- 443 • Biochemical studies (Tamaoka et al. 1994; Nilsberth et al. 2001; Sato et al. 2003) (ACMG
444 criterion PS3)
- 445 • The same amino acid is mutated to other amino acids by other segregating EOAD pathogenic
446 variants (Chartier-Harlin et al. 1991; Goate et al. 1991; Murrell et al. 2000), and others reviewed
447 in the AD&FTD Mutation Database (Cruts et al. 2012) (ACMG criterion PM5)
- 448 • The variant is located in a well-established functional domain at the epsilon cleavage site for
449 gamma secretase (Dimitrov et al. 2013) (and reviewed in (Holtzman et al. 2011)) (ACMG
450 criterion PM1)
- 451 • Absent from the gnomAD (Lek et al. 2016) and TOPMed Bravo population databases (NHLBI
452 2018) (ACMG criterion PM2)
- 453 • Predicted damaging by multiple computational methods (CADD (Kircher et al. 2014), PolyPhen-
454 2 (Adzhubei et al. 2010), and SIFT (Ng and Henikoff 2003)) (ACMG criterion PP3).

455

456 ***C9orf72 Expansion Carriers***

- 457 • Strong segregation with ALS and FTD (DeJesus-Hernandez et al. 2011; Renton et al. 2011)
458 (ACMG criterion PP1S).
- 459 • Extensive functional studies support the pathogenicity of this allele (key examples in (Chew et
460 al. 2015; Zhang et al. 2015), and recently reviewed in (Babic Leko et al. 2019; Vatsavayai et al.
461 2019)) (ACMG criterion PS3).
- 462 • *Note on the assumption that C9orf72 expansions will be absent from controls:* two studies have
463 assessed the frequency of C9orf72 expansions in healthy controls, both arriving at a frequency
464 of approximately 0.2% of individuals (Beck et al. 2013; Kaivola et al. 2019) (this would be

465 equivalent to approximately 1 carrier in our control set of 542 individuals). However, in one of
466 these studies, they also assessed for other neurologic diseases, and found that 4 of 6
467 individuals with *C9orf72* expansions (out of 3142) had another neurologic disease (Kaivola et al.
468 2019), leaving only 2 expansion carriers out of 3142 individuals in that study. Therefore, the
469 assumption that no repeat expansion carriers are present in the control set we selected where
470 individuals with a family history of any neurologic disease have been excluded is not
471 unreasonable.

472

473 **ARSA alleles**

474 In one *C9orf72* expansion carrier, we identified a possibly contributory combination of variants in
475 *ARSA*, associated with recessive metachromatic leukodystrophy (which can include dementia as a
476 symptom): one reported pathogenic variant that may maintain some residual activity (an “R” allele)
477 (NM_000487.5, c.256C>T, p.R86W), and one variant of uncertain significance (VUS) (NM_000487.5,
478 c.585G>T, p.W195C) that may be a pseudo-deficiency allele. Because we did not have phasing data
479 for these two variants and could not follow up with a biochemical test of enzyme activity (the patient
480 died between study enrollment and the observation of the variants in *ARSA*), the specific contribution of
481 these variants is unknown.

- 482 • Reported pathogenic variant that may maintain some residual activity (an “R” allele)
483 (NM_000487.5, c.256C>T, p.R86W) (Biffi et al. 2008; Cesani et al. 2016)
- 484 • Reported Variant of uncertain significance (VUS) (NM_000487.5, c.585G>T, p.W195C) that
485 may be a pseudo-deficiency allele (Xiong et al. 2015; Cesani et al. 2016; Dehghan Manshadi et
486 al. 2017)
- 487 • These alleles were reported together as a VUS, with special emphasis that this combination of
488 alleles may have no or little influence on disease given the presence of a *C9orf72* expansion
- 489 • <https://rarediseases.org/rare-diseases/metachromatic-leukodystrophy/>

490

491 **ABCA7 Loss-of-Function Alleles**

- 492 • We identified two loss-of-function variants in *ABCA7*: (NM_019112.3,
493 c.2126_2132delAGCAGGG, p.E709Afs*86) and *ABCA7* (NM_019112.3, c.5035G>T, p.E1679*
494 Loss-of-function variants in *ABCA7* have been solidly associated with AD by several
495 independent case-control studies (Cuyvers et al. 2015; Del-Aguila et al. 2015; Steinberg et al.
496 2015b; Allen et al. 2017; De Roeck et al. 2017; N'Songo et al. 2017).

497

498 **APOE ε4 allele**

- 499 • The APOE ε4 allele is definitively established by a plethora of studies to be associated with AD,
500 with a few key references noted here (Corder et al. 1993; Saunders et al. 1993; Farrer et al.
501 1997; Lambert et al. 2013; Yu et al. 2014; Qian et al. 2017).

502

503 **PSEN1 (NM_000021.3, c.103C>T, p.R35W)**

- 504 • Another VUS in *PSEN1* has been described at Arg35 that does not completely segregate with
505 disease (Rogaeva et al. 2001; Raux et al. 2005; Benitez et al. 2013).

506

507 **MAPT (NM_005910.5, c.1216C>T, p.R406W)**

- 508 • Strong segregation with EOAD in multiple studies (Reed et al. 1997; Rademakers et al. 2003;
509 Cruts et al. 2012) (ACMG criterion PP1S).
- 510 • Functional studies (Hasegawa et al. 1998; Hong et al. 1998; Krishnamurthy and Johnson 2004;
511 Zhang et al. 2004) (ACMG criterion PS3).
- 512 • Predicted damaging by multiple computational methods (CADD (Kircher et al. 2014), PolyPhen-
513 2 (Adzhubei et al. 2010), and SIFT (Ng and Henikoff 2003)) (ACMG criterion PP3).
- 514 • Altogether, the presence of two strong criteria and one supporting criterion result in the ACMG-
515 recommended assertion of “pathogenic.”

516

517 **CSF1R (NM_005211.3, c.2699G>A, p.R900K)**

- 518 • Critical domain of *CSF1R* where other pathogenic variants also cluster (Rademakers et al.
519 2011; Stabile et al. 2016) (ACMG criterion PM1)
- 520 • Absent from the gnomAD (Lek et al. 2016) and TOPMed Bravo population databases (ACMG
521 criterion PM2)
- 522 • This particular variant has been reported before along with segregation data (Kortvelyessy et al.
523 2015) (ACMG criterion PP1).
- 524 • Predicted damaging by multiple computational predictions (CADD, PolyPhen-2, and SIFT)
525 (ACMG criterion PP3).
- 526 • Taken together, the presence of two moderate criteria and two supporting criteria result in the
527 ACMG-recommended assertion of “likely pathogenic.”

528

529 **PLD3 variant (NM_012268.3, c.694G>A, p.V232M)**

530 While the *PLD3* variant described here has been controversial because of replication in some
531 but not all cohorts tested, we considered it a “likely risk variant” based on available evidence (Cruchaga
532 et al. 2014; Cacace et al. 2015; Cruchaga and Goate 2015b; Cruchaga and Goate 2015a; Heilmann et
533 al. 2015; Hooli et al. 2015; Lambert et al. 2015; van der Lee et al. 2015; Engelman et al. 2018). Rare
534 variants are not expected to replicate in all cohorts because of population effects and stochastic
535 sampling.

536

537 **VUS in APP (NM_000484.3, c.742G>A, p.D248N)**

538 This variant (*APP* (NM_000484.3, c.742G>A, p.D248N)) was returned to the patient as a VUS,
539 but with language indicating that, especially in the presence of the additional variants observed (*APOE*
540 $\epsilon 4$ homozygosity and the *PLD3* V232M variant), it may not contribute much, if at all, to symptoms.

541

542 **SORL1 M105T**

543 Because this variant lies in a critical functional domain for *SORL1*, the VPS10 domain (Pottier et
544 al. 2012; Caglayan et al. 2014; Louwersheimer et al. 2017), we computational modeled the effect of the

545 variant. Modelling suggests this is a highly conserved residue (Fig. 3A) where change to a Threonine
546 may create a PLK1 kinase site (Fig. 3B). PLK1 has known roles in the cell cycle, and is aberrantly
547 present in neurons of AD patients but not age-matched controls (Song et al. 2011), leading us to
548 speculate that presence of this variant in SORL1 may lead to faster progression of disease if this kinase
549 phosphorylates this residue, which could disrupt the amyloid- β clearance mechanism of the VPS10
550 domain (Kitago et al. 2015).

- 551 • Studies where *SORL1* M105T would have been observed, but no other carriers of *SORL1*
552 M105T were identified in either cases or controls (Vardarajan et al. 2015; Fernandez et al. 2016;
553 Verheijen et al. 2016; Bellenguez et al. 2017).

554

555 **TREM2**

556 TREM2 is a well-established risk factor for AD and FTD. References for the specific variants
557 described are as follows:

- 558 • *TREM2* (NM_018965.3, c.140G>A, p.R47H) (Guerreiro et al. 2013; Jonsson et al. 2013)
- 559 • *TREM2* (NM_018965.3, c.259G>A, p.D87N) (Guerreiro et al. 2013; Cuyvers et al. 2014; Ghani
560 et al. 2015; Jin et al. 2015; Ghani et al. 2016; Piccio et al. 2016)

561

562 **VPS13C Loss-of-Function Support**

- 563 • unpublished studies have reported an association between heterozygous loss-of-function
564 variant in *VPS13C* and FTD (Philtjens 2014; Picillo 2018)

565

566 **Variants of Uncertain Significance and Variants of Research Interest**

567 Variants denoted as “Variants of Uncertain Significance” described in the following section were
568 returned to patients because it would be possible, with limited additional information, for them to
569 become established as associated with the patients phenotype. Variants denoted as of “research
570 interest” in contrast were not returned to patients because it would take a great deal of evidence to
571 establish a definitive link to the patient’s phenotype, but there is limited literature evidence indicating
572 that it is important that we point them out to the field.

573

574 **A possible CADASIL case with two non-Cysteine variants in NOTCH3 (D45H and G52R)**
575 **spanning C49**

576 A patient with a differential diagnosis of cerebral amyloid angiopathy, leukodystrophy, or
577 CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and
578 Leukoencephalopathy) (Joutel et al. 1996) harbored two variants on the same allele in *NOTCH3*
579 (NM_000435.2, c.133G>C, p.D45H and NM_000435.2, c.154G>A, p.G52R). While these variants do
580 not induce a typically pathogenic alteration of a Cysteine, they do flank pathogenic variants at residue
581 Cys49 that have been reported with three different amino acid changes (Clinvar RCV000518559.1,
582 RCV000710993.1, RCV000518038.1, and (Joutel et al. 1996; Oki et al. 2007; Wang et al. 2011; Meng
583 et al. 2012)). Both of the variants we observe are in ClinVar as variants of uncertain significance
584 (RCV000518589.1 and RCV000516491.1). Furthermore, both variants are predicted damaging by
585 CADD (27.6 and 29.5) and SIFT, and one (D45H) is predicted damaging by PolyPhen-2. We speculate
586 that, given that these variants fall on the same haplotype, the presence of one or both of these variants
587 may affect the function of residue Cys49 or other nearby disease-associated Cys residues such as
588 Cys43 (Clinvar RCV000517549.1) or Cys55 (Clinvar RCV000710994.1 and RCV000516615.1).
589 Biochemical testing for CADASIL would be informative in this case, and this haplotype was returned as
590 a variant of uncertain significance with clear language in the report that biochemical testing should be
591 pursued.

592

593 **A case with a MAPT VUS**

594 A patient with unspecified dementia with an age-of-onset in the late 40s had a VUS returned in
595 *MAPT* (NM_005910.5, c.1174A>G, p.I392V). Family history information was incomplete for this patient,
596 precluding knowledge of if a dominant family history was present. The variant had a CADD score of
597 24.6, was absent from gnomAD (out of 135,743 non-TOPMed individuals), and was present only one
598 time in TOPMed Bravo (out of 62,784 individuals). The closest pathogenic variants are R406W (already
599 described) and G389R (Murrell et al. 1999; Ghetti et al. 2000; Pickering-Brown et al. 2000;
600 Bermingham et al. 2008; Rossi et al. 2008). Of note, these established pathogenic variants are present

601 four and three times in gnomAD, respectively, indicating that the rarity of the VUS observed here
602 justifies return to the patient as a VUS. The uncertainty around this variant was emphasized in the letter
603 to the patient.

