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Causal Association Between mTOR-Dependent Protein Levels and Alzheimer's Disease: A Mendelian Randomization Study

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Abstract.

Background: Most previous studies supported that the mammalian target of rapamycin (mTOR) is over-activated in Alzheimer's disease (AD) and exacerbates the development of AD. It is unclear whether the causal associations between the mTOR signaling-related protein and the risk for AD exist.

Objective: This study aims to investigate the causal effects of the mTOR signaling targets on AD.

Methods: We explored whether the risk of AD varied with genetically predicted AKT, RP-S6K, EIF4E-BP, eIF4E, eIF4A, and eIF4G circulating levels using a two-sample Mendelian randomization analysis. The summary data for targets of the mTOR signaling were acquired from published genome-wide association studies for the INTERVAL study. Genetic associations with AD were retrieved from the International Genomics of Alzheimer's Project. We utilized the inverse variance weighted as the primary approach to calculate the effect estimates.

Results: The elevated levels of AKT (OR = 0.910, 95%CI=0.840-0.986, $p = 0.02$) and RP-S6K (OR = 0.910, 95%CI=0.840-0.986, $p = 0.02$) may decrease the AD risk. In contrast, the elevated eIF4E levels (OR = 1.805, 95%CI=1.002-1.174, $p = 0.045$) may genetically increase the AD risk. No statistical significance was identified for levels of EIF4-BP, eIF4A, and eIF4G with AD risk ($p > 0.05$).

Conclusion: There was a causal relationship between the mTOR signaling and the risk for AD. Activating AKT and RP-S6K, or inhibiting eIF4E may be potentially beneficial to the prevention and treatment of AD.

Keywords: Alzheimer's disease, causal relationships, mammalian target of rapamycin, Mendelian randomization

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INTRODUCTION

Alzheimer's disease (AD) is a typical neurodegenerative disease characterized by morbidity and cognitive impairment in elderly people [1]. It makes up 60%–80% of all dementia cases [1], affecting over 50 million people worldwide [2]. Given the high prevalence and heavy economic burden, exploring more detailed pathogenesis to help reduce the risk of AD is urgently required [3]. Amyloid-β (Aβ) peptide and neurofibrillary tangles (NFTs) formed by hyperphosphorylated tau have been regarded as the major pathological markers of AD [4]. These protein affect dendritic integrity and lead to the death of nerve cells by interrupting the connections between nerve cells [4, 5]. Various targets and pathways participate and stimulate the composition of Aβ and tau, including the mammalian target of rapamycin (mTOR) signaling-related pathway [6].

mTOR, an atypical serine/threonine protein kinase, regulates several essential mammalian cell functions [7]. The mTOR signaling pathway has been repeatedly reported to participate in nervous system disease, especially AD. Additionally, mTOR complexes exist in two main multiprotein complexes, mTOR complex 1 and mTOR complex 2 (mTORC1 and mTORC2) [8] (Fig. 1). Both the upstream regulation and downstream outputs of mTORC1 are being more studied and well discussed compared with mTORC2 in AD [9]. Importantly, mTORC1 was more activated in the AD brains instead of mTORC2 by detecting levels of mTOR/p-mTOR and its downstream targets in autopsied brain hippocampal tissues obtained from AD patients [10]. Our study mainly focuses on the upstream and downstream targets of mTORC1 for the reason that they can contribute to messenger RNA translation and protein synthesis of Aβ and tau [7].

