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1 # Genetics of dementia in a Finnish cohort

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5 **Running title:** Genetics of dementia in Finnish families

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28 **Conflict of interest**

29 The authors declare that they have no conflict of interest.

30

Abstract

Alzheimer's disease (AD) and frontotemporal dementia (FTD) are the two most common neurodegenerative dementias. Variants in *APP*, *PSEN1* and *PSEN2* are typically linked to early-onset AD, and several genetic risk loci are associated with late-onset AD. Inherited FTD can be caused by hexanucleotide expansions in *C9orf72*, or variants in *GRN*, *MAPT* or *CHMP2B*. Several other genes have also been linked to FTD or FTD with motor neuron disease.

Here we describe a cohort of 60 Finnish families with possible inherited dementia. Our aim was to clarify the genetic background of dementia in this cohort by analysing both known dementia-associated genes and searching for rare or novel segregating variants with exome sequencing. *C9orf72* repeat expansions were detected in 12 (20%) of the 60 families, including, in addition to FTD, a family with neuropathologically verified AD. Twelve families (10 with AD and 2 with FTD) with representative samples from affected and unaffected subjects and without *C9orf72* expansions were selected for whole exome sequencing. Exome sequencing did not reveal any variants that could be regarded unequivocally causative of the disease in the families, but revealed potentially damaging variants in *UNC13C* and *MARCH4.*

Key words: Alzheimer's disease, frontotemporal dementia, *C9orf72*, *MARCH4*, *UNC13C*, *CLU*

52 **Introduction**

53 Alzheimer's disease (AD) and frontotemporal dementia (FTD) are the two most common
54 neurodegenerative dementias. AD is characterized by progressive loss of memory, typically
55 presenting with deficits in anterograde episodic memory. Other cognitive functions, such as
56 language, executive functions and visuospatial functions, deteriorate as the disease progresses
57 [1]. Most AD patients first develop symptoms after 65 years of age (late-onset AD, LOAD),
58 while less than 10% of patients present with early-onset AD (EOAD). Autosomal dominant
59 inheritance and rare cases of autosomal recessive inheritance are seen in the EOAD group,
60 due to variants in the amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*) and presenilin 2
61 (*PSEN2*) genes. Familial cases are also seen in LOAD with an estimated heritability of 58 to
62 79% [2]. More than 20 disease-associated loci have been detected in genome-wide association
63 studies (GWAS)[3-7] and meta-analyses [8] but, apart from the *APOE* ε4 and *TREM2* p.(R47H)
64 risk alleles, most of these only have a modest effect. Although rare variants in *APP*, *PSEN1*
65 and *PSEN2* have been detected in LOAD patients by targeted resequencing [9], variants
66 affecting function are rare in LOAD and its pathobiology reflects the interplay of
67 predisposing genetic variants and environmental factors. Sequencing studies have also shown
68 that rare variants can be found in dementia-associated loci identified through GWAS [10].

69 In contrast to AD, FTD is more commonly observed in patients younger than 65 years [11].
70 FTD may present with changes in personality and behaviour (behavioural-variant FTD) or
71 language difficulties (non-fluent variant primary progressive aphasia and semantic-variant
72 progressive aphasia) [11]. Up to 40% of patients have a positive family history with autosomal
73 dominant inheritance in 10% [12]. The most commonly mutated genes are *C9orf72* [13,14], *GRN*
74 [15,16] and *MAPT* [17], while rare variants in *TARDBP* [18], *FUS* [19,20], *VCP* [21], *CHMP2B* [22],
75 *UBQLN2* [23,24], *TBK1*[25], *SQSTM1* [26] and *CCNF* [27] have been detected in patients with either
76 FTD and motor neuron disease (FTD-MND) or pure FTD. *C9orf72* expansions are prevalent

77 in Finnish patients with FTD or ALS, accounting for 48% and 46% of familial FTD and ALS,

78 respectively, and 19% and 21% of sporadic FTD and ALS[13].

