Associations Between Genetic Variants in 19p13 and 19q13 Regions and Susceptibility to Alzheimer Disease: A Meta-Analysis

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Background: Alzheimer disease (AD) has become an epidemic within the growing elderly population and effective therapies of AD have not been discovered. Genetic factors accounted for over 70% of the incidence of AD and the disease-related polymorphisms are located on chromosome 19, which is one of several prominent chromosomes related to the development of AD. Many inconsistent associations between polymorphisms in ABCA7, CD33, and TOMM40 genes and the susceptibility to AD have been suggested by several independent studies.

Material/Methods: A comprehensive literature search for studies involving the association between gene polymorphisms and AD was performed, and we finally selected 3 genes (4 polymorphisms) for the meta-analysis: ABCA7 (rs3764650), CD33 (rs3865444), and TOMM40 (rs157580, rs2075650).

Results: A total of 25 articles investigating 3 genes (4 polymorphisms) were included in the meta-analysis. The pooled results of 4 polymorphisms were all significantly associated with the susceptibility to AD. The pooled effect of ABCA7 rs3764605 allele G was significantly associated with an increased the risk of AD (OR=1.20, 95% CI: 1.14–1.26, \( P \) value <0.001). Similarly, our evidence suggested that allele A of TOMM40 rs2075650 polymorphism was a risk factor for AD (OR=2.87, 95% CI: 2.46–3.34, \( P \) value <0.001). Alleles A of CD33 rs3865444 and A of TOMM40 rs157580 were both protective factors for AD onset (OR=0.94, 95% CI: 0.90–0.98, \( P \) value=0.003; OR=0.62, 95% CI: 0.57–0.66, \( P \) value <0.001).

Conclusions: Results from the meta-analysis revealed that the pooled ABCA7 rs376465, CD33 rs3865444, TOMM40 rs157580, and rs2075650 variants were significantly associated with the susceptibility to AD. However, the association differed significantly between Asian and Caucasian groups for SNPs of CD33 rs3865444, TOMM40 rs157580, and rs2075650.

MeSH Keywords: Alzheimer Disease • Chromosome Aberrations • Polymorphism, Single Nucleotide

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Background

Alzheimer disease (AD) is a complicated neurodegenerative disease common in elderly people [1] and it has become a major threat to the growing elderly population due to the lack of effective therapies [2]. Several strategies, including the prevention of amyloid buildup and the promotion of amyloid removal, have been tested in a range of clinical trials. However, none of these strategies showed significant efficacy [3–5]. More attention has been paid to those factors that affect the disease in its early stage and it is estimated that genetic risk factors contribute to approximately over 70% of the incidence of AD [6]. As a result of this, the identification of genetic risk factors enables us to understand the disease mechanism in a sensible way [7].

Until now, only apolipoprotein E ε4 (APOE4) allele has been confirmed to be associated with increased risk of AD development [8]. The APOE4 gene is located in chromosome 19, which is one of several prominent chromosomes associated with the development of AD [8]. Recently, other genes in chromosome 19 associated with altered risks of AD, including ABCA7 (ATP-Binding Cassette, sub-family A, member 7), CD33 (Siglec-3), and TOMM40 (Translocase of the Outer Mitochondrial Membrane 40) [9–11], have been identified by several large-scale genome-wide association studies focusing on disease-associated single-nucleotide polymorphisms (SNPs).

The important role of ABCA7 rs3764650 polymorphism (location: 19p13) in AD pathogenesis has been investigated by several studies that suggested different associations between gene SNP and AD [12–15]. Apart from that, the CD33 (location: 19q13) gene was observed to be associated with various immune functions, including cell adhesion, anti-inflammatory signaling, and endocytosis [16]. The protective allele A of rs3865444 in this gene has been revealed to be associated with a reduced risk of AD in genome-wide studies [9,10]. Furthermore, the TOMM40 gene is also located in the 19q13 region, which is approximately 15 kb closer to APOE. Therefore, the TOMM40 gene should be investigated carefully due to the linkage disequilibrium with the ε4 allele [8]. Relevant research also indicated that a kind of a mitochondrial protein is coded by the TOMM40 gene [17]. Moreover, the mitochondrial dysfunction is closely associated with aging, particularly for AD [18]. The genetic variants of rs157580 and rs2075650 in TOMM40 have been revealed to be associated with altered risk of AD in independent genome-wide studies since 2008 [19,20].

