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Polymorphism of the *OLR1* 3'UTR potential microRNA binding site and risk of Alzheimer's disease: a meta-analysis

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ABSTRACT. Alzheimer's disease (AD) is a progressive neurodegenerative disorder that contributes to dementia in the elderly population. Genome-wide linkage analysis has identified chromosome 12p as the AD-susceptible region, which includes lectin-like oxidized low-density lipoprotein receptor 1 (*OLR1*). The *OLR1* +1073 C/T single-nucleotide polymorphism is located in the 3'-untranslated region of the gene and may influence the binding of regulatory microRNAs (miRNAs) and OLR1 protein homeostasis. A number of studies have reported an association between this variant and AD. However, the results are controversial. A meta-analysis of case-control studies examining the relationship between the *OLR1* +1073 C/T single-nucleotide polymorphism and AD risk was performed. Five studies were selected that included 2419 cases and 2381 controls. The results revealed a significantly decreased AD risk in the recessive model (TT *vs* TC + CC: odds ratio (OR) = 0.79, 95% confidence interval (CI) = 0.65-0.96). The control group in one of the studies was in Hardy-Weinberg disequilibrium, so we performed additional meta-analysis excluding this study. The significance was much more pronounced in

the recessive model (TT *vs* TC + CC: OR = 0.72, 95%CI = 0.62-0.85). Using miRanda and RNA hybrid methods, the polymorphic allele was shown to influence the binding of various miRNAs. Our results suggested that the +1073 C/T polymorphism decreased the risk of AD. The polymorphic allele was also predicted to affect the binding site of many miRNAs, which may explain the relationship between the +1073 C/T variant and AD.

Key words: Alzheimer's disease; Meta-analysis; MicroRNA; Oxidized low density lipoprotein receptor 1; Polymorphism

INTRODUCTION

Alzheimer's disease is a progressive neurodegenerative disorder and is considered to be the most common contributor to dementia in the elderly population. Its pathological features include amyloid plaques, neurofibril tangles, and neuronal loss (Krstic and Knuesel, 2013). β-amyloid (Aβ) is derived from amyloid precursor protein (APP) and requires sequential processing by β-secretase and γ-secretase (Karran et al., 2011). β-secretase, also known as the β-site of APP cleaving enzyme 1, is a transmembrane aspartic protease. γ-secretase is a protein complex composed of presenelin 1 and presenelin 2, as well as other subunits. Mutation of APP and its cleavage enzymes presenelin 1 or presenelin 2 are typically found during early onset, autosomal-dominant familial AD (FAD). However, most AD patients are late onset and sporadic. The reasons and mechanisms for this are unclear.

Genome-wide linkage analysis has identified chromosome 12p as an AD-susceptible region, which includes lipoprotein receptor-related protein 1, α-2-macrogobulin (A2M), and lectin-like oxidized low-density lipoprotein receptor 1 (OLR1) (Pericak-Vance et al., 1997; Wu et al., 1998; Lee et al., 2008). OLR1 was initially cloned from vascular endothelial cells and was shown to be the receptor for oxidized low-density lipoprotein (Sawamura et al., 1997). OLR1 participates in the pathogenesis of hypertension, diabetes, and atherosclerosis (Ando and Fujita, 2004; Mango et al., 2011; Palmieri et al., 2013). It is also widely expressed in the brain (Yamanaka et al., 1998).

MicroRNAs (miRNAs) are approximately 22-nucleotide (nt) short noncoding RNA molecules that can regulate target gene expression at the posttranscriptional level (He and Hannon, 2004). Binding to the 3' untranslated region (UTR) of target mRNA, miRNAs can cause translational inhibition or mRNA cleavage and subsequent degradation. miRNAs exert important physiological and pathological roles. A large number of miRNAs have been shown to be dysregulated in the brain of AD patients and AD animal models (Geekiyanage and Chan, 2011; Long et al., 2012; Bhattacharyya and Bandyopadhyay, 2013). Thus, their correlated target expression is altered, contributing to the progression of AD.