604

605 **A case with APOE ϵ 4 Heterozygosity, ADAM10 I120T, and TTC3 V1893M**

606 A patient with corticobasal syndrome with onset in the early 50s and positive amyloid PET was
607 found to harbor two variants of research interest, but that did not reach the level of evidence needed for
608 return of the variants as a VUS. The variants were in *ADAM10* (NM_001110.3, c.359T>C, p.I120T) and
609 *TTC3* (NM_001001894.2, c.5557G>A, p.V1853M). The *ADAM10* variant had a borderline CADD score
610 of 14.3 and was not predicted damaging by PolyPhen-2 or SIFT. Furthermore, the variant was
611 observed in gnomAD 12 times. *ADAM10* has been proposed as a candidate gene for AD in prior
612 studies (Kim et al. 2009) including two variants in the same domain as the variant identified here, the
613 prodomain (Suh et al. 2013). Furthermore, variation in *ADAM10* recently reached genome-wide
614 significance for association with AD by GWAS (Marioni et al. 2018; Kunkle et al. 2019). However, we
615 have chosen to not return this variant in the absence of more information about effect size or
616 segregation. The *TTC3* variant also had a borderline CADD score (14.6) and was not predicted
617 damaging by PolyPhen-2 or SIFT. However, this variant was not observed in gnomAD or TOPMed
618 Bravo. A different *TTC3* variant (NM_001001894.2, c.3113C>G, p.S1038C) has been reported to
619 segregate with late-onset AD in one family (Kohli et al. 2016). However, since we lacked segregation
620 data for the variant we observed, we did not have enough evidence to consider the *TTC3* variant as
621 more than a variant of research interest, and thus did not return the variant to the patient.

622

623 **A case with APOE ϵ 4 Heterozygosity, SORL1 R416Q, and MME Y414C**

624 A case with mild dementia of uncertain etiology and symptoms consistent with neuropathy with
625 onset in the mid 50s had one *APOE* ϵ 4 allele along with variants in *SORL1* (NM_003105.5, c.1247G>A,
626 p.R416Q) and *MME* (NM_007289.2, c.1241A>G, p.Y414C). This *SORL1* variant has a CADD score of
627 34 and is also predicted damaging by PolyPhen-2 and SIFT. A link between *MME* and
628 neurodegeneration, including AD and neuropathy, has previously been proposed (Rey-Salgueiro et al.

629 2009; Auer-Grumbach et al. 2016; Depondt et al. 2016), but there was insufficient evidence for this
630 particular variant in *MME* or for the *SORL1* variant to justify return to the patient.

631

632 **A case with TM2D3 P69L and TNK1 H131Q**

633 A patient with mild dementia due to either AD or bvFTD with onset in the mid 50s had variants in
634 *TM2D3* (NM_078474.2, c.206C>T, p.P69L) and *TNK1* (NM_001251902.1, c.393C>G, p.H131Q). A
635 different variant in *TM2D3* has been nominated as AD-associated from an Icelandic cohort
636 (Jakobsdottir et al. 2016). Other variants in *TNK1* have been nominated as AD-associated from
637 analysis of Alzheimer's Disease Sequencing Project data (He et al. 2017). While neither of these
638 variants had sufficient evidence for return as risk variants, our observation of these variants in this
639 cohort adds evidence for the possible contribution of variants in these genes to disease.

640

641 **Secondary Finding**

642 One patient harbored a secondary pathogenic variant in *KCNQ1* (NM_000218.2, c.1552C>T,
643 R518*), associated with cardiac arrhythmias. This is a known founder effect variant from the Swedish
644 population that responds well to beta blockers (Winbo et al. 2014). The variant is a null variant in a
645 gene where loss-of-function is a known mechanism of disease (ACMG criterion PVS1) and is enriched
646 in cases vs. controls with an odds ratio >5 (ACMG criterion PS4) (Kapplinger et al. 2009). Furthermore,
647 the variant's effect is supported by well-established functional studies (Harmer et al. 2014) (ACMG
648 criterion PS3). Taken together, the presence of one very strong criterion and two strong criteria result in
649 the ACMG-recommended assertion of "pathogenic." Consistent with the study consent and protocol,
650 presence of this variant was reported to the patient.

651

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669 (MAYO), Mayo Parkinson's Disease controls, University of Miami, the Multi-Institutional Research in
670 Alzheimer's Genetic Epidemiology Study (MIRAGE), the National Cell Repository for Alzheimer's
671 Disease (NCRAD), the National Institute on Aging Late Onset Alzheimer's Disease Family Study (NIA-
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739 **ADNI:**

740 Michael Weiner, MD (UC San Francisco, Principal Investigator, Executive Committee); Paul Aisen, MD
741 (UC San Diego, ADCS PI and Director of Coordinating Center Clinical Core, Executive Committee,
742 Clinical Core Leaders); Ronald Petersen, MD, PhD (Mayo Clinic, Rochester, Executive Committee,
743 Clinical Core Leader); Clifford R. Jack, Jr., MD (Mayo Clinic, Rochester, Executive Committee, MRI
744 Core Leader); William Jagust, MD (UC Berkeley, Executive Committee; PET Core Leader); John Q.
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746 Toga, PhD (USC, Executive Committee, Informatics Core Leader); Laurel Beckett, PhD (UC Davis,
747 Executive Committee, Biostatistics Core Leader); Robert C. Green, MD, MPH (Brigham and Women's
748 Hospital, Harvard Medical School, Executive Committee and Chair of Data and Publication Committee);
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750 Morris, MD (Washington University St. Louis, Executive Committee, Neuropathology Core Leader);
751 Leslie M. Shaw (University of Pennsylvania, Executive Committee, Biomarkers Core Leader); Enchi
752 Liu, PhD (Janssen Alzheimer Immunotherapy, ADNI two Private Partner Scientific Board Chair); Tom
753 Montine, MD, PhD (University of Washington) ; Ronald G. Thomas, PhD (UC San Diego); Michael
754 Donohue, PhD (UC San Diego); Sarah Walter, MSc (UC San Diego); Devon Gessert (UC San Diego);
755 Tamie Sather, MS (UC San Diego,); Gus Jiminez, MBS (UC San Diego); Danielle Harvey, PhD (UC
756 Davis,); Michael Donohue, PhD (UC San Diego); Matthew Bernstein, PhD (Mayo Clinic, Rochester);
757 Nick Fox, MD (University of London); Paul Thompson, PhD (USC School of Medicine); Norbert Schuff,
758 PhD (UCSF MRI); Charles DeCarli, MD (UC Davis); Bret Borowski, RT (Mayo Clinic); Jeff Gunter, PhD
759 (Mayo Clinic); Matt Senjem, MS (Mayo Clinic); Prashanthi Vemuri, PhD (Mayo Clinic); David Jones, MD
760 (Mayo Clinic); Kejal Kantarci (Mayo Clinic); Chad Ward (Mayo Clinic); Robert A. Koeppe, PhD
761 (University of Michigan, PET Core Leader); Norm Foster, MD (University of Utah); Eric M. Reiman, MD
762 (Banner Alzheimer's Institute); Kewei Chen, PhD (Banner Alzheimer's Institute); Chet Mathis, MD
763 (University of Pittsburgh); Susan Landau, PhD (UC Berkeley); Nigel J. Cairns, PhD, MRCPATH
764 (Washington University St. Louis); Erin Householder (Washington University St. Louis); Lisa Taylor
765 Reinwald, BA, HTL (Washington University St. Louis); Virginia Lee, PhD, MBA (UPenn School of

766 Medicine); Magdalena Korecka, PhD (UPenn School of Medicine); Michal Figurski, PhD (UPenn School
767 of Medicine); Karen Crawford (USC); Scott Neu, PhD (USC); Tatiana M. Foroud, PhD (Indiana
768 University); Steven Potkin, MD UC (UC Irvine); Li Shen, PhD (Indiana University); Faber Kelley, MS,
769 CCRC (Indiana University); Sungeun Kim, PhD (Indiana University); Kwangsik Nho, PhD (Indiana
770 University); Zaven Kachaturian, PhD (Khachaturian, Radebaugh & Associates, Inc and Alzheimer's
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774 MD (Oregon Health and Science University); Betty Lind, BS (Oregon Health and Science University);
775 Raina Carter, BA (Oregon Health and Science University); Sara Dolen, BS (Oregon Health and Science
776 University); Lon S. Schneider, MD (University of Southern California); Sonia Pawluczyk, MD (University
777 of Southern California); Mauricio Beccera, BS (University of Southern California); Liberty Teodoro, RN
778 (University of Southern California); Bryan M. Spann, DO, PhD (University of Southern California);
779 James Brewer, MD, PhD (University of California San Diego); Helen Vanderswag, RN (University of
780 California San Diego); Adam Fleisher, MD (University of California San Diego); Judith L. Heidebrink,
781 MD, MS (University of Michigan); Joanne L. Lord, LPN, BA, CCRC (University of Michigan); Ronald
782 Petersen, MD, PhD (Mayo Clinic, Rochester); Sara S. Mason, RN (Mayo Clinic, Rochester); Colleen S.
783 Albers, RN (Mayo Clinic, Rochester); David Knopman, MD (Mayo Clinic, Rochester); Kris Johnson, RN
784 (Mayo Clinic, Rochester); Rachelle S. Doody, MD, PhD (Baylor College of Medicine); Javier Villanueva
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790 Carroll, RN, MSN (Washington University, St. Louis); Sue Leon, RN, MSN (Washington University, St.
791 Louis); Erin Householder, MS, CCRP (Washington University, St. Louis); Mark A. Mintun, MD
792 (Washington University, St. Louis); Stacy Schneider, APRN, BC, GNP (Washington University, St.
793 Louis); Angela Oliver, RN, BSN, MSG ; Daniel Marson, JD, PhD (University of Alabama Birmingham);

794 Randall Griffith, PhD, ABPP (University of Alabama Birmingham); David Clark, MD (University of
795 Alabama Birmingham); David Geldmacher, MD (University of Alabama Birmingham); John Brockington,
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797 Hillel Grossman, MD (Mount Sinai School of Medicine); Effie Mitsis, PhD (Mount Sinai School of
798 Medicine); Leyla deToledo-Morrell, PhD (Rush University Medical Center); Raj C. Shah, MD (Rush
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800 T. Greig, HP (Wien Center); Peggy Roberts, CNA (Wien Center); Marilyn Albert, PhD (Johns Hopkins
801 University); Chiadi Onyike, MD (Johns Hopkins University); Daniel D'Agostino II, BS (Johns Hopkins
802 University); Stephanie Kielb, BS (Johns Hopkins University); James E. Galvin, MD, MPH (New York
803 University); Dana M. Pogorelec (New York University); Brittany Cerbone (New York University);
804 Christina A. Michel (New York University); Henry Rusinek, PhD (New York University); Mony J de Leon,
805 EdD (New York University); Lidia Glodzik, MD, PhD (New York University); Susan De Santi, PhD (New
806 York University); P. Murali Doraiswamy, MD (Duke University Medical Center); Jeffrey R. Petrella, MD
807 (Duke University Medical Center); Terence Z. Wong, MD (Duke University Medical Center); Steven E.
808 Arnold, MD (University of Pennsylvania); Jason H. Karlawish, MD (University of Pennsylvania); David
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810 (University of Kentucky); Peter Hardy, PhD (University of Kentucky); Partha Sinha, PhD (University of
811 Kentucky); Elizabeth Oates, MD (University of Kentucky); Gary Conrad, MD (University of Kentucky);
812 Oscar L. Lopez, MD (University of Pittsburgh); MaryAnn Oakley, MA (University of Pittsburgh); Donna
813 M. Simpson, CRNP, MPH (University of Pittsburgh); Anton P. Porsteinsson, MD (University of
814 Rochester Medical Center); Bonnie S. Goldstein, MS, NP (University of Rochester Medical Center);
815 Kim Martin, RN (University of Rochester Medical Center); Kelly M. Makino, BS (University of Rochester
816 Medical Center); M. Saleem Ismail, MD (University of Rochester Medical Center); Connie Brand, RN
817 (University of Rochester Medical Center); Ruth A. Mulnard, DNSc, RN, FAAN (University of California,
818 Irvine); Gaby Thai, MD (University of California, Irvine); Catherine Mc Adams Ortiz, MSN, RN, A/GNP
819 (University of California, Irvine); Kyle Womack, MD (University of Texas Southwestern Medical School);
820 Dana Mathews, MD, PhD (University of Texas Southwestern Medical School); Mary Quiceno, MD
821 (University of Texas Southwestern Medical School); Ramon Diaz Arrastia, MD, PhD (University of