79 Based on the known functions of disease-associated genes, several pathways involved in the

80 pathogenesis of AD and FTD have been identified. In AD, these include the amyloid β

81 pathway, the immune system (*CLU*, *CR1*, *ABCA7*, *CD33*, *EPHA1*, the *MS4A* gene cluster),

82 synaptic activity (*PICALM*, *CD33*, *CD2AP*, *EPHA1*, *BIN1*) and lipid metabolism (*CLU*,

83 *ABCA7*) (reviewed in [28]). In FTD, the disease-implicated pathways include RNA processing

84 and transcription regulation (*C9orf72, TARDBP, FUS*), microtubule function (*MAPT*),

85 immune response (*GRN*), lysosome-mediated and ubiquitin-mediated protein degradation and

86 autophagy (*GRN, VCP, CHMP2B*) (reviewed in [11]).

87 Here we describe a cohort of 60 Finnish families with possible inherited dementia. Our aim

88 was to clarify the genetic background of dementia in this cohort by analysing both known

89 dementia-associated genes and searching for rare or novel segregating variants using exome

90 sequencing. We show that *C9orf72* hexanucleotide repeat expansions are common in this

91 cohort but variants affecting function of the other most common AD and FTD genes are not

92 accountable for the disease in these families. We also present rare variants that segregate with

93 AD and FTD in small families. Although no definite conclusion can be achieved regarding

94 the causal involvement of these rare variants, these should be taken into account in future

95 studies trying to identify the genetic cause of familial dementias.

96 **Subjects and methods**

97 **Study cohort**

98 The study cohort is comprised of affected and unaffected members from 60 Finnish families

99 with possible inherited dementia. The families were recruited from neurology clinics in the

100 Helsinki and Uusimaa hospital district (Southern Finland) and via an advertisement in a
101 national newspaper in the late 1990s. The recruitment method proved particularly successful,
102 resulting in 60 families suited for the study. A total of 364 blood-derived DNA samples (107
103 from affected patients and 257 from unaffected family members) were available from the
104 families. A prerequisite for participation was a positive family history with two or more
105 living first-degree family members affected by dementia.

106 The clinical diagnosis was AD in most families (n = 38), FTD in 10 families, dementia with
107 Lewy bodies (DLB) in one family and unspecified dementia in 11 families (Supplementary
108 table 1). The diagnoses were based on clinical findings and brain CT imaging studies.
109 Extensive neuropsychological studies had been performed for some of the patients. Liquor
110 biomarkers were not available at the time of patient recruitment and sample collection.
111 Neuropathological data was available from patients belonging to seven of the 60 families.
112 The ages at onset are listed in Supplementary table 2.

113 **Ethical aspects**

114 Informed consent was obtained from all participants. The study was approved by the Ethics
115 committee of Neurology department at HUCH (4.6.1997 and 11.1.2012) and the HUCH
116 Ethics Committee of Medicine (Dnro104/13/03/01/14). Approval for using patient tissue
117 specimens was given by Valvira (Dnro 2855/06.01.03.01/2012). Approval for using medical
118 records and autopsy reports of the patients living outside the HUS district was obtained from
119 National Institute for Health and Welfare (Dnro THL/701/5.05.00/2013).

120 **Methods**

121 EDTA blood samples were drawn after obtaining informed consent from the participants.
122 Both affected patients and unaffected family members were recruited for the study. DNA

123 was extracted using standard protocols. Overview of the study scheme is shown in Figure
124 1. *APOE* genotypes were determined by PCR and *Cfo*I digestion as described in
125 Myllykangas *et al.* [29]. Screening of *C9orf72* expansions was done by repeat primed PCR
126 as described by Renton *et al.* [13].

127 Expansion was defined by two criteria that had to be fulfilled: 1. Characteristic saw-tooth
128 pattern in repeat-primed PCR extending over 30 G_4C_2 repeats on capillary electrophoresis
129 of the PCR products, and 2. Lack of large allele (>30 repeats) amplicon in standard PCR
130 across the repeat region. Standard PCR across the repeat region was performed using
131 LongAmp Taq Reaction Buffer (New England Biolabs) with the following PCR primers
132 5'-GGA GGG AAA CAA CCG CAG CCT GTA G-3'and 5'-ATG CCG CCT CCT CAC
133 TCA CCC ACT CG-3', 1.8 M of Betaine. The PCR products were run on 2 % agarose
134 gels.