Considering these inconsistent associations between polymorphisms in the above genes and the development of AD, it is critical to carry out a meta-analysis in order to produce a consistent result. Additionally, differences in sample sizes and heterogeneous populations may be considered as potential sources of heterogeneity among individual studies. Therefore, we performed the present meta-analysis with increased statistical power using a well-established method to assess the association between genetic mutation and disease [21] through incorporating all available published data. This meta-analysis on all eligible related studies was performed to evaluate the association between polymorphism of ABCA7 rs3764650 or CD33 rs3865444 or TOMM40 rs157580/rs2075650 and the susceptibility to AD.

Material and Methods

Search Strategies

All of the potential eligible studies were screened based on the electronic databases PubMed, EMBASE, Medline, and China National Knowledge Internet (CNKI) up to 1 December 2014 using advanced searching strategies. Five MeSH terms, “Alzheimer Disease”, “Single-nucleotide Polymorphism”, “ABCA7”, “CD33” and “TOMM40”, were used to search for relevant articles. Systematic searching was performed using the combination of “Alzheimer Disease”, “Single-nucleotide Polymorphism (SNP)” and each one of “ABCA7”, “CD33” and “TOMM40” with other similar eligible terms. Additional studies were screened manually from the references contained in each eligible study.

Study selection and data extraction

The following 4 criteria were used to determine the inclusion of studies: (1) studies must assess the association between polymorphism of ABCA7 rs3764650 or CD33 rs3865444 or TOMM40 rs157580/rs2075650 and the susceptibility to AD; (2) case-control studies must be based on humans; (3) sufficient information should be accessible (e.g., the study sample size for each research group, allele or genotype frequencies, effect sizes, and other useful information); (4) the diagnose of AD should meet the clinical criteria set by the World Health Organization.

A predesigned data collection form was used by 2 independent reviewers to collect the following data: first author name, year of publication, country of research, ethnicity of study population, type of AD, mean age of the case and control groups, sex ratio in the case and control groups, and frequency distributions of allele and numbers of different genotypes in the case and control groups. If several subpopulations were presented in the original articles, then they were considered as separate studies in this meta-analysis. Finally, relevant studies were selected and key data were collected by 2 independent reviewers.

Statistical analysis

The associations between ABCA7 rs3764650 or CD33 rs3865444 or TOMM40 rs157580 or rs2075650 polymorphisms and the...
susceptibility to AD were quantified using odds ratios (ORs) with 95% confidence intervals (CIs). The final ORs and 95% CIs for each SNP were pooled from individual study ORs and 95% CIs. Pooled ORs and 95% CIs were estimated by allelic models. For each SNP, statistical heterogeneity among individual studies was inferred by Q test and I² statistic; these 2 heterogeneity tests were used to calculate the variability among individual studies and the combined I² metric was derived to assess the percentage of variation. Meta-analysis was first performed with the fixed-effects model. If the P value of the Q test was greater than 0.05 and the I² statistic result was less than 50% (P >0.05 and I² <50%), then there was no significant heterogeneity among individual studies and the fixed-effects model was suitable for analysis. On the other hand, if the P value of Q test was less than or equal to 0.05 or if the I² statistic result was greater than or equal to 50% (P ≤0.05 or I² ≥50%), then significant heterogeneity was presented in these studies [22] and the random-effects model was appropriate for meta-analysis. Furthermore, subgroup analyses were performed by ethnic groups (Caucasian and Asian) to explore the effects of ethnicity on the association between gene polymorphisms and the susceptibility to AD. Publication bias was indicated by the funnel plot and plot asymmetry was confirmed by the rank correlation test. If the P value of the rank correlation test was greater than 0.05, then there is no significant evidence of publication bias, and a symmetrical inverted funnel was approximately presented in the plot; otherwise, there was significant publication bias. The robustness of these statistical results was evaluated by the sensitivity analysis. A 2-sided P value of 0.05 was selected as the significance level and all statistical analyses were performed using R software (Version 3.1.2, Copyright(C) 2014 The R Foundation for Statistical Computing).

