

RESEARCH ARTICLE

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# Replication of *EPHA1* and *CD33* associations with late-onset Alzheimer's disease: a multi-centre case-control study

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## Abstract

**Background:** A recently published genome-wide association study (GWAS) of late-onset Alzheimer's disease (LOAD) revealed genome-wide significant association of variants in or near *MS4A4A*, *CD2AP*, *EPHA1* and *CD33*. Meta-analyses of this and a previously published GWAS revealed significant association at *ABCA7* and *MS4A*, independent evidence for association of *CD2AP*, *CD33* and *EPHA1* and an opposing yet significant association of a variant near *ARID5B*. In this study, we genotyped five variants (in or near *CD2AP*, *EPHA1*, *ARID5B*, and *CD33*) in a large (2,634 LOAD, 4,201 controls), independent dataset comprising six case-control series from the USA and Europe. We performed meta-analyses of the association of these variants with LOAD and tested for association using logistic regression adjusted by age-at-diagnosis, gender, and *APOE*  $\epsilon 4$  dosage.

**Results:** We found no significant evidence of series heterogeneity. Associations with LOAD were successfully replicated for *EPHA1* (rs11767557; OR = 0.87,  $p = 5 \times 10^{-4}$ ) and *CD33* (rs3865444; OR = 0.92,  $p = 0.049$ ), with odds ratios comparable to those previously reported. Although the two *ARID5B* variants (rs2588969 and rs494288) showed significant association with LOAD in meta-analysis of our dataset ( $p = 0.046$  and  $0.008$ , respectively), the associations did not survive adjustment for covariates ( $p = 0.30$  and  $0.11$ , respectively). We had insufficient evidence in our data to support the association of the *CD2AP* variant (rs9349407,  $p = 0.56$ ).

**Conclusions:** Our data overwhelmingly support the association of *EPHA1* and *CD33* variants with LOAD risk: addition of our data to the results previously reported (total  $n > 42,000$ ) increased the strength of evidence for these variants, providing impressive  $p$ -values of  $2.1 \times 10^{-15}$  (*EPHA1*) and  $1.8 \times 10^{-13}$  (*CD33*).

## Background

Following the identification of the *APOE*  $\epsilon 4$  allele as a risk factor for late-onset Alzheimer's disease (LOAD) in 1993 [1], consistent replication of subsequently identified candidates was not achieved until 2009, when two genome-wide association studies (GWAS) [2,3] identified associations of variants in or near *CLU*, *PICALM*, and *CRI* with LOAD, which were consistently replicated in multiple large, independent case-control studies

[4-17]. Subsequently, a variant near *BINI* was reported [4] to achieve genome-wide significant association in a later GWAS published in 2010 that also replicated well in follow-up studies [14-19]. These results demonstrate the utility of the hypothesis-free GWAS approach for identifying loci that associate with LOAD and the necessity of pooling samples and data from multiple centers to obtain resources with sufficient statistical power (GWAS typically  $> 14,000$ , follow-up typically total  $> 28,000$ ) to detect the modest ORs (e.g. 0.8/1.2) associated with these variants in GWAS and follow-up studies.

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Two recently published companion studies by Hollingworth *et al.* [20] and Naj *et al.* [17] performed meta-analysis of two large GWAS datasets ( $n > 75,000$ ). Besides *APOE*, *CLU*, *PICALM*, and *CRI*, the meta-analyses revealed association at *ABCA7* ( $p = 5 \times 10^{-21}$ ), *MS4A6A* ( $p = 1.2 \times 10^{-16}$ ), *MS4A4E* ( $p = 1.1 \times 10^{-10}$ ), *EPHA1* ( $p = 6 \times 10^{-10}$ ), *CD2AP* ( $p = 8.6 \times 10^{-9}$ ) and *CD33* ( $p = 1.6 \times 10^{-9}$ ). In addition, the two datasets revealed opposing association (Naj *et al.* OR = 0.93,  $p = 0.001$ ; Hollingworth *et al.* OR = 1.06,  $p = 0.03$ ) of the variant near *ARID5B* (rs2588969) with LOAD, suggesting potential heterogeneity at this locus. In this study, we genotyped the variants identified at the *CD2AP*, *EPHA1*, and *CD33* loci in our independent case-control dataset comprising six case-control series ( $n = 6,835$ ). To assess the opposing associations at the *ARID5B* locus, we also genotyped the two *ARID5B* variants included in the Hollingworth *et al.* study. Genotypes from our follow-up case-control series (Mayo 2) for variants in *ABCA7*, *MS4A6A* and *MA4A4E* were included in Stage 3 of the Hollingworth *et al.* study, so we have not included these three variants in this study. We have performed meta-analyses of five variants (at *CD2AP*, *EPHA1*, *ARID5B* and *CD33* loci) in our six case-control series, which showed no significant series heterogeneity. Furthermore, we have performed logistic regression analysis of our pooled series adjusting for covariates. Finally, we have used a Fisher's combined test to evaluate the significance of the association of these five variants in our data combined with the data in the Hollingworth *et al.* and Naj *et al.* studies.

## Results

We genotyped five variants (*CD2AP*; rs9349407, *EPHA1*; rs11767557, *ARID5B*; rs2588969 and rs4948288, *CD33*; rs3865444) in our independent follow-up case-control series (Mayo2) from three North American and three European Caucasian series. Detailed information about these samples is shown in Table 1 and genotype counts

are shown in Table 2. Samples used in this study do not overlap with those included in the Naj *et al.* study and have not been included in any of the published LOAD GWAS. The Mayo2 dataset included in the Hollingworth *et al.* publication only included genotypes for *ABCA7*, *MS4A6A* and *MA4A4E*.