135 We evaluated the pedigrees for the availability of samples from both affected and unaffected
136 individuals as well as the availability of neuropathological data. Twelve families without
137 *C9orf72* expansions (2 with FTD and 10 with AD) were selected for further genetic studies.
138 The FTD families were screened for variants in *GRN* and the AD families for variants in
139 exons 16 and 17 of *APP* and the coding regions of *PSEN1* and *PSEN2*. Exons and flanking
140 splice site regions were amplified by PCR and the purified PCR products were sequenced in
141 both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied
142 Biosystems, CA, USA). All primer sequences and PCR conditions are available upon request.

143 Large structural and copy number variants were excluded by using HumanOmniExpress
144 Bead Chip (Illumina, San Diego, CA, USA). Loci known to have copy number variants that
145 associate with dementia (such as APP and SNCA) were checked visually. In addition, the
146 data was analysed with CNVPartition in Genome Studio (Illumina, San Diego, CA, USA) to

147 detect large (> 50 kb) CNVs. The identified CNVs were checked against the Database of
148 Genomic Variants (DGV) [30].

149 Whole exome sequencing (WES) of selected individuals was done at University College
150 London (UCL, London, UK). Exome enrichment was performed using TruSeq Exome
151 Capture kit (Illumina, San Diego, CA, USA). Sequencing was performed on a HiSeq 2000
152 (Illumina, San Diego, CA, USA). Reads were aligned to GRCh37/hg19 using BWA, variants
153 called according to GATK best practice guidelines and annotated with ANNOVAR [31]. *In*
154 *silico* pathogenicity predictions of nonsynonymous variants were done with SIFT [32],
155 Polyphen2 [33], MutationTaster [34], MutationAssessor [35], and CADD [36]. Variants were filtered
156 against population databases (1000Genomes, ESP and ExAC) and prioritised based on
157 variant type (missense, nonsense, splice site, frameshift, non-frameshift) and predicted
158 pathogenicity. We concentrated on variants found in genes implicated in GWAS or genes that
159 are highly expressed in the brain. We also assessed the known functions of the genes of
160 interest.

161 Selected variants (shared by the affected members in each family but not present in the
162 unaffected family member, if appropriate sample was available) from WES were confirmed
163 with Sanger sequencing and their segregation tested in a family setting. These variants and
164 the associated phenotypes have been submitted to ClinVar
165 (https://www.ncbi.nlm.nih.gov/clinvar/) with accession numbers SCV000576395,
166 SCV000576396, SCV000576397, SCV000576398, and SCV000576399. We also checked
167 the frequencies of these selected variants in SISu, a database of sequence variants in Finns
168 (Sequencing Initiative Suomi project (SISu), Institute for Molecular Medicine Finland
169 (FIMM), University of Helsinki, Finland (URL: http://sisuproject.fi), SISu v4.1, accessed in
170 09/2016).

171

172 **Results**

173 *APOE* genotyping

174 *APOE* genotyping was performed for 364 samples from 60 families. There were 34
175 individuals homozygous for the risk *APOE* genotype ε4 (20/107 affected, 18.7%; 14/257
176 unaffected, 5.4%). A total of 166 individuals were heterozygotes *APOE* ε3/ε4 (56/107
177 affected, 52.3%; 110/257 unaffected, 42.8%). The most common allele, *APOE* ε3, is not
178 associated with an increased risk for AD, and it was detected in 28 of 107 (26.1%) affected
179 patients and in 123 of 257 (47.8%) unaffected individuals. Five unaffected individuals
180 (5/257, 1.99%) were *APOE* ε2/ε4 heterozygotes and one unaffected (1/257, 0.3%) was ε2/ ε3.
181 No genotype was obtained for seven samples (3 affected and 4 unaffected). The genotypes in
182 each family are shown in Supplementary table 2.