The *OLR1* +1073 C/T (rs1050283) single-nucleotide polymorphism is located in the 3'UTR of *OLR1* and may influence its regulatory miRNA binding and subsequent protein homeostasis. Recently, Wang et al. (2013) reported that an *OLR1* 3'UTR single-nucleotide polymorphism affected miR-370 targeting in *Bos taurus*. This mechanism is likely conserved in humans. Serpente et al. (2011) demonstrated an association between this specific variant and decreased OLR1 protein levels, which may be mediated by the miRNA has-miR369-3p.

Several studies have reported an association between *OLR1* +1073 C/T and AD (Luedecking-Zimmer et al., 2002; Lambert et al., 2003; D'Introno et al., 2005; Colacicco et al., 2009; Serpente et al., 2011); however, the results are controversial. To precisely define the role of this variant, we performed a meta-analysis of all published case-control studies. Our results suggested that the +1073 C/T polymorphism decreased AD risk. The polymorphic allele was also predicted to influence binding to *OLR1* mRNA for many miRNAs, which may explain the mechanism between the +1073 C/T variant and AD.

MATERIAL AND METHODS

Data sources and search strategy

In this study, the terms "oxidized low-density lipoprotein receptor 1 or OLR1 or lectin-like oxidized LDL receptor or LOX-1", "Alzheimer disease or AD", "polymorphism or mutation or variant", and the combined phrases were used to search various databases (PubMed, Web of Science, and Embase databases until October 2, 2013). References of related studies and reviews were manually searched for additional studies.

Inclusion and exclusion criteria

Included studies satisfied the following criteria: 1) evaluated the *OLR1* +1073 C/T polymorphism and AD risk; 2) case-control design; 3) AD was diagnosed according to the NINCDS-ADRDA criteria; and 4) sufficient data including sample size, genotype, and allele frequencies.

Exclusion criteria included: 1) duplicated studies; 2) no controls; and 3) no details regarding genotype frequencies. We did not consider unpublished literature.

Data extraction

Two reviewers extracted eligible studies independently based on the inclusion and exclusion criteria. Disagreements between the 2 reviewers were discussed further with the other reviewer to obtain a consensus.

Statistical analysis

Hardy-Weinberg equilibrium in the controls was tested using an online program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl), and $P < 0.05$ was considered to be a disequilibrium. The association between the *OLR1* +1073 C/T polymorphism and AD risk was measured using the odds ratio (OR) and 95% confidence interval (95%CI). Pooled ORs were calculated for the dominant model (TT + CT *vs* CC), recessive model (TT *vs* TC + CC), heterozygote comparison model (TC *vs* CC), homozygote comparison model (TT *vs* CC), and allele comparison model (T *vs* C). Heterogeneity was tested using the *Q* statistic. $P < 0.10$ was considered to be statistically significant for heterogeneity, and the random-effects model was used to estimate the pooled OR. Otherwise, the fixed-effects model was used. The significance of the pooled OR was determined using the *Z* test and $P < 0.05$ was considered to be statistically significant. All statistical tests were performed using the Revman 4.2 software using 2-sided P values.

Publication bias and sensitivity analysis

Publication bias was tested using Begg's funnel plots. In addition, sensitivity analysis was performed by sequential exclusion of individual studies to assess the stability of the results.

RESULTS

Study characteristics

The workflow according to the inclusion and exclusion criteria is shown in Figure 1. A total of 5 studies investigating *OLR1* +1073 T/C polymorphisms and AD susceptibility were selected for meta-analysis, which included 2419 cases and 2381 controls. Detailed characteristics of the studies, including author, year, country, sample size, and genotype distribution of each study are listed in Table 1. Hardy-Weinberg equilibrium of the genotype distribution in the controls was tested using an online program and these results are also shown in Table 1. The P value for Hardy-Weinberg equilibrium of 4 studies was greater than 0.05, but was 0.048 for the Leudecking-Zimmer et al. (2002) study. Thus, we performed the meta-analyses both including (Meta1) and excluding (Meta2) this study. In Meta2, without Leudecking-Zimmer et al. (2002) study, there were 1563 cases and 1678 controls.