Results

Study inclusion and characteristics

A total of 175 articles were initially identified based on the predefined searching strategies, including 71 articles for ABCA7, 60 articles for TOMM40, and 44 articles for CD33. Then 33 of the 175 articles were excluded because of duplication and 142 articles were screened manually for potential available information. After that, 119 of 142 articles were further excluded for several reasons. For example, some articles did not include essential information to evaluate the effect size and others did not have full-text available for review. Consequently, 23 case-control articles were included in

Supplementary Table 1. Characteristics of included studies for ABCA7 gene.

| ABCA7     | No. | First Author | Year | Country | Ethnicity | Case | Control | OR (95% CI)     |
|-----------|-----|--------------|------|---------|-----------|------|---------|----------------|
| rs3764650 T>G | 01  | Lambert      | 2009 | France  | Caucasians| 2025 | 5328    | 1.29 (1.15–1.45) |
|           | 02  | Harold       | 2009 | UK/Ireland | Caucasians| 2226 | 4704    | 1.29 (1.15–1.45) |
|           | 03  | Harold       | 2009 | Germany | Caucasians| 555  | 824     | 0.98 (0.76–1.27) |
|           | 04  | Harold       | 2009 | USA     | Caucasians| 551  | 960     | 1.20 (0.94–1.54) |
|           | 06  | Hollingworth (ADNI) | 2011 | USA     | Caucasians| 151  | 177     | 1.01 (0.58–1.76) |
|           | 07  | Hollingworth (GERAD2) | 2011 | UK      | Caucasians| 3262 | 3320    | 1.32 (1.15–1.52) |
|           | 09  | Hollingworth (deCODE) | 2011 | Iceland | Caucasians| 925  | 612     | 1.23 (0.93–1.63) |
|           | 10  | Hollingworth (AD-IG) | 2011 | USA     | Caucasians| 709  | 971     | 1.15 (0.83–1.59) |
|           | 11  | Hollingworth (EAD1) | 2011 | France  | Caucasians| 2751 | 2620    | 1.31 (1.15–1.49) |
|           | 12  | Hollingworth (CHARGE) | 2011 | Netherlands | Caucasians| 1239 | 10813   | 1.09 (0.92–1.29) |
|           | 13  | Hollingworth (MAYO2) | 2011 | USA     | Caucasians| 2490 | 4114    | 1.24 (1.10–1.40) |
|           | 14  | Tan          | 2012 | China   | Asians    | 612  | 612     | 1.04 (0.88–1.24) |
|           | 15  | Chung        | 2013 | Korea   | Asians    | 290  | 554     | 1.05 (0.85–1.29) |
|           | 16  | Miyashita    | 2013 | Japan   | Asians    | 891  | 844     | 1.13 (1.01–1.26) |
|           | 17  | Carrasquillo | 2014 | America | Caucasians| 132  | 2486    | 1.08 (0.69–1.69) |
|           | 18  | Liu          | 2014 | China   | Asians    | 350  | 283     | 1.10 (0.87–1.40) |
|           | 19  | Omoumi       | 2014 | Canada  | Caucasians| 580  | 524     | 1.32 (0.97–1.80) |
### Supplementary Table 2. Characteristics of included studies for CD33 gene.