183 *C9orf72*

184 All 60 families were included in the *C9orf72* hexanucleotide expansion screening.
185 Expansions were detected in 12 of the 60 families (20%). The distribution of expansions in
186 affected and unaffected individuals in each family is shown in Supplementary table 2.
187 Clinical diagnosis was FTD in seven families, AD or variant AD in four families and
188 degenerative dementia (ALS, DLB or AD resembling syndromes) in one family (Table 1).
189 The proportions of *C9orf72* expansions in each diagnosis group in shown in Figure 2. As
190 FTD or FTD/ALS are the typical clinical phenotypes associated with *C9orf72* expansions, we
191 only describe the five families with more atypical presentations in detail. No additional
192 information was available from family Fam-62.

193 Table 1. Families with *C9orf72* expansions. Two families (Fam-18 and Fam-73) marked with
194 an asterisk may have been initially misdiagnosed as AD. In Fam-25 (marked with two
195 asterisks) the neuropathological diagnoses are based on haematoxylin & eosin and ancillary
196 stainings. No immunohistochemical stainings for FTLD were available at the time of the
197 neuropathological examination.

Family ID	Clinical diagnosis	Neuropathological diagnosis
Fam-18	AD*+FTD	not available
Fam-22	FTD	not available
Fam-25	FTD	FTLD + AD-type lesions**
Fam-27	dementia (AD)	not available
Fam-31	degenerative dementia (ALS, DLB or AD resembling syndromes)	AD
Fam-33	FTD	not available
Fam-39	FTD	not available
Fam-50	AD	not available
Fam-62	AD	not available
Fam-71	FTD	not available
Fam-73	variant AD*	not available
Fam-77	FTD/ALS	not available

198

199 The index patient of family Fam-18 developed symptoms at 65 years. Clinical presentation
200 was compatible with AD, but CT and MRI studies showed frontal atrophy. Clinical data from
201 other family members was not available.

202 Patient records of two affected patients from family Fam-27 were available for review. Both
203 patients had clinical AD with late onset.

204 Neuropathological data of two affected patients from family Fam-31 were available. The
205 index patient had late-onset AD and the diagnosis was verified neuropathologically post
206 mortem in 1998 using the methods available at that time. Re-analysis of the archived
207 formalin-fixed, paraffin-embedded sample revealed Braak stage V tau-pathology and

208 CERAD stage B beta-amyloid load. TDP-43 staining was negative. p62 positive inclusions
209 were observed in the granular cerebellar cells. No DNA sample was available for study.
210 Formalin-fixed brain tissue from temporal lobe of another patient of the family was available
211 for immunohistochemistry. TDP-43 staining was negative, but moderate to severe tau-
212 pathology suggestive of AD was observed. This patient was shown to harbor a *C9orf72*
213 expansion and carried the *APOE* ε4/4 genotype.

214 Family Fam-50 included several affected individuals with onset of disease after 70 years of
215 age. DNA sample was available from one of them. One patient had visual hallucinations as
216 the first symptom and subsequently developed loss of concentration and memory deficit.
217 Neuropsychological examination was consistent with large-scale impairment and visual
218 defect.

219 The index patient of family Fam-73 was diagnosed with variant AD. However, brain SPECT
220 was suspective of FTD. The index patient's sister had been diagnosed with ALS. Thus, the
221 actual diagnostic spectrum of this family is consistent with FTD and ALS.

222 *Further studies on 12 families without C9orf72 expansions*

223 *Exclusion of GRN, APP, PSEN1, PSEN2*

224 Twelve families without *C9orf72* expansions and representative samples from both affected
225 and unaffected individuals were selected for further studies (Figure 1.). The clinical
226 diagnoses in these families were FTD (two families: Fam-13 and Fam-59) and AD (10
227 families: Fam-15, Fam-29, Fam-32, Fam-35, Fam-38, Fam-49, Fam-52, Fam-55, Fam-56,
228 and Fam-57). Sanger sequencing did not reveal any causal variants in *GRN* (FTD), *APP*
229 exons 16 and 17, or coding regions of *PSEN1* and *PSEN2* (AD families).

