

## Common variants at *ABCA7*, *MS4A6A/MS4A4E*, *EPHA1*, *CD33* and *CD2AP* are associated with Alzheimer's disease

We sought to identify new susceptibility loci for Alzheimer's disease through a staged association study (GERAD+) and by testing suggestive loci reported by the Alzheimer's Disease Genetic Consortium (ADGC) in a companion paper. We undertook a combined analysis of four genome-wide association datasets (stage 1) and identified ten newly associated variants with  $P \leq 1 \times 10^{-5}$ . We tested these variants for association in an independent sample (stage 2). Three SNPs at two loci replicated and showed evidence for association in a further sample (stage 3). Meta-analyses of all data provided compelling evidence that *ABCA7* (rs3764650, meta  $P = 4.5 \times 10^{-17}$ ; including ADGC data, meta  $P = 5.0 \times 10^{-21}$ ) and the *MS4A* gene cluster (rs610932, meta  $P = 1.8 \times 10^{-14}$ ; including ADGC data, meta  $P = 1.2 \times 10^{-16}$ ) are new Alzheimer's disease susceptibility loci. We also found independent evidence for association for three loci reported by the ADGC, which, when combined, showed genome-wide significance: *CD2AP* (GERAD+,  $P = 8.0 \times 10^{-4}$ ; including ADGC data, meta  $P = 8.6 \times 10^{-9}$ ), *CD33* (GERAD+,  $P = 2.2 \times 10^{-4}$ ; including ADGC data, meta  $P = 1.6 \times 10^{-9}$ ) and *EPHA1* (GERAD+,  $P = 3.4 \times 10^{-4}$ ; including ADGC data, meta  $P = 6.0 \times 10^{-10}$ ).

Alzheimer's disease is the most common form of dementia, with both environmental and genetic factors contributing to risk. Alzheimer's disease is genetically complex and shows heritability of up to 79% (ref. 1). Rare variants in three genes (*APP*, *PSEN1* and *PSEN2*)<sup>1</sup> cause disease in a minority of cases, but until recently, *APOE* (encoding apolipoprotein E) was the only gene known to increase disease risk for the common form of Alzheimer's disease with late onset<sup>2</sup>. In 2009, we published a genome-wide association study (GWAS) of Alzheimer's disease in a sample designated GERAD1 (Genetic and Environmental Risk in Alzheimer's Disease Consortium 1), in which we identified two new genome-wide significant susceptibility loci: clusterin (*CLU*;  $P = 8.5 \times 10^{-10}$ ) and the phosphatidylinositol-binding clathrin assembly protein gene (*PICALM*;  $P = 1.3 \times 10^{-9}$ ). We also observed more variants with  $P < 1 \times 10^{-5}$  than were expected by chance ( $P = 7.5 \times 10^{-6}$ )<sup>3</sup>. These included variants in *CRI* (the complement receptor 1 gene), *BINI* (the bridging integrator 1 gene) and the *MS4A* (membrane-spanning 4A gene) cluster. A second independent Alzheimer's disease GWAS<sup>4</sup> using the EADI1 (European Alzheimer's Disease Initiative 1) sample showed genome-wide significant evidence for association with *CLU* ( $P = 7.5 \times 10^{-9}$ ) and *CRI* ( $P = 3.7 \times 10^{-9}$ ) and support for association with *PICALM*

( $P = 3 \times 10^{-3}$ ). A combined analysis of the GERAD1 and EADI1 data yielded highly significant support for all three loci (*CLU* meta  $P = 6.7 \times 10^{-16}$ ; *PICALM* meta  $P = 6.3 \times 10^{-9}$ ; and *CRI* meta  $P = 3.2 \times 10^{-12}$ ). The associations in *CLU*, *PICALM* and *CRI* have since been replicated in several independent datasets<sup>5-8</sup>, have shown trends in another dataset<sup>9</sup> and have shown relationships with the neurodegenerative processes underlying disease<sup>10</sup>. In addition, members of this consortium have since reported genome-wide significant association for *BINI* ( $P = 1.6 \times 10^{-11}$ ) and support for *EPHA1* (encoding ephrin receptor A1) ( $P = 1.7 \times 10^{-6}$ )<sup>11</sup>.

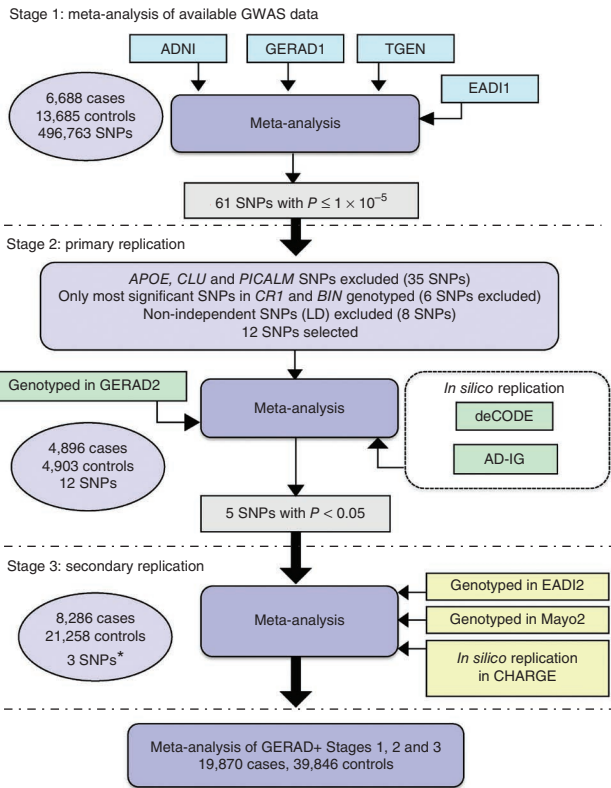
This study sought to identify new common susceptibility variants for Alzheimer's disease by first undertaking a three-stage association study based upon predominantly European samples (GERAD+; Fig. 1) and then by testing these samples for loci showing suggestive evidence for association in the ADGC GWAS<sup>12</sup>.