Numerous studies have made the relationships between upstream and downstream targets of mTOR and their interaction relatively straightforward [11]. mTORC1 is activated by growth factors, energy metabolism, nutrients and stress [12]. The phosphoinositide 3-kinase (PI3K)/ protein kinase B (AKT) pathway plays a vital role in transmitting growth factors (Insulin, IGF) signals to mTORC1 by regulating the phosphorylation of TSC1/2. The downstream targets of mTORC1 are unc-51-like kinase 1 (ULK1), transcription factor EB (TFEB), ribosomal protein S6 kinases (RP-S6K), and eukaryotic translation initiation factor 4E-binding protein (EIF4E-BPs). The former two are crucial protein for autophagy. ULK1 is a crucial part of the macroautophagy initiation complex. TFEB regulates the expression of lysosomal biogenesis and autophagy. Activating mTORC1 can restrain autophagy by phosphorylating ULK1 and TFEB [13]. The latter two play essential roles in the regulation of translation, which will be primarily discussed in this study. The activated mTORC1 activates RP-S6K, further controls cell growth via promoting ribosome biogenesis, and activating several key factors in translation [14]. EIF4E-BPs, composed of EIF4E-BP1, 2, and 3, isolate eukaryotic translation initiation factor 4E (eIF4E) and prevent assembling eIF4F complex, further inhibiting translation initiation and protein synthesis [15]. Only when mTORC1 inhibits EIF4E-BPs and then dissociated eIF4E combines with eIF4G and eIF4A to assemble the eIF4F complex can it mediates the recruitment of ribosomes to mRNA during translation [16]. Accordingly, the activated mTOR leads to the activation of RP-S6K and inhibition of EIF4E-BP, resulting in protein synthesis (Fig. 1).

At present, the causal associations between mTOR and AD are still debated. Hence, our study focuses on exploring the causal association of mTOR pathways with AD through an approach called Mendelian randomization (MR) because randomized controlled trials and observational studies are difficult to establish and prone to confounding, reverse causation, and various biases [17]. MR is a valuable and efficient way to detect unbiased causal effects [17]. A valid approach is to choose SNPs as instrumental variables (IVs) to mimic the randomized allocation of individuals to the exposure and thus, ensure comparability of groups any confounder. Besides, genetic variants of exposure are not subject to reverse causation as they are randomly allocated [18].

In this study, we investigated the six target levels of mTORC1 (AKT, RP-S6K EIF4E-BP, eIF4E, eIF4A, and eIF4G) using the MR approach to explore the causal relationships between these protein and AD.

METHODS

Genetic predictors of plasma RP-S6K, EIF4E-BP, eIF4E, eIF4A, and eIF4G

Genetic predictors of the data about mTOR-related gene exposure were retrieved from a recent genome-wide association study (GWAS) of results of the human plasma proteome from the INTERVAL study with 3301 healthy individuals of European descent (mean age 43.7 years, 48.9% females) [19] (Supplementary Table 1). The study performed the