230 *Exclusion of large structural and copy number variants by SNP microarray*

231 None of the 12 index patients had a duplication of *APP*. Neither did we identify any deletions

232 or duplications involving other known dementia-associated genes, such as *SNCA*.

233 *Whole exome sequencing*

234 Whole exome sequencing (WES) data was generated for at least two affected patients from

235 each of the 12 families. The oldest unaffected family members from whom a DNA sample

236 was available (7 families) were exome sequenced as controls. We concentrated on rare

237 variants identified in WES shared by the affected patients in each family but not seen in the

238 analysed healthy family members, when available (list of rare variants in each family can be

239 found in a Supplementary table 3). Confirmation and segregation analyses were done with

240 Sanger sequencing. A large number of shared rare variants were identified in each family, but

241 we concentrated on variants in GWAS hit genes or in genes with known functions possibly

242 relevant for neurodegeneration. The validated variants are listed in Table 2 and presented in

243 detail below.

244 Table 2. Rare variants identified in exome sequencing and validated in a family setting.

245 Minor allele frequencies (MAFs) of each variant in 1000Genomes, ESP, SiSU and ExAC are

246 shown. Different prediction programs (SIFT, PolyPhen, MutationTaster, MutationAssessor,

247 CADD) were used to estimate the deleteriousness of the variants.

Gene	*CLU*	*PCDH11X*	*UNC13C*	*MARCH4*	*MARCH4*
Family	Fam-56	Fam-15	Fam-49	Fam-59	Fam-13
clinical diagnosis	AD	AD	AD	FTD	FTD
genomic location (hg19)	27462662	91133518	54306424	217234945	217148338
chromosome location	8p21.1	Xq21.31	15q21.3	2q35	2q35
reference sequence	NM_001831.3	NM_001168360.4	NM_001080534.2	NM_020814.2	NM_020814.2
cDNA change	c.608C>T	c.2279A>T	c.1324_1326del	c.39G>C	c.631A>G
amino acid change	p.(Thr203Ile)	p.(Asp760Val)	p.(Lys443del)	p.(Trp13Cys)	p.(Lys211Glu)
rs identifier	rs41276297	rs781770086	rs746069739	rs145386484	rs756981946

SIFT	tolerated	Damaging	-	tolerated	damaging
PolyPhen	Benign	possibly damaging	-	benign	damaging
MutationTaster	Neutral	damaging	-	damaging	damaging
MutationAssessor	medium effect	neutral	-	neutral	neutral
CADD phred score	8.521	14.33	-	2.416	19.62
1000G	0.005	absent	Absent	0.000599042	absent
ESP	0.0027	absent	Absent	7.7e-05	absent
SISu	0.000698324	0.00017454	Singleton	0.00124611	singleton
ExAC (Finnish)	0.00121	singleton	Absent	0.001285	absent
ExAC (total)	0.001673	singleton	Absent	0.0002082	singleton
Significance	likely benign	possibly benign	possibly deleterious	likely benign	possibly deleterious

248

249 *WES findings in AD families*

250 *CLU*

251 A heterozygous *CLU* c.608C>T, p.(Thr203Ile) variant (rs41276297) was identified in two
252 affected patients of family Fam-56. The variant was not detected in the four unaffected
253 family members from whom a sample was available (Figure 3a). This variant is a previously
254 known, rare variant with a frequency of 0.00121 in Finnish samples in ExAC. Polyphen and
255 SIFT predicted no deleterious effect. This variant has also been detected in British AD
256 samples (reported as p.T255I) with a frequency of 0.003 as well as in unaffected controls
257 (frequency 0.006)[37]. One of the affected individuals also carried one *APOE* ε4 allele, while
258 the other was homozygous for ε3.