822 Texas Southwestern Medical School); Richard King, MD (University of Texas Southwestern Medical
823 School); Myron Weiner, MD (University of Texas Southwestern Medical School); Kristen Martin Cook,
824 MA (University of Texas Southwestern Medical School); Michael DeVous, PhD (University of Texas
825 Southwestern Medical School); Allan I. Levey, MD, PhD (Emory University); James J. Lah, MD, PhD
826 (Emory University); Janet S. Cellar, DNP, PMHCNS BC (Emory University); Jeffrey M. Burns, MD
827 (University of Kansas, Medical Center); Heather S. Anderson, MD (University of Kansas, Medical
828 Center); Russell H. Swerdlow, MD (University of Kansas, Medical Center); Liana Apostolova, MD
829 (University of California, Los Angeles); Kathleen Tingus, PhD (University of California, Los Angeles);
830 Ellen Woo, PhD (University of California, Los Angeles); Daniel H.S. Silverman, MD, PhD (University of
831 California, Los Angeles); Po H. Lu, PsyD (University of California, Los Angeles); George Bartzokis, MD
832 (University of California, Los Angeles); Neill R Graff Radford, MBBCH, FRCP (London) (Mayo Clinic,
833 Jacksonville); Francine Parfitt, MSH, CCRC (Mayo Clinic, Jacksonville); Tracy Kendall, BA, CCRP
834 (Mayo Clinic, Jacksonville); Heather Johnson, MLS, CCRP (Mayo Clinic, Jacksonville); Martin R.
835 Farlow, MD (Indiana University); Ann Marie Hake, MD (Indiana University); Brandy R. Matthews, MD
836 (Indiana University); Scott Herring, RN, CCRC (Indiana University); Cynthia Hunt, BS, CCRP (Indiana
837 University); Christopher H. van Dyck, MD (Yale University School of Medicine); Richard E. Carson, PhD
838 (Yale University School of Medicine); Martha G. MacAvoy, PhD (Yale University School of Medicine);
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840 Univ., Montreal Jewish General Hospital); Chris Hosein, Med (McGill Univ., Montreal Jewish General
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842 (Sunnybrook Health Sciences, Ontario); Curtis Caldwell, PhD (Sunnybrook Health Sciences, Ontario);
843 Ging Yuek Robin Hsiung, MD, MHSc, FRCPC (U.B.C. Clinic for AD & Related Disorders); Howard
844 Feldman, MD, FRCPC (U.B.C. Clinic for AD & Related Disorders); Benita Mudge, BS (U.B.C. Clinic for
845 AD & Related Disorders); Michele Assaly, MA Past (U.B.C. Clinic for AD & Related Disorders); Andrew
846 Kertesz, MD (Cognitive Neurology St. Joseph's, Ontario); John Rogers, MD (Cognitive Neurology St.
847 Joseph's, Ontario); Dick Trost, PhD (Cognitive Neurology St. Joseph's, Ontario); Charles Bernick, MD
848 (Cleveland Clinic Lou Ruvo Center for Brain Health); Donna Munic, PhD (Cleveland Clinic Lou Ruvo
849 Center for Brain Health); Diana Kerwin, MD (Northwestern University); Marek Marsel Mesulam, MD

850 (Northwestern University); Kristine Lipowski, BA (Northwestern University); Chuang Kuo Wu, MD, PhD
851 (Northwestern University); Nancy Johnson, PhD (Northwestern University); Carl Sadowsky, MD
852 (Premiere Research Inst (Palm Beach Neurology)); Walter Martinez, MD (Premiere Research Inst
853 (Palm Beach Neurology)); Teresa Villena, MD (Premiere Research Inst (Palm Beach Neurology));
854 Raymond Scott Turner, MD, PhD (Georgetown University Medical Center); Kathleen Johnson, NP
855 (Georgetown University Medical Center); Brigid Reynolds, NP (Georgetown University Medical Center);
856 Reisa A. Sperling, MD (Brigham and Women's Hospital); Keith A. Johnson, MD (Brigham and Women's
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858 Hospital); Jerome Yesavage, MD (Stanford University); Joy L. Taylor, PhD (Stanford University); Barton
859 Lane, MD (Stanford University); Allyson Rosen, PhD (Stanford University); Jared Tinklenberg, MD
860 (Stanford University); Marwan N. Sabbagh, MD (Banner Sun Health Research Institute); Christine M.
861 Belden, PsyD (Banner Sun Health Research Institute); Sandra A. Jacobson, MD (Banner Sun Health
862 Research Institute); Sherye A. Sirrel, MS (Banner Sun Health Research Institute); Neil Kowall, MD
863 (Boston University); Ronald Killiany, PhD (Boston University); Andrew E. Budson, MD (Boston
864 University); Alexander Norbash, MD (Boston University); Patricia Lynn Johnson, BA (Boston
865 University); Thomas O. Obisesan, MD, MPH (Howard University); Saba Wolday, MSc (Howard
866 University); Joanne Allard, PhD (Howard University); Alan Lerner, MD (Case Western Reserve
867 University); Paula Ogrocki, PhD (Case Western Reserve University); Leon Hudson, MPH (Case
868 Western Reserve University); Evan Fletcher, PhD (University of California, Davis Sacramento); Owen
869 Carmichael, PhD (University of California, Davis Sacramento); John Olichney, MD (University of
870 California, Davis Sacramento); Charles DeCarli, MD (University of California, Davis Sacramento); Smita
871 Kittur, MD (Neurological Care of CNY); Michael Borrie, MB ChB (Parkwood Hospital); T Y Lee, PhD
872 (Parkwood Hospital); Dr Rob Bartha, PhD (Parkwood Hospital); Sterling Johnson, PhD (University of
873 Wisconsin); Sanjay Asthana, MD (University of Wisconsin); Cynthia M. Carlsson, MD (University of
874 Wisconsin); Steven G. Potkin, MD (University of California, Irvine BIC); Adrian Preda, MD (University of
875 California, Irvine BIC); Dana Nguyen, PhD (University of California, Irvine BIC); Pierre Tariot, MD
876 (Banner Alzheimer's Institute); Adam Fleisher, MD (Banner Alzheimer's Institute); Stephanie Reeder,
877 BA (Banner Alzheimer's Institute); Vernice Bates, MD (Dent Neurologic Institute); Horacio Capote, MD

878 (Dent Neurologic Institute); Michelle Rainka, PharmD, CCRP (Dent Neurologic Institute); Douglas W.
879 Scharre, MD (Ohio State University); Maria Kataki, MD, PhD (Ohio State University); Anahita Adeli, MD
880 (Ohio State University); Earl A. Zimmerman, MD (Albany Medical College); Dzintra Celmins, MD
881 (Albany Medical College); Alice D. Brown, FNP (Albany Medical College); Godfrey D. Pearlson, MD
882 (Hartford Hosp, Olin Neuropsychiatry Research Center); Karen Blank, MD (Hartford Hosp, Olin
883 Neuropsychiatry Research Center); Karen Anderson, RN (Hartford Hosp, Olin Neuropsychiatry
884 Research Center); Robert B. Santulli, MD (Dartmouth Hitchcock Medical Center); Tamar J. Kitzmiller
885 (Dartmouth Hitchcock Medical Center); Eben S. Schwartz, PhD (Dartmouth Hitchcock Medical Center);
886 Kaycee M. Sink, MD, MAS (Wake Forest University Health Sciences); Jeff D. Williamson, MD, MHS
887 (Wake Forest University Health Sciences); Pradeep Garg, PhD (Wake Forest University Health
888 Sciences); Franklin Watkins, MD (Wake Forest University Health Sciences); Brian R. Ott, MD (Rhode
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890 Hospital); Stephen Salloway, MD, MS (Butler Hospital); Paul Malloy, PhD (Butler Hospital); Stephen
891 Correia, PhD (Butler Hospital); Howard J. Rosen, MD (UC San Francisco); Bruce L. Miller, MD (UC San
892 Francisco); Jacobo Mintzer, MD, MBA (Medical University South Carolina); Kenneth Spicer, MD, PhD
893 (Medical University South Carolina); David Bachman, MD (Medical University South Carolina);
894 Elizabeth Finger, MD (St. Joseph's Health Care); Stephen Pasternak, MD (St. Joseph's Health Care);
895 Irina Rachinsky, MD (St. Joseph's Health Care); John Rogers, MD (St. Joseph's Health Care); Andrew
896 Kertesz, MD (St. Joseph's Health Care); Dick Drost, MD (St. Joseph's Health Care); Nunzio Pomara,
897 MD (Nathan Kline Institute); Raymundo Hernando, MD (Nathan Kline Institute); Antero Sarrael, MD
898 (Nathan Kline Institute); Susan K. Schultz, MD (University of Iowa College of Medicine, Iowa City);
899 Laura L. Boles Ponto, PhD (University of Iowa College of Medicine, Iowa City); Hyungsub Shim, MD
900 (University of Iowa College of Medicine, Iowa City); Karen Elizabeth Smith, RN (University of Iowa
901 College of Medicine, Iowa City); Norman Relkin, MD, PhD (Cornell University); Gloria Chaing, MD
902 (Cornell University); Lisa Raudin, PhD (Cornell University); Amanda Smith, MD (University of South
903 Floriday: USF Health Byrd Alzheimer's Institute); Kristin Fargher, MD (University of South Floriday: USF
904 Health Byrd Alzheimer's Institute); Balebail Ashok Raj, MD (University of South Floriday: USF Health
905 Byrd Alzheimer's Institute)

906 **REFERENCES**

- 907 Abyzov A, Urban AE, Snyder M, Gerstein M. 2011. CNVnator: an approach to discover, genotype, and
908 characterize typical and atypical CNVs from family and population genome sequencing.
909 *Genome Res* **21**: 974–984.
- 910 Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev
911 SR. 2010. A method and server for predicting damaging missense mutations. *Nat Methods* **7**:
912 248–249.
- 913 Akimoto C, Volk AE, van Blitterswijk M, Van den Broeck M, Leblond CS, Lumbroso S, Camu W, Neitzel
914 B, Onodera O, van Rheenen W et al. 2014. A blinded international study on the reliability of
915 genetic testing for GGGGCC-repeat expansions in C9orf72 reveals marked differences in
916 results among 14 laboratories. *J Med Genet* **51**: 419–424.
- 917 Allen M, Lincoln SJ, Corda M, Watzlawik JO, Carrasquillo MM, Reddy JS, Burgess JD, Nguyen T,
918 Malphrus K, Petersen RC et al. 2017. ABCA7 loss-of-function variants, expression, and
919 neurologic disease risk. *Neurol Genet* **3**: e126.
- 920 Auer-Grumbach M, Toegel S, Schabhuttl M, Weinmann D, Chiari C, Bennett DLH, Beetz C, Klein D,
921 Andersen PM, Bohme I et al. 2016. Rare Variants in MME, Encoding Metalloprotease
922 Nephilysin, Are Linked to Late-Onset Autosomal-Dominant Axonal Polyneuropathies. *Am J Hum*
923 *Genet* **99**: 607–623.
- 924 Babic Leko M, Zupunski V, Kirincich J, Smilovic D, Hortobagyi T, Hof PR, Simic G. 2019. Molecular
925 Mechanisms of Neurodegeneration Related to C9orf72 Hexanucleotide Repeat Expansion.
926 *Behav Neurol* **2019**: 2909168.
- 927 Beck J, Poulter M, Hensman D, Rohrer JD, Mahoney CJ, Adamson G, Campbell T, Uphill J, Borg A,
928 Fratta P et al. 2013. Large C9orf72 hexanucleotide repeat expansions are seen in multiple
929 neurodegenerative syndromes and are more frequent than expected in the UK population. *Am J*
930 *Hum Genet* **92**: 345–353.
- 931 Bellenguez C, Charbonnier C, Grenier-Boley B, Quenez O, Le Guennec K, Nicolas G, Chauhan G,
932 Wallon D, Rousseau S, Richard AC et al. 2017. Contribution to Alzheimer's disease risk of rare