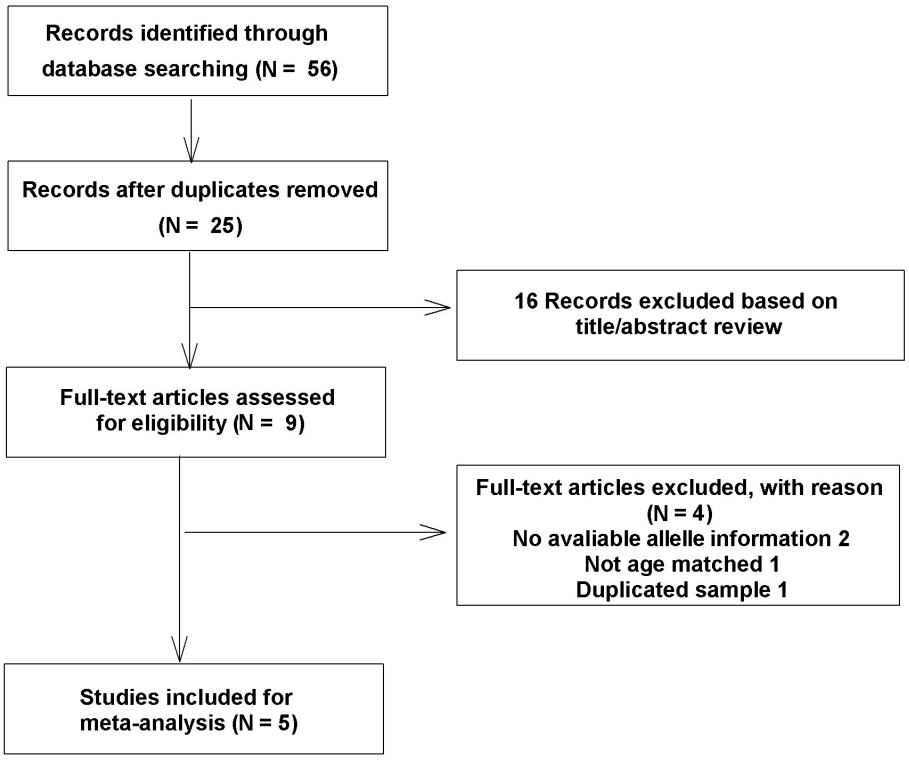

Figure 1. Workflow of the meta-analysis.

Table 1. Characteristics of studies included.

Author	Year	Country	Ethnicity	Genotype						P value (HWE)	Allele			
				Cases			Controls				Cases		Controls	
				CC	CT	TT	CC	CT	TT		C	T	C	T
Leudecking-Zimmer et al.	2002	USA	Caucasians	229	416	211	202	324	177	0.048	874	838	728	678
Lambert et al.	2003	France	Caucasians	178	302	118	158	322	183	0.48	658	538	638	688
Pritchard et al.	2004	Scotland	Caucasians	97	170	86	95	176	87	0.76	364	342	366	350
D'Introno et al.	2005	Italy	Caucasians	37	86	46	32	140	92	0.054	160	178	204	324
Serpente et al.	2011	Italy	Caucasians	80	249	114	79	180	134	0.19	409	477	338	448

HWE = Hardy-Weinberg equilibrium.

OLR1 +1073 T/C polymorphisms and AD susceptibility

In the Meta1 analysis, a significantly decreased AD risk was observed in the recessive model (TT *vs* TC + CC: OR = 0.79, 95%CI = 0.65-0.96, P = 0.02, Figure 2). However, no significant association was found for the homozygote comparison model (TT *vs* CC: OR = 0.76, 95%CI = 0.56-1.03, P = 0.08), allele comparison model (T *vs* C: OR = 0.87, 95%CI = 0.76-1.01, P = 0.06), dominant model (TT + TC *vs* CC: OR = 0.88, 95%CI = 0.69-1.13, P = 0.33), or heterozygote comparison model (TC *vs* CC: OR = 0.96, 95%CI = 0.75-1.22, P = 0.73). Interestingly, Serpente et al. (2011) found that AD patients carrying the CC+TC genotypes expressed lower levels of OLR1 than those carrying the TT genotype, supporting our results.

