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Genome-wide association analysis of age-at-onset in Alzheimer's disease

MI Kamboh[1], MM Barmada[1], FY Demirci[1], RL Minster[1], MM Carrasquillo[2], VS Pankratz[2], SG Younkin[2], AJ Saykin[3], The Alzheimer's Disease Neuroimaging Initiative[4], RA Sweet[5,6], E Feingold[1], ST DeKosky[7] and OL Lopez[6]

[1]*Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA;* [2]*Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL, USA;* [3]*Departments of Radiology and Imaging Sciences and Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA;* [4]*See Appendix;* [5]*Department of Psychiatry, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA;* [6]*Department of Neurology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA and* [7]*Office of the Dean and Department of Neurology, University of Virginia School of Medicine, Charlottesville, VA, USA*

The risk of Alzheimer's disease (AD) is strongly determined by genetic factors and recent genome-wide association studies (GWAS) have identified several genes for the disease risk. In addition to the disease risk, age-at-onset (AAO) of AD has also strong genetic component with an estimated heritability of 42%. Identification of *AAO* genes may help to understand the biological mechanisms that regulate the onset of the disease. Here we report the first GWAS focused on identifying genes for the AAO of AD. We performed a genome-wide meta-analysis on three samples comprising a total of 2222 AD cases. A total of ~2.5 million directly genotyped or imputed single-nucleotide polymorphisms (SNPs) were analyzed in relation to AAO of AD. As expected, the most significant associations were observed in the apolipoprotein E (*APOE*) region on chromosome 19 where several SNPs surpassed the conservative genome-wide significant threshold ($P < 5E-08$). The most significant SNP outside the *APOE* region was located in the *DCHS2* gene on chromosome 4q31.3 (rs1466662; $P = 4.95E-07$). There were 19 additional significant SNPs in this region at $P < 1E-04$ and the *DCHS2* gene is expressed in the cerebral cortex and thus is a potential candidate for affecting AAO in AD. These findings need to be confirmed in additional well-powered samples.
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Introduction

Alzheimer's disease (AD), a devastating neurodegenerative disease, is the most common form of dementia among the elderly. Genetically, AD is a complex and multifactorial disease with the possible involvement of multiple genes. The rare early-onset form of the disease usually follows an autosomal-dominant inheritance pattern and to date three genes have been identified: amyloid precursor protein (*APP*) and presenilin 1 and 2 (*PSEN1* and *PSEN2*). The common late-onset form of the disease is much more complex than the early-onset form and until recently the apolipoprotein E (*APOE*) gene was the only major genetic factor accounting for 20–29% of the risk for late-onset AD.[1,2] Recent large genome-wide association studies (GWAS) have identified nine additional genes for late-onset AD, including *CR1, BIN1, CLU* (a.k.a. *APOJ*), *PICALM, MS4A4/MS4A6E, CD2AP, CD33, EPHA1* and *ABCA7*.[3–7] There is high heritability for AD risk (up to 80%),[8] but the total risk attributable to all confirmed loci is about 50%, indicating the presence of additional risk genes for late-onset AD.

In addition to the disease risk, age-at-onset (AAO) of AD is also genetically influenced[9,10] with an estimated heritability of about 42%.[10] The *APOE* gene, in addition to affecting the risk of AD, also has a significant impact on AAO of AD and explains about 10% of its variation.[1] Several additional loci with effect sizes similar to or even greater than that of *APOE* have been suggested.[9] However, some additional genes implicated in AAO of AD have effect sizes far less than the effect of *APOE*.[11,12] Recently a GWAS implicated a locus on chromosome 14 with AAO of AD,[13] but it was not confirmed in a replication study.[14]

In this study we have used GWAS data from three independent samples in an effort to identify

Correspondence: Professor MI Kamboh, Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261, USA.
E-mail: kamboh@pitt.edu
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additional AAO loci in AD. The initial association analysis was performed in a new GWAS data derived from the University of Pittsburgh Alzheimer's Disease Research Center (ADRC), which was subsequently combined with two existing GWAS data from Mayo[15] and the Alzheimer's Disease Neuroimaging Initiative (ADNI)[16] for meta-analysis.