- 933 variants in TREM2, SORL1, and ABCA7 in 1779 cases and 1273 controls. *Neurobiol Aging* **59**:
934 220.e221–220.e229.
- 935 Benitez BA, Karch CM, Cai Y, Jin SC, Cooper B, Carrell D, Bertelsen S, Chibnik L, Schneider JA,
936 Bennett DA et al. 2013. The PSEN1, p.E318G variant increases the risk of Alzheimer's disease
937 in APOE-epsilon4 carriers. *PLoS Genet* **9**: e1003685.
- 938 Bermingham N, Cowie TF, Paine M, Storey E, McLean C. 2008. Frontotemporal dementia and
939 Parkinsonism linked to chromosome 17 in a young Australian patient with the G389R Tau
940 mutation. *Neuropathol Appl Neurobiol* **34**: 366–370.
- 941 Biffi A, Cesani M, Fumagalli F, Del Carro U, Baldoli C, Canale S, Gerevini S, Amadio S, Falautano M,
942 Rovelli A et al. 2008. Metachromatic leukodystrophy - mutation analysis provides further
943 evidence of genotype-phenotype correlation. *Clin Genet* **74**: 349–357.
- 944 Bis JC, Jian X, Kunkle BW, Chen Y, Hamilton-Nelson KL, Bush WS, Salerno WJ, Lancour D, Ma Y,
945 Renton AE et al. 2018. Whole exome sequencing study identifies novel rare and common
946 Alzheimer's-Associated variants involved in immune response and transcriptional regulation.
947 *Mol Psychiatry* doi:10.1038/s41380-018-0112-7.
- 948 Blauwendraat C, Wilke C, Simon-Sanchez J, Jansen IE, Reifschneider A, Capell A, Haass C, Castillo-
949 Lizardo M, Biskup S, Maetzler W et al. 2018. The wide genetic landscape of clinical
950 frontotemporal dementia: systematic combined sequencing of 121 consecutive subjects. *Genet*
951 *Med* **20**: 240–249.
- 952 Bowling KM, Thompson ML, Amaral MD, Finnila CR, Hiatt SM, Engel KL, Cochran JN, Brothers KB,
953 East KM, Gray DE et al. 2017. Genomic diagnosis for children with intellectual disability and/or
954 developmental delay. *Genome Med* **9**: 43.
- 955 Cacace R, Van den Bossche T, Engelborghs S, Geerts N, Laureys A, Dillen L, Graff C, Thonberg H,
956 Chiang HH, Pastor P et al. 2015. Rare Variants in PLD3 Do Not Affect Risk for Early-Onset
957 Alzheimer Disease in a European Consortium Cohort. *Hum Mutat* **36**: 1226–1235.
- 958 Caglayan S, Takagi-Niidome S, Liao F, Carlo AS, Schmidt V, Burgert T, Kitago Y, Fuchtbauer EM,
959 Fuchtbauer A, Holtzman DM et al. 2014. Lysosomal sorting of amyloid-beta by the SORLA
960 receptor is impaired by a familial Alzheimer's disease mutation. *Sci Transl Med* **6**: 223ra220.

- 961 Champion D, Charbonnier C, Nicolas G. 2019. SORL1 genetic variants and Alzheimer disease risk: a
962 literature review and meta-analysis of sequencing data. *Acta Neuropathol* doi:10.1007/s00401-
963 019-01991-4.
- 964 Cesani M, Lorioli L, Grossi S, Amico G, Fumagalli F, Spiga I, Filocamo M, Biffi A. 2016. Mutation
965 Update of ARSA and PSAP Genes Causing Metachromatic Leukodystrophy. *Hum Mutat* **37**:
966 16–27.
- 967 Chartier-Harlin MC, Crawford F, Houlden H, Warren A, Hughes D, Fidani L, Goate A, Rossor M,
968 Roques P, Hardy J et al. 1991. Early-onset Alzheimer's disease caused by mutations at codon
969 717 of the beta-amyloid precursor protein gene. *Nature* **353**: 844–846.
- 970 Chesi A, Staahl BT, Jovicic A, Couthouis J, Fasolino M, Raphael AR, Yamazaki T, Elias L, Polak M,
971 Kelly C et al. 2013. Exome sequencing to identify de novo mutations in sporadic ALS trios. *Nat*
972 *Neurosci* **16**: 851–855.
- 973 Chew J, Gendron TF, Prudencio M, Sasaguri H, Zhang YJ, Castanedes-Casey M, Lee CW, Jansen-
974 West K, Kurti A, Murray ME et al. 2015. Neurodegeneration. C9ORF72 repeat expansions in
975 mice cause TDP-43 pathology, neuronal loss, and behavioral deficits. *Science* **348**: 1151–1154.
- 976 Cirulli ET, Lasseigne BN, Petrovski S, Sapp PC, Dion PA, Leblond CS, Couthouis J, Lu YF, Wang Q,
977 Krueger BJ et al. 2015. Exome sequencing in amyotrophic lateral sclerosis identifies risk genes
978 and pathways. *Science* **347**: 1436–1441.
- 979 Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines
980 JL, Pericak-Vance MA. 1993. Gene dose of apolipoprotein E type 4 allele and the risk of
981 Alzheimer's disease in late onset families. *Science* **261**: 921–923.
- 982 Cruchaga C, Goate AM. 2015a. Cruchaga & Goate reply. *Nature* **520**: E5–6.
- 983 Cruchaga C, Goate AM. 2015b. Cruchaga & Goate reply. *Nature* **520**: E10.
- 984 Cruchaga C, Karch CM, Jin SC, Benitez BA, Cai Y, Guerreiro R, Harari O, Norton J, Budde J, Bertelsen
985 S et al. 2014. Rare coding variants in the phospholipase D3 gene confer risk for Alzheimer's
986 disease. *Nature* **505**: 550–554.
- 987 Cruts M, Theuns J, Van Broeckhoven C. 2012. Locus-specific mutation databases for
988 neurodegenerative brain diseases. *Hum Mutat* **33**: 1340–1344.

- 989 Cuyvers E, Bettens K, Philtjens S, Van Langenhove T, Gijssels I, van der Zee J, Engelborghs S,
990 Vandenbulcke M, Van Dongen J, Geerts N et al. 2014. Investigating the role of rare
991 heterozygous TREM2 variants in Alzheimer's disease and frontotemporal dementia. *Neurobiol*
992 *Aging* **35**: 726 e711–729.
- 993 Cuyvers E, De Roeck A, Van den Bossche T, Van Cauwenberghe C, Bettens K, Vermeulen S,
994 Mattheijssens M, Peeters K, Engelborghs S, Vandenbulcke M et al. 2015. Mutations in ABCA7
995 in a Belgian cohort of Alzheimer's disease patients: a targeted resequencing study. *Lancet*
996 *neurology* **14**: 814–822.
- 997 Dashnow H, Lek M, Phipson B, Halman A, Sadedin S, Lonsdale A, Davis M, Lamont P, Clayton JS,
998 Laing NG et al. 2018. STretch: detecting and discovering pathogenic short tandem repeat
999 expansions. *Genome Biol* **19**: 121.
- 1000 De Roeck A, Van den Bossche T, van der Zee J, Verheijen J, De Coster W, Van Dongen J, Dillen L,
1001 Baradaran-Heravi Y, Heeman B, Sanchez-Valle R et al. 2017. Deleterious ABCA7 mutations
1002 and transcript rescue mechanisms in early onset Alzheimer's disease. *Acta Neuropathol* **134**:
1003 475–487.
- 1004 Dehghan Manshadi M, Kamalidehghan B, Aryani O, Khalili E, Dadgar S, Tondar M, Ahmadipour F,
1005 Yong Meng G, Houshmand M. 2017. Four novel ARSA gene mutations with pathogenic impacts
1006 on metachromatic leukodystrophy: a bioinformatics approach to predict pathogenic mutations.
1007 *Ther Clin Risk Manag* **13**: 725–731.
- 1008 DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM,
1009 Finch NA, Flynn H, Adamson J et al. 2011. Expanded GGGGCC hexanucleotide repeat in
1010 noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* **72**: 245–
1011 256.
- 1012 Del-Aguila JL, Fernandez MV, Jimenez J, Black K, Ma S, Deming Y, Carrell D, Saef B, Alzheimer's
1013 Disease Neuroimaging I, Howells B et al. 2015. Role of ABCA7 loss-of-function variant in
1014 Alzheimer's disease: a replication study in European-Americans. *Alzheimers Res Ther* **7**: 73.
- 1015 Depondt C, Donatello S, Rai M, Wang FC, Manto M, Simonis N, Pandolfo M. 2016. MME mutation in
1016 dominant spinocerebellar ataxia with neuropathy (SCA43). *Neurol Genet* **2**: e94.

- 1017 Desikan RS, Fan CC, Wang Y, Schork AJ, Cabral HJ, Cupples LA, Thompson WK, Besser L, Kukull
1018 WA, Holland D et al. 2017. Genetic assessment of age-associated Alzheimer disease risk:
1019 Development and validation of a polygenic hazard score. *PLoS Med* **14**: e1002258.
- 1020 Dimitrov M, Alattia JR, Lemmin T, Lehal R, Fligier A, Houacine J, Hussain I, Radtke F, Dal Peraro M,
1021 Beher D et al. 2013. Alzheimer's disease mutations in APP but not gamma-secretase
1022 modulators affect epsilon-cleavage-dependent AICD production. *Nat Commun* **4**: 2246.
- 1023 Dolzhenko E, van Vugt J, Shaw RJ, Bekritsky MA, van Blitterswijk M, Narzisi G, Ajay SS, Rajan V,
1024 Lajoie BR, Johnson NH et al. 2017. Detection of long repeat expansions from PCR-free whole-
1025 genome sequence data. *Genome Res* **27**: 1895–1903.
- 1026 Engelman CD, Darst BF, Bilgel M, Vasiljevic E, Kosciak RL, Jedynak BM, Johnson SC. 2018. The effect
1027 of rare variants in TREM2 and PLD3 on longitudinal cognitive function in the Wisconsin Registry
1028 for Alzheimer's Prevention. *Neurobiol Aging* **66**: 177.e171–177.e175.
- 1029 Escott-Price V, Sims R, Bannister C, Harold D, Vronskaya M, Majounie E, Badarinarayan N,
1030 Gerad/Perades, consortia I, Morgan K et al. 2015. Common polygenic variation enhances risk
1031 prediction for Alzheimer's disease. *Brain* **138**: 3673–3684.
- 1032 Farhan SMK, Gendron TF, Petrucelli L, Hegele RA, Strong MJ. 2018. OPTN p.Met468Arg and ATXN2
1033 intermediate length polyQ extension in families with C9orf72 mediated amyotrophic lateral
1034 sclerosis and frontotemporal dementia. *Am J Med Genet B Neuropsychiatr Genet* **177**: 75–85.
- 1035 Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA,
1036 Risch N, van Duijn CM. 1997. Effects of age, sex, and ethnicity on the association between
1037 apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer
1038 Disease Meta Analysis Consortium. *JAMA* **278**: 1349–1356.
- 1039 Fernandez MV, Black K, Carrell D, Saef B, Budde J, Deming Y, Howells B, Del-Aguila JL, Ma S, Bi C et
1040 al. 2016. SORL1 variants across Alzheimer's disease European American cohorts. *Eur J Hum*
1041 *Genet* **24**: 1828–1830.
- 1042 Finckh U, Kuschel C, Anagnostouli M, Patsouris E, Pantos GV, Gatzonis S, Kapaki E, Davaki P,
1043 Lamszus K, Stavrou D et al. 2005. Novel mutations and repeated findings of mutations in
1044 familial Alzheimer disease. *Neurogenetics* **6**: 85–89.

- 1045 Geier EG, Bourdenx M, Storm NJ, Cochran JN, Sirkis DW, Hwang JH, Bonham LW, Ramos EM, Diaz
1046 A, Van Berlo V et al. 2019. Rare variants in the neuronal ceroid lipofuscinosis gene MFSD8 are
1047 candidate risk factors for frontotemporal dementia. *Acta Neuropathol* **137**: 71–88.
- 1048 Ghani M, Lang AE, Zinman L, Nacmias B, Sorbi S, Bessi V, Tedde A, Tartaglia MC, Surace EI, Sato C
1049 et al. 2015. Mutation analysis of patients with neurodegenerative disorders using NeuroX array.
1050 *Neurobiol Aging* **36**: 545 e549–514.
- 1051 Ghani M, Sato C, Kakhki EG, Gibbs JR, Traynor B, St George-Hyslop P, Rogaeva E. 2016. Mutation
1052 analysis of the MS4A and TREM gene clusters in a case-control Alzheimer's disease data set.
1053 *Neurobiol Aging* **42**: 217.e217–217.e213.
- 1054 Ghetti B, Murrell JR, Zolo P, Spillantini MG, Goedert M. 2000. Progress in hereditary tauopathies: a
1055 mutation in the Tau gene (G389R) causes a Pick disease-like syndrome. *Ann N Y Acad Sci*
1056 **920**: 52–62.
- 1057 Giannoccaro MP, Bartoletti-Stella A, Piras S, Pession A, De Massis P, Oppi F, Stanzani-Maserati M,
1058 Pasini E, Baiardi S, Avoni P et al. 2017. Multiple variants in families with amyotrophic lateral
1059 sclerosis and frontotemporal dementia related to C9orf72 repeat expansion: further
1060 observations on their oligogenic nature. *J Neurol* **264**: 1426–1433.
- 1061 Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N,
1062 James L et al. 1991. Segregation of a missense mutation in the amyloid precursor protein gene
1063 with familial Alzheimer's disease. *Nature* **349**: 704–706.
- 1064 Goldman JS, Farmer JM, Wood EM, Johnson JK, Boxer A, Neuhaus J, Lomen-Hoerth C, Wilhelmsen
1065 KC, Lee VM, Grossman M et al. 2005. Comparison of family histories in FTLN subtypes and
1066 related tauopathies. *Neurology* **65**: 1817–1819.
- 1067 Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, Cruchaga C, Sassi C, Kauwe
1068 JS, Younkin S et al. 2013. TREM2 variants in Alzheimer's disease. *N Engl J Med* **368**: 117–127.
- 1069 Harmer SC, Mohal JS, Royal AA, McKenna WJ, Lambiase PD, Tinker A. 2014. Cellular mechanisms
1070 underlying the increased disease severity seen for patients with long QT syndrome caused by
1071 compound mutations in KCNQ1. *Biochem J* **462**: 133–142.