259 *PCDH11X*

260 Affected members of family Fam-15 carried a heterozygous c.2279A>T, p.(Asp760Val)
261 variant in *PCDH11X*. This variant (rs781770086) is present as a singleton in ExAC and in
262 SISu. Sanger sequencing confirmed the variant in the two affected patients (II:4 and III:4).
263 However, segregation analysis showed that the variant was also present in two currently

264 unaffected individuals (III:2 and III:5) and in one individual (III: 6) with unclear status. The
265 remaining two unaffected family members (II:7 and III:7) did not carry the variant (Figure
266 3b). Only one of the affected individuals carried *APOE* ε4.

267 *UNC13C*

268 In family Fam-49, a heterozygous 3-bp deletion in *UNC13C*, c.1324_1326del, p.(443del) was
269 detected in two affected patients. The variant was not seen in two unaffected family members
270 (Figure 3c). This in-frame deletion variant (rs746069739) is present as a singleton in ExAC
271 and in SISu (low-quality). In addition to the *UNC13C* variant, both affected individuals also
272 carried one *APOE* ε4 allele.

273 *WES findings in FTD families*

274 *MARCH4*

275 Two affected patients from the FTD family Fam-13 carried a heterozygous c.631A>G,
276 p.(Lys211Glu) variant, (rs756981946) in *MARCH4*. This variant was absent from the
277 unaffected family members (Figure 3d). The *APOE* genotypes of the two affected individuals
278 of Fam-13 were ε3/4 and ε3/3.Two affected members of the second FTD family, Fam-59,
279 carried a heterozygous c.39C>G variant, p.(Trp13Cys) (rs145386484) in *MARCH4*.
280 Segregation analysis showed that this variant was also present in 7 currently unaffected
281 family members (ages 45 – 73 years) and absent in other 9 unaffected family members
282 (Figure 3e). All studied individuals in Fam-59 were homozygous for *APOE* ε3.

283 *MARCH4* p.(Lys211Glu) variant is present in ExAC as a singleton in a non-Finnish European
284 sample and in SISu as a singleton (identifier: rs756981946). In silico predictions gave the
285 following results: Polyphen2 predicted the variant to be probably damaging (score 0.995),
286 SIFT tolerated (score 0.29), MutationTaster damaging (score 1.000), MutationAssessor

287 medium effect (score 2.22), CADD Phred-like scaled C-score was 19.62. These data
288 demonstrate that the variant is extremely rare and suggest that it might alter the normal
289 function of *MARCH4*.

290 The p.(Trp13Cys) variant is more common as it is reported in ExAC with a frequency of
291 0.001285 in Finnish samples. It has also been detected in other populations: European
292 (3/56914), South Asian (8/13234), African (3/7972) and Latino (1/9762). SIFT predicted this
293 variant to be tolerated (score 0.29), Polyphen2 benign (score 0.00), MutationTaster damaging
294 (score 0.981), MutationAssessor neutral (score -0.55), and CADD Phred-like scaled C-score
295 was 2.416. These predictions along with the fact that it was present in individuals over 70
296 years of age suggest that p.(Trp13Cys) might be a rare neutral variant.

297 **Discussion**

298 In contrast to early-onset AD, late-onset Alzheimer's disease is rarely caused by segregating
299 variants in families. The strongest identified risk factor is the *APOE* ε4 allele. In few cases,
300 variants in *APP*, *PSEN1* and *PSEN2* have been reported in LOAD families[9]. GWAS studies
301 have identified approximately 20 loci associated with predisposition to AD but finding
302 variants that actually have a biological effect has proven difficult. In FTD, variants in
303 *C9orf72*, *MAPT* and *GRN* account for up to 60% of familial cases while variants in other
304 genes are rare [11].

305 In addition to the ALS/FTD entity, *C9orf72* expansions have been linked to several other
306 clinical manifestations including AD, Parkinson's disease and Huntington's disease
307 phenocopies (reviewed in [38]). We detected *C9orf72* expansions in 7/60 (11.6%) families with
308 either FTD or FTD/ALS but also in 3/60 (5%) families with clinical AD. In one family, Fam-
309 31, neuropathological examinations disclosed moderate to severe AD tau-pathology, and no
310 TDP-43-positive inclusions were seen. However, p62-positive inclusions were present in the