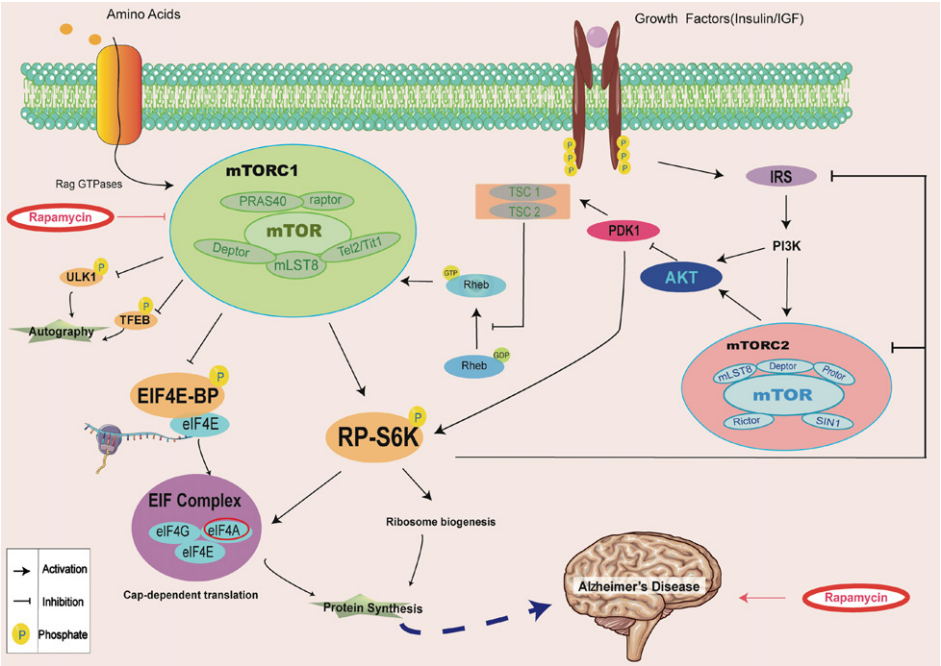

Fig. 1. Upstream and downstream regulations of mTOR and the function with AD. Both mTORC1 and mTORC2 are activated by growth factor and PI3K-dependent signaling. PI3K/AKT activates mTORC1 via inhibitory phosphorylation of TSC1/2. AKT serves as an essential connecting node, activating mTORC1 through PI3K signaling or downstream of mTORC2 activation. mTORC1 is hyperactive in AD in most previous studies, further inhibits autophagy initiation, and promotes ribosome biogenesis and mRNA translation through phosphorylation of the ULK, TFEB, RP-S6Ks, and EIF4E-BPs, respectively. Rapamycin can reduce the hyperactivity of mTORC1 and regulate mTORC1 in proper function. mTOR, mammalian target of rapamycin; AD, Alzheimer's disease; ULK1, unc-51-like kinase 1; TFEB, transcription factor EB; PI3K, phosphatidylinositol 3-kinase; RP-S6Ks, ribosomal protein S6 kinases; EIF4E-BPs, eukaryotic translation initiation factor 4E-binding protein.

SomaLogic method to assess 3,622 plasma protein, which enhances the sensitivity of the proteomic assay [20], and adjusted genetic associations for several factors, including age, sex, duration between blood collecting and processing, and the first three principal components of ancestry from multi-dimensional scaling [19].

Genetic associations with Alzheimer's disease

The AD-related GWAS data were obtained from the International Genomics of Alzheimer's Project (IGAP) [21]. The genetic associations with AD were measured in 63926 individuals gathered from 46 case-control cohorts in the discovery sample, which consisted of 21,982 clinically diagnosed cases (mean age 72.9, 61.3% females) and 41,944 cognitively normal controls (mean age 72.4, 57.1% females) (Supplementary Table 1). The cases were confined to LOAD (onset at 65 years of age or older) as diagnosed by clinical assessment, MRI- or autopsy-confirmed, and diagnoses from health care records.

Selection of instrumental variables

In our MR analysis, IVs need to meet three critical assumptions: 1) SNPs of mTOR were strongly associated with AD; 2) SNPs of mTOR were independent of the confounders of mTOR and AD; 3) SNPs of mTOR were related to AD only via mTOR [17, 22].

We used all SNPs independently ($r^2 < 0.001$) and significantly ($p < 5 \times 10^{-6}$) predicting an exposure. F statistics were utilized to assure the strength of the SNPs [23]. If the F statistic was greater than 10, the SNPs selected were regarded as valid IVs, and results based on those ought not to suffer considerably from weak IVs [22]. Only the SNPs whose F statistics were more than 10 would be remained. The process of selection of IVs was presented in Fig. 2. Besides, to guarantee meeting the three basic assumptions above, we also analyzed in PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/), a database involving comprehensive information on the connection of genotype and phenotype, to identify whether these SNPs were related with the possible

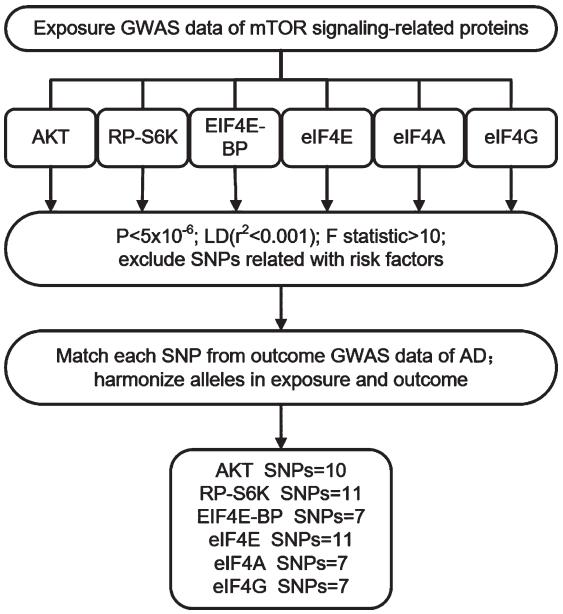

Fig. 2. A flow diagram showing the process of instrumental variants selection. GWAS, genome-wide association study; mTOR, mammalian target of rapamycin; AKT, protein kinase B; RP-S6K, Ribosomal Protein-S6 kinase; EIF4E-BP, eukaryotic translation initiation factor 4E-binding protein; eIF4E, eukaryotic translation initiation factor 4E; eIF4A, eukaryotic translation initiation factor 4A; eIF4G, eukaryotic translation initiation factor 4 G; LD, linkage disequilibrium; SNP, single nucleotide polymorphism; AD, Alzheimer's disease.

risk factors, including immune cells [24], platelets [25], obesity [26], education degree [27], and diabetes [28], and removed SNPs correlated with any of these potential aspects.