- 1072 Hasegawa M, Smith MJ, Goedert M. 1998. Tau proteins with FTDP-17 mutations have a reduced ability
1073 to promote microtubule assembly. *FEBS Lett* **437**: 207–210.
- 1074 He Z, Zhang D, Renton AE, Li B, Zhao L, Wang GT, Goate AM, Mayeux R, Leal SM. 2017. The Rare-
1075 Variant Generalized Disequilibrium Test for Association Analysis of Nuclear and Extended
1076 Pedigrees with Application to Alzheimer Disease WGS Data. *Am J Hum Genet* **100**: 193–204.
- 1077 Heilmann S, Drichel D, Clarimon J, Fernandez V, Lacour A, Wagner H, Thelen M, Hernandez I, Fortea
1078 J, Alegret M et al. 2015. PLD3 in non-familial Alzheimer's disease. *Nature* **520**: E3–5.
- 1079 Holt JM, Wilk B, Birch CL, Brown DM, Gajapathy M, Moss AC, Sosonkina N, Wilk MA, Anderson JA,
1080 Harris JM et al. 2019. VarSight: Prioritizing Clinically Reported Variants with Binary
1081 Classification Algorithms. *bioRxiv* doi:10.1101/532440 %J bioRxiv: 532440.
- 1082 Holtzman DM, Morris JC, Goate AM. 2011. Alzheimer's disease: the challenge of the second century.
1083 *Sci Transl Med* **3**: 77sr71.
- 1084 Hong M, Zhukareva V, Vogelsberg-Ragaglia V, Wszolek Z, Reed L, Miller BI, Geschwind DH, Bird TD,
1085 McKeel D, Goate A et al. 1998. Mutation-specific functional impairments in distinct tau isoforms
1086 of hereditary FTDP-17. *Science* **282**: 1914–1917.
- 1087 Hooli BV, Lill CM, Mullin K, Qiao D, Lange C, Bertram L, Tanzi RE. 2015. PLD3 gene variants and
1088 Alzheimer's disease. *Nature* **520**: E7–8.
- 1089 Ikezu T, Chen C, DeLeo AM, Zeldich E, Fallin MD, Kanaan NM, Lunetta KL, Abraham CR, Logue MW,
1090 Farrer LA. 2018. Tau Phosphorylation is Impacted by Rare AKAP9 Mutations Associated with
1091 Alzheimer Disease in African Americans. *J Neuroimmune Pharmacol* **13**: 254–264.
- 1092 Jakobsdottir J, van der Lee SJ, Bis JC, Chouraki V, Li-Kroeger D, Yamamoto S, Grove ML, Naj A,
1093 Vronskaya M, Salazar JL et al. 2016. Rare Functional Variant in TM2D3 is Associated with Late-
1094 Onset Alzheimer's Disease. *PLoS Genet* **12**: e1006327.
- 1095 Jin SC, Carrasquillo MM, Benitez BA, Skorupa T, Carrell D, Patel D, Lincoln S, Krishnan S,
1096 Kachadoorian M, Reitz C et al. 2015. TREM2 is associated with increased risk for Alzheimer's
1097 disease in African Americans. *Mol Neurodegener* **10**: 19.

- 1098 Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson PV, Snaedal J, Bjornsson S, Huttenlocher
1099 J, Levey AI, Lah JJ et al. 2013. Variant of TREM2 associated with the risk of Alzheimer's
1100 disease. *N Engl J Med* **368**: 107–116.
- 1101 Joutel A, Corpechot C, Ducros A, Vahedi K, Chabriat H, Mouton P, Alamowitch S, Domenga V,
1102 Cecillion M, Marechal E et al. 1996. Notch3 mutations in CADASIL, a hereditary adult-onset
1103 condition causing stroke and dementia. *Nature* **383**: 707–710.
- 1104 Kaivola K, Kiviharju A, Jansson L, Rantalainen V, Eriksson JG, Strandberg TE, Laaksovirta H, Renton
1105 AE, Traynor BJ, Myllykangas L et al. 2019. C9orf72 hexanucleotide repeat length in older
1106 population: normal variation and effects on cognition. *Neurobiol Aging*
1107 doi:10.1016/j.neurobiolaging.2019.02.026.
- 1108 Kapplinger JD, Tester DJ, Salisbury BA, Carr JL, Harris-Kerr C, Pollevick GD, Wilde AA, Ackerman MJ.
1109 2009. Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients
1110 referred for the FAMILION long QT syndrome genetic test. *Heart Rhythm* **6**: 1297–1303.
- 1111 Kim M, Suh J, Romano D, Truong MH, Mullin K, Hooli B, Norton D, Tesco G, Elliott K, Wagner SL et al.
1112 2009. Potential late-onset Alzheimer's disease-associated mutations in the ADAM10 gene
1113 attenuate {alpha}-secretase activity. *Hum Mol Genet* **18**: 3987–3996.
- 1114 Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. 2014. A general framework for
1115 estimating the relative pathogenicity of human genetic variants. *Nat Genet* **46**: 310–315.
- 1116 Kitago Y, Nagae M, Nakata Z, Yagi-Utsumi M, Takagi-Niidome S, Mihara E, Nogi T, Kato K, Takagi J.
1117 2015. Structural basis for amyloidogenic peptide recognition by sorLA. *Nat Struct Mol Biol* **22**:
1118 199–206.
- 1119 Kohli MA, Cukier HN, Hamilton-Nelson KL, Rolati S, Kunkle BW, Whitehead PL, Zuchner SL, Farrer LA,
1120 Martin ER, Beecham GW et al. 2016. Segregation of a rare TTC3 variant in an extended family
1121 with late-onset Alzheimer disease. *Neurol Genet* **2**: e41.
- 1122 Kortvelyessy P, Krageloh-Mann I, Mawrin C, Heinze HJ, Bittner D, Wieland I, Zenker M, Nestor P.
1123 2015. Hereditary diffuse leukoencephalopathy with spheroids (HDLS) with a novel CSF1R
1124 mutation and spinal cord involvement. *J Neurol Sci* **358**: 515–517.

- 1125 Krishnamurthy PK, Johnson GV. 2004. Mutant (R406W) human tau is hyperphosphorylated and does
1126 not efficiently bind microtubules in a neuronal cortical cell model. *J Biol Chem* **279**: 7893–7900.
- 1127 Kunkle BW Grenier-Boley B Sims R Bis JC Damotte V Naj AC Boland A Vronskaya M van der Lee SJ
1128 Amlie-Wolf A et al. 2019. Genetic meta-analysis of diagnosed Alzheimer's disease identifies
1129 new risk loci and implicates A β , tau, immunity and lipid processing. *Nat Genet* **51**: 414–430.
- 1130 Lambert JC, Grenier-Boley B, Bellenguez C, Pasquier F, Campion D, Dartigues JF, Berr C, Tzourio C,
1131 Amouyel P. 2015. PLD3 and sporadic Alzheimer's disease risk. *Nature* **520**: E1.
- 1132 Lambert JC Ibrahim-Verbaas CA Harold D Naj AC Sims R Bellenguez C DeStafano AL Bis JC
1133 Beecham GW Grenier-Boley B et al. 2013. Meta-analysis of 74,046 individuals identifies 11 new
1134 susceptibility loci for Alzheimer's disease. *Nat Genet* **45**: 1452–1458.
- 1135 Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS,
1136 Hill AJ, Cummings BB et al. 2016. Analysis of protein-coding genetic variation in 60,706
1137 humans. *Nature* **536**: 285–291.
- 1138 Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform.
1139 *Bioinformatics* **25**: 1754–1760.
- 1140 Liu G, Boot B, Locascio JJ, Jansen IE, Winder-Rhodes S, Eberly S, Elbaz A, Brice A, Ravina B, van
1141 Hilten JJ et al. 2016. Specifically neuropathic Gaucher's mutations accelerate cognitive decline
1142 in Parkinson's. *Ann Neurol* **80**: 674–685.
- 1143 Logue MW, Schu M, Vardarajan BN, Farrell J, Bennett DA, Buxbaum JD, Byrd GS, Ertekin-Taner N,
1144 Evans D, Foroud T et al. 2014. Two rare AKAP9 variants are associated with Alzheimer's
1145 disease in African Americans. *Alzheimers Dement* **10**: 609–618 e611.
- 1146 Louwersheimer E, Cohn-Hokke PE, Pijnenburg YA, Weiss MM, Sistermans EA, Rozemuller AJ,
1147 Hulsman M, van Swieten JC, van Duijn CM, Barkhof F et al. 2017. Rare Genetic Variant in
1148 SORL1 May Increase Penetrance of Alzheimer's Disease in a Family with Several Generations
1149 of APOE-varepsilon4 Homozygosity. *J Alzheimers Dis* **56**: 63–74.
- 1150 Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen WM. 2010. Robust relationship
1151 inference in genome-wide association studies. *Bioinformatics* **26**: 2867–2873.

- 1152 Marioni RE, Harris SE, Zhang Q, McRae AF, Hagenaars SP, Hill WD, Davies G, Ritchie CW, Gale CR,
1153 Starr JM et al. 2018. GWAS on family history of Alzheimer's disease. *Transl Psychiatry* **8**: 99.
- 1154 Mata IF, Samii A, Schneer SH, Roberts JW, Griffith A, Leis BC, Schellenberg GD, Sidransky E, Bird
1155 TD, Leverenz JB et al. 2008. Glucocerebrosidase gene mutations: a risk factor for Lewy body
1156 disorders. *Arch Neurol* **65**: 379–382.
- 1157 McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytisky A, Garimella K, Altshuler D,
1158 Gabriel S, Daly M. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing
1159 next-generation DNA sequencing data. *Genome res* **20**: 1297–1303.
- 1160 Meng H, Zhang X, Yu G, Lee SJ, Chen YE, Prudovsky I, Wang MM. 2012. Biochemical
1161 characterization and cellular effects of CADASIL mutants of NOTCH3. *PLoS One* **7**: e44964.
- 1162 Murrell J, Farlow M, Ghetti B, Benson MD. 1991. A mutation in the amyloid precursor protein
1163 associated with hereditary Alzheimer's disease. *Science* **254**: 97–99.
- 1164 Murrell JR, Hake AM, Quaid KA, Farlow MR, Ghetti B. 2000. Early-onset Alzheimer disease caused by
1165 a new mutation (V717L) in the amyloid precursor protein gene. *Arch Neurol* **57**: 885–887.
- 1166 Murrell JR, Spillantini MG, Zolo P, Guazzelli M, Smith MJ, Hasegawa M, Redi F, Crowther RA, Pietrini
1167 P, Ghetti B et al. 1999. Tau gene mutation G389R causes a tauopathy with abundant pick body-
1168 like inclusions and axonal deposits. *J Neuropathol Exp Neurol* **58**: 1207–1226.
- 1169 N'Songo A, Carrasquillo MM, Wang X, Burgess JD, Nguyen T, Asmann YW, Serie DJ, Younkin SG,
1170 Allen M, Pedraza O et al. 2017. African American exome sequencing identifies potential risk
1171 variants at Alzheimer disease loci. *Neurol Genet* **3**: e141.
- 1172 Nalls MA, Duran R, Lopez G, Kurzawa-Akanbi M, McKeith IG, Chinnery PF, Morris CM, Theuns J,
1173 Crosiers D, Cras P et al. 2013. A multicenter study of glucocerebrosidase mutations in dementia
1174 with Lewy bodies. *JAMA Neurol* **70**: 727–735.
- 1175 Ng PC, Henikoff S. 2003. SIFT: Predicting amino acid changes that affect protein function. *Nucleic
1176 Acids Res* **31**: 3812–3814.
- 1177 NHLBI UoMa. 2018. The NHLBI Trans-Omics for Precision Medicine (TOPMed) Whole Genome
1178 Sequencing Program. BRAVO variant browser.