Materials and methods

University of Pittsburgh ADRC sample

Genomic DNA from 1440 AD cases was genotyped using the Illumina HumanOmni1-Quad (San Diego, CA, USA) as part of the stage 1 discovery sample. All subjects were Caucasian Americans. AD cases (mean (AAO) 72.8 ± 6.5 years; 62.9% women; 23.5% autopsy confirmed) were derived from the University of Pittsburgh ADRC, all of whom met the National Institute of Neurological and Communication Disorders and Stroke (NINCDS) and Alzheimer's Disease and Related Disorders Association (ADRDA) criteria for probable or definite AD. The University of Pittsburgh ADRC follows a standard evaluation protocol, including medical history, general medical and neurological examinations, psychiatric interview, neuropsychological testing and magnetic resonance imaging scan. All subjects were recruited with informed consent, and the study was approved by the University of Pittsburgh Institutional Review Board.

Mayo sample

The Mayo AD GWAS comprised 844 AD cases between the ages of 60–80 (mean AAO: 74 ± 4.8 years; 57.2% women), which were previously genotyped using the Illumina HumanHap300 BeadChip.[15] AD diagnosis was established using the NINCDS–ADRDA criteria.

The Alzheimer's Disease Neuroimaging Initiative (ADNI) sample

AD GWAS dataset consists of 188 AD cases with a clinical diagnosis of AD at baseline visit that were genotyped using the Illumina 610-Quad BeadChip.[16] AD cases were between the ages of 55–90 (mean AAO: 71.9 ± 8.1 years; 44.6% women) and met the NINCDS–ADRDA criteria. Details of the clinical evaluation and sample characterization are described elsewhere.[17,18] The ADNI data used in this report were obtained from the ADNI database (adni.loni.ucla.edu). The initial goal of ADNI was to recruit 800 adults, ages 55–90, to participate in research on the sensitivity and specificity of neuroimaging and other biomarkers for detecting and monitoring AD pathology *in vivo*. In ADNI, $\sim$200 cognitively normal older individuals and 400 people with amnestic MCI were followed for 3 years and 200 people with mild early stage AD followed for 2 years. For up-to-date information, see http://www.adni-info.org.

Genotyping and quality control of genotype data

The University of Pittsburgh ADRC sample of 1440 AD cases was genotyped using the Illumina Omni1-Quad chip (containing probes for 1 016 423 single-nucleotide polymorphisms (SNPs) and/or copy-number variations) at the Feinstein Institute of Medical Research (Manhasset, NY, USA). Genotypes for two *APOE* SNPs, rs429358 (*E***4*) and rs7412 (*E***2*) were determined either as previously described[19] or using TaqMan SNP genotyping assays. Exclusion criteria for individual samples included high genotype failure rate (106 individuals were removed because of a genotype failure rate $>2\%$), and cryptic relatedness (43 individuals were removed because they displayed an average degree of sharing (identity by state, or IBS) >0.4 with other members of the data set). An additional 101 cases that passed the stringent quality control criteria were excluded because of the uncertainty in AAO. Exclusion criteria for markers included minor allele frequency (189 727 SNPs were removed because of MAF $<1\%$), deviation from Hardy–Weinberg expectations (2239 SNPs gave a HWE test P-value $\leqslant$1E-06), and high genotype failure rates (22 385 SNPs were removed because of genotype failure rates $>2\%$). The final ADRC sample after all exclusions consisted of 1190 cases genotyped at 803 322 SNPs.

Population stratification

Population stratification testing was done using a multi-dimensional scaling-based method using all SNPs, as implemented in PLINK.[20] In all four components were conservatively determined to be relevant to the determination of population origin based on visual examination of principle component plots.