The first stage of this study comprised a meta-analysis of four Alzheimer's disease GWAS datasets (6,688 affected individuals (cases) and 13,685 controls) including: the GERAD1 (ref. 3), EADI1 (ref. 4), Translational Genomics Research Institute (TGEN1)<sup>13</sup> and the Alzheimer's Disease Neuroimaging Initiative (ADNI)<sup>14</sup> datasets. SNPs which remained significant at  $P \leq 1 \times 10^{-5}$  were then tested for replication in the second stage of this study, comprising 4,896 cases and 4,903 controls, including genotyping of the GERAD2 sample and *in silico* replication in the deCODE and German Alzheimer's Disease Integrated Genome Research Network (AD-IG) GWAS datasets. In stage 3, previously unidentified SNPs showing significant evidence of replication in stage 2 were then tested for association in a sample comprising 8,286 cases and 21,258 controls, which included new genotyping in the EADI2 (ref. 4) and Mayo2 samples and *in silico* replication in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) sample<sup>11</sup>. Sample descriptions and characteristics can be found in the **Supplementary Note and Supplementary Table 1**.

In stage 1, we identified 61 SNPs associated with Alzheimer's disease at  $P \leq 1 \times 10^{-5}$  following meta-analysis of 496,763 SNPs in the GERAD1, TGEN1, ADNI and EADI1 samples (**Supplementary Table 2 and Supplementary Note**). Ten SNPs at newly associated loci and two SNPs at previously identified susceptibility loci that surpassed the  $P \leq 1 \times 10^{-5}$  threshold were selected for further analysis (see below). One SNP, rs610932 (stage 1  $P = 1.8 \times 10^{-8}$ ) at the *MS4A* (encoding membrane spanning 4A) gene cluster, surpassed the threshold for genome-wide significance ( $P < 5.0 \times 10^{-8}$ )<sup>15</sup>. We also observed strong evidence for association at *ABCA7* (encoding ATP-binding cassette, sub-family A, member 7; rs3764650; stage 1  $P = 2.6 \times 10^{-7}$ ).

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**Figure 1** GERAD+ study design. \*Data for rs744373 and rs3818361 in the CHARGE consortium have been presented elsewhere<sup>15</sup>, as has data for rs381861 in the EADI2 samples<sup>4</sup>; as such these SNPs were not included in stage 3.

When selecting SNPs for testing in stage 2, we excluded known susceptibility loci that had previously been tested in GERAD2, and we limited our analysis of *BIN1* and *CRI1*, which had not been tested in GERAD2, to the most significant SNPs at each locus (Supplementary Table 2). Following pruning for linkage disequilibrium (LD), 12 SNPs were taken forward for replication in stage 2 (10 SNPs, excluding *BIN1* and *CRI1*).

Five of the 12 SNPs tested in stage 2 showed significant evidence for replication using a Bonferroni-adjusted threshold for significance of  $P = 4.2 \times 10^{-3}$  (Table 1 and Supplementary Table 3). In addition to SNPs at *BIN1* and *CRI1*, one SNP within *ABCA7* (rs3764650, stage 2  $P = 1.9 \times 10^{-5}$ ) and two SNPs at the *MS4A* gene cluster (rs610932, stage 2  $P = 1.6 \times 10^{-3}$ ; and rs670139, stage 2  $P = 1.1 \times 10^{-3}$ ) showed evidence of replication in stage 2. We tested the three SNPs implicating new risk loci for association in the stage 3 sample and showed further evidence of replication (rs3764650,

stage 3  $P = 2.9 \times 10^{-7}$ ; rs610932, stage 3  $P = 2.1 \times 10^{-5}$ ; and rs670139, stage 3  $P = 3.2 \times 10^{-3}$ ; Table 1 and Supplementary Table 3).

We conducted an inverse variance weighted meta-analysis of data from stages 1, 2 and 3 (Table 1 and Supplementary Table 3). This provided strong evidence for association with rs3764650 at *ABCA7* (meta  $P = 4.5 \times 10^{-17}$ ) and two SNPs at the *MS4A* gene cluster: rs610932 (meta  $P = 1.8 \times 10^{-14}$ ) and rs670139 (meta  $P = 1.4 \times 10^{-9}$ ). When combining GERAD+ and ADGC results (after removing overlapping samples), *ABCA7* had  $P = 5.0 \times 10^{-21}$  (odds ratio (OR) = 1.22). The two SNPs at the *MS4A* gene cluster, rs610932 and rs670139, showed  $P = 1.2 \times 10^{-16}$  (OR = 0.91) and  $P = 1.1 \times 10^{-10}$  (OR = 1.08), respectively, in the combined analysis of the GERAD+ and ADGC results. It is noteworthy that the most significant ADGC SNP at the *MS4A* locus is in LD with our top SNP (rs4938933 with rs610932,  $r^2 = 0.62$ ,  $D' = 0.86$ ), and thus both datasets may be detecting the same underlying signal.