| rs3965444 C>A | No. | First Author | Year | Country | Ethnicity | Case | Control | OR (95% CI) |
|----------------|-----|--------------|------|---------|-----------|------|---------|-------------|
|              | 01  | Logue (Logue) | 2011 | USA     | Caucasians | 513  | 496     | 0.95 (0.70–1.29) |
|              | 02  | Hollingworth (GERAD1) | 2011 | Europe  | Caucasians | 3333 | 1225     | 0.91 (0.82–1.00) |
|              | 03  | Hollingworth (EADI1) | 2011 | Europe  | Caucasians | 2025 | 5328     | 0.89 (0.82–0.99) |
|              | 04  | Hollingworth (deCODE) | 2011 | Europe  | Caucasians | 925  | 612      | 0.85 (0.68–1.04) |
|              | 05  | Naj (ADGC-GWAS) | 2011 | USA     | Caucasians | 8309 | 7366     | 0.88 (0.84–0.93) |
|              | 06  | Naj (ADGC-REP) | 2011 | USA     | Caucasians | 3531 | 3565     | 0.92 (0.85–0.99) |
|              | 07  | Carrasquillo (Jacksonville) | 2011 | USA     | Caucasians | 492  | 920      | 0.91 (0.77–1.08) |
|              | 08  | Carrasquillo (Rochester) | 2011 | USA     | Caucasians | 312  | 1577     | 0.93 (0.77–1.12) |
|              | 09  | Carrasquillo (Autopsy) | 2011 | USA     | Caucasians | 298  | 97       | 0.90 (0.64–1.27) |
|              | 10  | Carrasquillo (Norway) | 2011 | Europe  | Caucasians | 327  | 541      | 0.98 (0.80–1.21) |
|              | 11  | Carrasquillo (Poland) | 2011 | Europe  | Caucasians | 467  | 187      | 1.20 (0.92–1.57) |
|              | 12  | Carrasquillo (ARUK) | 2011 | Europe  | Caucasians | 642  | 730      | 0.95 (0.81–1.11) |
|              | 13  | Deng | 2012 | China  | Asians     | 190  | 193      | 0.48 (0.35–0.66) |
|              | 14  | Chung | 2013 | Korea  | Asians     | 290  | 554      | 0.70 (0.53–0.92) |
|              | 15  | Tan | 2013 | China  | Asians     | 612  | 612      | 1.44 (1.18–1.76) |
|              | 16  | Lambert (ADGC) | 2013 | Europe  | Caucasians | 10273 | 10892   | 0.89 (0.86–0.93) |
|              | 17  | Lambert (CHARGE) | 2013 | Europe  | Caucasians | 1315 | 12968    | 1.00 (0.92–1.10) |
|              | 18  | Lambert (EADI) | 2013 | Europe  | Caucasians | 2243 | 6017     | 0.90 (0.83–0.97) |
|              | 19  | Lambert (GERAD) | 2013 | Europe  | Caucasians | 3177 | 7277     | 0.89 (0.77–1.02) |
|              | 20  | Lambert | 2013 | Austria | Caucasians | 210  | 829      | 1.08 (0.82–1.42) |
|              | 21  | Lambert | 2013 | Belgium | Caucasians | 878  | 661      | 0.95 (0.79–1.14) |
|              | 22  | Lambert | 2013 | Finland | Caucasians | 422  | 562      | 1.08 (0.89–1.30) |
|              | 23  | Lambert | 2013 | Germany | Caucasians | 972  | 2378     | 1.00 (0.89–1.13) |
|              | 24  | Lambert | 2013 | Greece  | Caucasians | 256  | 229      | 0.79 (0.52–1.20) |
|              | 25  | Lambert | 2013 | Hungary | Caucasians | 125  | 100      | 0.94 (0.60–1.47) |
|              | 26  | Lambert | 2013 | Italy   | Caucasians | 1729 | 720      | 1.12 (0.97–1.30) |
|              | 27  | Lambert | 2013 | Spain   | Caucasians | 2121 | 1921     | 0.94 (0.85–1.04) |
|              | 28  | Lambert | 2013 | Sweden  | Caucasians | 797  | 1506     | 0.99 (0.87–1.12) |
|              | 29  | Lambert | 2013 | UK      | Caucasians | 490  | 1066     | 1.00 (0.84–1.19) |
|              | 30  | Lambert | 2013 | USA     | Caucasians | 572  | 1340     | 0.93 (0.80–1.09) |
|              | 31  | Miyashita | 2013 | Japan  | Asians     | 891  | 844      | 1.04 (0.92–1.18) |
|              | 32  | Omoumi | 2014 | Canada  | Caucasians | 580  | 524      | 0.76 (0.63–0.91) |
|              | 33  | Walker | 2014 | USA     | Caucasians | 97   | 96       | 1.13 (0.74–1.70) |
the analysis. [2,6,8–12,14,20,23–36]. Studies performed by Hollingworth [9], Naj [10], Harold [20], and Lambert [29] were considered as independent studies because they included different AD subgroup analyses by different countries. Similarly, studies by Naj [10] and Carrasquillo [23] were also treated as several independent studies because they were 2-stage studies including discovery and replication. Therefore, 25 published articles were included in this meta-analysis.

The detailed characteristics of these studies are presented in the Supplementary Tables 1–3. All the research subjects came from Europe, Asia, and North America. A total of 19,739 cases and 39,746 controls were included for **ABCA7** polymorphism studies. Studies for **CD33** comprised 49,414 cases and 73,933 controls, whereas studies for **TOMM40** comprised 11,490 cases and 16,094 controls. The study populations and structures varied among all studies and only a few studies stated that the study populations were matched by race and environmental factors. The characteristics of the studies are presented in the Supplementary Tables 1–3.