Meta-analyses of allelic association in the six Mayo2 series performed using a DerSimonian-Laird random effects model (Figure 1) revealed a significant pooled OR for the *EPHA1* variant (Figure 1b; OR = 0.88,  $p = 0.008$ ) comparable to that previously published by Naj *et al.* (OR = 0.87) and by Hollingworth *et al.* (OR = 0.90). As shown in Figure 1c and 1d, we also observed significant association for both *ARID5B* variants (rs2588969, OR = 1.08,  $p = 0.046$ ; rs4948288, OR = 1.11,  $p = 0.008$ ) with ORs comparable to those reported by Hollingworth *et al.* (OR = 1.06 and 1.07, respectively) and in the opposing direction to those reported by Naj *et al.* for rs2588969 (Stage 1+2 OR = 0.93,  $p = 7.7 \times 10^{-4}$ ). As shown in Figure 1a and 1e, we did not observe significant association for *CD2AP* (OR = 0.98,  $p = 0.76$ ) or *CD33* (OR = 0.96,  $p = 0.32$ ) in our meta-analyses. Breslow-Day tests provided no significant evidence that the ORs for any of these variants were heterogeneous among our series (all  $p > 0.25$ ), as shown in Figure 1. The variant with the most heterogeneity was *CD2AP* (rs9349407) where the estimated percentage of variation due to heterogeneity across studies ( $I^2$ ) was 25.1% (95% CI 0%-70%) suggesting the presence of some heterogeneity for that variant.

To adjust for important covariates, we included age-at-diagnosis/entry, sex and *APOE*  $\epsilon 4$  dosage in logistic regression analyses of all five variants in each of the six Mayo2 series; in our analysis of all Mayo2 series combined, series was included as an additional covariate. Table 3 shows the results for the six Mayo2 series combined (Mayo follow-up) as well as for each of the six individual Mayo2 series. For the purpose of comparison, we have also included in Table 3 the published GWAS

**Table 1 Details of the Mayo2 samples used in this study and genotype counts**

Series	Number of samples			Mean Age (SD)		% Female		% $\epsilon 4+$	
	AD	CON	Total	AD	CON	AD	CON	AD	CON
Jacksonville	507	967	1,474	80.0 (6.7)	81.7 (7.6)	61.9	56.3	60.2	21.8
Rochester	317	1,638	1,955	85.8 (4.5)	80.3 (5.2)	62.1	54.6	42.3	22.4
Autopsy	312	102	414	87.4 (4.8)	86.0 (4.3)	67.6	52.0	61.2	14.7
Norway	346	555	901	80.2 (7.3)	75.3 (6.8)	69.9	59.8	63.0	24.1
Poland	483	188	671	76.7 (4.8)	73.0 (5.9)	66.3	76.6	56.4	19.0
ARUK	669	751	1,420	75.6 (8.2)	76.2 (7.3)	55.6	49.9	58.0	24.4

The number of LOAD patients (AD) and controls (CON), mean age-at-diagnosis, percentage that are female and percentage that possess at least one copy of the *APOE*  $\epsilon 4$  allele are given for each individual series. Mean age is given as age at diagnosis/entry with the standard deviation (SD) from the mean in parentheses. None of the samples comprising the Jacksonville, Rochester and autopsy-confirmed Mayo Clinic or ARUK series (comprising Bristol, Leeds, Manchester, Nottingham, Oxford and Southampton), which were included in this follow-up study overlap with those used in the Naj *et al.* study and have not been included in any of the published LOAD GWAS. The Mayo2 dataset included in the Hollingworth *et al.* publication only included genotypes for *ABCA7*, *MS4A6A* and *MA4A4E*.

**Table 2 Genotype counts for each of the six Mayo2 series**

Series	CD2AP (rs9349407)		EPHA1 (rs11767557)		ARID5B (rs2588969)		ARID5B (rs4948288)		CD33 (rs3865444)	
	GG/GC/CC	GG/GC/CC	TT/TC/CC	TT/TC/CC	CC/CA/AA	CC/CA/AA	GG/GA/AA	GG/GA/AA	CC/CA/AA	CC/CA/AA
	AD	CON	AD	CON	AD	CON	AD	CON	AD	CON
Jacksonville	254/197/41	497/369/56	339/143/ 19	612/301/44	188/226/81	379/400/149	164/233/99	351/426/148	251/200/41	446/386/88
Rochester	170/126/17	843/640/117	198/102/9	985/518/69	100/159/48	623/755/226	92/172/50	581/748/250	148/134/30	715/692/170
Autopsy	156/110/19	49/44/7	205/97/5	61/28/10	118/148/42	50/38/14	115/142/43	38/43/17	141/125/32	42/44/11
Norway	177/131/16	273/205/41	212/113/ 13	337/185/26	129/165/44	215/250/78	115/156/53	184/268/88	153/139/35	248/236/57
Poland	235/193/40	100/70/11	297/140/ 20	108/52/9	153/243/77	65/91/29	160/222/84	62/96/26	224/204/39	96/83/8
ARUK	341/243/55	363/317/53	386/191/ 20	439/234/37	236/313/ 101	271/367/102	208/326/ 122	259/351/122	289/286/67	329/307/94
Total	1333/1000/ 188	2125/1645/ 285	1637/786/ 86	2542/1318/ 195	924/1254/ 393	1603/1901/ 598	854/1251/ 451	1475/1932/ 651	1206/1088/ 244	1876/1748/ 428

The genotype counts (major allele homozygotes/heterozygotes/minor allele homozygotes) for CD2AP (rs9349407), EPHA1 (rs11767557), ARID5B (rs2588969 and rs4948288) and CD33 (rs3865444) variants are given for each individual series.

results for the same variants. Adjustment for covariates revealed comparable ORs to those obtained in the meta-analyses, with improved p-values for the EPHA1 (OR = 0.87,  $p = 5 \times 10^{-4}$ ), CD33 (OR = 0.92,  $p = 0.049$ ) and CD2AP (OR = 0.97,  $p = 0.56$ ) loci. However, the associations of the ARID5B variants were no longer significant following adjustment for covariates (rs2588969: OR = 1.05,  $p = 0.30$ , rs4948288: OR = 1.07,  $p = 0.11$ ) suggesting that these associations may be dependent upon the series, age-at-diagnosis/entry, sex and/or APOE  $\epsilon$  4 dosage of the individual.

In order to estimate the overall association of these five variants in our data combined with the previously published associations, we used Fisher's method to combine the p-values for all associations (Table 3; Mayo2/ADGC/Hollingworth). We found that adding our data to those previously reported, increased the strength of evidence for all variants as LOAD risk modifiers (CD2AP:  $p = 6.5 \times 10^{-11}$ , EPHA1:  $p = 2.1 \times 10^{-15}$ , ARID5B rs2588969:  $p = 2.3 \times 10^{-9}$ , ARID5B rs4948288:  $p = 4.0 \times 10^{-4}$ , CD33:  $p = 1.8 \times 10^{-13}$ ).

