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Suppression of Wnt/β-catenin signaling is associated with downregulation of Wnt1, PORCN and Rspo2 in Alzheimer's disease

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Abstract

Wnt and R-spondin (Rspo) proteins are two major types of endogenous Wnt/β-catenin signaling agonists. While Wnt/β-catenin signaling is greatly diminished in Alzheimer's disease (AD), it remains to be elucidated whether the inhibition of this pathway is associated with dysregulation of Wnt and Rspo proteins. By analyzing temporal cortex RNA-seq data of the human postmortem brain samples, we found that *WNT1* and *RRPO2* were significantly downregulated in human AD brains. In addition, the expression of Wnt acyltransferase porcupine (*PORCN)*, which is essential for Wnt maturation and secretion, was greatly deceased in these human AD brains. Interestingly, the lowest levels of *WNT1*, *PORCN* and *RSPO2* expression were found in human AD brains carrying two copies of *APOE4* allele, the strongest genetic risk factor of late-onset AD. Importantly, there were positive correlations among the levels of *WNT1*, *PORCN* and *RSPO2* expression in human AD brains. Supporting observations in humans, Wnt1, PORCN and Rspo2 were downregulated and Wnt/β-catenin signaling was diminished in the 5xFAD amyloid model mice. In human *APOE*-targeted replacement mice, downregulation of *WNT1*, *PORCN* and *RSPO2* expression was positively associated with aging and *APOE4* genotype. Finally, *WNT1* and *PORCN* expression and Wnt/β-catenin signaling were inhibited in human *APOE4* iPSC-derived astrocytes when compared to the isogenic *APOE3* iPSC-derived astrocytes. Altogether, our findings suggest that the dysregulations of Wnt1, PORCN and Rspo2 could be coordinated

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Authors' contributions

Y.L. developed the research concept and designed the experiments. G.B. and C.-C.L. contributed to scientific discussions. J.R.M., N.W., J.Z., W.L., L.L. and Y.L. conducted the experiments and/or data analyses. N. Z. provided RNA samples of apoE-TR mice. Y.R. and T.C.I. performed analyses of the RNA-seq data of the Mayo Clinic temporal cortex human postmortem brain samples. The paper was drafted by Y.L., and all authors read, edited, and approved the manuscript.

Ethics Approval: All animal experiments were conducted in accordance with NIH guidelines for the care and use of laboratory animals and were reviewed and approved by the Mayo Clinic Institutional Animal Care and Use Committee (IACUC).

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together to diminish Wnt/β-catenin signaling in aging- and *APOE4*-dependent manners in the AD brain.

Keywords

Wnt signaling; Wnt1; Rspo2; PORCN; apoE4; Alzheimer's disease

Introduction

Wnt proteins are secreted glycoproteins which can activate β-catenin-dependent (canonical) signaling through binding to both Frizzled (Fzd) and co-receptor low-density lipoprotein receptor-related protein 6 (LRP6) or β-catenin-independent (non-canonical) signaling through binding to Fzd and other co-receptors such as ROR2 and RYK [1]. Generally, activation of non-canonical Wnt signaling can suppress canonical Wnt signaling through multiple mechanisms [2–4]. Alzheimer's disease (AD) is the most common age-dependent neurodegenerative disorder characterized by the pathological accumulation of amyloid-β (Aβ) plaques and tau-containing neurofibrillary tangles (NFTs) [5, 6]. Mounting evidence indicates that deregulated Wnt/β-catenin signaling plays an important role in the AD pathogenesis [7–9]. Critically, inhibition of Wnt/β-catenin signaling is associated with synaptic loss, neuronal loss, neurodegeneration, Aβ production and deposition, tau hyperphosphorylation, neuroinflammation and blood-brain barrier (BBB) disruption [8].

Wnt/β-catenin signaling is greatly diminished via multiple mechanisms in AD [8, 10–12]. Particularly, the level of Wnt co-receptor LRP6 is decreased in AD brains [10] and two *LRP6* SNPs and an alternative splice variant, which result in downregulation of Wnt/β-catenin signaling, are associated with an increased risk of developing AD [13, 14]. In addition, apoE4, whose gene is the strongest genetic risk factor of late-onset AD [15–17], interacts with and decreases cell surface abundance of LRP6 in astrocytes [18], and inhibits Wnt/β-catenin signaling in neuronal LRP6-expressing PC-12 cells [19]. Moreover, Wnt antagonist Dickkopf-1 (Dkk1), which binds to LRP6 on the cell surface, is elevated in postmortem AD brains and brains from transgenic mouse models for AD [20, 21], and there is a pathogenic-positive feedback loop between Dkk1 level and Aβ production [22]. Therefore, downregulation of Wnt/β-catenin signaling can occur on the cell surface of neurons and other cells in AD brains.