### Supplementary Table 3. Characteristics of included studies for **TOMM40** gene.

| No. | First Author         | Year | Country          | Ethnicity | Case  | Control | OR (95% CI) |
|-----|----------------------|------|------------------|-----------|-------|---------|-------------|
| 01  | Takei                | 2009 | Japan            | Asians    | 539   | 700     | 0.62 (0.53–0.73) |
| 02  | Harold               | 2009 | UK/Ireland       | Caucasians| 2227  | 4833    | 0.66 (0.61–0.71) |
| 03  | Harold               | 2009 | Germany          | Caucasians| 555   | 823     | 0.67 (0.57–0.80) |
| 04  | Harold               | 2009 | USA              | Caucasians| 1159  | 2187    | 0.57 (0.51–0.64) |
| 05  | Carrasquillo (Stage I) | 2009 | USA              | Caucasians| 844   | 1255    | 0.51 (0.45–0.59) |
| 06  | Carrasquillo (Stage II) | 2009 | USA              | Caucasians| 1547  | 1209    | 0.58 (0.52–0.65) |
| 07  | Naj (Discovery)      | 2010 | USA              | Caucasians| 931   | 1104    | 0.66 (0.57–0.75) |
| 08  | Naj (Replication)    | 2010 | USA              | Caucasians| 1242  | 1737    | 0.49 (0.36–0.68) |
| 09  | Cervantes            | 2011 | Spain            | Caucasians| 955   | 869     | 0.74 (0.63–0.88) |
| 10  | Valant               | 2012 | USA              | Caucasians| 308   | 215     | 0.48 (0.36–0.64) |
| 11  | Ma                   | 2013 | China            | Asians    | 787   | 791     | 0.72 (0.57–0.92) |
| 12  | Bagnoli              | 2013 | Italy            | Caucasians| 282   | 269     | 0.72 (0.57–0.92) |

| No. | First Author         | Year | Country          | Ethnicity | Case  | Control | OR (95% CI) |
|-----|----------------------|------|------------------|-----------|-------|---------|-------------|
| 01  | Harold               | 2009 | UK/Ireland       | Caucasians| 2227  | 4832    | 2.48 (2.27–2.70) |
| 02  | Harold               | 2009 | Germany          | Caucasians| 555   | 824     | 2.31 (1.92–2.77) |
| 03  | Harold               | 2009 | USA              | Caucasians| 1159  | 2187    | 2.68 (2.37–3.02) |
| 04  | Carrasquillo (Stage I) | 2009 | USA              | Caucasians| 844   | 1255    | 2.89 (2.49–3.36) |
| 05  | Carrasquillo (Stage II) | 2009 | USA              | Caucasians| 1547  | 1209    | 3.93 (3.39–4.57) |
| 06  | Naj (Discovery)      | 2010 | USA              | Caucasians| 931   | 1104    | 2.96 (2.50–3.50) |
| 07  | Naj (Replication)    | 2010 | USA              | Caucasians| 1242  | 1737    | 5.72 (3.63–9.02) |
| 08  | Cervantes            | 2011 | Spain            | Caucasians| 955   | 869     | 2.94 (2.31–3.74) |
| 09  | Valant               | 2012 | USA              | Caucasians| 308   | 215     | 3.39 (2.40–4.80) |
| 10  | Schott               | 2012 | UK               | Caucasians| 114   | 102     | 4.30 (2.81–7.06) |
| 11  | Ma                   | 2013 | China            | Asians    | 787   | 791     | 1.52 (1.19–1.94) |
| 12  | Chung                | 2013 | Korea            | Asians    | 290   | 554     | 3.57 (2.51–5.06) |
| 13  | Bagnoli              | 2013 | Italy            | Caucasians| 280   | 272     | 2.10 (1.46–3.01) |
| 14  | Omoumi               | 2014 | Canada           | Caucasians| 580   | 524     | 2.58 (2.04–3.25) |
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### CD33, rs3764650