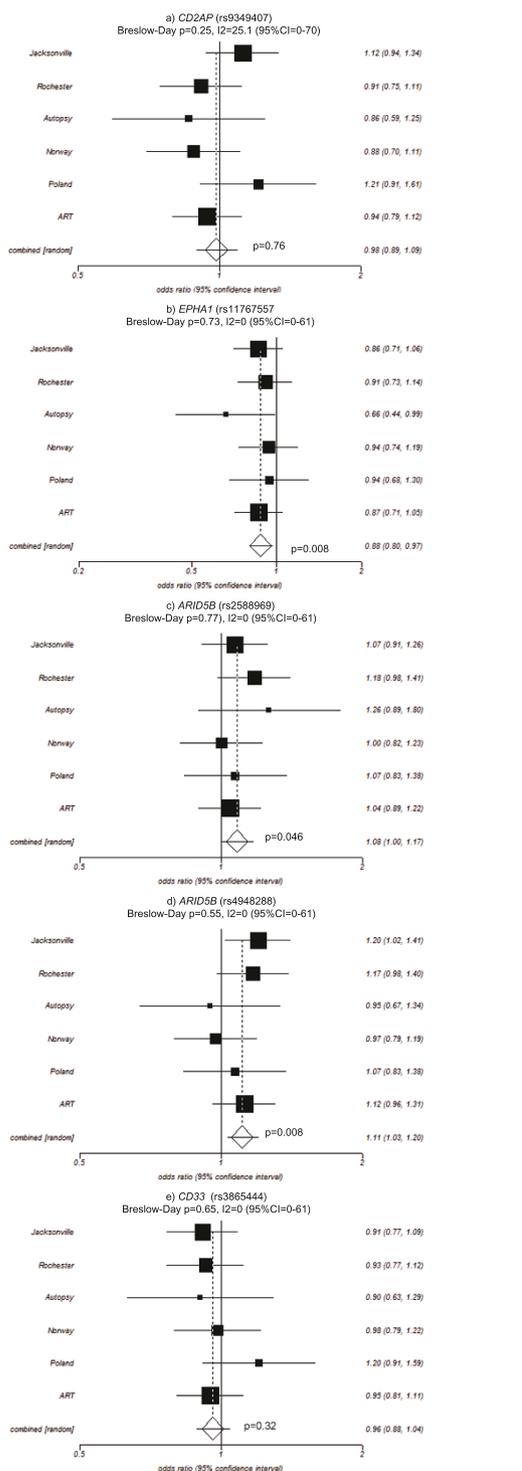
## Discussion

We report here successful replication of the association of two variants with LOAD in a large ( $n = 6,835$ ), independent case-control study; rs11767557, which is located 3 kb upstream of EPHA1 ( $p = 5 \times 10^{-4}$ ) and rs3865444, which is located 373 bp upstream of CD33 ( $p = 0.049$ ). The ORs we observed in our meta-analyses (EPHA1 = 0.88, CD33 = 0.96) were comparable to those reported by both Naj *et al.* (EPHA1 = 0.87, CD33 = 0.89) and by Hollingworth *et al.* (EPHA1 = 0.90, CD33 = 0.89) such that the estimated p-values for association of these variants in all data ( $n > 42,000$ ) were an impressive  $2.1 \times 10^{-15}$  for EPHA1 and  $1.8 \times 10^{-13}$  for CD33.

Although our meta-analyses showed successful replication of the association of the ARID5B variants rs2588969 (OR = 1.08,  $p = 0.046$ ) and rs4948288 (OR = 1.11,  $p = 0.008$ ) with a direction of association consistent with that reported by Hollingworth *et al.* (OR = 1.06 and 1.07, respectively), the associations did not survive adjustment for age-at-diagnosis/entry, sex and APOE  $\epsilon$  4 status ( $p = 0.30$  and 0.11, respectively). This covariate-dependent association could explain the opposing association reported by Naj *et al.* in their discovery (OR = 0.88) and replication (OR = 1.05) datasets for rs2588969; the only ARID5B variant they tested. Therefore, while estimation of the p-values for association of the ARID5B variants in all datasets combined were highly significant (rs2588969;  $p = 2.3 \times 10^{-9}$  and rs4948288;  $p = 4.0 \times 10^{-4}$ ), interpretation of these associations should be treated with caution and should take into account the age-at-diagnosis/entry, sex and APOE  $\epsilon$  4 dosage of the populations. Finally, although the estimated p-value for association of rs9349407 (located in intron 1 of CD2AP) in all datasets was  $6.5 \times 10^{-11}$ , there was no evidence for association of this variant in our dataset alone (OR = 0.97,  $p = 0.56$ ).

Our Mayo2 collection of case-control series studies provided a total of 2,634 LOAD and 4,201 controls. Combining across studies to perform global tests of significance for additive genotypic trend tests gave us 80% power to detect ORs ranging from 1.17 (or 0.855) for variants with a minor allele frequency (MAF) of 0.2 to 1.13 (or 0.883) for variants with a MAF of 0.45 in controls. The study provided approximately 60% power to detect the OR of 1.11 that we report for CD2AP (MAF = 0.27).

Case-control studies such as this are not designed to ascertain whether the variants with reported association with LOAD risk are the functional variant but



**Figure 1 Forest plots for meta-analysis of *CD2AP*, *EPHA1*, *ARID5B*, and *CD33* variants in our six Mayo2 case-control series.** ORs (boxes) and 95% CI (whiskers) are plotted for each population and shown on the right of each plot. Combined OR is the overall OR calculated by the meta-analysis using a random effects model. P-values are provided for the combined ORs and Breslow-Day tests of heterogeneity. I<sup>2</sup> gives an estimate of between studies variance.

they can identify a linkage disequilibrium (LD) block within which a truly functional variant may reside. Our results indicate that the *EPHA1* and *CD33* variants represent excellent candidates for targeted deep sequencing or high density genotyping in order to define the locus further, followed by subsequent functional studies of nearby genes to elucidate the mechanism behind these associations. With the exception of rs9349407, which lies within intron 1 of *CD2AP*, all of these variants lie within intergenic regions but for ease of the reader, we have thus far only referred to the nearest gene for each variant. This by no means signifies that these variants (or the functional variants in LD with them) are assumed to affect the expression or function of the nearest gene but may affect other nearby genes. Until it is known which gene underlies these associations, all nearby genes should be included in follow-up functional investigation (all genes that reside within 100 kb of these variants are listed in Additional file 1, Table S1).