Imputation

Genotype posterior probabilities were imputed with MACH v.1.0, on all three GWAS data using haplotypes from the HapMap CEU v3 data release as a reference sample. The imputation generated data for >3 million SNPs that were subsequently filtered to exclude SNPs with $r^2<0.3$ and eventually 2 543 888 were included in the final analysis.

Association analyses

SNP analyses were conducted using a linear regression framework implemented in PLINK,[20] using covariates of sex, and the four principle components of population stratification identified above. A Bonferroni-adjusted significance level of $P<$5E-08 was employed to determine genome-wide significance following meta-analysis. Significance values from linear regression analyses were used for ranking purposes only, and so were not adjusted for multiple testing. After performing analyses for each SNP in each study sample individually, a meta-analysis was performed to obtain pooled estimates of the effect of these SNPs on AAO of AD across all study groups. Meta-analysis was done using a fixed-effects

methodology, as implemented in PLINK. Heterogeneity testing was accomplished using Cochran's *Q* statistic, summarized as the I^2 statistic (the percentage of total variation across studies that is due to heterogeneity rather than chance).[21,22] All analyses were done in R and/or PLINK using methods that correctly employ the imputed posterior probabilities for all genotypes at each SNP.

Results

Initially we performed association analysis of AAO in our new GWAS data on the University of Pittsburgh ADRC sample, where a total of 1440 AD cases were genotyped using the Illumina Human Omni-Quad1. After standard quality control filters for both genotypes and samples and imputing for unobserved genotypes, a total of ~2.5 million SNPs were examined in 1190 AD cases for association analysis. Association of SNPs with AAO was tested using linear regression of the quantitative phenotype that included sex and the first four principal components as covariates.

Figure 1a shows the quantile–quantile plot for comparison of observed and expected *P*-values distribution in the ADRC sample that demonstrates no evidence of significant population stratification but strong evidence of genetic associations. Figure 2 shows the genome-wide *P*-values in the ADRC sample in a Manhattan plot. As expected, the most significant associations were observed in the *APOE* region on chromosome 19 where five SNPs surpassed the genome-wide significant threshold of $P < 5E\text{-}08$ in the ADRC sample. The most significant SNP was rs429358 (*E*4*) in the *APOE* gene ($P = 3.37E\text{-}18$) followed by rs6857 in *TOMM40* ($P = 4.85E\text{-}15$) rs4420638 in *APOC1* ($P = 4.14E\text{-}14$), rs157582 in *TOMM40* ($P = 1.91E\text{-}13$) and rs2075650 in *TOMM40* ($P = 2.17E\text{-}10$). The regional association plot including all SNPs in the *APOE* region is given in Supplementary Figure 1. After removing SNPs in the *APOE* region, a deviation of *P*-values from the null distribution remained in the quantile–quantile plot, although within the 95% confidence interval of the expectation (Figure 1b). As no other SNPs outside the APOE region were genome-wide significant, we performed a

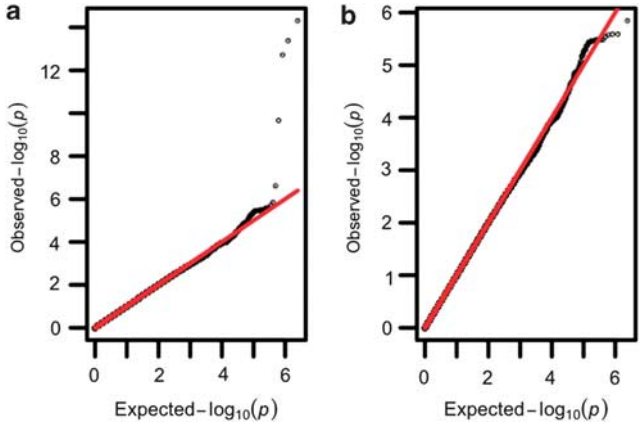

Figure 1 Quantile–quantile plots showing the observed versus the expected *P*-values in the University of Pittsburgh Alzheimer's Disease Research Center (ADRC) sample including all single-nucleotide polymorphisms (SNPs) (**a**) and after removing SNPs in the apolipoprotein E (*APOE*) region (**b**). The red line shows the distribution under the null-hypothesis.