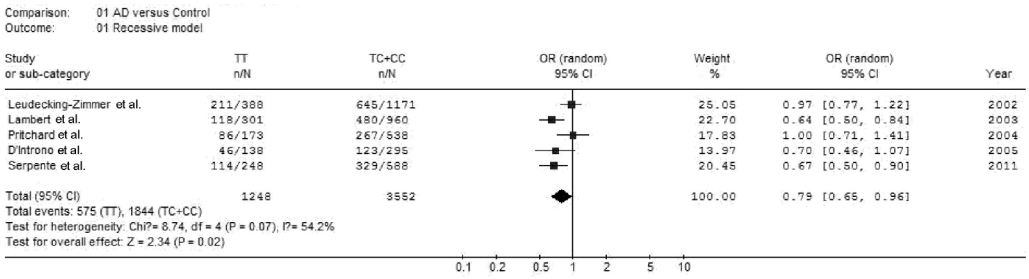

Figure 2. Forrest plot of *OLR1*+1073 C/T and AD risk in the recessive model in Meta1 (TT *vs* TC + CC).

The control group in Leudecking-Zimmer's study was in Hardy-Weinberg disequilibrium, so we performed meta-analysis (Meta2) excluding this study. In Meta2, we found significantly decreased AD risks in the recessive model (TT *vs* TC + CC: OR = 0.72, 95%CI = 0.62-0.85, P < 0.0001, Figure 3), further confirming our conclusion. In addition, a correlation was also detected for the homozygote comparison model (TT *vs* CC: OR = 0.69, 95%CI = 0.50-0.95, P = 0.02) and allele comparison model (T *vs* C: OR = 0.83, 95%CI = 0.75-0.91, P = 0.0001) in Meta2.

Sensitivity analysis

Sensitivity analysis was performed with 1 study excluded each time. In Meta1, which

included all 5 studies, the pooled OR for the recessive model was statistically similar after excluding 3 single studies (Luedecking-Zimmer et al., 2002; Lambert et al., 2003; Pritchard et al., 2004). However, there was no significant correlation after sequentially omitting the other 2 studies (D'Introno et al., 2005; Serpente et al., 2011).

For Meta2, which did not include Leudecking-Zimmer's study, the results were stable for the recessive model while omitting single studies.

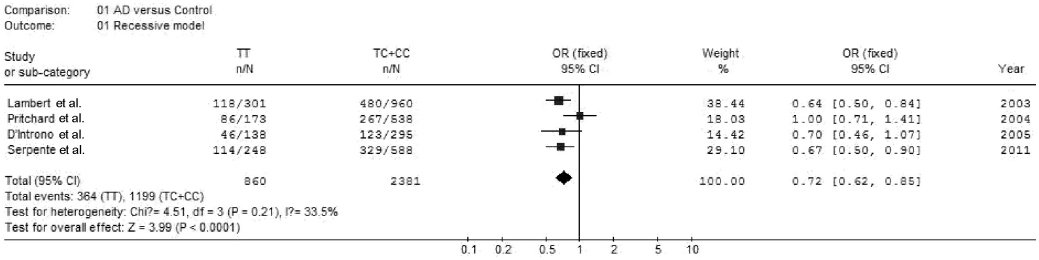

Figure 3. Forrest plot of *OLR1*+1073 C/T and AD risk in the recessive model in Meta2 (TT *vs* TC + CC).

Publication bias

Begg's funnel plots were used to determine publication bias. The shape of the funnel plots for the recessive models both in Meta1 and Meta2 showed no obvious asymmetry for all of the studies (Figures 4 and 5).