## Conclusions

Taken along with our previous publications [5,18,20,21], we have now performed follow-up association studies of 25 of the top GWAS-identified candidate LOAD genes and successfully replicated the association of eleven variants (in or near *ABCA7*, *BIN1*, *CD33*, *CLU*, *CR1*, *EPHA1*, *GAB2*, *LOC651924*, *MS4A6A/4E* and *PICALM*), eight of which are currently ranked in the top ten (after *APOE*) on AlzGene. This recent success in replicating genetic association highlights the utility of multiple, large case-control follow-up studies to confirm the novel associations reported by large GWAS, thus confirming them as good candidate genes for functional follow-up studies.

## Methods

### Ethics statement

Approval was obtained from the ethics committee or institutional review board of each institution responsible for the ascertainment and collection of samples. Written informed consent was obtained for all individuals that participated in this study.

### Case-control subjects

The Mayo2 case-control series consisted of Caucasian subjects from the United States ascertained at the Mayo Clinic Jacksonville, Mayo Clinic Rochester, or through the Mayo Clinic Brain Bank. Additional Caucasian subjects from Europe were obtained from Norway [22], Poland [23], and from six research institutes in the United Kingdom that are part of the Alzheimer's Research UK (ARUK) Network. Although the ARUK samples used in this follow-up do not overlap with

**Table 3 Association of CD2AP, EPHA1, ARID5B, and CD33 variants with LOAD in the initial studies (ADGC and GERAD+) and Mayo2 follow-up series**

Study	N <sup>a</sup>		MAF <sup>b</sup>		Association test	
	Cases	Controls	Cases	Controls	OR (95% CI)	p-value
<b>CD2AP-rs9349407-C (minor allele)</b>						
ADGC Discovery (Stage 1)	8,309	7,366			1.14 (1.08-1.21)	<b>1.2 × 10<sup>-6</sup></b>
ADGC Replication (Stage 2)	3,531	3,565			1.07 (0.98-1.17)	<b>0.12</b>
ADGC combined analysis (Stages 1+2)	11,840	10,931			1.12 (1.07-1.18)	<b>1.0 × 10<sup>-6</sup></b>
Hollingworth <i>et al.</i> (GERAD + Consortia)	6,283	7,165			1.11 (1.04-1.18)	<b>8 × 10<sup>-4</sup></b>
Mayo2 <sup>c</sup>	2,521	4,055	0.27	0.27	0.97 (0.89-1.07)	0.56
Jacksonville	492	922	0.28	0.26	1.10 (0.91-1.33)	0.34
Rochester	313	1,600	0.26	0.27	0.88 (0.70-1.09)	0.24
Autopsy	285	100	0.26	0.29	0.98 (0.65-1.47)	0.92
Norway	324	519	0.25	0.28	0.81 (0.62-1.06)	0.13
Poland	468	181	0.29	0.25	1.04 (0.77-1.42)	0.79
ARUK	639	733	0.28	0.29	0.97 (0.81-1.16)	0.72
ADGC/Hollingworth <sup>d</sup>	18,123	18,096				1.2 × 10 <sup>-10</sup>
Mayo2/ADGC/Hollingworth <sup>e</sup>	20,644	22,151				6.5 × 10 <sup>-11</sup>
<b>EPHA1-rs11767557-C (minor allele)</b>						
ADGC Discovery (Stage 1)	8,309	7,366			0.85 (0.80-0.90)	<b>7.3 × 10<sup>-8</sup></b>
ADGC Replication (Stage 2)	3,531	3,565			0.94 (0.86-1.03)	0.17
ADGC combined analysis (Stages 1+2)	11,840	10,931			0.87 (0.83-0.92)	<b>2.4 × 10<sup>-7</sup></b>
Hollingworth <i>et al.</i> (GERAD + Consortia)	6,283	12,935			0.90 (0.85-0.95)	<b>3.4 × 10<sup>-4</sup></b>
Mayo2 <sup>c</sup>	2,509	4,055	0.19	0.21	0.87 (0.78-0.96)	<b>5.5 × 10<sup>-4</sup></b>
Jacksonville	501	957	0.18	0.20	0.86 (0.70-1.06)	0.17
Rochester	309	1,572	0.19	0.21	0.89 (0.69-1.13)	0.33
Autopsy	307	99	0.17	0.24	0.66 (0.43-1.02)	0.06
Norway	338	548	0.21	0.22	0.94 (0.71-1.24)	0.67
Poland	457	169	0.20	0.21	0.93 (0.66-1.31)	0.67
ARUK	597	710	0.19	0.22	0.85 (0.69-1.04)	0.12
ADGC/Hollingworth <sup>d</sup>	18,123	18,096				4.2 × 10 <sup>-12</sup>
Mayo2/ADGC/Hollingworth <sup>e</sup>	20,632	27,921				2.1 × 10 <sup>-15</sup>
<b>ARID5B-rs2588969-A (minor allele)</b>						
ADGC Discovery (Stage 1)	8,309	7,366			0.88 (0.84-0.93)	<b>1.1 × 10<sup>-6</sup></b>
ADGC Replication (Stage 2)	3,531	3,565			1.05 (0.97-1.13)	0.23
ADGC combined analysis (Stages 1+2)	11,840	10,931			0.93 (0.89-0.97)	<b>0.001</b>
Hollingworth <i>et al.</i> (GERAD + Consortia)	6,283	7,165			1.06 (1.01-1.13)	<b>0.03</b>
Mayo2 <sup>c</sup>	2,571	4,102	0.40	0.38	1.05 (0.96-1.14)	0.30
Jacksonville	495	928	0.39	0.38	1.04 (0.88-1.23)	0.63
Rochester	307	1,604	0.42	0.38	1.12 (0.92-1.37)	0.26
Autopsy	308	102	0.38	0.32	1.24 (0.86-1.79)	0.24
Norway	338	543	0.37	0.37	1.05 (0.83-1.33)	0.69
Poland	473	185	0.42	0.40	0.91 (0.68-1.20)	0.49
ARUK	650	740	0.40	0.39	1.05 (0.88-1.24)	0.61
ADGC/Hollingworth <sup>d</sup>	18,123	18,096				7.6 × 10 <sup>-9</sup>
Mayo2/ADGC/Hollingworth <sup>e</sup>	20,694	22,198				2.3 × 10 <sup>-9</sup>
<b>ARID5B-rs4948288-A (minor allele)</b>						
ADGC Discovery (Stage 1)	8,309	7,366				
ADGC Replication (Stage 2)	3,531	3,565				
ADGC combined analysis (Stages 1+2)	11,840	10,931				
Hollingworth <i>et al.</i> (GERAD + Consortia)	6,992	13,472			1.07 (1.03-1.15)	<b>3.6 × 10<sup>-3</sup></b>
Mayo2 <sup>c</sup>	2,556	4,058	0.42	0.40	1.07 (0.99-1.16)	0.11
Jacksonville	496	925	0.43	0.39	1.13 (0.96-1.34)	0.14