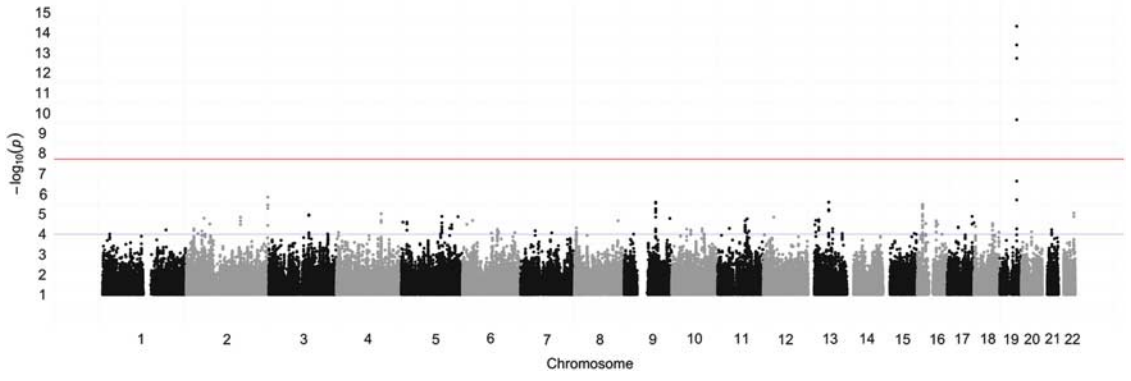

Figure 2 Manhattan plots showing the genome-wide *P*-values in the University of Pittsburgh Alzheimer's Disease Research Center (ADRC) sample after adjusting for sex and principle components. Red line indicates genome-wide significant level ($P < 5E\text{-}08$) and blue line indicates suggestive associations ($P < 1E\text{-}04$) in the ADRC sample.

