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CD33 in Alzheimer's Disease

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Abstract The amyloid-beta peptide (Aβ) cascade hypothesis posits that Aβ accumulation is the fundamental initiator of Alzheimer's disease (AD), and mounting evidence suggests that impaired Aβ clearance rather than its overproduction is the major pathogenic event for AD. Recent genetic studies have identified cluster of differentiation 33 (CD33) as a strong genetic locus linked to AD. As a type I transmembrane protein, CD33 belongs to the sialic acid-binding immunoglobulin-like lectins, mediating the cell–cell interaction and inhibiting normal functions of immune cells. In the brain, CD33 is mainly expressed on microglial cells. The level of CD33 was found to be increased in the AD brain, which positively correlated with amyloid plaque burden and disease severity. More importantly, CD33 led to the impairment of microglia-mediated clearance of Aβ, which resulted in the formation of amyloid plaques in the brain. In this article, we review the recent epidemiological findings of CD33 that related with AD and discuss the levels and pathogenic roles of CD33 in this disease. Based on the contributing effects of CD33 in AD pathogenesis, targeting CD33 may provide new opportunities for AD therapeutic strategies.

Keywords Alzheimer's disease · CD33 · Aβ · Genetics · Microglia · Pathogenesis · Therapy

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Introduction

Alzheimer's disease (AD) is a complex and multifactorial neurodegenerative disease, which is characterized by the formation of extracellular amyloid plaques containing the amyloid-beta peptide (Aβ) within the parenchyma of the brain [1]. Emerging evidence indicates that impaired Aβ clearance rather than its overproduction is the central pathogenic event in AD, as the clearance rates for Aβ are declined in AD patients, while its production rates stay unchanged compared with those in healthy controls [2]. Recent genome-wide association studies (GWASs) have identified cluster of differentiation 33 (CD33) as a strong genetic locus associated with late-onset AD (LOAD) in white populations [3–5], and these findings have been successfully replicated in other ethnic groups [6–11] (Table 1). CD33 is a type I transmembrane protein belonging to the sialic acid-binding immunoglobulin-like lectins (Siglecs) family, which is thought to mediate the cell–cell interaction and to inhibit normal functions of immune cells [12]. In the brain, CD33 is mainly expressed on microglial cells, and compelling evidence implicating that CD33 facilitates Aβ-related pathology in AD by impairing microglia-mediated Aβ clearance [13–16]. In this article, we review the recent epidemiological findings of CD33 that related with LOAD. The evidence about the levels and pathogenic functions of CD33 in AD has also been discussed. Moreover, we present the advances and challenges in targeting CD33 for AD therapeutic strategies.

Structure, Localization, and Physiological Function of CD33

CD33 gene maps on chromosome 19q13.33 in humans, encoding a 67-kD protein CD33 (Fig. 1), and CD33 protein consists of an extracellular N-terminal V-set immunoglobulin domain responsible for sialic acid recognition, followed by a C2-type immunoglobulin repeat [12, 17]. Intracellularly,

Table 1 Epidemiological studies reported significant associations of CD33 variants with AD

No. and type of subjects	SNPs	OR (95 % CI)	*P* value	Population type	Refs
GWAS (8,309 cases and 7,366 controls)	rs3865444	0.88 (0.84–0.93)	8.2×10^{-7}	Stage 1: ADGC GWAS includes ACT/eMERGE, ADC, ADNI, GenADA, UM/VU/MSSM, OHSU, NIA-LOAD, and TGEN2.	[4]
Follow-up (3,531 cases and 3,565 controls)	rs3865444	0.91 (0.85–0.99)	0.021	Stage 2: follow-up replication includes Mayo Clinic, ROSMAP, UP, and WU.	
Combined (11,840 cases and 10,931 controls)	rs3865444	0.89 (0.86–0.93)	1.1×10^{-7}		
GWAS (6,283 cases and 7,165 controls)	rs3865444	0.89 (0.84–0.95)	2.2×10^{-4}	GERAD + Consortia includes GERAD1, EADI1, deCODE, and AD-IG.	[5]
2,634 cases and 4,201 controls	rs3865444	0.92 (0.84–1.00)	0.049	Mayo2 samples includes Jacksonville, Rochester, Autopsy-confirmed, Norway, Poland, and ARUK	[6]
290 cases and 554 controls	rs3865444	0.70 (0.51–0.96)	0.0258	Korean	[7]
190 cases and 193 controls	rs3865444	2.084 (1.526–2.846)[a]	<0.001[a]	Han Chinese	[8]
612 cases and 612 controls	rs3865444	1.492 (1.188–1.873)	0.017	North Han Chinese	[9]
Family-based GWASs (1,376 samples from 410 families)	rs3826656	NA	4×10^{-6}	NIMH	[3]
Follow-up (2,361 samples from 875 families)	rs3826656	NA	0.007	NIA LOAD, NCRAD, and CAG	
Combined (3,737 samples from 1,285 families)	rs3826656	NA	6×10^{-6}		
191 cases and 180 controls	rs3826656	0.479 (0.263–0.870)	0.015	North Han Chinese	[10]
1,968 cases and 3,928 controls	rs114282264	0.61 (0.47–0.81)	6×10^{-4}	African Americans	[11]