**Table 3 Association of CD2AP, EPHA1, ARID5B, and CD33 variants with LOAD in the initial studies (ADGC and GERAD+) and Mayo2 follow-up series (Continued)**

Rochester	314	1,579	0.43	0.40	1.08 (0.89-1.32)	0.43
Autopsy	300	98	0.38	0.39	0.91 (0.63-1.32)	0.61
Norway	324	540	0.40	0.41	1.06 (0.83-1.34)	0.64
Poland	466	184	0.42	0.40	0.90 (0.68-1.20)	0.48
ARUK	656	732	0.43	0.41	1.13 (0.96-1.33)	0.14
Mayo2/ADGC/Hollingworth <sup>e</sup>	9,548	17,530				$4.0 \times 10^{-4}$
<b>CD33 -rs3865444-A (minor) allele</b>						
ADGC Discovery (Stage 1)	8,309	7,366			0.88 (0.84-0.93)	$8.2 \times 10^{-7}$
ADGC Replication (Stage 2)	3,531	3,565			0.91 (0.85-0.99)	<b>0.02</b>
ADGC combined analysis (Stages 1+2)	11,840	10,931			0.89 (0.86-0.93)	$1.1 \times 10^{-7}$
Hollingworth et al (GERAD + Consortia)	6,283	7,165			0.89 (0.84-0.95)	$2.2 \times 10^{-4}$
Mayo2 <sup>c</sup>	2538	4052	0.31	0.32	0.92 (0.84-1.00)	$4.9 \times 10^{-2}$
Jacksonville	492	920	0.29	0.31	0.82 (0.68-0.98)	<b>0.03</b>
Rochester	312	1,577	0.31	0.33	0.88 (0.72-1.08)	0.23
Autopsy	298	97	0.32	0.34	0.84 (0.57-1.24)	0.39
Norway	327	541	0.32	0.32	0.89 (0.70-1.14)	0.37
Poland	467	187	0.30	0.26	1.00 (0.72-1.37)	0.99
ARUK	642	730	0.33	0.34	0.98 (0.83-1.17)	0.85
ADGC/Hollingworth <sup>d</sup>	18,123	18,096				$3.6 \times 10^{-12}$
Mayo2/ADGC/Hollingworth <sup>e</sup>	20,661	22,148				$1.8 \times 10^{-13}$

Abbreviations: MAF, minor allele frequency; OR, odds ratio for the minor allele; 95% CI, 95% confidence interval

<sup>a</sup>The numbers shown for the series in the Naj *et al.* and Hollingworth *et al.* studies refer to the complete set analyzed. The numbers for the Mayo follow-up data refer to the number of samples successfully genotyped.

<sup>b</sup>MAFs were not reported for LOAD and control groups in the Naj *et al.* or Hollingworth *et al.* studies.

<sup>c</sup>The results shown here for the Mayo2 follow-up dataset combined and for the subseries were obtained using logistic regression adjusted for age, sex and APOE  $\epsilon$  4 dosage. The Mayo2 follow-up dataset reported here is independent of that which was incorporated in the GWAS reported by Hollingworth *et al.* The results for each of the Mayo follow-up subseries (Jacksonville, Rochester, Autopsy-confirmed, Norway, Poland and ARUK) are listed immediately below the results for the Mayo2 follow-up dataset combined.

<sup>d</sup>Indicates Fisher's combined p-value for each individual GWAS in the Naj *et al.* study (Combined) and the Hollingworth *et al.* study.

<sup>e</sup>Indicates Fisher's combined p-value for each individual GWAS in the Naj *et al.* study (Combined), the Hollingworth *et al.* study and Mayo2 independent follow-up series.

those employed in the original GWAS publication by Hollingworth *et al.*, the same subject/sample ascertainment methodology was followed. The ARUK series included here are from Bristol, Leeds, Manchester, Nottingham, Oxford and Southampton. Since the Manchester cohort only consisted of LOAD cases, the Manchester cases were combined with subjects in the Nottingham series.

### Genotyping

All genotyping was performed at the Mayo Clinic in Jacksonville using TaqMan<sup>®</sup> SNP Genotyping Assays in an ABI PRISM<sup>®</sup> 7900HT Sequence Detection System with 384-Well Block Module from Applied Biosystems, California, USA. The genotype data was analyzed using the SDS software version 2.2.3 (Applied Biosystems, California, USA).

### Statistical Analyses

Meta-analysis of allelic association and Breslow-Day tests were performed using StatsDirect v2.5.8 software.

Meta-analyses were performed using the results from each individual case-control series. Summary ORs and 95% CI were calculated using the DerSimonian and Laird (1986) random-effects model [24]. Breslow-Day tests were used to test for heterogeneity between populations. PLINK software [25] (<http://pngu.mgh.harvard.edu/purcell/plink/>) was used to perform logistic regression analysis under an additive model adjusting for age-at-diagnosis, sex and APOE  $\epsilon$  4 dose as covariates. In our analysis of all series combined, series was included as an additional covariate. Since genotype counts were not reported for series included in the Naj *et al.* or Hollingworth *et al.* studies, we employed a Fisher combined test to combine p-values across series. Power calculations, based on a Mantel-Haenszel chi-square test that pooled across six different study groups, were obtained to estimate the detectable odds ratios for an ordinal effect using a range of minor allele frequencies spanning those expected from the candidate variants.