This study also provides additional independent support for association with *CRI1* (stage 2  $P = 1.4 \times 10^{-3}$ ) and *BIN1* (stage 2  $P = 3.8 \times 10^{-5}$ ; see Table 1 for meta-analysis.) We did not observe interaction between *APOE* and the new variants identified in this study, and indeed, we did not find evidence of epistasis between any of the genome-wide significant variants identified to date (*ABCA7*, *MS4A*, *BIN1*, *CRI1*, *PICALM*, *CLU* or *APOE*) (Supplementary Table 4a). Likewise, adjusting for the presence of at least one *APOE*  $\epsilon 4$  allele had little effect on the results of the analysis of the three newly associated variants (Supplementary Table 4b). We also found no evidence for association between these loci and age at onset of Alzheimer's disease (rs3764650,  $P = 0.17$ ; rs670139,  $P = 0.38$ ; rs610932,  $P = 0.95$ ; rs744373,  $P = 0.87$ ; and rs3818361,  $P = 0.58$ ).

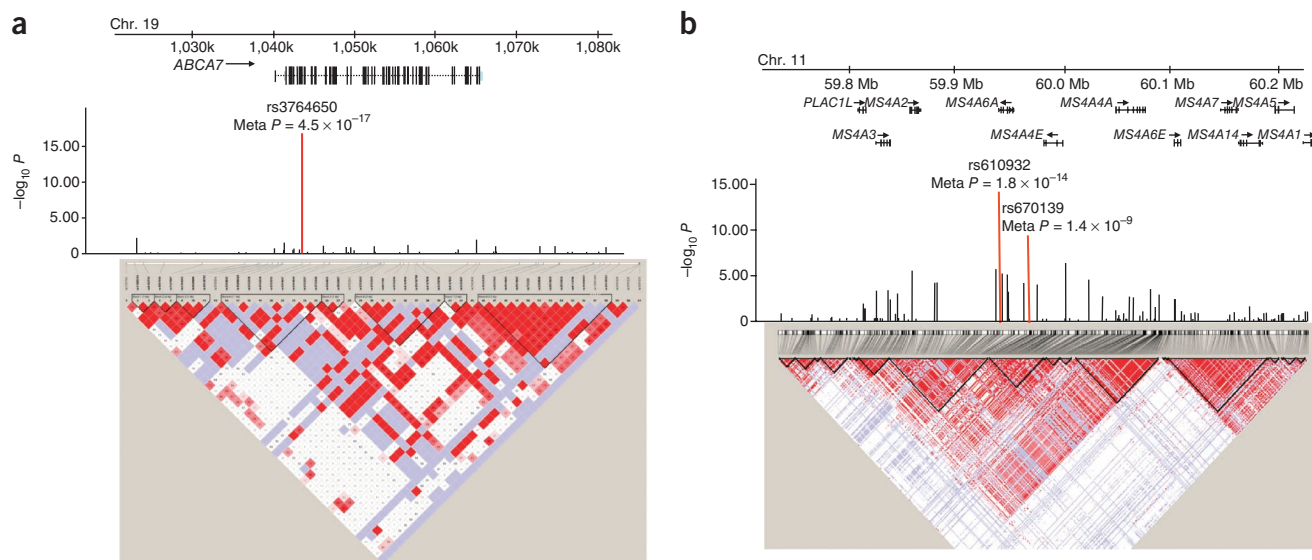
This study therefore identifies two new Alzheimer's disease susceptibility loci, which replicate over a number of independent case-control samples. The first of these is the *ABCA7* (encoding ATP-binding cassette, sub-family A, member 7) locus (Fig. 2a). The associated marker is rs3764650, which is located in intron 13. This SNP was the only variant in the gene that passed our stage 1 criterion, which is not unexpected given the low levels of LD between this SNP and others included in the GWAS. However, in a preliminary attempt to identify an associated functional variant at the *ABCA7* locus, we genotyped the GERAD2 sample for rs3752246, a non-synonymous SNP in exon 32 of the gene, which showed the highest LD with rs3764650 out of all HapMap *ABCA7*-coding variants based on  $r^2$  values ( $r^2 = 0.36$ ,  $D' = 0.89$ ). This variant (which was not genotyped in stage 1) was also associated with Alzheimer's disease (GERAD2  $P = 1 \times 10^{-3}$ , OR = 1.17). rs3752246 encodes a glycine to alanine substitution at position 1527 of the protein, which is predicted to be a benign change<sup>16</sup> and is unlikely to be the relevant functional variant. We used data from two published expression quantitative trait loci (eQTL) datasets (derived from lymphoblastoid cell lines<sup>17</sup> and brain<sup>18</sup>) to determine if rs3764650 is associated with the expression of *ABCA7*. However,