Statistical analysis

We eliminated SNPs with incompatible alleles in the exposure and the outcome. For instance, an SNP would be removed if the effect/non-effect alleles for exposure and outcome were A/G and A/C [29]. After harmonizing the GWAS datasets of mTOR signaling pathway-associated targets and AD, we utilized several MR methods from TwoSampleMR packages (version 0.5.6) in R software (version 4.0.5) to estimate the causal effects of the six upstream and downstream targets of mTOR on AD. Multiple methods were performed as they differed from each other on the basis of sensitivity to power, heterogeneity, and bias. IVW was selected as the primary analysis method, which could assume balanced pleiotropy. Other MR methods, such as MR-Egger and weighted median, were used to increase the IVW estimates despite being less efficient (wider CIs) [30].

Sensitivity analysis consisted of assessing the heterogeneity and pleiotropy and leave-one-out analysis. Detecting underlying pleiotropy and the heterogeneity for MR estimates were severely important in MR studies. Firstly, we conducted Cochran's Q test to check the heterogeneity representing potential horizontal pleiotropy [31]. This was a weighted sum of the squared distances of the variant-specific estimates from the overall IVW estimate. Additionally, we implemented MR-Egger regression to detect and determine whether there remained some violations of the standard IVs assumptions [32]. Moreover, the intercept obtained from the MR-Egger regression can be an indicator for assessing directional pleiotropy, which could influence causal estimates [33]. Mendelian Randomization Pleiotropy RESidual Sum and Outlier methods (MR-PRESSO) were used to detect and correct directional pleiotropy [34]. The leave-one-out analysis evaluated the effect of outlying and pleiotropic genetic variants [33].

Given the 6 MR estimates, a Bonferroni-corrected p-value below 0.008 (0.05/6) was considered significant, and $p < 0.05$ was regarded nominally significant. Results were presented in odd ratios (ORs) and 95% confidence intervals (CIs) per genetically predicted standard deviation (1-SD) unit increase in each target. As this study was based on publicly available summary data, no ethical approval is must-have.

RESULTS

We included six upstream and downstream targets of mTOR in our MR analysis. After selection and quality control, the numbers of IVs of each target (AKT, RP-S6K, EIF4E-BP, eIF4E, eIF4A, and eIF4G) for our MR analyses were 10, 11, 7, 11, 7, and 7 respectively (Fig. 2). All harmonized IVs with their characteristics associated with mTOR-related targets and AD were listed in Supplementary Tables 2, 3, 4, 5, 6, and 7. The ORs and 95%CI were shown in Fig. 3. Three MR test methods estimating the association of SNP effect sizes on each target for those on AD were plotted in Fig. 4, and each line represents the estimated results per method. Results for potential pleiotropy and heterogeneity assessments of mTOR-dependent protein levels with the risk of AD were shown in Supplementary Table 8.

As for crucial upstream targets of mTOR, AKT was found a causal effect on the risk of AD (IVW:

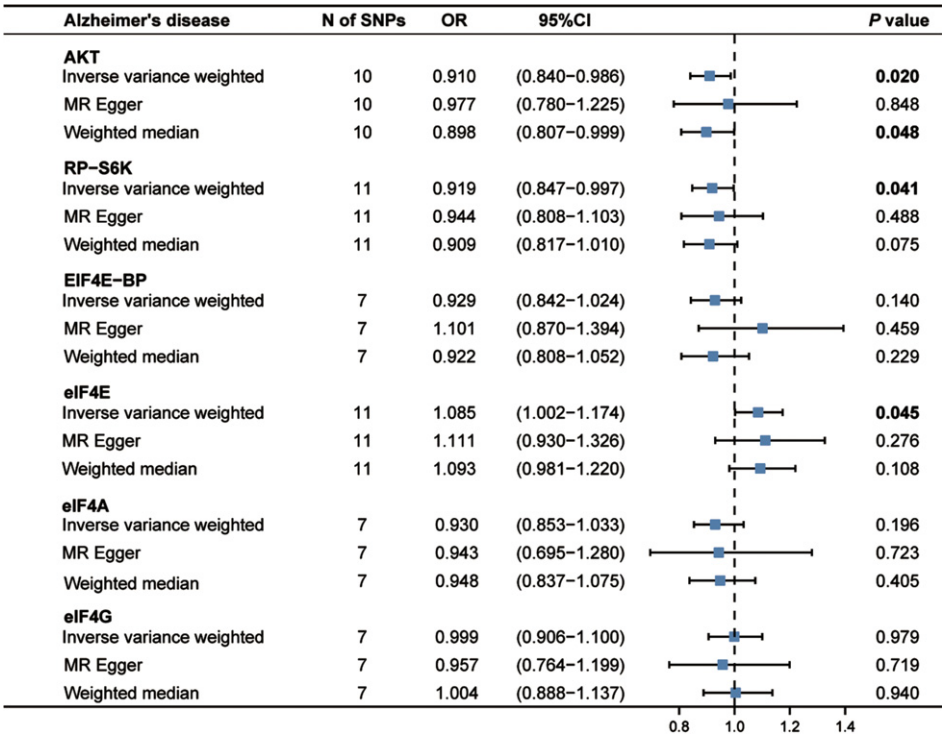

Fig. 3. ORs for estimates of the relationship between genetically predicted mTOR signaling pathway-related targets and AD. ORs, Odds ratios; N of SNPs, number of single nucleotide polymorphisms; CI, confidence interval; AKT, protein kinase B; RP-S6K, Ribosomal Protein-S6 kinase; EIF4E-BP, eukaryotic translation initiation factor 4E-binding protein; eIF4E, eukaryotic translation initiation factor 4E; eIF4A, eukaryotic translation initiation factor 4A; eIF4G, eukaryotic translation initiation factor 4 G; AD, Alzheimer's disease.

OR = 0.910, 95%CI=0.840-0.986, $p = 0.020$; Fig. 3). Using weighted median method also found the same association with significant statistic (OR = 0.898, 95% CI = 0.807-0.999, $p = 0.048$; Fig. 3). MR-Egger pointed toward similar direction of effect despite not statistically significant (Fig. 3). Similarly, there was association of genetically predicted RP-S6K with the risk of AD using the IVW approach (OR = 0.919, 95%CI=0.847-0.997, $p = 0.041$) with similar estimates from the weighted median and MR-Egger (Figs. 3 and 4). Meanwhile, a suggestive association between eIF4E cap-dependent translation factor and AD was detected using IVW method (OR = 1.805, 95%CI=1.002-1.174, $p = 0.045$; Fig. 3). The same tendency of risk estimates were gained from the MR-Egger and weighted median approaches without statistical significance (Figs. 3 and 4). In addition, for all estimations, neither heterogeneity in the Cochran's Q test nor underlying pleiotropy in the MR-Egger regression analysis was identified (Supplementary Table 8). The leave-one-out analysis indicated that none of the SNPs affected the association (Supplementary Figure 1).

We did not find the other three targets associated with the risk of AD. They were EIF4E-BP (IVW: OR = 0.929, 95% CI = 0.842-1.024, $p = 0.140$), eIF4A (IVW: OR = 0.930, 95% CI = 0.855–1.033, $p = 0.196$), and eIF4G (IVW: OR = 0.999, 95% CI = 0.906–1.100, $p = 0.979$) (Fig. 3).

DISCUSSION

Through performing 2SMR analysis, our study estimated causal relationships between several mTOR-related targets and the risk for AD. Specifically, AKT and RP-S6K were protective factors of AD, and eIF4E was identified as a risk factor for AD, whereas EIF4E-BP, eIF4G, and eIF4A were of no significance. Our study, for the first time, used a 2SMR method to reveal the possible causal relationships between mTOR and AD from the selected GWAS data. Our findings were consistent with recent reports and support the causal association of these targets, especially AKT, RP-S6K, and eIF4E, with the risk of AD. EIF4-BP, eIF4A, and eIF4G are also vital targets in the mTOR signaling in the main-