- 1179 Nilsberth C, Westlind-Danielsson A, Eckman CB, Condrón MM, Axelman K, Forsell C, Stenb C,
1180 Luthman J, Teplow DB, Younkin SG et al. 2001. The 'Arctic' APP mutation (E693G) causes
1181 Alzheimer's disease by enhanced Abeta protofibril formation. *Nat Neurosci* **4**: 887–893.
- 1182 Oki K, Nagata E, Ishiko A, Shimizu A, Tanaka K, Takahashi K, Tabira T, Katayama T, Suzuki N. 2007.
1183 Novel mutation of the Notch3 gene in a Japanese patient with CADASIL. *Eur J Neurol* **14**: 464–
1184 466.
- 1185 Philtjens SG, I.; Van Langenhove, T.; van der Zee, J.; Engelborghs, S.; Vandenbulcke, M.;
1186 Vandenbergh, R.; Santens, P.; De Deyn, P.P.; Van Broeckhoven, C.; Cruts, M.; BELNEU
1187 consortium. 2014. 9th International Conference on Frontotemporal Dementias Vancouver,
1188 Canada; October 23–25, 2014: Next generation sequencing identifies mutations in VPS13C
1189 associated with decreased expression of endogenous protein in Frontotemporal lobar
1190 degeneration. *Am J Neurodegener Dis* **3**: 1–375.
- 1191 Piccio L, Deming Y, Del-Aguila JL, Ghezzi L, Holtzman DM, Fagan AM, Fenoglio C, Galimberti D,
1192 Borroni B, Cruchaga C. 2016. Cerebrospinal fluid soluble TREM2 is higher in Alzheimer disease
1193 and associated with mutation status. *Acta Neuropathol* **131**: 925–933.
- 1194 Picillo MG, M.; Erro, R.; Dati, G.; Vallelunga, A.; Ceravolo, R.; Nicoletti, V.; Nicoletti, A.; Zappia, M.;
1195 Pellicchia, M.T.; Valente, E.M.; Barone, P. . 2018. Merging parkinsonism with dementia:
1196 application of a genetic panel in familial parkinsonism
1197 associated with cognitive impairment. *4° Congresso Accademia LIMPE-DISMOV, Centro Congressi A*
1198 *Roma Lifestyle Hotel* **24**: 26.
- 1199 Pickering-Brown S, Baker M, Yen SH, Liu WK, Hasegawa M, Cairns N, Lantos PL, Rossor M, Iwatsubo
1200 T, Davies Y et al. 2000. Pick's disease is associated with mutations in the tau gene. *Ann Neurol*
1201 **48**: 859–867.
- 1202 Pottier C, Bieniek KF, Finch N, van de Vorst M, Baker M, Perkersen R, Brown P, Ravenscroft T, van
1203 Blitterswijk M, Nicholson AM et al. 2015. Whole-genome sequencing reveals important role for
1204 TBK1 and OPTN mutations in frontotemporal lobar degeneration without motor neuron disease.
1205 *Acta Neuropathol* **130**: 77–92.

- 1206 Pottier C, Hannequin D, Coutant S, Rovelet-Lecrux A, Wallon D, Rousseau S, Legallic S, Paquet C,
1207 Bombois S, Pariente J et al. 2012. High frequency of potentially pathogenic SORL1 mutations in
1208 autosomal dominant early-onset Alzheimer disease. *Mol Psychiatry* **17**: 875–879.
- 1209 Pottier C Ren Y Perkerson RB, 3rd Baker M Jenkins GD van Blitterswijk M DeJesus-Hernandez M van
1210 Rooij JGJ Murray ME Christopher E et al. 2019. Genome-wide analyses as part of the
1211 international FTL-D-TDP whole-genome sequencing consortium reveals novel disease risk
1212 factors and increases support for immune dysfunction in FTL-D. *Acta Neuropathol*
1213 doi:10.1007/s00401-019-01962-9.
- 1214 Prokop JW, Lazar J, Crapitto G, Smith DC, Worthey EA, Jacob HJ. 2017. Molecular modeling in the
1215 age of clinical genomics, the enterprise of the next generation. *J Mol Model* **23**: 75.
- 1216 Qian J, Wolters FJ, Beiser A, Haan M, Ikram MA, Karlawish J, Langbaum JB, Neuhaus JM, Reiman
1217 EM, Roberts JS et al. 2017. APOE-related risk of mild cognitive impairment and dementia for
1218 prevention trials: An analysis of four cohorts. *PLoS Med* **14**: e1002254.
- 1219 Rademakers R, Baker M, Nicholson AM, Rutherford NJ, Finch N, Soto-Ortolaza A, Lash J, Wider C,
1220 Wojtas A, DeJesus-Hernandez M et al. 2011. Mutations in the colony stimulating factor 1
1221 receptor (CSF1R) gene cause hereditary diffuse leukoencephalopathy with spheroids. *Nat*
1222 *Genet* **44**: 200–205.
- 1223 Rademakers R, Dermaut B, Peeters K, Cruts M, Heutink P, Goate A, Van Broeckhoven C. 2003. Tau
1224 (MAPT) mutation Arg406Trp presenting clinically with Alzheimer disease does not share a
1225 common founder in Western Europe. *Hum Mutat* **22**: 409–411.
- 1226 Raghavan NS, Brickman AM, Andrews H, Manly JJ, Schupf N, Lantigua R, Wollock CJ, Kamalakaran S,
1227 Petrovski S, Tosto G et al. 2018. Whole-exome sequencing in 20,197 persons for rare variants
1228 in Alzheimer's disease. *Ann Clin Transl Neurol* **5**: 832–842.
- 1229 Rausch T, Zichner T, Schlattl A, Stütz AM, Benes V, Korb J. 2012. DELLY: structural variant
1230 discovery by integrated paired-end and split-read analysis. *Bioinformatics* **28**: i333–i339.
- 1231 Raux G, Guyant-Marechal L, Martin C, Bou J, Penet C, Brice A, Hannequin D, Frebourg T, Campion D.
1232 2005. Molecular diagnosis of autosomal dominant early onset Alzheimer's disease: an update. *J*
1233 *Med Genet* **42**: 793–795.

- 1234 Reed LA, Grabowski TJ, Schmidt ML, Morris JC, Goate A, Solodkin A, Van Hoesen GW, Schelper RL,
1235 Talbot CJ, Wragg MA et al. 1997. Autosomal dominant dementia with widespread neurofibrillary
1236 tangles. *Ann Neurol* **42**: 564–572.
- 1237 Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta
1238 H, van Swieten JC, Myllykangas L et al. 2011. A hexanucleotide repeat expansion in C9ORF72
1239 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* **72**: 257–268.
- 1240 Rey-Salgueiro L, Pontevedra-Pombal X, Alvarez-Casas M, Martinez-Carballo E, Garcia-Falcon MS,
1241 Simal-Gandara J. 2009. Comparative performance of extraction strategies for polycyclic
1242 aromatic hydrocarbons in peats. *J Chromatogr A* **1216**: 5235–5241.
- 1243 Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E
1244 et al. 2015. Standards and guidelines for the interpretation of sequence variants: a joint
1245 consensus recommendation of the American College of Medical Genetics and Genomics and
1246 the Association for Molecular Pathology. *Genet Med* **17**: 405–424.
- 1247 Rogaeva EA, Fafel KC, Song YQ, Medeiros H, Sato C, Liang Y, Richard E, Rogaev EI, Frommelt P,
1248 Sadovnick AD et al. 2001. Screening for PS1 mutations in a referral-based series of AD cases:
1249 21 novel mutations. *Neurology* **57**: 621–625.
- 1250 Rossi G, Marelli C, Farina L, Laura M, Maria Basile A, Ciano C, Tagliavini F, Pareyson D. 2008. The
1251 G389R mutation in the MAPT gene presenting as sporadic corticobasal syndrome. *Mov Disord*
1252 **23**: 892–895.
- 1253 Sassi C, Ridge PG, Nalls MA, Gibbs R, Ding J, Lupton MK, Troakes C, Lunnon K, Al-Sarraj S, Brown
1254 KS et al. 2016. Influence of Coding Variability in APP-Abeta Metabolism Genes in Sporadic
1255 Alzheimer's Disease. *PLoS One* **11**: e0150079.
- 1256 Sato T, Dohmae N, Qi Y, Kakuda N, Misonou H, Mitsumori R, Maruyama H, Koo EH, Haass C, Takio K
1257 et al. 2003. Potential link between amyloid beta-protein 42 and C-terminal fragment gamma 49-
1258 99 of beta-amyloid precursor protein. *J Biol Chem* **278**: 24294–24301.
- 1259 Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL,
1260 Gusella JF, Crapper-MacLachlan DR, Alberts MJ et al. 1993. Association of apolipoprotein E

- 1261 allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* **43**: 1467–
1262 1472.
- 1263 Schormair B, Kemlink D, Mollenhauer B, Fiala O, Machetanz G, Roth J, Berutti R, Strom TM, Haslinger
1264 B, Trenkwalder C et al. 2018. Diagnostic exome sequencing in early-onset Parkinson's disease
1265 confirms VPS13C as a rare cause of autosomal-recessive Parkinson's disease. *Clin Genet* **93**:
1266 603–612.
- 1267 Shimohama S, Kamiya S, Fujii M, Ogawa T, Kanamori M, Kawamata J, Imura T, Taniguchi T,
1268 Yagisawa H. 1998. Mutation in the pleckstrin homology domain of the human phospholipase C-
1269 delta 1 gene is associated with loss of function. *Biochem Biophys Res Commun* **245**: 722–728.
- 1270 Sims R van der Lee SJ Naj AC Bellenguez C Badarinarayan N Jakobsdottir J Kunkle BW Boland A
1271 Raybould R Bis JC et al. 2017. Rare coding variants in PLCG2, ABI3, and TREM2 implicate
1272 microglial-mediated innate immunity in Alzheimer's disease. *Nat Genet* **49**: 1373–1384.
- 1273 Song B, Davis K, Liu XS, Lee HG, Smith M, Liu X. 2011. Inhibition of Polo-like kinase 1 reduces beta-
1274 amyloid-induced neuronal cell death in Alzheimer's disease. *Aging (Albany NY)* **3**: 846–851.
- 1275 Stabile C, Taglia I, Battisti C, Bianchi S, Federico A. 2016. Hereditary diffuse leukoencephalopathy with
1276 axonal spheroids (HDLS): update on molecular genetics. *Neurol Sci* **37**: 1565–1569.
- 1277 Steinberg KM, Yu B, Koboldt DC, Mardis ER, Pamphlett R. 2015a. Exome sequencing of case-
1278 unaffected-parents trios reveals recessive and de novo genetic variants in sporadic ALS. *Sci*
1279 *Rep* **5**: 9124.
- 1280 Steinberg S, Stefansson H, Jonsson T, Johannsdottir H, Ingason A, Helgason H, Sulem P, Magnusson
1281 OT, Gudjonsson SA, Unnsteinsdottir U et al. 2015b. Loss-of-function variants in ABCA7 confer
1282 risk of Alzheimer's disease. *Nat Genet* **47**: 445–447.
- 1283 Suh J, Choi SH, Romano DM, Gannon MA, Lesinski AN, Kim DY, Tanzi RE. 2013. ADAM10 missense
1284 mutations potentiate beta-amyloid accumulation by impairing prodomain chaperone function.
1285 *Neuron* **80**: 385–401.
- 1286 Tamaoka A, Odaka A, Ishibashi Y, Usami M, Sahara N, Suzuki N, Nukina N, Mizusawa H, Shoji S,
1287 Kanazawa I et al. 1994. APP717 missense mutation affects the ratio of amyloid beta protein