## Additional material

**Additional file 1: Table S1. Genes located within 100 kb of the five variants tested in this study.** Chr, chromosome. Base pair positions (bp) are relative to the NCBI Human Genome build 36.1. The position of the variant relative to the gene is given as 5' (upstream from the gene's transcription start site) or 3' (downstream from the gene's last exon). Distance indicates the number of base pairs from the variant position to the gene's nearest exon.

### Abbreviations

ABCA7: ATP-binding cassette, sub-family A (ABC1), member 7; AD: Alzheimer's disease; ADGC: Alzheimer's disease Genetic Consortium; APOE: apolipoprotein E; ARID5B: AT rich interactive domain 5B (MRF1-like); ARUK: Alzheimer's Research United Kingdom; BIN1: bridging integrator 1; Bp: base pair; CD2AP: CD2-associated protein; CD33: CD33 molecule; CI: confidence interval; CLU: clusterin; CR1: complement component (3 b/4 b) receptor 1 (Knops blood group); EPHA1: EPH receptor A1; GAB2: GRB2-associated binding protein 2; GERAD: Genetic and Environmental Risk in Alzheimer's Disease Consortium; GWAS: genome-wide association study; kb: kilobases; LD: linkage disequilibrium; LOAD: late-onset Alzheimer's disease; MAF: minor allele frequency; M54A4A: membrane-spanning 4-domains, subfamily A, member 4; OR: odds ratio; PICALM: phosphatidylinositol binding clathrin assembly protein; SD: standard deviation.

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### Authors' contributions

*Study concept and design:* MMC and SGY. *Sample Collection and Diagnosis:* ARUK, DWD, JOA, MB, NRG-R, RCP, SBS, and ZKW. *Genotyping:* MMC and TAH. *DNA Sample Preparation:* GDB, ML and ZFG. *Analysis and interpretation of data:* JEC, KM, MMC, OB, SGY and VSP. *Drafting of the manuscript:* MMC and OB. *Critical revision of the manuscript for important intellectual content:* KM, MMC, OB, SGY and VSP. *Study supervision:* KM, MMC and SGY. All authors have read and approve the final manuscript.

### Competing interests

The authors declare that they have no competing interests.

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### References

- Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-MacLachlan DR, Alberts MJ, Hulette C, Crain B, Goldgaber D, Roses AD: **Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease.** *Neurology* 1993, **43**:1467-1472.
- Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, Pahwa JS, Moskva V, Dowzell K, Williams A, Jones N, Thomas C, Stretton A, Morgan AR, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Morgan K, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Love S, Kehoe PG, Hardy J, Mead S, Fox N, Rossor M, Collinge J, Maier W, Jessen F, Schürmann B, van den Bussche H, Heuser I, Kornhuber J, Wilfang J, Dichgans M, Frölich L, Hampel H, Hüll M, Rujescu D, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Mühleisen TW, Nöthen MM, Moebus S, Jöckel KH, Klopp N, Wichmann HE, Carrasquillo MM, Pankratz VS, Younkin SG, Holmans PA, O'Donovan M, Owen MJ, Williams J: **Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease.** *Nat Genet* 2009, **41**:1088-1093.
- Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B, Letenneur L, Bettens K, Berr C, Pasquier F, Fiévet N, Barberger-Gateau P, Engelborghs S, De Deyn P, Mateo I, Franck A, Helisalmi S, Porcellini E, Hanon O, European Alzheimer's Disease Initiative Investigators, de Pancorbo MM, Lendon C, Dufouil C, Jaillard C, Leveillard T, Alvarez V, Bosco P, Mancuso M, Panza F, Nacmias B, Bossù P, Piccardi P, Annoni G, Seripa D, Galimberti D, Hannequin D, Licastro F, Soininen H, Ritchie K, Blanché H, Dartigues JF, Tzourio C, Gut I, Van Broeckhoven C, Alperovitch A, Lathrop M, Amouyel P: **Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease.** *Nat Genet* 2009, **41**:1094-1099.
- Seshadri S, Fitzpatrick AL, Ikram MA, DeStefano AL, Gudnason V, Boada M, Bis JC, Smith AV, Carrasquillo MM, Lambert JC, Harold D, Schrijvers EM, Ramirez-Lorca R, Debette S, Longstreth WT Jr, Janssens AC, Pankratz VS, Dartigues JF, Hollingworth P, Aspelund T, Hernandez I, Beiser A, Kuller LH, Koudstaal PJ, Dickson DW, Tzourio C, Abraham R, Antunez C, Du Y, Rotter JJ, Aulchenko YS, Harris TB, Petersen RC, Berr C, Owen MJ, Lopez-Arrieta J, Varadarajan BN, Becker JT, Rivadeneira F, Nalls MA, Graff-Radford NR, Campion D, Auerbach S, Rice K, Hofman A, Jonsson PV, Schmidt H, Lathrop M, Mosley TH, Au R, Psaty BM, Uitterlinden AG, Farrer LA, Lumley T, Ruiz A, Williams J, Amouyel P, Younkin SG, Wolf PA, Launer LJ, Lopez OL, van Duijn CM, Breteler MM, CHARGE Consortium, GERAD1 Consortium, EADI1 Consortium: **Genome-wide analysis of genetic loci associated with Alzheimer disease.** *JAMA* 2010, **303**:1832-1840.