The Wnt/β-catenin signaling pathway is highly regulated at the cell surface by multiple secreted proteins including two major groups of endogenous Wnt signaling agonists Wnt and R-spondin (Rspo) proteins [1, 23, 24]. In addition, the maturation, secretion, and activity of Wnt proteins are regulated by post-translational modifications via stearoyl CoA desaturase (SCD), acyltransferase porcupine (PORCN), and carboxylesterase Notum [24]. While Wnt/β-catenin signaling is diminished in AD brains [8, 10, 11], it remains to be elucidated whether the suppression of Wnt/β-catenin signaling is associated with dysregulation of Wnt and Rspo proteins in AD. In this study, we examined the expression of Wnts, Rspos and Wnt post-translational modification enzymes in human AD brains, 5xFAD mice, apoE-targeted replacement (TR) mice, and human iPSC-derived astrocytes.

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Our findings suggest that dysregulation of Wnts, PORCN and Rspo2 attributes to the inhibition of Wnt/β-catenin signaling in the AD brain.

Materials and Methods

Analysis of the expression of Wnt-related genes using RNA-seq database for AD

We used RNA-seq data of 80 AD and 73 controls from the Mayo Clinic temporal cortex human postmortem brain samples. The tissue processing, RNA extraction, RNA sequencing, quality control and data normalization were previously described [25, 26]. Conditional Quantile Normalization (CQN) was previously performed on the raw gene counts to correct for GC bias and gene length differences and to obtain similar quantile-by-quantile distributions of gene expression levels across [26]. Based on the bi-modal distribution of the CQN normalized and log2-transformed reads per kb per million (RPKM), we removed genes whose average expressions in AD and control were both < 1, leaving 23,149 expressed genes. Differential gene expression analyses were performed using Partek Genomics Suite (Partek Inc., St. Louis, MO). Gene expression between groups were compared using Analyses of Variance models (ANOVA) while correcting for RNA integrity number (RIN), which significantly contributed to the variation of the gene expression values (mean F ratio > 1.5).

5xFAD mice and apoE-TR mice

5xFAD mice that overexpress human APP and PS1 with five familial AD mutations were from Jackson Labs and were kept in a heterozygote state in a C57BL/6 background. ApoE3-TR and apoE4-TR mice in which murine *Apoe* gene locus is replaced with human *APOE3* or *APOE4* genes were obtained from Taconic Biosciences [27]. All experiments were conducted in accordance with NIH guidelines for the care and use of laboratory animals and were reviewed and approved by the Mayo Clinic Institutional Animal Care and Use Committee (IACUC). 5xFAD mice (sex mixed) at 14 months of age were sacrificed. Cortex from left hemisphere was snap-frozen in liquid nitrogen immediately and stored at −80°C for further RNA extraction and qPCR and protein extraction for Western blotting. RNA extraction from cortex of female apoE-TR mice at age of 3 and 24 months was described previously [28].

Culture of iPSC-derived astrocytes

The preparation of human parental iPSC line for *APOE* ε4/ε4 (Pat-E4/E4) and its isogenic iPSC line for *APOE* ε3/ε3 (Iso-E3/3) were described previously [29, 30]. The differentiation of human iPSCs into astrocytes and culture of iPSC-derived astrocytes were performed essentially as described previously [31].

Quantitative Real-Time PCR

The real-time primer sets of mouse *WNT1* (PPM03491C-200), *PORCN* (PPM05433A-200), *RSPO2* (PPM32746A-200), *ACTIN* (PPM02945B-200) and *GAPDH* (PPM02946E-200) were purchased from Qiagen. RNA was extracted from mouse cortex samples via the Trizol/chloroform method as described [32]. Reverse transcription of RNA was performed with iScript Reverse Transcription Supermix (Bio-Rad), and real-time qPCR was conducted with

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Universal SYBR Green Supermix (Bio-Rad) using an iCycler thermocycler (Bio-Rad). The relative amount of *WNT1*, *PORCN* and *RSPO2* mRNA was normalized to *GAPDH* or *ACTIN* level as a housekeeping gene, and the data were analyzed according to the 2−ΔΔCT method.