- 1288 species (A beta 1-42/43 and a beta 1-40) in familial Alzheimer's disease brain. *J Biol Chem* **269**:
1289 32721–32724.
- 1290 Tarasov A, Vilella AJ, Cuppen E, Nijman IJ, Prins P. 2015. Sambamba: fast processing of NGS
1291 alignment formats. *Bioinformatics* **31**: 2032–2034.
- 1292 Tsuang D, Leverenz JB, Lopez OL, Hamilton RL, Bennett DA, Schneider JA, Buchman AS, Larson EB,
1293 Crane PK, Kaye JA et al. 2012. GBA mutations increase risk for Lewy body disease with and
1294 without Alzheimer disease pathology. *Neurology* **79**: 1944–1950.
- 1295 van Blitterswijk M, Mullen B, Wojtas A, Heckman MG, Diehl NN, Baker MC, DeJesus-Hernandez M,
1296 Brown PH, Murray ME, Hsiung GY et al. 2014. Genetic modifiers in carriers of repeat
1297 expansions in the C9ORF72 gene. *Mol Neurodegener* **9**: 38.
- 1298 van Blitterswijk M, van Es MA, Hennekam EA, Dooijes D, van Rheenen W, Medic J, Bourque PR,
1299 Schelhaas HJ, van der Kooi AJ, de Visser M et al. 2012. Evidence for an oligogenic basis of
1300 amyotrophic lateral sclerosis. *Hum Mol Genet* **21**: 3776–3784.
- 1301 van der Lee SJ, Holstege H, Wong TH, Jakobsdottir J, Bis JC, Chouraki V, van Rooij JG, Grove ML,
1302 Smith AV, Amin N et al. 2015. PLD3 variants in population studies. *Nature* **520**: E2–3.
- 1303 Vardarajan BN, Zhang Y, Lee JH, Cheng R, Bohm C, Ghani M, Reitz C, Reyes-Dumeyer D, Shen Y,
1304 Rogaeva E et al. 2015. Coding mutations in SORL1 and Alzheimer disease. *Ann Neurol* **77**:
1305 215–227.
- 1306 Vatsavayai SC, Nana AL, Yokoyama JS, Seeley WW. 2019. C9orf72-FTD/ALS pathogenesis: evidence
1307 from human neuropathological studies. *Acta Neuropathol* **137**: 1–26.
- 1308 Verheijen J, Van den Bossche T, van der Zee J, Engelborghs S, Sanchez-Valle R, Llado A, Graff C,
1309 Thonberg H, Pastor P, Ortega-Cubero S et al. 2016. A comprehensive study of the genetic
1310 impact of rare variants in SORL1 in European early-onset Alzheimer's disease. *Acta*
1311 *Neuropathol* **132**: 213–224.
- 1312 Vissers LE, de Ligt J, Gilissen C, Janssen I, Stehouwer M, de Vries P, van Lier B, Arts P, Wieskamp
1313 N, del Rosario M et al. 2010. A de novo paradigm for mental retardation. *Nat Genet* **42**: 1109–
1314 1112.

- 1315 Wang Z, Yuan Y, Zhang W, Lv H, Hong D, Chen B, Liu Y, Luan X, Xie S, Wu S. 2011. NOTCH3
1316 mutations and clinical features in 33 mainland Chinese families with CADASIL. *J Neurol*
1317 *Neurosurg Psychiatry* **82**: 534–539.
- 1318 Winbo A, Stattin EL, Nordin C, Diamant UB, Persson J, Jensen SM, Rydberg A. 2014. Phenotype,
1319 origin and estimated prevalence of a common long QT syndrome mutation: a clinical,
1320 genealogical and molecular genetics study including Swedish R518X/KCNQ1 families. *BMC*
1321 *Cardiovasc Disord* **14**: 22.
- 1322 Wingo TS, Lah JJ, Levey AI, Cutler DJ. 2012. Autosomal recessive causes likely in early-onset
1323 Alzheimer disease. *Arch Neurol* **69**: 59–64.
- 1324 Xi R, Lee S, Xia Y, Kim TM, Park PJ. 2016. Copy number analysis of whole-genome data using BIC-
1325 seq2 and its application to detection of cancer susceptibility variants. *Nucleic Acids Res* **44**:
1326 6274–6286.
- 1327 Xiong HY, Alipanahi B, Lee LJ, Bretschneider H, Merico D, Yuen RK, Hua Y, Gueroussov S, Najafabadi
1328 HS, Hughes TR et al. 2015. RNA splicing. The human splicing code reveals new insights into
1329 the genetic determinants of disease. *Science* **347**: 1254806.
- 1330 Yu JT, Tan L, Hardy J. 2014. Apolipoprotein E in Alzheimer's disease: an update. *Annu Rev Neurosci*
1331 **37**: 79–100.
- 1332 Zhang B, Higuchi M, Yoshiyama Y, Ishihara T, Forman MS, Martinez D, Joyce S, Trojanowski JQ, Lee
1333 VM. 2004. Retarded axonal transport of R406W mutant tau in transgenic mice with a
1334 neurodegenerative tauopathy. *J Neurosci* **24**: 4657–4667.
- 1335 Zhang K, Donnelly CJ, Haeusler AR, Grima JC, Machamer JB, Steinwald P, Daley EL, Miller SJ,
1336 Cunningham KM, Vidensky S et al. 2015. The C9orf72 repeat expansion disrupts
1337 nucleocytoplasmic transport. *Nature* **525**: 56–61.
- 1338 Zhu M, Need AC, Han Y, Ge D, Maia JM, Zhu Q, Heinzen EL, Cirulli ET, Pelak K, He M et al. 2012.
1339 Using ERDS to infer copy-number variants in high-coverage genomes. *Am J Hum Genet* **91**:
1340 408-421.
- 1341

1342 **FIGURE LEGENDS**

1343

1344 **Figure 1. Summary of genomic analysis results for 32 patients with early-onset or familial**
1345 **dementia.** Pathogenic variants were observed in APP (x2), C9orf72 (x3), and MAPT (x3). A likely
1346 pathogenic variant was observed in CSF1R. Four patients were APOE ϵ 4 homozygous, with three of
1347 these patients also harboring additional risk variants in GBA, PLD3, and TREM2. Three patients were
1348 APOE ϵ 4 heterozygous and had additional risk variants in AKAP9, SORL1, and TREM2. Two patients
1349 had variants of uncertain significance (VUS) in MAPT and NOTCH3. For six patients, the only
1350 returnable finding was APOE ϵ 4 heterozygosity. Eight patients had no returnable findings.

1351

1352 **Figure 2. Neuroimaging findings in a CSF1R variant carrier. (A,B)** Frontal-predominant, mildly
1353 asymmetric (R>L) white matter hyperintensities on axial FLAIR images. **(C,D)** Global cerebral atrophy
1354 on coronal and axial MPRAGE images. Radiological orientation with patient's R side displayed on L.

1355

1356 **Figure 3. Molecular modeling of the effect of the M105T variant on SORL1. (A)** Conservation
1357 analysis of the SORL1 gene sequence was performed across open reading frame sequences of 135
1358 species. Scores at each codon were assessed with 100% conservation receiving a score of 1, with
1359 addition of a score for codon selection (score of 0 if dN-dS of site is below mean, 0.25 for sites with
1360 values above the mean to one standard deviation above the mean, 0.5 for sites greater than one
1361 standard deviation but below two standard deviations, one for sites greater than two standard
1362 deviations). A score of two is maximal, suggesting an amino acid that is 100% conserved with codon
1363 wobble indicative of a high selection rate at the position. The values were then placed on a 21-codon
1364 sliding window (combining values for 10 codons before and after each position) to identify conserved
1365 motifs within the gene. **(B)** Model of SORL1 protein (assessed with YASARA2). Colors are based on
1366 135 species alignments fed into ConSurf such that colors indicate: gray=not conserved,
1367 yellow=conserved hydrophobic, red=conserved polar acidic, blue=conserved polar basic,
1368 green=conserved hydrophilic. Note that the M105T variant leads to a predicted gain of a PLK1 kinase
1369 target site in SORL1.

1370

1371 **Figure 4. Multiple variants in neurodegeneration-associated genes are often observed in early-**
1372 **onset dementia, with a critical role for rare variants acting in combination with APOE ϵ 4.** Note:
1373 for all panels, ϵ 4/ ϵ^* indicates either ϵ 4/ ϵ 3 or ϵ 4/ ϵ 2 (mostly ϵ 4/ ϵ 3). Also for all panels, cases N=31 (32
1374 probands excluding 1 sibling from an affected sibling pair) and controls N=542. **(A)** Qualifying candidate
1375 alleles associated with neurodegeneration (see text for criteria) are highly enriched in cases ($p=9.2 \times 10^{-$
1376 12 by exact conditional Cochran-Armitage trend test). **(B)** Presence of APOE ϵ 4 alone, in the absence of
1377 any other qualifying variants, is not enriched in cases ($p=0.57$ by exact conditional Cochran-Armitage
1378 trend test). **(C)** Presence of APOE ϵ 4 along with at least one qualifying rare variant (including
1379 Mendelian variants) is highly enriched in cases ($p=1.0 \times 10^{-9}$ by exact conditional Cochran-Armitage
1380 trend test). **(D)** Presence of APOE ϵ 4 along with at least one qualifying rare variant (excluding
1381 Mendelian variants) is highly enriched in cases ($p=1.4 \times 10^{-6}$ by exact conditional Cochran-Armitage
1382 trend test). The odds ratio for Presence of one APOE ϵ 4 allele along with one qualifying rare variant vs.
1383 controls is 5.5 ($p=0.01$ by Fisher's exact test, 95% CI 1.2–19.3). The odds ratio for Presence of two
1384 APOE ϵ 4 alleles along with one qualifying rare variant vs. controls is 39.1 ($p=9.8 \times 10^{-5}$ by Fisher's exact
1385 test, 95% CI 5.3–447.5).

1386

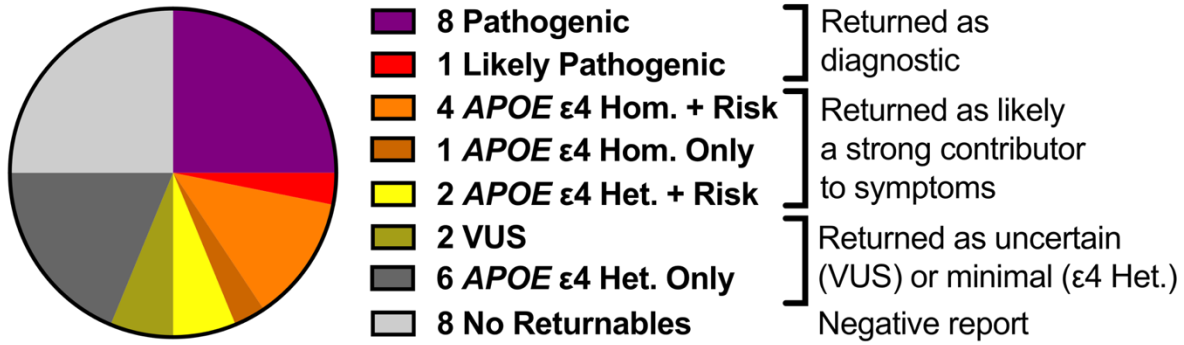
1387 **Table 1:** Variant Table. Note that many individuals had multiple candidate contributory variants, which
1388 is not captured when considering variants individually. For an expanded table that indicates multiple
1389 candidate variants, see **Supplemental Table 1**.

1390

1391 **Supplemental Table 1:** Phenotype and variant table. Prior clinical diagnosis category, age of onset
1392 range, family history score, **Figure 1** category, and variant information listed in **Table 1** for each
1393 proband are listed along with information on which variants were returned to patients and which did not
1394 have sufficient evidence for return but are of research interest. Note that some detailed information
1395 such as sex, age of onset to the year, self-reported ethnicity, and detailed phenotype and family history
1396 information has been excluded to protect the identity of participants but is available along with raw data
1397 via controlled access to qualified researchers.