311 cerebellum, consistent with the *C9orf72* expansion. Several earlier reports have described
312 *C9orf72* expansions in either clinically diagnosed [39-42] or neuropathologically confirmed AD
313 [43]. It is possible that the AD pathology is at least partly attributable to *APOE* as the one
314 affected individual with *C9orf72* expansion and AD-type neuropathology was homozygous
315 for the *APOE* ε4 allele (Supplementary table 2). Previous work has shown that *C9orf72*
316 expansions are seen in ~30% of Finnish FTD patients [13] and in 48.1% of familial FTD [44]. Our
317 results confirm this finding and suggest that *C9orf72* expansions may manifest as clinical AD
318 and some patients may also show concomitant AD pathology at the neuropathological
319 examination. Previous studies on *C9orf72* expansions in AD patients have suggested that the
320 clinical or neuropathological classification as AD may have been incorrect, and this appeared
321 to be the case in some of our families with clinical diagnosis of AD.

322 No variants in *APP* exons 16 and 17 or the coding regions of *PSEN1* and *PSEN2* were
323 observed in the 10 AD families selected from our cohort. Whole-genome genotyping also
324 showed no clearly causative CNVs. Both results are in agreement with previous studies. Only
325 a few *PSEN1* variants have been reported in Finnish AD families: two families carry the
326 'Cotton-wool' variant, Δ9Finn (c.869_955del) [45], p.(Met146Val) has been reported in a
327 Swedish family of Finnish descent [46,47] and p.(Pro264Leu) in one family [48]. Screening of
328 *APP*, *PSEN1* and *PSEN2* in a cohort of 140 EOAD patients revealed no variants that might
329 affect function [49]. In addition, duplication of *APP* was not detected in a cohort of 64 Finnish
330 EOAD patients [50].

331 GRN sequencing and exome sequencing did not reveal any pathogenic variants in the two
332 FTD families without *C9orf72* expansions. In agreement with our results, previous work
333 suggests that GRN variants are rare among Finnish FTD patients [51].

334 Exome sequencing revealed rare, potentially relevant variants in five families. Two variants
335 were in genes previously linked to AD (*CLU* and *PCDH11X*) while three variants were in
336 genes (*UNC13C* and *MARCH4*) that have not been directly linked to dementia but could be
337 important in maintaining normal neuronal function.

338 In 2010, a large GWAS study indicated that *PCDH11X* was linked to LOAD in a combined
339 American Caucasian cohort[52]. However, subsequent studies in different populations failed to
340 confirm the findings of Carrasquillo *et al.* [53-56] Recently, Jiao *et al.* reported a SNP in
341 *PCDH11X* to confer a risk to LOAD [57]. Thus, the possible role of *PCDH11X* in AD
342 susceptibility is still somewhat unclear. Our results show that the rare p.(Asp760Val) variant
343 is present in all affected individuals of family Fam-15 but also in two asymptomatic
344 individuals and in one subject with unclear status.

345 The role of *CLU* as an AD risk gene has been established in independent datasets [3,4]. We
346 noted co-segregation of a rare *CLU* variant and dementia in an AD family (Fam-56). Even
347 though rare non-synonymous and small insertion/deletion variants have been reported to
348 increase AD risk[58,59], the p.(Thr203Ile) variant is predictably not deleterious, but at present
349 we cannot exclude its possible role in AD risk.

350 Two AD patients from family Fam-49 shared a 3bp in-frame deletion in *UNC13C*. The
351 *UNC13C* gene is highly expressed in brain. Experimental evidence from cat and mouse
352 models have suggested that its mammalian homologue, *Munc13-3*, has a role in controlling
353 critical-period neuronal plasticity in visual cortex.[60,61] Gene expression studies in human AD
354 and control brain samples showed increased *UNC13C* expression in hippocampal CA3
355 compared to CA1 in Alzheimer patients. This implicates that *UNC13C* might have a
356 neuroprotective role in the brain [62]. The rare variant found in family 49 removes one amino

357 acid residue but does not disturb the reading frame. Both affected patients were also
358 heterozygous for the *APOE* ε4 allele, a likely risk factor in this family.