**Table 1** Results of the GERAD+ study

SNP	Closest gene	Chr.	MAF	Stage 1 <sup>a</sup>			Stage 2 <sup>b</sup>			Stage 3 <sup>c</sup>			Meta-analysis of GERAD+ and stage 1, 2 and 3 <sup>d</sup>			Meta-analysis of GERAD+ and ADGC		
				<i>P</i>	OR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>	OR	95% CI
rs3764650	<i>ABCA7</i>	19	0.10	$2.6 \times 10^{-7}$	1.22	1.13–1.32	$1.9 \times 10^{-5}$	1.28	1.14–1.44	$2.9 \times 10^{-7}$	1.22	1.13–1.32	$4.5 \times 10^{-17}$	1.23	1.18–1.30	$5.0 \times 10^{-21}$	1.23	1.17–1.28
rs610932	<i>MS4A6A</i>	11	0.42	$1.8 \times 10^{-8}$	0.88	0.85–0.92	$1.6 \times 10^{-3}$	0.90	0.84–0.96	$2.1 \times 10^{-5}$	0.91	0.87–0.95	$1.8 \times 10^{-14}$	0.90	0.87–0.92	$1.2 \times 10^{-16}$	0.91	0.88–0.93
rs670139	<i>MS4A4E</i>	11	0.41	$1.0 \times 10^{-5}$	1.11	1.06–1.16	$1.1 \times 10^{-3}$	1.11	1.04–1.19	$3.2 \times 10^{-3}$	1.06	1.02–1.11	$1.4 \times 10^{-9}$	1.09	1.06–1.12	$1.1 \times 10^{-10}$	1.08	1.06–1.11
rs3818361	<i>CRI1</i>	1	0.19	$3.2 \times 10^{-12}$	1.21	1.14–1.27	$1.4 \times 10^{-3}$	1.14	1.05–1.23	NA	NA	NA	$3.7 \times 10^{-14}$	1.18	1.13–1.24	NA	NA	NA
rs744373	<i>BIN1</i>	2	0.29	$1.5 \times 10^{-10}$	1.17	1.11–1.22	$3.8 \times 10^{-5}$	1.17	1.08–1.25	NA	NA	NA	$2.6 \times 10^{-14}$	1.17	1.12–1.21	NA	NA	NA

Chr., chromosome; MAF, minor allele frequency.

<sup>a</sup>GERAD1, EADI1, ADNI and TGEN1, less than 6,688 cases and 13,685 controls. <sup>b</sup>GERAD2, deCODE and AD-IG, 4,896 Alzheimer's disease cases and 4,903 controls. <sup>c</sup>EADI2, CHARGE and Mayo2, less than 8,286 Alzheimer's disease cases and 21,258 controls. <sup>d</sup>GERAD1 and GERAD2, EADI1 and EADI2, ADNI, TGEN1, deCODE, AD-IG, CHARGE and Mayo2, less than 19,870 Alzheimer's disease cases and 39,846 controls.



**Figure 2** Schematic of the associated variants reported in reference to (a) *ABCA7* and (b) chromosomal region chr11: 59.81Mb–60.1Mb harboring members of the *MS4A* gene cluster. Chromosome positions are shown at the top of the schematics (UCSC Feb 2009). Gene schematic: horizontal arrows indicate directions of transcription, black boxes indicate gene exons and the untranslated region. The  $-\log_{10} P$  of the SNPs analyzed in stage 1 are shown in the chart and graph. The GERAD+ stage 1, 2 and 3 meta-analysis  $P$  values for rs3764650 (*ABCA7*), rs610932 (*MS4A6A*) and rs670139 (*MS4A4E*) are indicated by the red lines. The  $D'$  LD block structure of *ABCA7* plus the surrounding region and chr11: 59.81Mb–60.1Mb according to the CEPH HapMap data are provided at the bottom of each schematic with lines indicating where each SNP genotyped on the Illumina 610-quad chip is represented.

we observed no association (**Supplementary Table 5**). Further work will be required to identify the causal variant(s) at this locus.

Second, we implicate the *MS4A* (encoding membrane-spanning 4A) gene cluster (**Fig. 2b**). The association spans an LD block of 293 kb (chr11: 59,814,287–60,107,105) and includes 6 of 16 known genes comprising the membrane-spanning 4-domains subfamily A (encoded by *MS4A*). These are *MS4A2*, *MS4A3*, *MS4A4A*, *MS4A4E*, *MS4A6A* and *MS4A6E*. The associated SNPs are found in the 3' untranslated region of *MS4A6A* (rs610932) and the intergenic region between *MS4A4E* and *MS4A6A* (rs670139). rs610932 showed nominally significant association with expression levels of *MS4A6A* in cerebellum and temporal cortex ( $0.01 < P < 0.05$ ; **Supplementary Table 5**) but not in the frontal cortex, pons or lymphoblastoid cell lines. The non-synonymous SNP that was most strongly associated with the genome-wide significant variants was rs2304933. This SNP was analyzed in stage 1 but showed weaker evidence for association ( $P = 0.006$ ) than the genome-wide significant variant at this locus in the same sample. **Figure 3** shows forest plots depicting association in the different datasets for SNPs at the *ABCA7* (rs3764650) and *MS4A* (rs610932 and rs670139) loci.