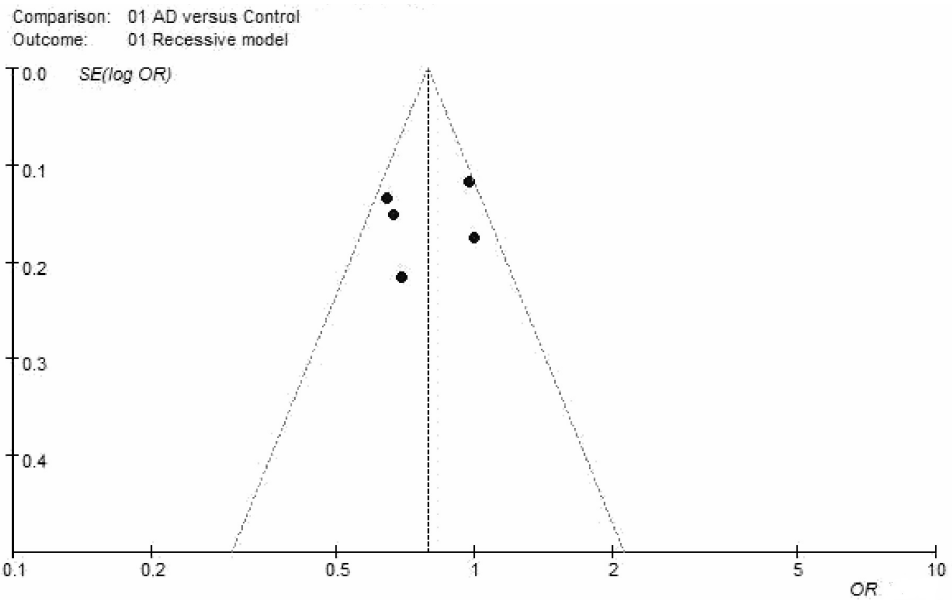

Figure 4. Funnel plots of *OLR1*+1073 C/T polymorphism in the recessive model in Meta1 (TT *vs* TC + CC).

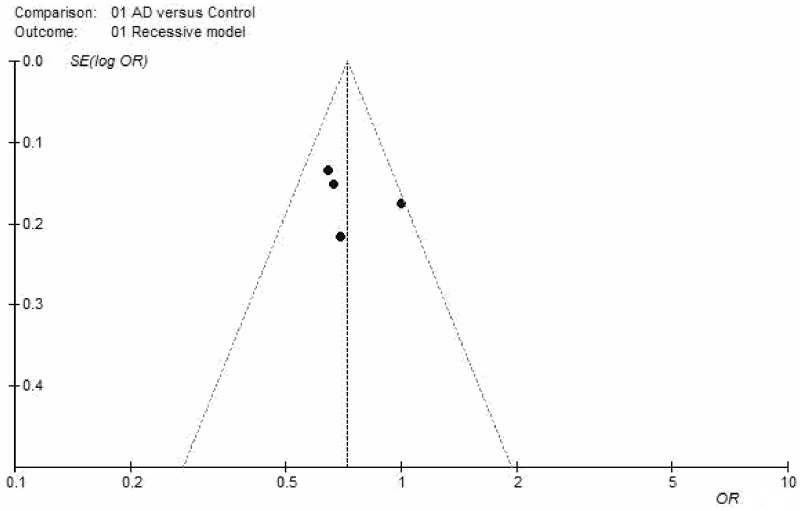

Figure 5. Funnel plots of *OLR1*+1073 C/T polymorphism in the recessive model in Meta2 (TT *vs* TC + CC).