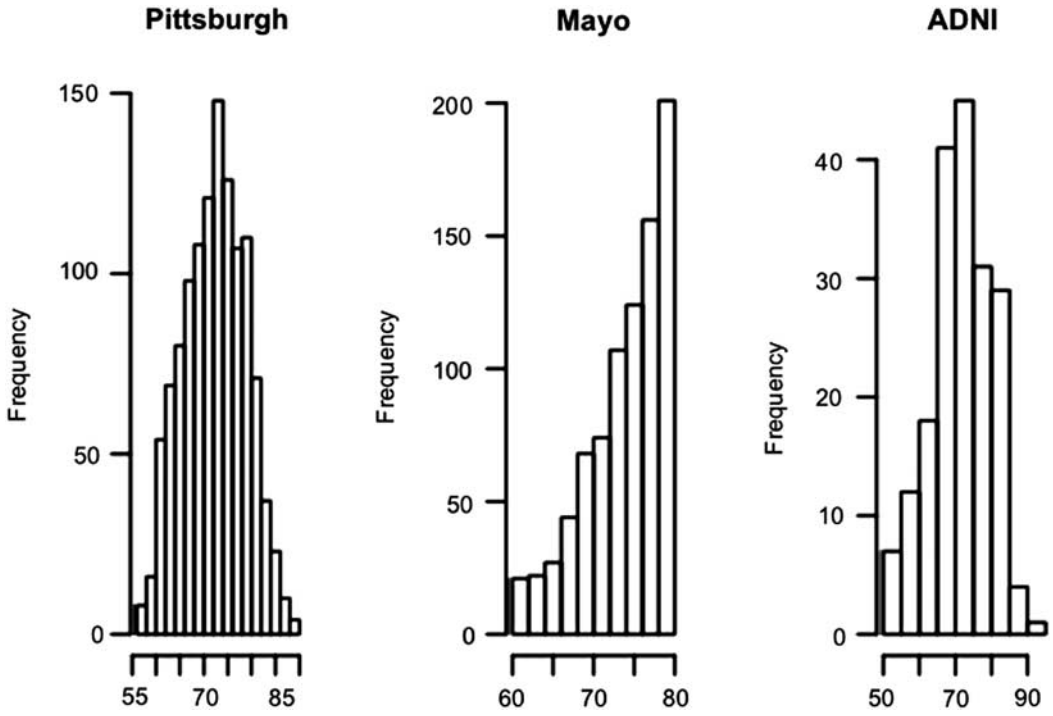

Figure 3 Distribution of age-at-onset in the University of Pittsburgh Alzheimer's Disease Research Center, Mayo and Alzheimer's Disease Neuroimaging Initiative (ADNI) samples.

meta-analysis by combining the University of Pittsburgh ADRC GWAS data with the Mayo ($n=844$) and ADNI ($n=188$) GWAS data. Figure 3 shows the distribution of AAO in the three samples. Although the AAO distribution is almost normal in the ADRC and ADNI samples, it is skewed towards older AAO. The quantile–quantile plot with the meta-analysis results is shown in Supplementary Figure 2. The meta-analysis of three samples yielded 11 non-*APOE* loci with $P<1E-05$ (Table 1). All SNPs with $P<1E-04$ in the meta-analysis are presented in the Supplementary Table.

The association of all top 11 non-*APOE* significant SNPs was consistent in direction in the three samples as reflected in β-values. Furthermore, 10 of the top 11 non-*APOE* SNPs were directly genotyped or had proxy genotyped SNPs with $P<1E-04$ on Illumina arrays (see Table 1 and Supplementary Table), thus eliminating potential spurious associations due to imputation artifact. The most significant SNP outside the *APOE* region, rs1466662, was located in the *DCHS2* gene on chromosome 4q31.3 at position 155.57 Mb ($P=4.95E-07$). There were 19 additional significant SNPs in this region at $P<1E-04$ a. Figure 4 shows the regional plot for SNPs within 500 kb on either side of the *DCHS2* index SNP and the meta *P*-values for markers, which had $P<IE-04$ in the meta-analysis. Chromosome 4 harbors another potential region for AAO at position 32.88 Mb ($P=4.13E-06$ for top SNP rs10517270) and this region had the most number of significant SNPs ($n=25$) at $P<1E-04$ (Supplementary Figure 3). There is no known gene in this region. The regional plots for the remaining nine top loci associated with AAO are shown in Supplementary Figures 4–12. In order to remove any variation in AAO owing to the established effect of *APOE*, we also analyzed the data after adjusting for the effect of *APOE*4*, but found no appreciable difference in *P*-values for the non-*APOE* loci (Table 1), indicating that their effects are independent of *APOE*.

Discussion

The focus of almost all reported GWAS related to AD has been on identifying genes for the disease risk. However, in addition to the disease risk, AAO of AD has also strong genetic component with an estimated heritability of 42%.[9,10] Here we report the first GWAS focused on identifying genes for the AAO of AD. We performed a genome-wide meta-analysis on three GWAS data sets comprising a total of 2222 cases. Only the *APOE/TOMM40/APOC1* region demonstrated genome-wide significant association with AAO, which is due to the previously well-established association of the *APOE* *4 SNP, as this demonstrated the most significant association in the discovery sample.

The present GWAS for AAO has identified 11 suggestive loci that although do not meet the strict criteria for genome-wide significant, their consistent and directional associations in three independent samples suggest that they are worthy of follow-up in additional studies. This is supported further by the fact that many of these suggestive loci contain genes