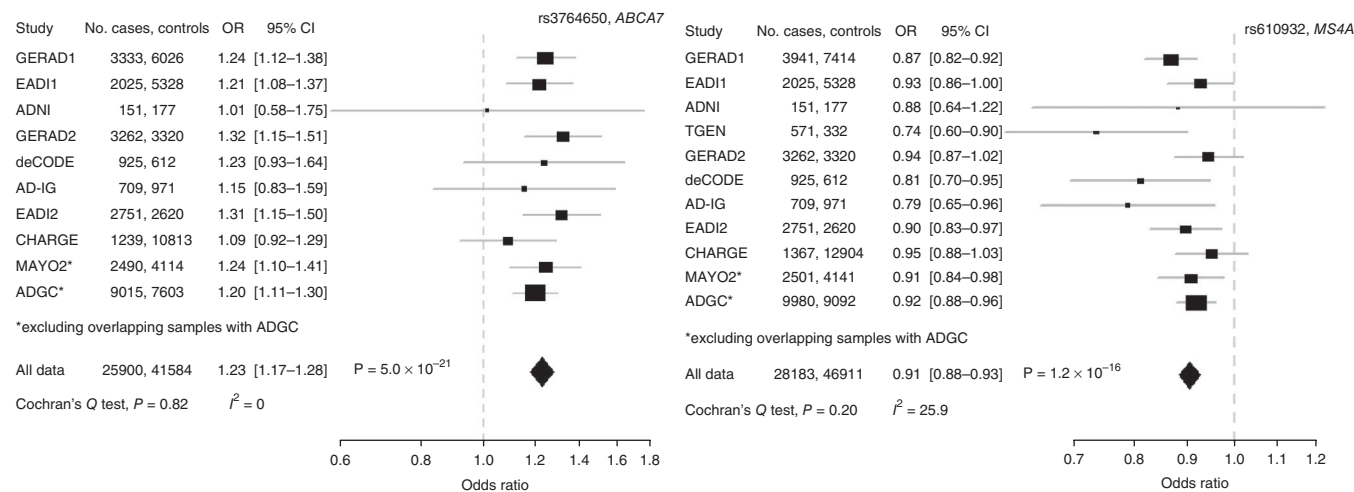
We also sought to follow up four additional loci showing suggestive evidence for association with Alzheimer's disease ( $1 \times 10^{-6} \geq P > 5 \times 10^{-8}$ ) from the ADGC GWAS<sup>12</sup>. These loci included those in *CD33*, *EPHA1*, *CD2AP* and *ARID5B*. It should be noted that evidence for suggestive association with *EPHA1* and *CD33* has been reported previously. Members of this collaboration were the first to report *EPHA1* as showing suggestive evidence of association with Alzheimer's disease (rs11771145,  $P = 1.7 \times 10^{-6}$ ; LD with ADGC rs11767557,  $r^2 = 0.28$ ,  $D' = 0.75$ )<sup>11</sup>, which included the GERAD1 and EADI1 samples reported on here. Similarly, researchers from another study were the first to show suggestive evidence for *CD33* (rs3826656,  $P = 4.0 \times 10^{-6}$ ; LD with ADGC rs3865444,  $r^2 = 0.13$ ,  $D' = 1.0$ )<sup>19</sup>.

We combined data from the GERAD+ dataset, comprising the GERAD1, EADI1, deCODE and AD-IG GWAS datasets (up to 6,992 cases and 13,472 controls), using inverse variance meta-analysis.

We included the TGEN1, ADNI and Mayo1 datasets in the ADGC discovery set and were thus excluded from these particular analyses. We observed support for association with *CD2AP* (rs9349407,  $P = 8.0 \times 10^{-4}$ , OR = 1.11), *CD33* (rs3865444,  $P = 2.2 \times 10^{-4}$ , OR = 0.89) and *EPHA1* (rs11767557,  $P = 3.4 \times 10^{-4}$ , OR = 0.90).

When these data were combined with the ADGC data, we observed genome-wide evidence for association with Alzheimer's disease (rs9349407, GERAD+ and ADGC meta  $P = 8.6 \times 10^{-9}$ , OR = 1.11; rs3865444, GERAD+ and ADGC meta  $P = 1.6 \times 10^{-9}$ , OR = 0.91; rs11767557, GERAD+ and ADGC meta  $P = 6.0 \times 10^{-10}$ , OR = 0.90). We observed nominally significant evidence of association with *ARID5B* (rs2588969,  $P = 3.3 \times 10^{-2}$ , OR = 1.06); however, the direction of effect was opposite to that reported by ADGC<sup>12</sup> and was not significant overall (GERAD+ and ADGC meta  $P = 3.6 \times 10^{-1}$ , OR = 0.99). See **Table 2** for results of the GERAD+ and ADGC combined analyses and **Supplementary Table 6** for results of additional SNPs at these loci.

Taken together, these results show compelling evidence for an additional five Alzheimer's disease susceptibility loci. *ABCA7* encodes an ATP-binding cassette (ABC) transporter. The ABC transporter superfamily has roles in transporting a wide range of substrates across cell membranes<sup>20</sup>. *ABCA7* is highly expressed in brain, particularly in the hippocampal CA1 neurons<sup>21</sup> and microglia<sup>22</sup>. *ABCA7* is involved in the efflux of lipids from cells to lipoprotein particles. Notably, the main lipoproteins in brain are APOE followed by CLU. We observed no evidence for epistatic interactions between the three genetic loci (**Supplementary Table 4a**), however, this is not a prerequisite for biological interaction between these molecules. In addition, *ABCA7* has been shown to regulate APP processing and inhibit  $\beta$ -amyloid secretion in cultured cells overexpressing *APP* (ref. 23). *ABCA7* also modulates phagocytosis of apoptotic cells by macrophages mediated through the C1q complement receptor protein on the apoptotic cell surface<sup>23</sup>. *ABCA7* is an ortholog of *Caenorhabditis elegans ced-7*, the product of which is known to clear apoptotic cells, and the high levels of expression of *ABCA7* in microglia are consistent with such a role.