Bioinformatic analysis of miRNA and *OLR* +1073 C/T interaction

We analyzed the 3'UTR of *OLR1* using miRanda and RNA hybrid analysis. We found that several miRNAs could target the *OLR* +1073 T/C variant site, including miR-196a, miR-196b, miR-369-3p, miR-552, and miR-655. The mutation decreased the binding ability of miR-196a, miR-196b, and miR-552, as shown in Figure 6. The +1073 T/C variant did not influence

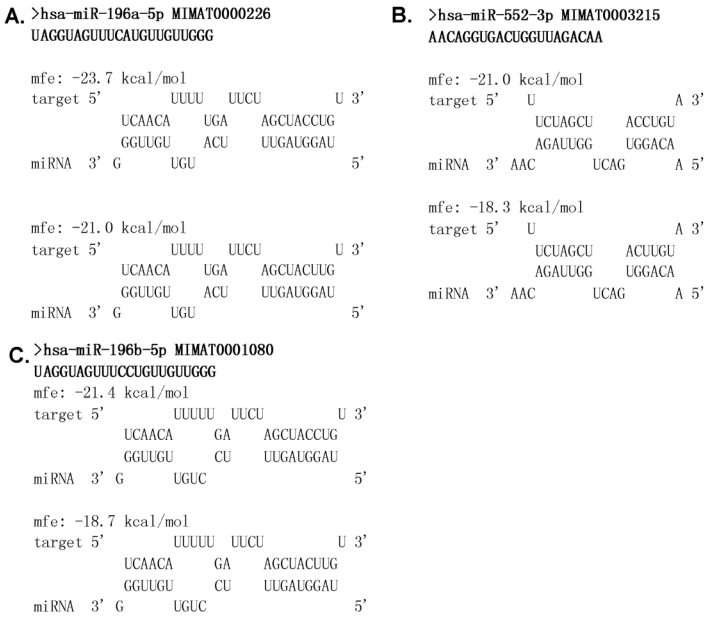

Figure 6. +1073 C/T polymorphism directly influences the interaction between miRNA and the *OLR1* 3'UTR according to RNA hybrid analysis.

the base pairing of miR-369-3p and miR-655 with the *OLR1* +1073 site (Figure 7). However, the secondary structure of the miRNA-mRNA hybrid and the ability of miRNA inhibition of target mRNA may be affected. OLR1 levels were found to be much lower in CC+TC carriers than in those with the TT genotype in AD patients, with no significant difference in miR-396-3p expression (Serpente et al., 2011). Furthermore, they found a negative correlation between *OLR1* and miR-396-3p gene expression in AD patients carrying the CC+TC genotypes.

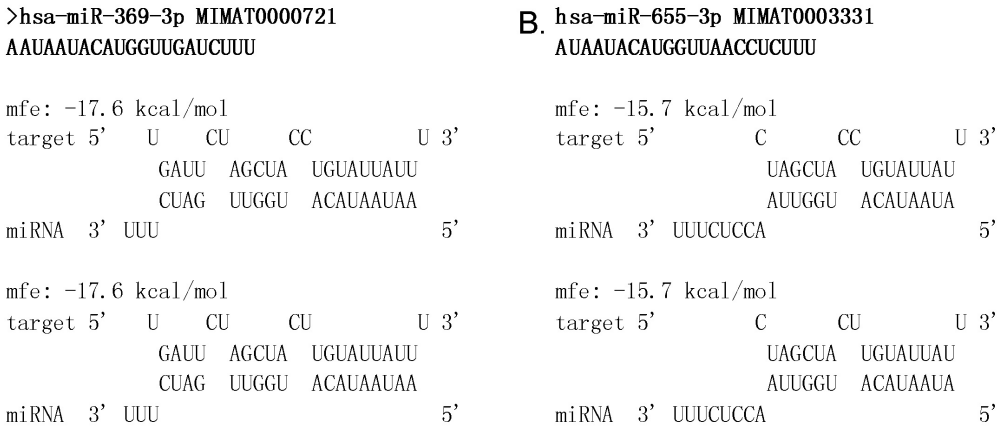

Figure 7. +1073 C/T polymorphism influences the nucleotide adjacent to miRNA-mRNA hybrids.