ACT/eMERGE the Adult Changes in Thought/Electronic Medical Records and Genetics study, *ADC* Alzheimer's Disease Centers cohort, *ADGC* Alzheimer's Disease Genetics Consortium, *AD-IG* German Alzheimer's Disease Integrated Genome Research Network, *ADNI* Alzheimer's Disease Neuroimaging Initiative cohort, *ARUK* Alzheimer's Research United Kingdom, *CAG* Consortium on Alzheimer's Genetics, *CI* confidence interval, *EADI* European AD Initiative Consortium, *GenADA* Genotype-Phenotype Associations in Alzheimer's Disease Study, *GERAD* Genetic and Environmental Risk in Alzheimer's Disease Consortium, *GWAS* genome-wide association study, *NCRAD* National Cell Repository for Alzheimer's Disease, *NIA-LOAD* National Institute on Aging Late-Onset Alzheimer's Disease cohort, *NIMH* National Institute of Mental Health genetics initiative study, *OHSU* Oregon Health and Science University cohort, *OR* odds ratio, *ROSMAP* the Rush University Religious Orders Study/Memory and Aging Project, *SNP* single-nucleotide polymorphism, *TGEN* Translational Genomics Research Institute cohort, *UM/VU/MSSM* University of Miami/Vanderbilt University/Mount Sinai School of Medicine cohort, *UP* University of Pittsburgh, *WU* Washington University

[a] Calculated from the raw data provided by the authors

human CD33 has two conserved cytoplasmic tyrosine-based motifs: a membrane-proximal immunoreceptor tyrosine-based inhibitory motif (ITIM) and a membrane-distal ITIM-like motif. However, the ITIM is lacking in mouse CD33 (Fig. 2a) [18]. Both ITIM and ITIM-like motif are involved in inhibitory signal transduction via the recruitment of SHP [Src homology 2 (SH2) domain-containing protein tyrosine phosphatase]-1 and SHP-2 tyrosine phosphatases as well as other SH2 domain-containing

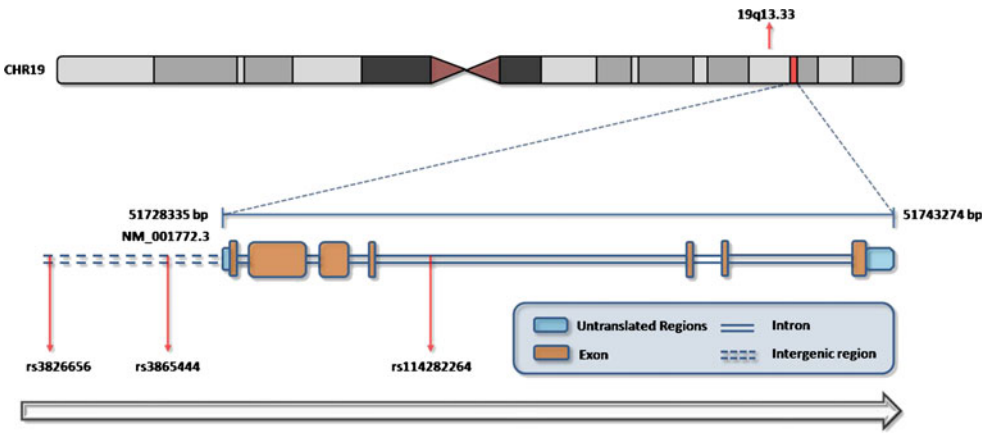

Fig. 1 Schematic of CD33 gene. The CD33 gene structure spans 51,728,335–51,743,274 bp on chromosome 19q13.33 (hg19) and encodes seven exons (represented by *orange boxes*). Note that the single-nucleotide polymorphisms in CD33 that have a significant association with AD risk are highlighted on this figure