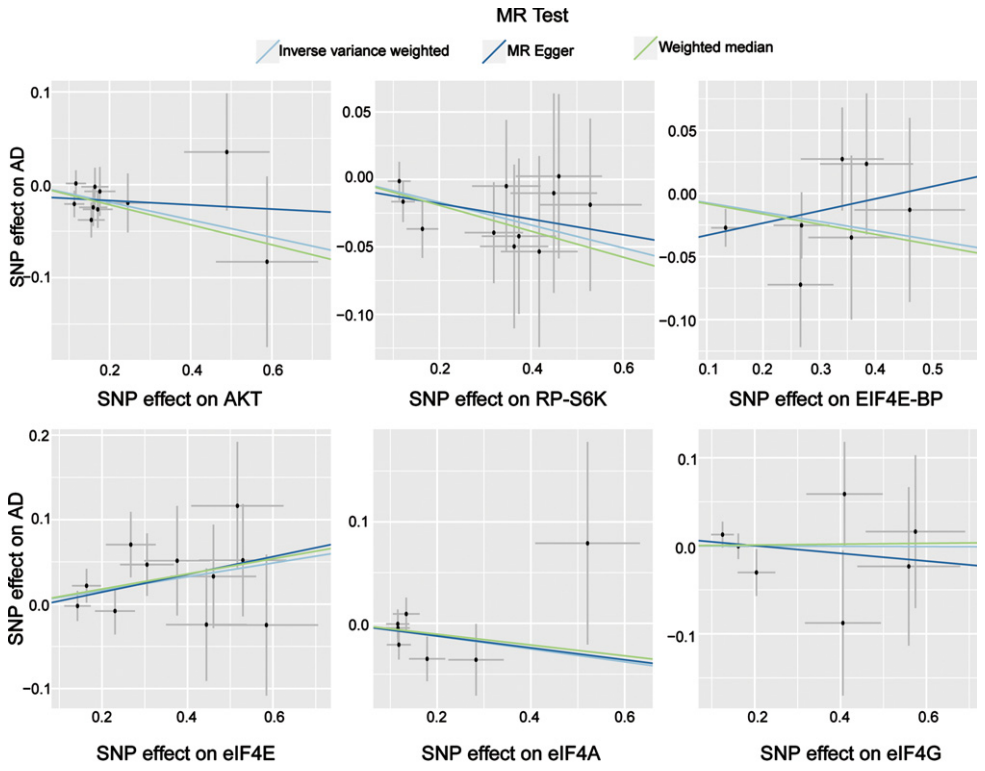

Fig. 4. Scatterplot of SNP effects on mTOR signaling pathway-related targets versus AD. The different slopes of three lines represented the estimated Mendelian randomization effect from three MR tests. Each point in the scatterplot represents an instrumental variable, and the crossing lines on each point reflect the 95% confidence interval. The abscissa is the effect of SNP on the exposures (AKT, RP-S6K, EIF4E-BP, eIF4E, eIF4A, and eIF4G), and the ordinate is the effect of SNP on the outcome (AD). AKT, protein kinase B; RP-S6K, Ribosomal Protein-S6 kinase; EIF4E-BP, eukaryotic translation initiation factor 4E-binding protein; eIF4E, eukaryotic translation initiation factor 4E; eIF4A, eukaryotic translation initiation factor 4A; eIF4G, eukaryotic translation initiation factor 4 G; SNP, single nucleotide polymorphism; AD, Alzheimer's disease.

stream study [35] despite no association in the current study.

The amyloid cascade hypothesis, NFTs, and neuroinflammatory have been the three main foci of AD research. We investigated how mTOR influences the abnormal development of Aβ oligomers, tau protein phosphorylation, and nervous inflammation in AD patients. In most studies, abnormally upregulated mTOR signaling in AD can drive the synthesis of Aβ and tau, decreasing the clearance of these protein [11]. Meanwhile, it is indicated that elevated Aβ deposits, in turn, hyperactivate mTOR, which hyperphosphorylated tau, forming NFTs [36]. Aβ deposits and NFTs lead to nerve inflammation and further blood-brain barrier impairment, where mTOR signaling also acts as an essential factor [37, 38].