Table 1 Genetic loci associated with age-at-onset of AD with $P < E.05$

Chromosome	*Top SNP*[a]	*Position bp*	*Total SNPs*[b]	*Nearest gene*	*Pittsburgh sample*		*Mayo sample*		*ADNI sample*		*Meta-analysis*	*Homogeneity test*[c]	*Meta APO-E adjusted*	*Homogeneity test*
					β	P	*β*	P	*β*	P	P		P	
19	rs4420638	50.11	7 (5)	*APOE/TOMM40/APOC1*	2.20	4.14E-14	0.70	2.47E-02	−0.05	9.83E-01	1.11E-12	84.6	1.32E-05	0
4q31	rs1466662	155.57	20 (4)	*DCHS2*	−0.96	1.19E-03	−1.00	1.37E-04	−0.58	7.59E-01	4.95E-07	0	2.35E-06	0
12q24	rs17429217	115.78	4 (1)	*HRK/RNFT2*	2.44	4.00E-03	2.84	2.48E-04	4.76	5.03E-01	2.57E-06	0	3.18E-06	0
3p14	rs704454	64.90	2 (1)	*ADAMTS9*	0.50	8.32E-02	1.22	4.60E-06	2.94	1.54E-01	2.60E-06	53.4	1.55E-05	53.5
9p24	rs2034764	2.73	11 (4)	*KCNV2/VLDLR*	0.88	1.11E-03	0.76	1.62E-03	2.52	2.36E-01	3.82E-06	0	4.34E-06	0
4p15	rs10517270	32.88	25 (6)	—	−3.31	2.67E-04	−3.95	1.13E-02	−18.66	4.08E-02	4.13E-06	34.3	5.87E-06	45.9
6p21	rs2104362	33.93	16 (5)	*LEMD2/MLN/MIR1275*	0.84	5.87E-03	0.90	8.36E-04	4.04	6.79E-02	6.91E-06	6.83	5.03E-06	2.5
16q24	rs12933233	85.67	2 (1)	—	0.79	8.46E-03	0.91	5.63E-04	2.92	1.35E-01	7.64E-06	0	1.99E-05	0
18q21	rs1037757	54.90	6 (2)	*LOC390958/Sec11C*	−4.56	2.93E-05	−2.26	3.69E-02	−3.45	7.10E-01	8.29E-06	10.7	1.36E-05	0
15q25	rs3743162	83.23	5 (4)	*ZNF592/ALPK3/SLC28A1*	−0.88	5.70E-03	−1.03	6.02E-04	−1.84	4.96E-01	8.68E-06	0	9.99E-05	0
2q37	rs753855	231.77	3 (2)	*PSMD1/HTR2B/ARMC9*	0.91	7.00E-04	0.69	4.75E-03	1.13	5.36E-01	9.82E-06	0	4.09E-06	0
14q31	rs17764668	79.31	1 (0)	*NRXN3*	1.14	3.65E-03	1.15	1.14E-03	2.72	4.37E-01	9.84E-06	0	2.08E-05	0

Abbreviations: AD, Alzheimer's disease; ADNI, Alzheimer's disease neuroimaging initiative; APOE, apolipoprotein E; SNP, single-nucleotide polymorphism.
[a]SNP with lowest *P*-value in a given gene region after meta-analysis.
[b]Total genotyped or imputed significant SNPs with $P < 1E-04$ in a given region after meta-analysis. The number of genotyped SNPs is given in parentheses.
[c]Heterogeneity testing was accomplished using Cochran's *Q* statistic, summarized as the I2 statistic.[21,22]
Values closer to 0 indicate no heterogeneity, whereas larger numbers indicate increasing degrees of heterogeneity between studies.