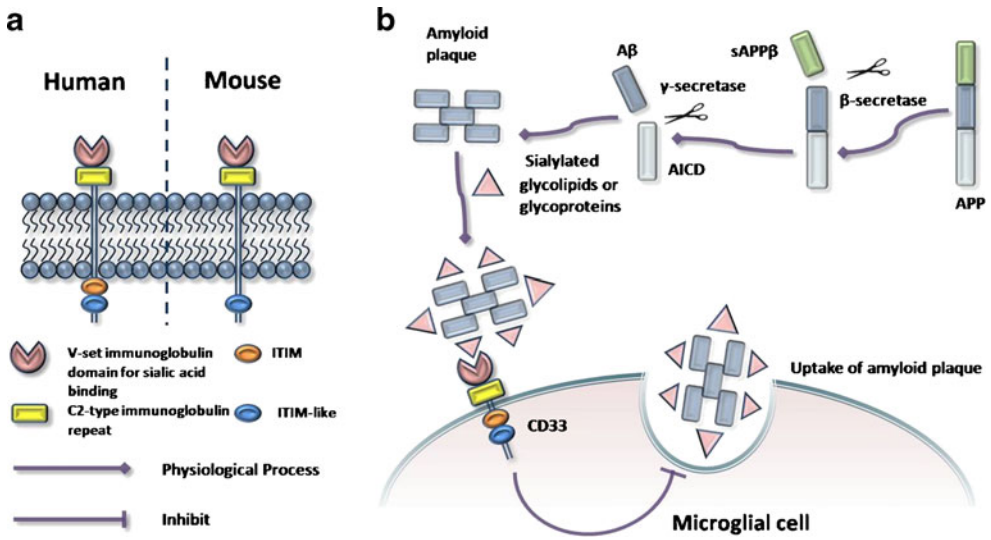

Fig. 2 Structure of CD33 and its relation with Aβ pathology in AD. **a** CD33 consists of an extracellular N-terminal V-set immunoglobulin domain responsible for sialic acid recognition, followed by a C2-type immunoglobulin repeat. Intracellularly, human CD33 has two conserved cytoplasmic tyrosine-based motifs: a membrane-proximal ITIM and a membrane-distal ITIM-like motif. However, the ITIM is lacking in mouse CD33. **b** Binding with sialic acid is necessary for CD33 to exert its inhibitory effects on microglia-mediated Aβ uptake [37]. In the AD brain, amyloid plaques are often aggregated with several proteins and lipids which are highly sialylated [38–40]. Hence, the binding of sialylated glycoproteins and glycolipids on amyloid plaques to CD33 expressed on microglial cells may account for the immune avoidance for amyloid plaques in AD, which avoided the uptake and clearance by microglial cells

effector proteins [19, 20]. In mammals, CD33 has been found to be expressed on hematopoietic and phagocytic cells, including hematopoietic progenitors, myelomonocytic precursors, macrophages, monocytes, dendritic cells, and microglial cells [21, 22]. With respect to its functions, CD33 is known to participate in adhesion processes of human primary immune cells. CD33 is able to bind to high-affinity sialoglycans on target cells, mediating cell–cell interaction [23]. Similarly, CD33 can interact with sialylated pathogens and viruses, which are decorated on their surface with host-derived or self-synthesized sialoglycans. Binding of this pathogen to CD33 via sialylated ligands could facilitate their endocytosis and clearance by host phagocytes or, as opposed, could mediate their internalization to spread infection [24]. Interestingly, the endocytic function of CD33 has been exploited for cell-directed therapies in acute myeloid leukemia (AML) [25]. Another important role of CD33 is its ability to inhibit immune cell functions [26]. CD33 appeared to inhibit the human monocyte production of pro-inflammatory cytokines, such as interleukin (IL)-1β, tumor necrosis factor alpha (TNF-α), and IL-8 via phosphatidylinositol 3-kinase and p38 mitogen-activated protein kinase-dependent pathway [27]. Conversely, reduction of CD33 resulted in the increased secretion of TNF-α in human monocyte [28]. CD33 has also been reported to regulate cell growth and survival, through the inhibition of proliferation and induction of apoptosis. Activation of CD33 on $CD34^+$ myeloid progenitors as well as CD33 on monocyte-derived dendritic cells by a specific antibody markedly inhibited their growth [29]. In addition, engagement of CD33 expressed on AML cells led to their apoptosis [30].