359 A rare segregating missense variant in *MARCH4* was identified in the FTD family Fam-13.
360 *MARCH4* is a member of membrane-associated RING-CH family of ubiquitin E3 ligases.
361 These ligases function in the last step of ubiquitination by recruiting the ubiquitin carrying E2
362 enzyme and transferring ubiquitin from E2 to the target protein[63]. *MARCH4* is predominantly
363 expressed in the adult human brain [64]. The ubiquitin-related protein degradation pathway has
364 been implicated in many neurodegenerative diseases, including FTD. Recent work by
365 Williams and coworkers described variants in a component of the ubiquitin E3 ligase
366 complex, *CCNF*, in a large ALS/FTD family and a few singleton patients [27]. Although the
367 *MARCH4* variant segregated with FTD in our small family, we cannot exclude the possibility
368 that we merely identified a rare neutral variant in a gene with function that could fit in the
369 model of FTD pathogenesis.

370 *C9orf72* repeat expansions are common among Finnish FTD patients and our results indicate
371 that expansions may also be seen in patients with clinical and neuropathological diagnoses of
372 AD. Our results suggest that unknown genetic factors are likely to be responsible for a
373 proportion of familial dementia in the Finnish cohort, but definitely causal or risk variants in
374 novel genes are yet to be identified. Exome sequencing is an efficient way to search for rare
375 coding variants, but thus far only few segregating risk variants (e.g. *TREM2* p.(Arg47His) [65]
376 and *TTC3* p.(Ser1038Cys) [66]) have been described in LOAD families. Our results
377 corroborate the view that even in large LOAD families with multiple affected individuals the
378 disease is likely caused by combination of multiple genetic and environmental risk factors.
379 The *APOE* ε4 risk allele can be assumed to account for multiple affected individuals in
380 several of the AD families in our study.

381 We detected rare segregating coding variants in *UNC13C* in an AD family and in *MARCH4*
382 in an FTD family. However, replication in larger familial and case-control datasets and
383 functional assays would be needed to prove their causality. The limitation or our study is the
384 relatively small number of patients. Thus, we could only aim to find highly penetrant
385 pathogenic variants. In addition, exome sequencing does not enable the identification of non-
386 coding variants that might affect splicing or gene expression.

387 While our exome sequencing approach failed to identify any clearly causal variants in the 12
388 families, we believe that the rare variants found in our cohort will be of interest for other
389 dementia researchers. Thus, we presented all the variants and genes of potential interest in the
390 hope this may be useful for future studies and can facilitate analyses in other families and
391 datasets.

392

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402

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628 **Legends to the figures**

629 Figure 1. Schematic presentation of the study describing the workflow of genetic
630 examinations. WES = whole exome sequencing.

631 Figure 2. Proportions of *C9orf72* expansions in clinical frontotemporal dementia (FTD),
632 Alzheimer's disease (AD) and unspecified dementia in a cohort of 60 families. DLB =
633 dementia with Lewy bodies.

634 Figure 3. Pedigrees of the families with rare variants verified by Sanger sequencing. DNA
635 samples were available from individuals marked with an asterisk. *APOE* genotypes are also
636 marked in the pedigree.

637 a. Family Fam-56 with the *CLU* p.(Thr203Ile) variant. Heterozygous variant (-/+),
638 homozygous wild-type allele (-/-).

639 b. Family Fam-15 with the *PCDH11X* p.(Asp760Val) variant. Heterozygous variant (-/+),
640 homozygous wild-type allele (-/-), hemizygous variant (+), hemizygous wild-type allele (-).

641 c. Family Fam-49 with the *UNC13C* p.(443del) variant. Heterozygous variant (-/+),
642 homozygous wild-type allele (-/-).

643 d. Family Fam-13 with the *MARCH4* p.(Lys211Glu) variant. Heterozygous variant (-/+),
644 homozygous wild-type allele (-/-).

645 e. Family Fam-59 with the *MARCH4* p.(Trp13Cys) variant. Heterozygous variant (-/+),
646 homozygous wild-type allele (-/-).

647