DISCUSSION

OLR1 is the receptor for oxidized low-density lipoprotein and is widely expressed in the brain and spinal cord. Its expression can be induced by oxidative stress, inflammatory cytokines, and oxidized low-density lipoprotein (Mehta et al., 2006). These stimuli are typically reported as hallmarks in AD patients (Agostinho et al., 2010). OLR1 is activated by interactions with ligands and may facilitate cells within the brain to release cytokines and combat amyloid peptide neurotoxicity. As a result, adequate expression of OLR1 may be important for preventing AD. In addition, OLR1 is abundantly expressed in vascular cells and may play a role in cerebral amyloid angiopathy (CAA), which is correlated with AD progress (Shi et al., 2006).

The +1073 C/T polymorphism site is located in the 3'UTR of *OLR1*, where miRNAs typically bind and exert their posttranscriptional regulatory effects. We analyzed the sequence flanking this site using miRanda-mirSVR and RNA hybrid methods. The results indicated that several miRNAs could target the DNA sequence flanking this site, including miR-196a, miR-196b, miR-369-3p, miR-552, and miR-655. The +1073 C/T polymorphism decreased the binding activity of miR-196a, miR-196b, and miR-552. In addition, it influenced the nucleotide adjacent to the base pairs between the *OLR1* 3'UTR and miR-369-3p or miR-655, which may affect the structures of miRNA-mRNA duplexes and interactions with protein factors essential for miRNA targeting. Interestingly, miR-196a and miR-196b are expressed in the brain and play roles in neurodegenerative disorders, Huntington disease, and glioblastoma (Guan et

al., 2010; Cheng et al., 2013). miR-655 has been reported to significantly inhibit endogenous and exogenous APP expression (Delay et al.,2011). Consistent with our results, Serpente et al. (2011) found that miR-369-3p may target *OLR1* based on targetscan analysis. Thus, we hypothesize that the +1073 C/T variant influences the expression of *OLR1* by impairing miRNA targeting.

Our hypothesis is supported by several lines of experimental evidence. Lambert et al. (2003) reported that the *OLR1* mRNA level was significantly lower in AD patients than in controls. Using peripheral blood mononuclear cells isolated from AD patients, Serpente et al. (2011) demonstrated significantly decreased expression levels of *OLR1* in carriers of the CC+CT genotype compared to in TT carriers. In addition, there was no difference in miR-369-3p transcription levels between cases and controls. The +1073 C/T variant may play a protective role in AD progression by deteriorating the inhibitory effects of miRNA on *OLR1* and increase *OLR1* expression. The detailed mechanism requires further investigation involving *in vitro* and *in vivo* experiments.

There is also another single-nucleotide polymorphism site within the *OLR1* 3'UTR known as +1071 T/A, which is only 2 nucleotides away from the +1073 C/T site (Luedecking-Zimmer et al., 2002). Thus, it may work together with the +1073 variant to influence miRNA targeting. The association between +1071 T/A and AD was tested for 3 groups (Lambert et al., 2003; Pritchard et al., 2004; D'Introno et al., 2005). However, they found no relationship between the +1071 T/A polymorphism and AD risk. Further studies including a larger number of cases are required to confirm this result.

Leudecking-Zimmer et al. (2002) reported that the relationship between the *OLR1* 3'UTR polymorphism and AD risk depended on APOE4 status. The *OLR1*+1073 C/T polymorphism was found to play protective roles in non-APOE4 carriers, but had deteriorative effects in APOE4 carriers. However, this finding was disputed by many other scientists (Lambert et al., 2003; D'Introno et al., 2005; Colacicco et al., 2009; Serpente et al., 2011). We could not conduct further analysis to confirm the influence of APOE4 on the association between the *OLR* 3'UTR variant and AD because this data was not available from the published studies. In addition, all studies included primarily Caucasian subjects, and other ethnicities were not investigated. Subgroup analysis should be performed in the future when a sufficient number of studies become available.

Our meta-analysis demonstrated that *OLR1* +1073 C/T is associated with a decreased risk of AD. The mechanism may depend on the decreased interaction between the *OLR1* 3'UTR and miRNAs. Further studies involving a larger number of cases and more ethnicities are required to confirm our data. We will also investigate the molecular functions of *OLR1* in AD using *in vitro* and *in vivo* experiments in the future.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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