Genetics of CD33 Gene in AD

In last 5 years, three independent GWASs have identified CD33 as a strong genetic locus linked to LOAD (Table 1) [3–5]. The main single-nucleotide polymorphisms (SNPs) associated with LOAD are rs3865444 and rs3826656, which are located 373 and 1,722 bp upstream of CD33 coding region, respectively.

In regard to rs3865444, the minor allele has been identified as a protective allele in two GWASs in white populations [4, 5], and this result has been successfully replicated in cohorts from Europe, North America, and Korea [6, 7]. By contrast, the T allele of rs3865444 appears to be a risky rather than a protective allele in AD in Han Chinese population, as demonstrated by studies from Deng et al. and our group [8, 9]. In fact, the minor allele frequency of rs3865444 in Han Chinese cohort was 17 % [9], which was significant lower than that of the white populations (30 %) [4]. Besides, some epidemiological researchers suggested that the rs3865444 polymorphism may be in linkage disequilibrium (LD) with a functional polymorphism that causes stronger influences on AD risk, and the degrees of LD among these variants usually differ across populations [31], which may be responsible for the contradictory findings on the association between rs3865444 and AD. Another possible explanation is that the association between rs3865444 and AD was influenced by environmental and lifestyle factors in different populations, which led to the phenomenon that rs3865444 conferred a risk for AD in some populations while provided protective effects against AD in other populations [32]. Aside from rs3865444, Bertram et al.

found that the minor allele of rs3826656 conferred a risk for AD in a large family-based GWAS containing 1,376 samples from 410 European families [3]. The association between rs3826656 and AD was unable to replicate in a more recent GWAS by Naj and colleagues [4]. However, it should be noted that only stage 1 analysis (containing 8,309 AD patients and 7,366 cognitively normal controls) was conducted in the study of Naj, not the entire sample, so this finding was not yet conclusive. Nevertheless, in contrast to the findings in Caucasians, Yuan et al. reported that the minor allele of rs3826656 was associated with a reduced risk of AD in Han Chinese population with apolipoprotein E (APOE) ε4 alleles [10]. It is worth noting that rs3826656 is only 1,348 bp from rs3865444, and they are in a complete linkage with each other in both Caucasian and Han Chinese populations (for Caucasian population, D'=1.0, r^2=0.11; for Han Chinese population, D'=1, r^2=0.47). The relatively low r^2 may be accountable by the vastly different minor allele frequencies of these two SNPs [for Caucasian population, rs3826656= 19.5 % and rs3865444=31.9 %; for Han Chinese population, rs3826656=29.9 % and rs3865444=17.6 %; frequencies taken from the HapMap database (release no. 28, August 2010, http://www.hapmap.org)].

More recently, a novel SNP rs114282264 that is located in the intron region of CD33 has been identified to be significantly associated with AD in African Americans [11], and this association requires to be confirmed in the other ethnic groups in the future.

CD33 Levels in AD

In the brain, the levels of CD33 protein were increased in AD patients, as revealed by the increase in the count of CD33-immunoreactive microglial cells [13]. Regarding the transcription level, CD33 mRNA was revealed to be dramatically increased in AD, suggesting a potential upregulation of CD33 transcription in microglial cells [13]. These findings were consistent with a recent study from Karch et al., which demonstrated a significant correlation between mRNA levels of CD33 and Iba-1 (a biomarker of microglial cells) in brain tissue from the parietal lobe of AD cases. In addition, when normalized to Iba-1 expression, the CD33 mRNA expression was revealed to correlate with disease status and Clinical Dementia Rating scores [14].