5. Carrasquillo MM, Belbin O, Hunter TA, Ma L, Bisceglia GD, Zou F, Crook JE, Pankratz VS, Dickson DW, Graff-Radford NR, Petersen RC, Morgan K, Younkin SG: **Replication of CLU, CR1, and PICALM associations with Alzheimer disease.** *Arch Neurol* 2010, **67**:961-964.
6. Zhang Q, Yu JT, Zhu QX, Zhang W, Wu ZC, Miao D, Tan L: **Complement receptor 1 polymorphisms and risk of late-onset Alzheimer's disease.** *Brain Res* 2010, **1348**:216-221.
7. Corneveaux JJ, Myers AJ, Allen AN, Pruzin JJ, Ramirez M, Engel A, Nalls MA, Chen K, Lee W, Chewning K, Villa SE, Meechoovet HB, Gerber JD, Frost D, Benson HL, O'Reilly S, Chibnik LB, Shulman JM, Singleton AB, Craig DW, Van Keuren-Jensen KR, Dunckley T, Bennett DA, De Jager PL, Heward C, Hardy J, Reiman EM, Huentelman MJ: **Association of CR1, CLU and PICALM with Alzheimer's disease in a cohort of clinically characterized and neuropathologically verified individuals.** *Hum Mol Genet* 2010, **19**:3295-3301.
8. Kamboh MI, Minster RL, Demirci FY, Ganguli M, Dekosky ST, Lopez OL, Barmada MM: **Association of CLU and PICALM variants with Alzheimer's disease.** *Neurobiol Aging*, advance online publication: 4 Jun 2010.
9. Yu JT, Li L, Zhu QX, Zhang Q, Zhang W, Wu ZC, Guan J, Tan L: **Implication of CLU gene polymorphisms in Chinese patients with Alzheimer's disease.** *Clin Chim Acta* 2010, **411**:1516-1519.
10. Jun G, Naj AC, Beecham GW, Wang LS, Buros J, Gallins PJ, Buxbaum JD, Ertekin-Taner N, Fallin MD, Friedland R, Inzelberg R, Kramer P, Rogava E, St George-Hyslop P, Alzheimer's Disease Genetics Consortium, Cantwell LB, Dombroski BA, Saykin AJ, Reiman EM, Bennett DA, Morris JC, Lunetta KL, Martin ER, Montine TJ, Goate AM, Blacker D, Tsuang DW, Beekly D, Cupples LA, Hakonarson H, Kukull W, Foroud TM, Haines J, Mayeux R, Farrer LA, Pericak-Vance MA, Schellenberg GD: **Meta-analysis Confirms CR1, CLU, and PICALM as Alzheimer Disease Risk Loci and Reveals Interactions With APOE Genotypes.** *Arch Neurol* 2010, **67**:1473-1484.
11. Yu JT, Song JH, Ma T, Zhang W, Yu NN, Xuan SY, Tan L: **Genetic association of PICALM polymorphisms with Alzheimer's disease in Han Chinese.** *J Neurol Sci* 2011, **300**:78-80.
12. Schjeide BM, Schnack C, Lambert JC, Lill CM, Kirchheiner J, Tumani H, Otto M, Tanzi RE, Lehrach H, Amouyel P, von Arnim CA, Bertram L: **The role of clusterin, complement receptor 1, and phosphatidylinositol binding clathrin assembly protein in Alzheimer disease risk and cerebrospinal fluid biomarker levels.** *Arch Gen Psychiatry* 2011, **68**:207-213.
13. Brouwers N, Van Cauwenbergh C, Engelborghs S, Lambert JC, Bettens K, Le Bastard N, Pasquier F, Montoya AG, Peeters K, Mattheijssens M, Vandenberghe R, De Deyn PP, Cruts M, Amouyel P, Sleegers K, Van Broeckhoven C: **Alzheimer risk associated with a copy number variation in the complement receptor 1 increasing C3b/C4b binding sites.** *Mol Psychiatry*, advance online publication: 15 March 2011.
14. Lee JH, Cheng R, Barral S, Reitz C, Medrano M, Lantigua R, Jimenez-Velazquez IZ, Rogava E, St George-Hyslop PH, Mayeux R: **Identification of Novel Loci for Alzheimer Disease and Replication of CLU and BIN1 in Caribbean Hispanic Individuals.** *Arch Neurol* 2011, **68**:320-328.
15. Lambert JC, Zelenika D, Hiltunen M, Chouraki V, Combarros O, Bullido MJ, Tognoni G, Fiévet N, Boland A, Arosio B, Coto E, Del Zompo M, Mateo I, Frank-Garcia A, Helisalmi S, Porcellini E, Pilotto A, Forti P, Ferri R, Delepine M, Scarpini E, Siciliano G, Solfrizzi V, Sorbi S, Spalletta G, Ravaglia G, Valdivieso F, Alvarez V, Bosco P, Mancuso M, Panza F, Nacmias B, Bossù P, Piccardi P, Annoni G, Seripa D, Galimberti D, Licastro F, Lathrop M, Soininen H, Amouyel P: **Evidence of the association of BIN1 and PICALM with the AD risk in contrasting European populations.** *Neurobiol Aging* 2011, **32**: 756e11-756e15.
16. Wijsman EM, Pankratz ND, Choi Y, Rothstein JH, Faber KM, Cheng R, Lee JH, Bird TD, Bennett DA, Diaz-Arrastia R, Goate AM, Farlow M, Ghetti B, Sweet RA, Foroud TM, Mayeux R, NIA-LOAD/NCRAD Family Study Group: **Genome-wide association of familial late-onset Alzheimer's disease replicates BIN1 and CLU and nominates CUGBP2 in interaction with APOE.** *PLoS Genet* 2011, **7**:e1001308.