Upper regulators like PI3K/AKT signaling axis play a crucial role in AD patients. We found that AKT could lower the risk of AD, which was consistent with previous studies. In some experiments, PI3K/AKT is involved in many signaling pathways delaying the progression of AD, such as PI3K/Akt/Nrf2/HO-1 [39], PI3K/Akt/GSK-3 β [40], and PI3K/AKT/FoxO3a [41]. Via PI3K/AKT signaling axis, these experiments reduced oxidative stress, neurodegeneration, neuroinflammation and cognitive impairment in AD mouse/mice. Accordingly, AKT can be regarded as one of the treatment targets for AD.

In our study, AD would be at a lower risk with higher levels of RP-S6K. Chiku et al. knocked down S6K of Drosophila homolog and found that the level of pSer262-tau increased. Their study indicated that activation of S6K significantly decreased the levels of toxic tau and acted as a protective role in suppressing neurodegeneration [42]. However, in terms of previous mainstream studies, it seemed to increase the risk of AD. It has been reported that over-activating mTORC1 in AD activates RP-S6K, which upregulates excessive tau mRNA translation

[43]. Examination of brain tissue from AD patients revealed that phosphorylated S6K was associated with increased tau protein [44]. No matter how RP-S6K influences the development of AD, we confirm that RP-S6K is genetically related to the risk of AD.

As mentioned earlier, EIF4E-BP and its downstream targets, involving eIF4E, eIF4A, and eIF4G, are essential indicators of mTORC1. We discovered a causal relationship between eIF4E and the risk for AD, though no causal associations of EIF4E-BP, eIF4G, and eIF4A were observed in AD. We found that eIF4E played a risk factor in the development of AD, which was in line with previous studies about the hypothesis of over-activation of mTOR. The mTOR signaling stimulates 4EBP1 phosphorylation and then leads to the formation of the eIF4F complex [45], which is positively correlated with total tau and p-tau [46].

MR is a relatively superior and convenient method for exploring the understanding of cause-and-effect relationships between disease and molecule targets, providing a probable reference for clinical trials. As we know, rapamycin, a mTORC1 inhibitor, is relatively well established in animal studies and proved effective for patients at the early age of AD. A phase I study of rapamycin is on the go (NCT042009). Several previous studies have reported that rapamycin and other methods of inhibiting mTORC1 effectively improve AD in different well-established mouse models of AD by reducing Aβ levels [47], inhibiting the abnormal phosphorylation of tau protein [48], restoring brain functions for asymptomatic *APOE4* carriers [49], clearing chronic nervous inflammation [50], and breaking the negative feedback mechanism on insulin signaling to improve synaptic protein synthesis [51]. As we found, these three mTOR-dependent circulating proteins (AKT, RP-S6K, eIF4E) have causal effects on AD, arguably supporting the approach to AD therapy using mTOR as a therapeutic target.

The current study still had several limitations. First, the GWAS dataset of EIF4E-BP mainly concerns 4E-BP2 because 4E-BP1-related GWAS data were not found. Most related studies were conducted with 4E-BP1, although 4E-BP2 could be the most abundant isoform. For example, 4E-BP2 was the most plentiful in the brain [14]. This was one of our most significant limitations. Secondly, this study was based on genetic predictors of upstream and downstream protein of mTOR whose correspondence with biological markers of AD. Given that the GWAS datasets about Aβ and tau are hard to obtain, we did not analyze MR between pathological features of AD and mTOR-dependent protein levels. Thirdly, in spite of age having been adjusted in the original GWAS datasets papers [19, 21], the case of AD in IGAP is an older age group, which could cause selection bias [52]. Finally, because there were only a few SNPs selected through the conventional GWAS threshold ($p < 5 \times 10^{-8}$), we relaxed the threshold ($p < 5 \times 10^{-6}$) to obtain more IVs to perform MR analysis, as portrayed in other mTOR MR studies [53].

Conclusions

Although further studies are needed to discover the detailed role of mTOR signaling-related targets in AD, our findings support a shared genetic background between mTOR signaling-related targets and the risk for AD. Activating AKT and RP-S6K, or inhibiting eIF4E may be potentially beneficial to the prevention and treatment of AD.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

DATA AVAILABILITY

The Proteomics-GWAS data are open in their database (https://www.phpc.cam.ac.uk/ceu/protein/). The genetic association data on six mTOR-dependent protein and the risk of AD in this study can be found at the IEU Open GWAS Project (IEU OpenGWAS project (mrcieu.ac.uk)).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JAD-230128.

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