As mentioned above, most of the genetic studies indicated a protective role of minor (T) allele of the CD33 SNP rs3865444 in AD [4–7]. The mechanism by which T allele protects against AD has been uncovered recently, as T allele was associated with reduced CD33 protein levels on microglial cells both in the AD and control subject. Coincidentally, a recent study from Bradshaw et al. demonstrated that the risk allele (C) of rs3865444 was associated with elevated CD33 levels, as a sevenfold increase in CD33 expression on monocytes was observed in young cognitively normal individuals carrying the CC genotype versus those carrying the TT genotype. Meanwhile, this finding has been successfully replicated in old person either with or without AD [15]. It is worth noting that T allele is not associated with significant alterations in CD33 mRNA levels in those carriers [13]. One reasonable explanation is that the rs3865444 SNP is in linkage disequilibrium with functional variant(s) located in the coding region, which influences mRNA translation rather than its stability.

Taken together, these findings suggested a possible association of CD33 levels with the etiology and pathogenesis of AD.

CD33, Phagocytes, and Aβ Pathology

Amyloid hypothesis have emphasized the crucial role of Aβ pathology in the pathogenesis of AD, which is thought to drive a pathologic cascade including hyperphosphorylation of tau protein and neuroinflammation, ultimately leading to cognitive impairment [1]. Accumulating evidence has indicated a strong association of CD33 with Aβ pathology in AD progression. In a recent study from Griciuc et al., the T allele of SNP rs3865444, which led to the reduction of CD33 levels in the brain, was found to be linked with decreased amyloid plaque burdens in the brain cortex of AD patients [13]. In agreement with this finding, Bradshaw and colleagues showed that the C allele of SNP rs3865444, which caused the elevation in CD33 levels, was associated with a greater burden of fibrillar amyloid in older asymptomatic individuals and with neuritic amyloid plaques in the brains of older individuals at autopsy [15]. More direct evidence on the association between CD33 and Aβ pathology has been gathered from animal models of AD, as amyloid precursor protein/presenilin 1 (APP/PS1) transgenic mice lacking CD33 exhibited significant lower Aβ levels as well as reduced amyloid plaque burden in the brain [13]. These observations clearly indicated a pathogenic role of CD33 in facilitation of Aβ pathology. More importantly, deletion of CD33 in APP/PS1 transgenic mice did not alter the APP processing or the levels of pro-inflammatory cytokines in the brain, implying that CD33 contributed to Aβ pathology by interfering Aβ clearance rather than promoting its generation [13].

Microglial cells are considered to be the main phagocytes in the brain, which plays a nuanced and complex role in the progression of AD [33]. Despite of its detrimental role in exacerbation of neuroinflammation, microglial cells is also known to the uptake and degradation of Aβ, which prevents the formation of amyloid plaque in the AD brain [34]. In view of the fact that CD33 is mainly expressed on the surface of phagocytes including microglial cells, some researches

inferred that CD33 may facilitate amyloid pathology via impairing the microglia-mediated clearance of Aβ. Considering the fact that microglial cells are replenished partly by the immigration of circulating monocytes under pathological conditions including AD [35, 36], Bradshaw and colleagues [15] employed circulating monocytes to study the role of CD33 in Aβ clearance. They found that a higher expression of CD33 on the surface of circulating monocytes directly inhibited their abilities to phagocytose Aβ. In addition, they also observed that CD33 levels positively correlated with the mean proportion of terminally activated microglial cells in the inferior temporal lobe, which is an early target of Aβ pathology in the brain. This observation can be explained by the hypothesis that a higher CD33 levels inhibited the functions of microglial cells in Aβ uptake, which subsequently led to the accumulation of less functional microglial cells in plaque-associated brain regions.