| Authors, country | Year | Cases | Controls | OR (95% CI) |
|------------------|------|-------|----------|-------------|
| Lambert, France  | 2009 | 2025  | 5328     | 1.29 [1.15, 1.45] |
| Harold, UK/Ireland | 2009 | 2226  | 4704     | 1.29 [1.15, 1.45] |
| Harold, Germany  | 2009 | 555   | 824      | 0.98 [0.76, 1.27] |
| Harold, USA      | 2009 | 521   | 960      | 1.20 [0.94, 1.54] |
| Holingworth (ANDI), USA | 2011 | 151  | 177      | 1.01 [0.58, 1.76] |
| Holingworth (GERAD2), USA | 2011 | 3262 | 3320     | 1.32 [1.15, 1.52] |
| Holingworth (deCODE), Iceland | 2011 | 925 | 612     | 1.23 [0.93, 1.63] |
| Holingworth (AD4G), USA | 2011 | 709  | 971      | 1.15 [0.83, 1.59] |
| Holingworth (EADI1), France | 2011 | 2751 | 2620     | 1.31 [1.15, 1.49] |
| Holingworth (CHARGE), The Netherlands | 2011 | 1239 | 10813    | 1.09 [0.92, 1.29] |
| Holingworth (MAYO2), USA | 2011 | 2490 | 4114     | 1.24 [1.10, 1.40] |
| Tan, China       | 2012 | 612   | 612      | 1.04 [0.88, 1.23] |
| Chung, Korea      | 2013 | 290   | 554      | 1.05 [0.85, 1.29] |
| Miyashita, Japan  | 2013 | 891   | 844      | 1.13 [1.01, 1.26] |
| Carrasquillo, America | 2014 | 132  | 2486     | 1.08 [0.69, 1.69] |
| Liu, China        | 2014 | 350   | 283      | 1.10 [0.87, 1.40] |
| Omosumi, Canada   | 2014 | 580   | 524      | 1.32 [0.97, 1.80] |

### ABCA7, rs3764650

| Authors, country | Year | Cases | Controls | OR (95% CI) |
|------------------|------|-------|----------|-------------|
| Lambert, France  | 2009 | 2025  | 5328     | 1.29 [1.15, 1.45] |
| Harold, UK/Ireland | 2009 | 2226  | 4704     | 1.29 [1.15, 1.45] |
| Harold, Germany  | 2009 | 555   | 824      | 0.98 [0.76, 1.27] |
| Harold, USA      | 2009 | 521   | 960      | 1.20 [0.94, 1.54] |
| Holingworth (ANDI), USA | 2011 | 151  | 177      | 1.01 [0.58, 1.76] |
| Holingworth (GERAD2), USA | 2011 | 3262 | 3320     | 1.32 [1.15, 1.52] |
| Holingworth (deCODE), Iceland | 2011 | 925 | 612     | 1.23 [0.93, 1.63] |
| Holingworth (AD4G), USA | 2011 | 709  | 971      | 1.15 [0.83, 1.59] |
| Holingworth (EADI1), France | 2011 | 2751 | 2620     | 1.31 [1.15, 1.49] |
| Holingworth (CHARGE), The Netherlands | 2011 | 1239 | 10813    | 1.09 [0.92, 1.29] |
| Holingworth (MAYO2), USA | 2011 | 2490 | 4114     | 1.24 [1.10, 1.40] |
| Tan, China       | 2012 | 612   | 612      | 1.04 [0.88, 1.23] |
| Chung, Korea      | 2013 | 290   | 554      | 1.05 [0.85, 1.29] |
| Miyashita, Japan  | 2013 | 891   | 844      | 1.13 [1.01, 1.26] |
| Carrasquillo, America | 2014 | 132  | 2486     | 1.08 [0.69, 1.69] |
| Liu, China        | 2014 | 350   | 283      | 1.10 [0.87, 1.40] |
| Omosumi, Canada   | 2014 | 580   | 524      | 1.32 [0.97, 1.80] |

## Figure 1.

The forest plot of ABCA7 genetic variation with AD.

## Figure 2.

The forest plot of CD33 genetic variation with AD.
META-ANALYSIS

Subgroup analysis

Table 1 shows that all polymorphisms except rs3764650 in ABCA7 (P=0.362, I²=20.42%) had significant heterogeneity among individual studies. Therefore, subgroup analyses by ethnicity (Asian and Caucasian) indicated significantly different ORs and 95% CIs for polymorphisms of rs157580, rs2075650, and rs3865444. For instance, CD33 rs3865444 and TOMM40 rs2075650 polymorphisms in the Asian group did not have significant association with the susceptibility to AD (OR=0.86, 95% CI=0.58–1.27; OR=2.45, 95% CI=0.45–4.45).