1398 **Figure 1:**

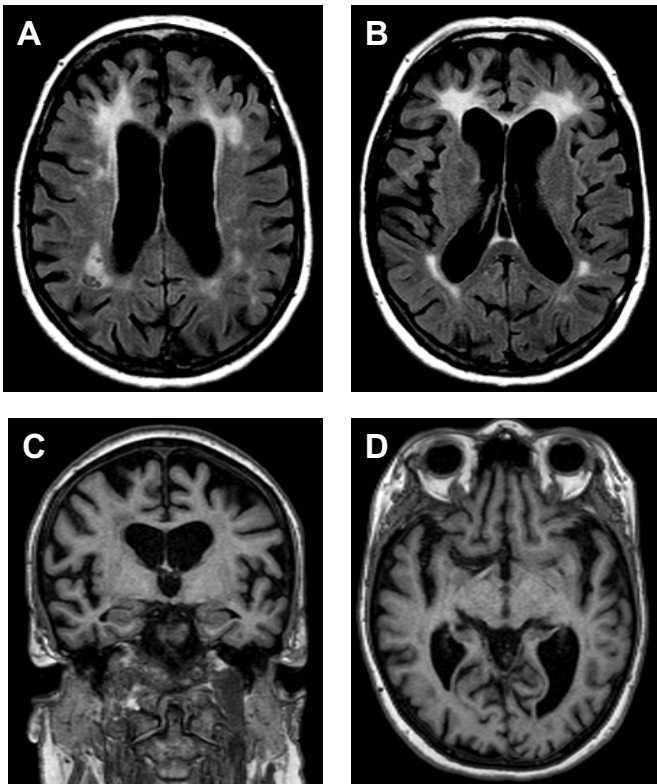
Case Level Strongest Findings for 32 Probands



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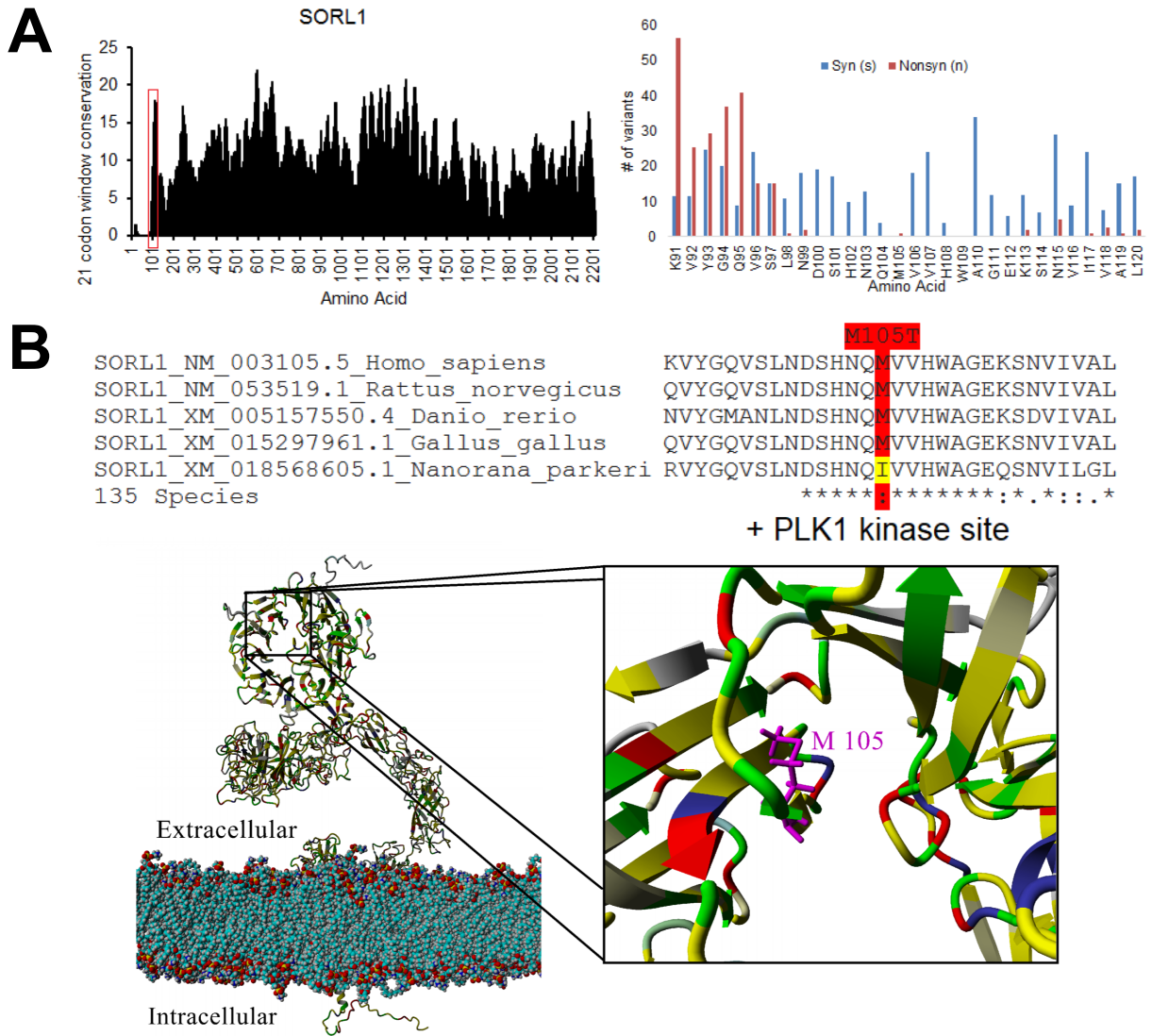
1401 **Figure 2:**



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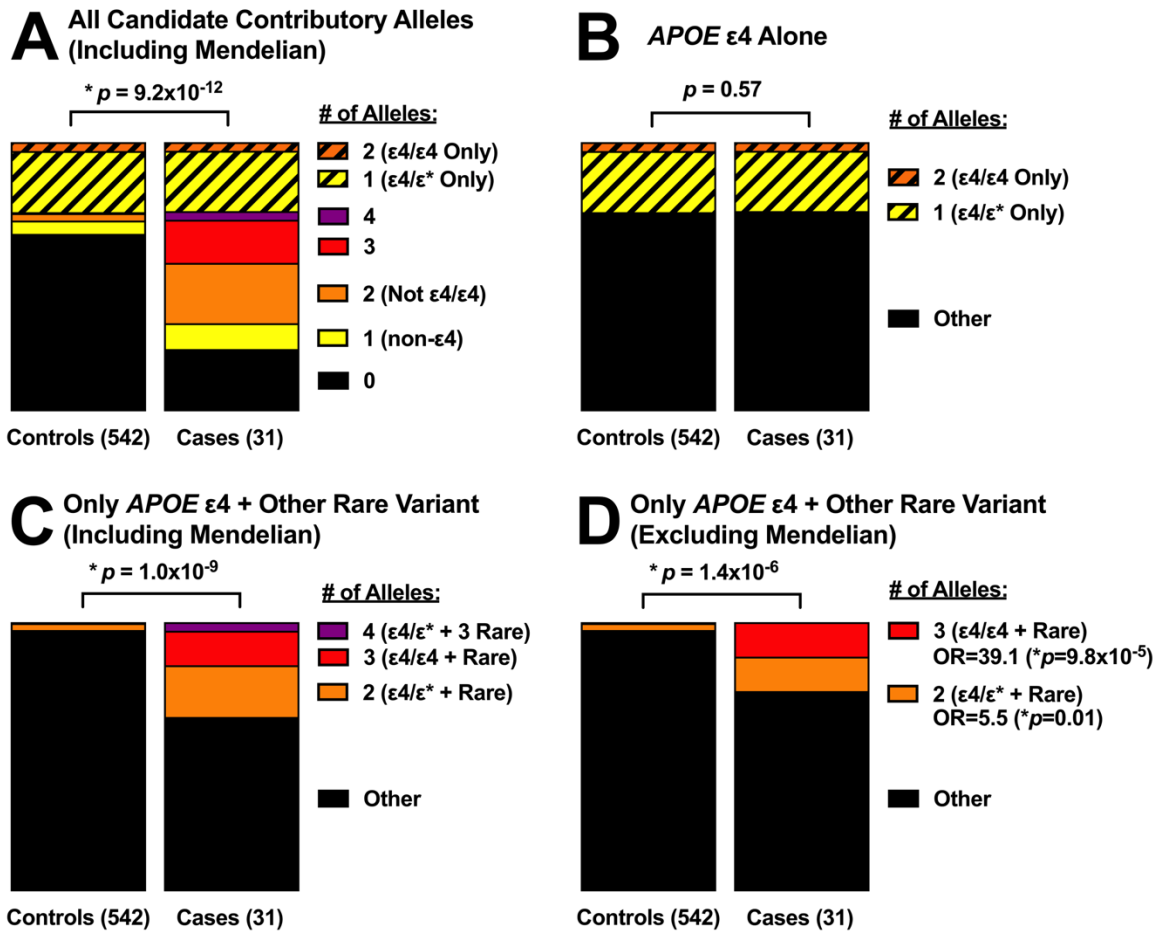
1404 **Figure 3:**



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1407 **Figure 4:**



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Table 1:

Gene	Chrom.	HGVS DNA	HGVS Protein	Variant Type	Predicted Effect	dbSNP ID	Genotype
<i>APP</i>	21	NM_000484.3:c.2149G>T	V717F	SNV	Missense	rs63750264	Heterozygous
<i>C9orf72</i>	9	NM_001256054.1:c.-45+163_-45+168GGGGCC[(24_?)]	NA	Insertion	Repeat Expansion	rs143561967	Heterozygous
<i>ABCA7</i>	19	NM_019112.3:c.5035G>T	E1679*	SNV	Stop Gained	rs770510230	Heterozygous
<i>APOE</i>	19	NM_000041.3:c.388T>C	C130R	SNV	Missense	rs429358	Het & Hom
<i>APOE</i>	19	NM_000041.3:c.526C>T	R176C	SNV	Missense	rs7412	Ref. (w/ Above Alt = ε4)
<i>PSEN1</i>	14	NM_000021.3:c.103C>T	R35W	SNV	Missense	rs746691776	Heterozygous
<i>ABCA7</i>	19	NM_019112.3:c.2126_2132delAGCAGGG	E709Afs*86	Deletion	Frameshift	rs547447016	Heterozygous
<i>ARSA</i>	22	NM_000487.5:c.256C>T	R86W	SNV	Missense	rs199476352	Compound Het
<i>ARSA</i>	22	NM_000487.5:c.585G>T	W195C	SNV	Missense	rs6151415	Compound Het
<i>MAPT</i>	17	NM_005910.5:c.1216C>T	R406W	SNV	Missense	rs63750424	Heterozygous
<i>APP</i>	21	NM_000484.3:c.1090C>T	L364F	SNV	Missense	rs749453173	Heterozygous
<i>GRID2IP</i>	7	NM_001145118.1:c.429+2T>G	NA	SNV	Splice	rs1413118387	Heterozygous
<i>CSF1R</i>	5	NM_005211.3:c.2699G>A	R900K	SNV	Missense	NA (private)	Heterozygous
<i>PLD3</i>	19	NM_012268.3:c.694G>A	V232M	SNV	Missense	rs145999145	Heterozygous
<i>APP</i>	21	NM_000484.3:c.742G>A	D248N	SNV	Missense	rs200103591	Heterozygous
<i>ABI3</i>	17	NM_016428.2:c.290T>A	V97E	SNV	Missense	NA (private)	Heterozygous
<i>SORL1</i>	11	NM_003105.5:c.314T>C	M105T	SNV	Missense	rs982581946	Heterozygous
<i>TREM2</i>	6	NM_018965.3:c.140G>A	R47H	SNV	Missense	rs75932628	Heterozygous
<i>TREM2</i>	6	NM_018965.3:c.259G>A	D87N	SNV	Missense	rs142232675	Heterozygous
<i>AKAP9</i>	7	NM_005751.4:c.7638A>G	I2546M	SNV	Missense	rs144662445	Heterozygous
<i>GBA</i>	1	NM_000157.3:c.1448T>C	L483P	SNV	Missense	rs421016	Heterozygous
<i>VPS13C</i>	15	NM_020821.2:c.10954C>T	R3652*	SNV	Stop Gained	rs138846118	Heterozygous
<i>VPS13C</i>	15	NM_020821.2:c.1988delC	T663Nfs*2	Deletion	Frameshift	rs1019238429	Heterozygous
<i>PLCD1</i>	3	NM_006225.3:c.631C>T	R211W	SNV	Missense	rs752156828	Heterozygous
<i>NOTCH3</i>	19	NM_000435.2:c.133G>C	D45H	SNV	Missense	rs142031490	Compound Het
<i>NOTCH3</i>	19	NM_000435.2:c.154G>A	G52R	SNV	Missense	rs148166997	Compound Het
<i>MAPT</i>	17	NM_005910.5:c.1174A>G	I392V	SNV	Missense	rs991713081	Heterozygous
<i>ADAM10</i>	15	NM_001110.3:c.359T>C	I120T	SNV	Missense	rs144890810	Heterozygous
<i>TTC3</i>	21	NM_001320703.1:c.5677G>A	V1893M	SNV	Missense	NA (private)	Heterozygous
<i>SORL1</i>	11	NM_003105.5:c.1247G>A	R416Q	SNV	Missense	rs377550239	Heterozygous
<i>MME</i>	3	NM_007289.2:c.1241A>G	Y414C	SNV	Missense	rs202095767	Heterozygous
<i>TM2D3</i>	15	NM_078474.2:c.206C>T	P69L	SNV	Missense	rs140152371	Heterozygous
<i>TNK1</i>	17	NM_001251902.1:c.393C>G	H131Q	SNV	Missense	rs767381816	Heterozygous
<i>KCNQ1</i>	11	NM_000218.2:c.1552C>T	R518*	SNV	Stop Gained	rs17215500	Heterozygous

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