Western blotting

Mouse cortex samples of 5xFAD mice and non-transgenic littermates were lysed with Tris-buffered saline containing 1% Triton X-100, protease inhibitor (Roche Diagnostics GmbH, Mannheim, Germany) and PHOSSTOP phosphatase inhibitor (Roche Diagnostics GmbH, Mannheim, Germany). Equal quantities of protein were subjected to SDS-PAGE under reducing conditions. Following transfer to immobilon-P transfer membrane, successive incubation with a primary antibody was carried out at 4°C overnight. The membranes were then either probed with LI-COR IRDye secondary antibodies and imaged using Odyssey infrared detection instrument (LI-COR) or incubated with horseradish peroxidase-conjugated secondary antibodies, and the immunoreactive proteins were detected by SuperSignal West Femto Chemiluminescent Substrate (Pierce). Films showing immunoreactive bands were scanned by HP Scanjet 5590 (Hewlett Packard, Palo Alto, CA). The primary antibodies and their dilutions used in this study are as follows: anti-Wnt1 (Fisher Healthcare, 365800, 1:500), anti-Rspo2 (Fisher Healthcare, 17781–1-AP, 1:400), anti-β-catenin (BD Biosciences, 610154, 1:1000), anti-β-actin (Sigma Life Sciences, A2228, 1:5000), anti-α-tubulin (Sigma Life Sciences, T9026, 1:8000).

Statistical analyses

Statistical analyses were performed with the GraphPad Prism 9 software unless noted otherwise, and all data were presented as mean values ± SEM unless elsewise indicated. For data with normal distributions, an unpaired t test was performed for comparison of two groups, and one-way ANOVA or two-way ANOVA was used with Tukey's multiple comparison test as a post-hoc test. When the data are not normally distributed, Mann-Whitney tests or Kruskal-Wallis tests with Dunn's multiple comparison tests were used. *, $P < 0.5$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

Results and Discussion

Dysregulation of *WNT1*, *WNT6*, *PORCN* and *RSPO2* expression in human AD brains

There are 19 Wnt proteins expressed in mammalian cells and many of them activate Wnt/β-catenin signaling [33]. To examine the expression of various Wnts in the AD brain, we analyzed temporal cortex RNA sequencing data of 80 AD and 73 controls from the Mayo Clinic human postmortem brain samples [25, 26]. We found 11 expressed *WNT* genes in the datasets. Among them, *WNT1* and *WNT6* displayed the most significant changes in human AD brains compared to controls (Fig. 1a–1c), although *WNT5B*, *WNT9A* and *WNT10B* were also statistically significantly different (Supplemental Fig. 1a–1c). While the level of *WNT1* was significantly decreased in human AD brains, the level of *WNT6* was significantly increased in human AD brains compared to the control cases (Fig. 1b & 1c). Wnt1 is a typical canonical Wnt [34, 35]. However, Wnt6 generally functions as a non-canonical Wnt ligand in the central nervous system [36, 37], albeit

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Wnt6 is able to activate both the canonical and non-canonical Wnt signaling pathways [38, 39]. It is generally believed that activation of non-canonical Wnt signaling results in inhibition of canonical Wnt signaling via multiple mechanisms, although it is not a universal phenomenon occurring in all non-canonical Wnts and any cell types [2–4]. Nevertheless, dysregulation of Wnt1 and Wnt6 could coordinately result in the suppression of Wnt/β-catenin signaling in the AD brain.

Post-translational attachment of palmitoleate to a conserved Ser in Wnt proteins is an essential step in the processing of Wnt ligand secretion. SCD, PORCN and Notum are three key enzymes that control Wnt acylation/deacylation [24, 40]. At the endoplasmic reticulum, SCD generates a monounsaturated fatty acid substrate for PORCN, and PORCN transfers the fatty acid to Wnt proteins [41]. At the extracellular matrix, Notum serves as a Wnt antagonist to deacylate Wnt proteins [42]. While the expression of *SCD* and *NOTUM* was not significantly changed (Supplemental Fig. 2), the expression of *PORCN* was significantly downregulated in human AD (Fig. 1d).