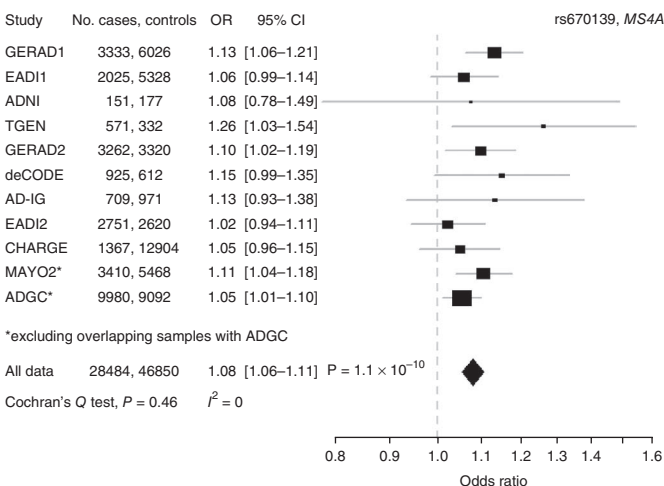


**Figure 3** Forest plots showing association in the different datasets for SNPs at the *ABCA7* (rs3764650) and *MS4A* (rs610932 and rs670139) loci.

The genes in the *MS4A* cluster on chromosome 11 have a common genomic structure with all other members of the family, including transmembrane domains, indicating that they are likely to be part of a family of cell-surface proteins<sup>24</sup>. *MS4A2* encodes the  $\beta$  subunit of high affinity IgE receptors<sup>25</sup>. The remaining genes in the LD block have no known specific functions.

CD33 is a member of the sialic-acid-binding immunoglobulin-like lectins (Siglec) family, which is thought to promote cell-cell interactions and regulate functions of cells in the innate and adaptive immune systems<sup>26</sup>. Most members of the Siglec family, including CD33, act as endocytic receptors, mediating endocytosis through a mechanism independent of clathrin<sup>27</sup>. CD2AP (CD2-associated protein) is a scaffold adaptor protein<sup>28</sup> that associates with cortactin, a protein also involved in the regulation of receptor-mediated endocytosis<sup>29</sup>. It is striking that these two new susceptibility genes for Alzheimer's disease, and the recently established susceptibility genes *PICALM* and *BIN1*, are all implicated in cell-cell communication and transduction of molecules across the membrane. *EPHA1* is a member of the ephrin receptor subfamily. Ephrins and Eph receptors are membrane-bound proteins which play roles in cell and axon guidance<sup>30</sup> and in synaptic development and plasticity<sup>31</sup>. However, *EPHA1* is expressed mainly in epithelial tissues<sup>32</sup> where it regulates cell morphology and motility<sup>33</sup>. Additional roles in apoptosis<sup>34</sup> and inflammation<sup>35</sup> have also been proposed.

Our study has identified variants at *ABCA7* and the *MS4A* gene cluster associated with susceptibility to Alzheimer's disease with replication over a number of independent case-control samples. We also provide independent support for three loci showing suggestive



evidence in a companion paper<sup>12</sup>: loci in *CD33*, *CD2AP* and *EPHA1*, which, when the data were combined, showed genome-wide levels of significance. Finally, we provide further replication evidence for *BIN1* and *CR1* loci as Alzheimer's disease susceptibility loci. What is striking about our findings is the emerging consistency in putative function of the genes identified. Five of the recently identified Alzheimer's disease susceptibility loci in *CLU*, *CR1*, *ABCA7*, *CD33* and *EPHA1* have putative functions in the immune system; *PICALM*, *BIN1*, *CD33* and *CD2AP* are involved in processes at the cell membrane, including endocytosis, and *APOE*, *CLU* and *ABCA7* are involved in lipid processing. It is conceivable that these processes would play strong roles in neurodegeneration and A $\beta$  clearance from the brain. These findings therefore provide new impetus for focused studies aimed at understanding the pathogenesis of Alzheimer's disease.

**Table 2** Results of the combined analysis of the GERAD+ consortia with the ADGC GWAS  $\times$

SNP	Closest gene	Chr.	MAF	LD with the top ADGC SNP at each loci		GERAD+ consortia <sup>a</sup>					GERAD+ and ADGC meta-analysis		
				$r^2$	$D'$	Cases	Controls	$P$	OR	95% CI	$P$	OR	95% CI
rs9349407 <sup>b</sup>	<i>CD2AP</i>	6	0.29	N/A	N/A	6,283	7,165	$8.0 \times 10^{-4}$	1.11	1.04-1.18	$8.6 \times 10^{-9}$	1.11	1.07-1.15
rs9296559 <sup>c</sup>	<i>CD2AP</i>	6	0.29	0.71	0.95	6,283	7,165	$1.5 \times 10^{-3}$	1.10	1.04-1.17	NA	NA	NA
rs11767557	<i>EPHA1</i>	7	0.21	N/A	N/A	6,283	12,935	$3.4 \times 10^{-4}$	0.90	0.85-0.95	$6.0 \times 10^{-10}$	0.90	0.86-0.93
rs2588969 <sup>b</sup>	<i>ARID5B</i>	10	0.40	N/A	N/A	6,283	7,165	$3.3 \times 10^{-2}$	1.06 <sup>c</sup>	1.01-1.13	$3.6 \times 10^{-1}$	0.99	0.95-1.02
rs4948288	<i>ARID5B</i>	10	0.26	0.55	0.78	6,992	13,472	$3.6 \times 10^{-3}$	1.07 <sup>c</sup>	1.03-1.15	NA	NA	NA
rs3865444 <sup>d</sup>	<i>CD33</i>	19	0.31	N/A	N/A	6,283	7,165	$2.2 \times 10^{-4}$	0.89	0.84-0.95	$1.6 \times 10^{-9}$	0.91	0.88-0.93

Chr., chromosome; MAF, minor allele frequency; LD, linkage disequilibrium.