Publication bias and sensitivity analysis

Funnel plots were constructed to assess publication bias for each genetic variants group (Figure 4A: ABCA7 rs3764650, Figure 4B: CD33 rs3865444, Figure 4C: TOMM40 rs2075650, Figure 4D: TOMM40 rs157580). P value of the rank correlation test for each polymorphism was greater than 0.05 (Table 1: ABCA7 rs3764650, P value=0.43; CD33 rs3865444, P value=0.79; TOMM40 rs157580, P value=0.94; TOMM40 rs2075650, P value=0.23), which suggested that there was no significant publication bias.
in each polymorphism study. The sensitivity analyses results revealed that no individual study significantly affected the overall value of ORs and 95% CIs.

**Discussion**

In this meta-analysis, a systematic overview of case-control studies for assessing the association between genetic variants and the susceptibility to AD was performed. All the eligible databases were searched electronically and the potential articles were screened to be included in this meta-analysis. Eventually, 25 articles investigating 4 SNPs of 3 AD candidate genes were included in this meta-analysis. The pooled results showed significant association between the 4 SNPs (rs3764650, rs157580, rs2075650, and rs3865444) and the susceptibility to AD. Two SNPs (rs3764650 and rs2075650) were found to be significantly associated with increased risk of AD, whereas the other 2 SNPs (rs3865444 and rs157580) were significantly associated with a decreased risk of AD. In addition, subgroup analyses suggested that the association between SNPs and the susceptibility to AD was significantly different between the Asian and Caucasian groups for SNP of rs157580, rs2075650, and rs3865444. Abca7 is a new member of the A-subfamily, which is also an integral transmembrane adenosine triphosphates-binding cassette transporter [37]. The biogenesis of high-density lipoprotein was mediated by its protein and combined with helical apolipoproteins and cellular lipid [37]. Moreover, cholinergic dysfunction, amyloid precursor protein processing, and Ab production of deposition, which are the neuropathologic landmarks of AD, are all regulated by local lipid homeostasis integrity [38]. Using reverse transcription-PCR analysis and Northern blot, previous studies concluded that the Abca7 gene is expressed in human brain, particularly on the CA1 hippocampal neurons [39–41]. Therefore, functions of the Abca7 gene were considered to have important

### Table 1. Meta-analysis of four polymorphisms and AD susceptibility.

| Gene      | SNP            | OR (95% CI)* | *P-value | TAU² | I²   | P heterogeneity | P funnel plot asymmetry | Ethnicity     |
|-----------|----------------|--------------|----------|------|------|----------------|-------------------------|---------------|
| ABCA7     | rs3764650      | 1.20 (1.14–1.26) | <0.001   | 0.002 | 20.42% | 0.3615       | 0.433                   | 1.24 (1.19–1.31) | 1.10 (1.01–1.19) |
| CD33      | rs3865444      | 0.94 (0.90–0.98) | 0.003    | 0.007 | 63.91% | <0.0001      | 0.794                   | 0.93 (0.90–0.95) | 0.86 (0.58–1.27)  |
| TOMM40    | rs157580       | 0.62 (0.57–0.66) | <0.001   | 0.009 | 61.97% | 0.0031       | 0.945                   | 0.61 (0.56–0.66) | 0.65 (0.56–0.73)  |
|           | rs2075650      | 2.87 (2.46–3.34) | <0.001   | 0.067 | 88.05% | <0.0001      | 0.233                   | 2.84 (2.54–3.13) | 2.45 (0.45–4.45)  |

* Pooled odds ratios and 95% confidence intervals

**Figure 4.** Funnel plot for publication bias of all included studies by genetic mutations.
associations with the susceptibility to AD. Furthermore, the results of our meta-analysis confirmed that rs3764650 allele G of ABCA7 was associated with a 20% increase in the risk of AD development. In addition, the evidence suggested that the association between SNP (rs157580, rs2075650, and rs3865444) and the susceptibility to AD differed significantly between the Asian and Caucasian groups. Unfortunately, there was no available data investigating how genetic variants in Africans affect the susceptibility to AD, whereas another SNP, rs115550680 in ABCA7, has been discovered to be significantly associated with AD in African-Americans [42]. Based on Haplovew, polymorphism of rs115550680 is in linkage disequilibrium with rs3764650. Among these 19 studies selected for our analysis, only 7 provided exact genotype numbers; therefore, meta-analysis was carried out using the allelic model only.