The observations from the study of Bradshaw [15] have been proven by Griciuc and colleagues [13] using microglial cells. In mouse primary microglial cells lacking CD33, increased uptake of Aβ was observed to be relative to wide-type (WT) cells. Intriguingly, there is no difference in Aβ degradation rate between CD33-deficient and WT cells. By contrast, BV2 microglial cells overexpressing WT CD33 protein exhibited a significant impairment in their capacity to uptake Aβ, but the ability to degrade Aβ remained unaffected. These findings were further confirmed by transfecting microglial cells with an ubiquitylation-defective CD33 mutant ($CD33^{K7R}$), which led to enhanced cell surface expression of CD33. As expected, microglial cells expressing $CD33^{K7R}$ exhibited a dramatic reduction in capacity of Aβ uptake when compared with WT cells [13]. Taken together, the findings from Bradshaw et al. [15] and Griciuc et al. [13] indicated that CD33 impaired Aβ clearance via directly inhibiting its uptake by microglial cells. As discussed above, CD33 could exert their physiological functions by interacting with sialic acids [12]. To explore the requirement of sialic acid binding in the CD33-mediated inhibition on microglial uptake of Aβ, Griciuc et al. [13] transfected BV2 microglial cells with a CD33 mutant that lead to the deficiency in the sialic acid-binding V-type immunoglobulin-like domain ($CD33^{\Delta V\text{-}Ig}$ protein). Cells expressing the $CD33^{\Delta V\text{-}Ig}$ protein demonstrated no impairment in their capacity to uptake Aβ, indicating that sialic acid binding is required for the inhibition of CD33 on microglia-mediated uptake of Aβ [13].

Moreover, the above observations could be linked to a recent proposed hypothesis that amyloid plaque is able to evade the clearance of microglial cells with the help of CD33-related Siglecs, which subsequently leads to neurodegeneration [37]. In the AD brain, amyloid plaques are often aggregated with several proteins and lipids which are highly sialylated, such as clusterin, APOE proteins, and gangliosides [38–40]. As discussed above, sialic acid binding is necessary for the inhibition of CD33 and its related Siglecs on Aβ uptake. Hence, the binding of sialylated glycoproteins and glycolipids on amyloid plaques to CD33 expressed on microglial cells may account for the immune avoidance for amyloid plaques in AD, which avoided the uptake and clearance by microglial cells [37] (Fig. 2b).

CD33 as a Potential Therapeutic Target for AD

The Aβ cascade hypothesis posits that accumulation of Aβ is the fundamental initiator of AD [1, 41]. Meanwhile, it is widely believed that impaired Aβ clearance is a major pathogenic event for LOAD [2, 42]. As mentioned earlier, microglial cells have been found to play critical roles in Aβ clearance in the brain, and the recent study from Griciuc et al. [13] demonstrated that CD33 contributed to the pathogenesis of AD by impairing microglia-mediated clearance of Aβ. Meanwhile, deletion of CD33 both in vitro and in vivo led to the increased Aβ clearance and reduced Aβ levels. Remarkably, CD33 deletion in vivo was viable, as CD33 knockout mice were fertile and exhibited no anatomical defect, implying that targeting CD33 may be a safety option in AD treatment. It should be noted that therapies targeting CD33 have already been developed in AML, as approximately 90 % of AML patients have myeloblasts expressing CD33 [43]. Naked humanized anti-CD33 and calicheamicin-conjugated humanized murine anti-CD33 antibodies have been developed and tested in several phase III clinical trials of AML [25, 44]. Meanwhile, development of a CD33 antibody that is able to cross the blood–brain barrier (BBB) is technically feasible, as a chimeric antibody in which the CD33 antibody is fused to a monoclonal antibody against the human insulin receptor was found to facilitate the receptor-mediated passage of the chimera across the BBB [45]. Alternatively, development of small compounds, such as sialic acid-based antagonists that specifically target CD33 and inhibit its function should also be considered as a therapeutic strategy for AD.

Conclusions and Future Perspectives

CD33 is a type I transmembrane protein expressed on brain microglial cells, exerting its inhibitory effects on microglia-mediated Aβ uptake. To date, three SNPs in CD33 have been identified to be significantly associated with LOAD risk, and these findings ought to be further confirmed in the other ethnic groups. In the future, more in-depth genetic screenings should be performed to uncover functional variants in CD33 that related to LOAD. In addition to the inhibition on Aβ clearance, the other biological mechanisms by which CD33 contributed to the pathogenesis of AD should also be investigated,

such as its role in neuroinflammation and neuronal apoptosis. Asides from the CD33 in the brain, the plasma level of CD33 in AD patient and its correlation with this disease should be evaluated by future studies, as a better understanding of CD33 action both in the brain and in the periphery will hopefully lead to the development of novel therapeutics for the prevention and treatment of AD.

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Conflict of interest The authors declare no conflicts of interest.

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