<sup>a</sup>GERAD1, EADI1, deCODE and AD-IG. <sup>b</sup>Results generated from imputed data. The results from the top genotyped SNP are also shown. See **Supplementary Table 6** for full details. <sup>c</sup>Opposite direction of effect to that reported by Naj *et al.*<sup>12</sup>. <sup>d</sup>Data imputed in the deCODE dataset.

URLs. ADNI database, <http://www.loni.ucla.edu/ADNI>; ADNI investigators, [http://adni.loni.ucla.edu/wp-content/uploads/how\\_to\\_apply/ADNI\\_Authorship\\_List.pdf](http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Authorship_List.pdf).

## METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturegenetics/>.

*Note: Supplementary information is available on the Nature Genetics website.*

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J. Williams directed this study, assisted by M.J.O. and M.O.D. and was also helped by P.H., R.S., A.G., R.A., L.J. and D. Harold. J. Williams, P.H. and D.H. took primary responsibility for drafting the manuscript, assisted by R.S., A.G., R.A., M.O.D. and M.J.O. All authors contributed to the sample collection, sample preparation, genotyping and/or conduct of the GWAS upon which this study is based. J. Williams, R.A., P.H., R.S., A.G., C.W., J. Chapman, K.D., N.J., A.S., C. Thomas, S. Lovestone, J.P., P. Proitsi, M.K.L., C. Brayne, D.C.R., M.G., B.L., A.L., K. Morgan, K.S.B., P.A.P., D. Craig, B.M., S.T., C.H., D.M., A.D.S., S. Love, P.G.K., J.H., S. Mead, N.C.F., M. Rossor, J. Collinge, W.M., F.J., B.S., E.R., R.H., H.K., H.v.d.B., I.H., J.K., J. Wiltfang, M. Dichgans, L.F., H.H., M. Hüll, J.G., A.M.G., D.R., I.G., J.S.K.K., C.C., P.N., J.C.M., K. Mayo, K. Sleegers, K.B., S.E., P.P.D., C.V.B., G.L., N.J.B., H.G., A.M., M.T., T.W.M., M.M.N., S. Moebus, K.-H.J., N.K. and H.-E.W. contributed to clinical sample collection, ascertainment, diagnosis and preparation of samples from the independent GERAD2 sample genotyped as part

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## ONLINE METHODS

**Sample ascertainment, diagnostic criteria and genotyping.** Stage 1 comprised a meta-analysis of four Alzheimer's disease GWAS datasets (6,688 cases and 13,685 controls) including: GERAD1 (ref. 3), EADI1 (ref. 4), TGEN1 (ref. 13) and ADNI<sup>14</sup>. All Alzheimer's disease cases were diagnosed according to National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)<sup>36</sup>, The Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) or the Consortium to Establish a Registry for Alzheimer's Disease (CERAD)<sup>37</sup> criteria for either probable or definite Alzheimer's disease. Alzheimer's disease cases were predominantly female (62.4%). The mean age at disease onset and mean age at ascertainment of cases with Alzheimer's disease were 71.6 years and 77.3 years, respectively. Stage 1 included a total of 7,915 aged ( $\geq 60$  years) screened controls (59.9% female; mean age at collection, 74.5 years) and 5,770 population based unscreened controls from the GERAD1 study (50.8% female, mean age at collection, 48.6 years). The complete genome-wide meta-analysis results of stage 1 are available to researchers upon application.

Stage 2 included 4,896 cases and 4,903 controls, which comprised individual genotyping of the GERAD2 sample and *in silico* replication in the deCODE and AD-IG GWAS datasets. All cases were diagnosed according to NINCDS-ADRDA<sup>36</sup>, DSM-IV or CERAD<sup>37</sup> criteria for possible, probable or definite Alzheimer's disease. Alzheimer's disease cases were predominantly female (63.4%). The mean age at disease onset and the mean age at ascertainment in cases with Alzheimer's disease were 72.3 years and 76.8 years, respectively. The stage 2 control group (55.1% female, mean age at ascertainment, 70.0 years) were predominantly aged ( $\geq 60$  years) and screened for dementia (77.2%). Stage 3 comprised 8,286 cases and 21,258 controls, which included new genotyping in the EADI2 (ref. 4) and Mayo2 samples and *in silico* replication in the CHARGE sample<sup>11</sup>. All individuals included in these analyses provided written consent or assent to take part in the genetic association studies. We obtained ethical approval to use these samples to search for susceptibility genes for Alzheimer's disease (MREC 04/09/030; Amendment 2 and 4; approved 27 July 2007). Full descriptions of all samples, genotyping methods and quality control measures can be found in the **Supplementary Note**. Clinical characteristics of all samples can be found in **Supplementary Table 1**.