17. Naj AC, Jun G, Beecham GW, Wang LS, Vardarajan BN, Buros J, Gallins PJ, Buxbaum JD, Jarvik GP, Crane PK, Larson EB, Bird TD, Boeve BF, Graff-Radford NR, De Jager PL, Evans D, Schneider JA, Carrasquillo MM, Ertekin-Taner N, Younkin SG, Cruchaga C, Kauwe JS, Nowotny P, Kramer P, Hardy J, Huentelman MJ, Myers AJ, Barmada MM, Demirci FY, Baldwin CT, Green RC, Rogava E, St George-Hyslop P, Arnold SE, Barber R, Beach T, Bigio EH, Bowen JD, Boxer A, Burke JR, Cairns NJ, Carlson CS, Carney RM, Carroll SL, Chui HC, Clark DG, Corneveaux J, Cotman CW, Cummings JL, DeCarli C, DeKosky ST, Diaz-Arrastia R, Dick M, Dickson DW, Ellis WG, Faber KM, Fallon KB, Farlow MR, Ferris S, Frosch MP, Galasko DR, Ganguli M, Gearing M, Geschwind DH, Ghetti B, Gilbert JR, Gilman S, Giordani B, Glass JD, Growdon JH, Hamilton RL, Harrell LE, Head E, Honig LS, Hulette CM, Hyman BT, Jicha GA, Jin LW, Johnson N, Karlawish J, Karydas A, Kaye JA, Kim R, Koo EH, Kowall NW, Lah JJ, Levey AI, Lieberman AP, Lopez OL, Mack WJ, Marson DC, Martiniuk F, Mash DC, Masliah E, McCormick WC, McCurry SM, McDavid AN, McKee AC, Mesulam M, Miller BL, Miller CA, Miller JW, Parisi JE, Perl DP, Peskind E, Petersen RC, Poon WW, Quinn JF, Rajbhandary RA, Raskind M, Reisberg B, Ringman JM, Roberson ED, Rosenberg RN, Sano M, Schneider LS, Seeley W, Shelanski ML, Slifer MA, Smith CD, Sonnen JA, Spina S, Stern RA, Tanzi RE, Trojanowski JQ, Troncoso JC, Van Deerlin VM, Vinters HV, Vonsattel JP, Weintraub S, Welsh-Bohmer KA, Williamson J, Woltjer RL, Cantwell LB, Dombroski BA, Beekly D, Lunetta KL, Martin ER, Kamboh MI, Saykin AJ, Reiman EM, Bennett DA, Morris JC, Montine TJ, Goate AM, Blacker D, Tsuang DW, Hakonarson H, Kukull WA, Foroud TM, Haines JL, Mayeux R, Pericak-Vance MA, Farrer LA, Schellenberg GD: **Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease.** *Nat Genet* 2011, **43**:436-441.
18. Carrasquillo MM, Belbin O, Hunter TA, Ma L, Bisceglia GD, Zou F, Crook JE, Pankratz VS, Sando SB, Aasly JO, Barcikowska M, Wszolek ZK, Dickson DW, Graff-Radford NR, Petersen RC, Morgan K, Younkin SG: **Replication of BIN1 Association with Alzheimer's Disease and Evaluation of Genetic Interactions.** *J Alzheimers Dis*, advance online publication: 14 Feb 2011.
19. Hu X, Pickering E, Liu YC, Hall S, Fournier H, Katz E, DeChairo B, John S, Van Eerdewegh P, Soares H: **Meta-analysis for genome-wide association study identifies multiple variants at the BIN1 locus associated with late-onset Alzheimer's disease.** *PLoS one* 2011, **6**:e16616.
20. Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, Abraham R, Hamshere ML, Pahwa JS, Moskva V, Dowzell K, Jones N, Stretton A, Thomas C, Richards A, Ivanov D, Widdowson C, Chapman J, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Beaumont H, Warden D, Wilcock G, Love S, Kehoe PG, Hooper NM, Vardy ER, Hardy J, Mead S, Fox NC, Rossor M, Collinge J, Maier W, Jessen F, Ruther E, Schürmann B, Heun R, Kölsch H, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frölich L, Hampel H, Gallacher J, Hüll M, Rujescu D, Giegling I, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Williams R, Deloukas P, Al-Chalabi A, Shaw CE, Tsoalaki M, Singleton AB, Guerreiro R, Mühleisen TW, Nöthen MM, Moebus S, Jöckel KH, Klopp N, Wichmann HE, Pankratz VS, Sando SB, Aasly JO, Barcikowska M, Wszolek ZK, Dickson DW, Graff-Radford NR, Petersen RC, Alzheimer's Disease Neuroimaging Initiative, van Duijn CM, Breteler MM, Ikram MA, DeStefano AL, Fitzpatrick AL, Lopez O, Launer LJ, Seshadri S, CHARGE consortium, Berr C, Campion D, Epelbaum J, Dartigues JF, Tzourio C, Alperovitch A, Lathrop M, EADI1 consortium, Feulner TM, Friedrich P, Riehle C, Krawczak M, Schreiber S, Mayhaus M, Nicolhaus S, Wagenpfeil S, Steinberg S, Stefansson H, Stefansson K, Snaedal J, Björnsson S, Jonsson PV, Chouraki V, Genier-Boley B, Hiltunen M, Soininen H, Combarros O, Zelenika D, Delepine M, Bullido MJ, Pasquier F, Mateo I, Frank-Garcia A, Porcellini E, Hanon O, Coto E, Alvarez V, Bosco P, Siciliano G, Mancuso M, Panza F, Solfrizzi V, Nacmias B, Sorbi S, Bossù P, Piccardi P, Arosio B, Annoni G, Seripa D, Pilotto A, Scarpini E, Galimberti D, Brice A, Hannequin D, Licastro F, Jones L, Holmans PA, Jonsson T, Riemschneider M, Morgan K, Younkin SG, Owen MJ, O'Donovan M, Amouyel P, Williams J: **Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease.** *Nat Genet* 2011, **43**:429-435.
21. Belbin O, Carrasquillo MM, Crump M, Culley OJ, Hunter TA, Ma L, Bisceglia G, Zou F, Allen M, Dickson DW, Graff-Radford NR, Petersen RC, Morgan K, Younkin SG: **Investigation of 15 of the top candidate genes for late-onset Alzheimer's disease.** *Hum Genet* 2011, **129**:273-282.
22. Sando SB, Melquist S, Cannon A, Hutton ML, Sletvold O, Saltvedt I, White LR, Lydersen S, Aasly JO: **APOE epsilon 4 lowers age at onset and is a high risk factor for Alzheimer's disease; a case control study from central Norway.** *BMC Neurol* 2008, **8**:9.
23. Klimkowicz-Mrowiec A, Marona M, Wolkow P, Maruszak A, Styczynska M, Barcikowska M, Zekanowski C, Szczudlik A, Slowik A: **Interleukin-1 gene**

-511 CT polymorphism and the risk of Alzheimer's disease in a Polish population. *Dement Geriatr Cogn Disord* 2009, **28**:461-464.

24. DerSimonian R, Laird N: **Meta-analysis in clinical trials.** *Control Clin Trials* 1986, **7**:177-188.
25. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC: **PLINK: a tool set for whole-genome association and population-based linkage analyses.** *Am J Hum Genet* 2007, **81**:559-575.

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