As a member of the sialic acid-binding immunoglobulin-like lectins (Siglec) family, the CD33 gene is located on chromosome 19q13.3 region [43]. Previous research suggested that the main function of CD33 related to Siglecs is immunological regulation, which is involved in the microglial cleansing process. Studies also suggested that the amyloid plaques in AD can be prevented [44,45]. CD33 rs3865444 polymorphism was first suggested by GWAS studies as a risk factor for AD [9,10]. Moreover, Griciuc et al. reported that the increased expressions of CD33 in microglial cells were observed in AD patients [46]. However, inconsistent associations between rs386444 polymorphism and the susceptibility to AD were suggested by several independent case-control studies. On the other hand, our meta-analysis results revealed that rs3865444 allele A contributed significantly to reduced risk of AD. However, the effect of rs3865444 allele A on the susceptibility to AD differed significantly between the Asian and Caucasian groups, as indicated by the subgroup analyses. Although the rs3865444 risk allele (C) was found to have greater cell surface expression of CD33 in African-Americans, the complete data were not available [47]. Among included studies on rs3865444, only 4 were based on Asians. Therefore, we strongly recommend that further studies should be carried out on Africans and South Asians to confirm the association between rs3865444 SNP and the susceptibility to AD in other ethnic groups.

Tom40 is a subunit of the translocase of the outer membrane (TOM), which is encoded by the TOMM40 gene. Tom40 is located in the mitochondria and is involved in transporting cytoplasmic peptides and proteins during mitochondrial biogenesis [48]. The role of Tom40 in regulating protein traffic across the outer mitochondrial membrane appears to be important in the development of AD, perhaps because of the unique bioenergetic requirements of neurons [49,50]. Mitochondrial dysfunction and oxidative imbalance have been linked to neuronal cell death and AD [48]. Two polymorphisms of TOMM40 were first found to be significantly associated with AD in genome-wide studies. However, inconsistent associations were reported by basic case-control studies due to different study methods and target populations. To identify the genetic association between SNPs and susceptibility to AD, genome-wide analysis is a viable method with great statistical power. However, genotype data were only available in Asians and Caucasians and further studies need to be performed in other ethnic groups to assess the effect of ethnicity on the overall association between gene SNP and the development of AD.

To the best of our knowledge, this is the first study to investigate 4 SNPs of 3 different genes located on the same chromosome – chromosome 19. The present meta-analysis has several limitations. First, some articles without sufficient genotype information were excluded and this may have introduced selection bias and reduced the statistical power. Second, the meta-analysis method is not able to cope with different study designs and population structures, which might contribute to the heterogeneity among individual studies.

Conclusions

Significant associations between the 4 SNPs (rs3764650, rs157580, rs2075650, and rs3865444) and susceptibility to AD were suggested by our study. Meta-analysis based on 19 739 cases and 39 746 controls confirmed that ABCA7 rs3764650 allele G is a risk factor of AD in both Caucasians and Asians. Furthermore, meta-analysis including 49 414 cases and 73 933 controls suggest that CD33 rs3865444 allele A is a protective factor of AD in Caucasians but this association was not significant in Asians. In addition, meta-analyses of 2 polymorphisms in TOMM40 (rs157580 and rs2075650) gene yielded contrary results; SNP of rs157580 was significantly associated with a reduced risk of AD, whereas SNP of rs2075650 was significantly associated with an increased risk of AD, but the association between the Asian and Caucasian groups was significantly different, as indicated by the heterogeneity test. Subgroup analysis by ethnicity was limited by the small sample size in Asians. Therefore, we recommend that well-designed studies with larger sample sizes should be performed. Genetic variants and other factors, such as individual biological characteristics and environmental factors, particularly in African and Asian populations, should be investigated together to assess the interaction between different factors that may significantly affect development of AD.

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Conflict of interest

The authors have declared that no competing interests exist.
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