The Rspo protein family is a group of Wnt signaling agonists which synergize with Wnt proteins to activate Wnt/β-catenin signaling [43]. This family consists of four members (Rspo1–4) that are structurally similar and share 40–60% homology [44]. Rspo proteins function as ligands of the orphan receptors LGR4, LGR5 and LGR6 [45–48], and Rspo-LGR complexes neutralize the ubiquitin ligases RNF43 and ZNRF3 to make Wnt receptor Fzd and Wnt co-receptor LRP5/6 available at the cell surface for the activation of the Wnt/β-catenin signaling pathway [49, 50]. By analyzing RNA-seq data of the brain samples, we found that the expression of *RSPO2*, but not *RSPO1*, *RSPO3* and *RSPO4*, was significantly downregulated in human AD brains (Fig. 1e & Supplemental Fig. 3a–3c).

Having demonstrated that the expression levels of *WNT1*, *PORCN* and *RSPO2* are downregulated in human AD brains, we then examined the potential associations among the expression levels of these genes. It was found that the *PORCN* mRNA levels were positively correlated not only with the *WNT1* mRNA levels, but also with the *RSPO2* mRNA levels in human AD brains (Fig. 2a & 2c). Moreover, the *RSPO2* mRNA levels were also positively associated with the *WNT1* mRNA levels in human AD brains (Fig. 2b). These findings suggest that the dysregulations of *PORCN*, *WNT1* and *RSPO2* expression are coordinated together to dimmish Wnt/β-catenin signaling in human AD brains.

We then examined whether the expression of non-canonical *WNT6* is associated with *WNT1*, *PORCN* and *RSPO2*. It was found that the *WNT6* mRNA levels were negatively correlated with the expression levels of *WNT1*, *PORCN* and *RSPO2* (Fig. 2d–2f). These findings suggest that upregulation of *WNT6* expression could coordinate with the downregulation of *WNT1*, *PORCN* and *RSPO2* expression to diminish Wnt/β-catenin signaling in human AD brains.

AXIN2, DKK1 and NKD1 are three well-known negative feedback regulators of Wnt/β-catenin signaling [51–58]. While *DKK1* was not available in the datasets and *AXIN2* was not significantly changed, the expression of *NKD1* was significantly increased in human AD brains compared to controls (Supplemental Fig. 4a & 4b). In addition, the *NKD1* mRNA

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levels were negatively associated with the mRNA levels of *RSPO2*, *Wnt1* and *PORCN* and positively associated with the *WNT6* mRNA levels in human AD brains (Supplemental Fig. 4c–4f). Like Wnt target DKK1, NKD1 can also function as an antagonist of Wnt/β-catenin signaling [55–58]. It is well established that DKK1 is elevated in postmortem AD brains and brains from transgenic mouse models for AD [20, 21], and that there is a pathogenic-positive feedback loop with amyloid beta (Aβ) stimulating DKK1 expression, thereby promoting synapse loss and driving further Aβ production [22]. It will be interesting to determine whether there is also a pathogenic-positive feedback loop with Aβ stimulating NKD1 expression in AD brain in the future.

Downregulation of Wnt/β-catenin signaling in 5xFAD amyloid model mice

The 5xFAD mouse model expresses human APP and PS1 with five familial AD mutations and is a widely used Alzheimer mouse model with amyloid pathology [59]. 5xFAD mice display an age-dependent increase of amyloid load and significant neuron loss in cortical Layer 5 at 12 months of age [60]. To further characterize the dysregulation of Wnt/β-catenin signaling in AD, we studied the expression of *WNT1*, *PORCN* and *RSPO2* in 5xFAD mice at 14 months of age. While the mRNA levels of *WNT1* and *RSPO2* were not changed, the expression of *PORCN* was significantly decreased in 5xFAD mice (Fig. 3a–3c), suggesting that amyloidosis causes PORCN, but not Wnt1 and Rspo2, downregulation at the transcriptional level in 5xFAD mice. In addition, Western blotting analyses revealed that Wnt1 and Rspo2 were greatly downregulated in 5xFAD mice (Fig. 3d–3f), suggesting that amyloidosis causes Wnt1 and Rspo2 downregulation at the post-transcriptional level in 5xFAD mice. Consequently, we also found that the level of β-catenin was significantly decreased in 5xFAD mice (Fig. 3g), indicating that Wnt/β-catenin signaling is inhibited in 5xFAD mice.