**Stage 1: combined analysis of four Alzheimer's disease GWAS.** An inverse variance-weighted fixed effects meta-analysis was used to test for association with Alzheimer's disease in the GERAD1, ADNI and TGEN datasets. The *P* values from this meta-analysis were then combined with the publicly available *P* values from the EADI1 study using Fisher's combined probability test. The combined analysis tested 496,795 autosomal SNPs. These SNPs passed quality control in each of the GERAD1, ADNI and EADI1 GWAS. We successfully imputed 457,509 of these SNPs in the TGEN sample (which, unlike the other studies, employed the Affymetrix 500K array). In the combined analysis, 67 SNPs were associated with Alzheimer's disease at  $P \leq 1 \times 10^{-5}$  (**Supplementary Table 2**). Full summary statistics were obtained from the EADI consortium for these 67 SNPs, and the analysis was repeated using inverse variance-weighted meta-analysis. Sixty-one SNPs remained significantly associated with Alzheimer's disease at  $P \leq 1 \times 10^{-5}$ . In selecting SNPs for replication in stage 2, we chose to exclude variants at the *APOE* locus (26 SNPs), as this is a known susceptibility gene for Alzheimer's disease, and also variants at the *CLU* and *PICALM* loci (1 SNP and 8 SNPs, respectively), as these data were already reported in the GERAD2 sample<sup>3</sup>. We restricted genotyping of *CRI* and *BINI* SNPs to the most significant markers at each locus, as they had not previously been tested in the stage 2 sample (6 SNPs excluded). We excluded 8 SNPs that were in high LD with the other SNPs selected for genotyping (**Supplementary Table 2**).

**Stage 2, 3 and final meta-analyses.** Both new genotyping data (GERAD2) and *in silico* replication (deCODE and Reimenschneider GWAS datasets) were included in stage 2. Data from these three samples were combined using an inverse-variance-weighted fixed effects meta-analysis (total sample of up to 4,896 cases and 4,903 controls). We employed a Bonferroni-adjusted threshold for significance, accounting for 12 tests, of  $P = 4.2 \times 10^{-3}$ . For the three new

SNPs that showed evidence for association in stage 2 ( $P < 4.2 \times 10^{-3}$ ), summary data (including ORs and variances) from the EADI2, Mayo2 and CHARGE and EADI2 studies were combined in an inverse-variance-weighted fixed effects meta-analysis (total sample of up to 8,286 cases and 21,258 controls), employing a Bonferroni adjusted threshold for significance, accounting for three tests, of  $P = 0.0167$ .

Finally, we combined full summary data (including ORs and variances) from all datasets (GERAD1 and GERAD2, EAD1 and EAD2, ADNI, TGEN, deCODE, AD-IG, Mayo2 and CHARGE) using an inverse-variance-weighted fixed effects meta-analysis (total sample of up to 19,870 cases and 39,846 controls). For meta-analyses at all stages, a Cochran's *Q* test was performed and *I*<sup>2</sup> was calculated to assess heterogeneity. The total sample size tested for each SNP is shown in **Supplementary Table 7**. Summary statistics for all datasets are shown in **Supplementary Table 8**.

**Secondary analyses.** We tested the genome-wide significant SNPs for relationships with age at onset. To this end, age at onset (in years) was used as the dependent variable in a linear regression analysis, and an additive model was assumed. Covariates were included in the logistic regression analysis to allow for country of origin. We found no evidence for association between these loci and age at onset of Alzheimer's disease (rs3764650,  $P = 0.17$ ; rs670139,  $P = 0.38$ ; and rs610932,  $P = 0.95$ ). We also tested the SNPs for association with Alzheimer's disease while adjusting for the presence of at least one *APOE*  $\epsilon 4$  allele within a logistic regression framework. Inclusion of presence or absence of *APOE*  $\epsilon 4$  as a covariate had little effect on the results (**Supplementary Table 5b**). Finally, we tested for genetic interactions between all pairwise combinations of genome-wide significant SNPs in *APOE*, *CLU*, *PICALM*, *BINI*, *CRI*, *ABCA7* and *MS4A*. Logistic regression analyses were performed including terms for covariates, main effects of each SNP, and a SNP  $\times$  SNP interaction term

$$\ln(P/(1-P)) = \alpha + \beta X_A + \gamma X_B + \delta Y_1 + \epsilon Y_2 + \zeta Y_3 + \eta Y_4 + \theta Y_5 + \iota Y_6 + \kappa X_A X_B$$

where *P* is the probability of having the disease; *X*<sub>A</sub> and *X*<sub>B</sub> correspond to variables representing the number of minor alleles at SNPs A and B respectively; *Y*<sub>1</sub> to *Y*<sub>6</sub> correspond to the covariates included in the main analysis (country of origin and principal components);  $\alpha$  is a constant; and  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\zeta$ ,  $\eta$ ,  $\theta$ ,  $\iota$  and  $\kappa$  are regression coefficients. We tested whether the regression coefficient that represents the interaction term,  $\kappa$ , equals zero or not. *P* values for this 1-degree-of-freedom test are presented in **Supplementary Table 5a**.

**Expression quantitative trait loci (eQTL) analysis.** Expression profiles were analyzed within two eQTL datasets. The first, published by Stranger and colleagues<sup>17</sup>, consists of gene expression profiles generated using RNA extracted from lymphoblastoid cell lines generated from 60 unrelated European CEU HapMap individuals. Expression analysis was performed using Illumina's commercial whole genome expression array, the Sentrix Human-6 Expression BeadChip. The second eQTL dataset<sup>18</sup> analyzed 143 neurologically normal subjects of European ancestry. Frozen tissue samples were obtained from four brain regions (cerebellum, pons, and frontal and temporal cortices) for each subject. Genotyping was performed using Infinium HumanHap550 BeadChips (Illumina). Expression analysis was performed using Illumina HumanRef-8 Expression BeadChips. Genotype data were used as presented in the original publications<sup>17,18,38</sup>. The expression data were normalized and log transformed as described in the original papers<sup>17,18,38</sup>. eQTLs were tested by linear regression of normalized expression level on SNP genotypes (coded as the number of minor alleles at each SNP: 0, 1 or 2).

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