that are expressed in the brain, and thus they may be relevant to AAO of AD. Our top SNP (rs1466662) is located in the *DCHS2* gene on 4q13 that is expressed in cerebral cortex.[23] Interestingly, another potential region on chromosome 4 (the fifth top SNP on 4p15), although gene poor, is located in the same broad region recently implicated in differential response to antipsychotic drugs in schizophrenia patients.[24] The second top SNP (rs17429217) is located in the *HRK* (also known as *DP5*) gene on 12q24, which is a member of the pro-apoptotic-only Bcl2 family and is abundantly expressed in the brain, especially in hippocampus.[25] The third top SNP on 3p14 (rs704454) is near the *ADAMTS9* gene, which is expressed in the spinal cord and brain along with other tissues,[26] and the later is a susceptibility gene for type 2 diabetes.[27] The fourth top SNP on 9p24 (rs2034764) is in the *KCNV2* gene that is adjacent to the VLDL-receptor (*VLDLR*) gene, which is expressed in the brain and binds to APOE,[28] and genetic variation in *VLDLR* has previously been linked to dementia.[29] The fifth top SNP (rs2104362) resides in an intergenic region on 6p21 that contains a nearby micro RNA gene *MIR1275* of unknown function. The last top SNP (rs17764668) is located in the *NRXN3* (neurexin 3) gene on 14q13 at ~80 Mb. *NRXN3* is expressed in the brain and previous studies have identified this gene to be associated with addiction and obesity.[30,31] Previously Bertram *et al*.[13] reported that a SNP (rs11159647) located at ~84 Mb in this region of 14q13 was associated with AAO of AD. However, this SNP did not show significant association in any of our three samples ($P = 0.335$, 0.075 and 0.301 in the ADRC, Mayo and ADNI samples, respectively; $P = 0.446$ in stage 3 meta-analysis). Other members of the neurexin gene family have been associated with AD, including structural neuroimaging phenotypes in ADNI,[32] where *NXPH1* (rs6463843) was related to decreased gray matter density on magnetic resonance imaging in individuals with the T/T relative to the G/G genotype. In particular, AD patients homozygous for the T allele showed differential vulnerability to right hippocampal atrophy as indicated by a SNP × diagnosis interaction.

The potential significance of the other four regions in the present study, not discussed above, is not clear as the 16q24 SNP is not located in a known gene and the known neighboring genes on both sides of this SNP are ~400 kb away. Although the other three regions on 18q21, 15q25 and 2q37 have known genes, their role in AD is not clear.

In conclusion, this study has confirmed the established association of the *APOE* locus with AAO of AD by revealing several genome-wide significant SNPs in this region. Although we did not identify any non-*APOE* SNPs meeting a conservative threshold of genome-wide significance, we have identified 11 suggestive loci, many of them harboring potential biological candidate genes that warrant follow-up in additional samples.

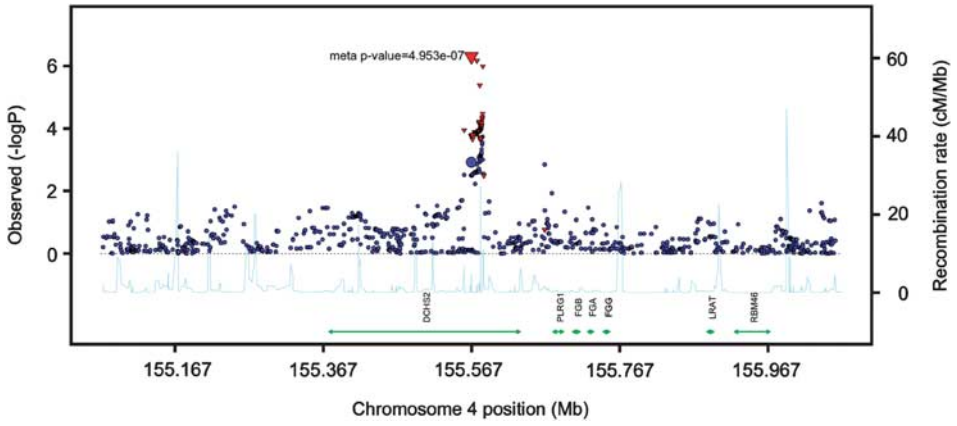

Figure 4 Regional association plot on chromosome 4, including the best single-nucleotide polymorphism (rs1466662) for age-at-onset of Alzheimer's disease in the *DCHS2* gene in the University of Pittsburgh Alzheimer's Disease Research Center data (blue dots), and meta-analysis (red triangles).

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)

Appendix

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