Dysregulation of *WNT1*, *WNT6*, *PORCN* and *RSPO2* expression displays an *APOE* genotype-dependent manner in human AD brains

Human apolipoprotein E (*APOE*) gene has three polymorphic alleles (ε2, ε3 and ε4) resulted from two single-nucleotide polymorphisms in the coding region and the ε4 allele of the *APOE* gene is the strongest genetic risk factor for late-onset AD [61–63]. It has been reported that apoE4 can directly decrease the membrane availability of Wnt co-receptor LRP6 in human astrocytes [18], and inhibit Wnt/β-catenin signaling in neuronal cells [19]. To determine whether apoE4 inhibits Wnt/β-catenin signaling via downregulation of Wnt and Rspo as well in AD brains, we further analyzed the expression of *WNTs*, *RSPOs* and genes of Wnt acylation/diacylation enzymes in the temporal cortex human postmortem brain samples. We found that the expression levels of *WNT1*, *PORCN* and *RSPO2* displayed a similar *APOE* genotype-dependent manner with the lowest levels of *WNT1*, *PORCN* and *RSPO2* found in human AD brains carrying two *APOE* ε4 alleles (Fig. 4a, 4b, 4d & 4e). Moreover, the expression of non-canonical *WNT6* also displayed an *APOE* genotype-dependent manner with the highest level of *WNT6* expression found in human AD brains carrying two *APOE* ε4 alleles (Fig. 4c). Together, these results suggest that apoE4 could contribute to the downregulation of Wnt/β-catenin signaling in AD.

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The Wnt/β-catenin signaling pathway can be regulated by the RSPO-LGR-RNF43/ZNRF3 module [49, 50]. It is interesting to note that both *LGR4* and *ZNRF3* were significantly upregulated in human AD brains (Supplemental Fig.3d & 3g), while *LGR5* and *RNF43* were not changed and *LGR6* was found unavailable in the datasets (Supplemental Fig. 3e & 3h). Moreover, the expression of *ZNRF3*, but not *LGR4*, displayed a *APOE* genotype-dependent manner with the highest level of *ZNRF3* found in human AD brains carrying two *APOE* ε4 alleles (Supplemental Fig. 3f & 3i). In addition, the *ZNRF3* mRNA levels were negatively associated with the mRNA levels of *RSPO2*, *Wnt1* and *PORCN* and positively associated with the *WNT6* mRNA levels in human AD brains (Supplemental Fig. 5), suggesting that *ZNRF3* could also play a role in dysregulation of Wnt/β-catenin signaling in AD.

Interestingly, the expression levels of *WNT5B*, *WNT7A* and *WNT10B* did not display an *APOE* genotype-dependent manner, although their expression levels were statistically significantly changed (Supplemental Fig. 1), further suggesting that the dysregulation of *WNT1*, *WNT6*, *PORCN* and *RSPO2* is more significant than that of *WNT5B*, *WNT7A* and *WNT10B* in the AD brain.

Downregulation of *WNT1*, *PORCN* and *RSPO2* in aged apoE-targeted replacement (TR) mice

ApoE-TR mice, also known as humanized apoE mice, have the targeted replacement of mouse *Apoe* gene with each of the human *APOE* gene alleles. These mice are widely used to investigate the function(s) of apoE in physiologically relevant conditions [28, 64, 65]. Having demonstrated that downregulation of the *WNT1*, *PORCN* and *RSPO2* expression displays an *APOE* genotype-dependent manner, we then studied the expression of these genes in apoE3-TR and apoE4-TR mice at 3 and 24 months of age. It was found that the *WNT1* expression in apoE4-TR mice was significantly decreased at 24 months of age when compared to the age-matched apoE3-TR mice (Fig. 5a). Moreover, the *WNT1* expression in both apoE3-TR and apoE4-TR mice at 24 months of age was significantly lower than that at 3 months of age (Fig. 5a). For *PORCN*, its expression was significantly lower in apoE4-TR mice at 3 months of age when compared to apoE3-TR mice, although there was no further suppression in apoE4-TR mice at 24 months of age (Fig. 5b). For *RSPO2*, while there were no *APOE* genotype effects on its expression, this gene was significantly downregulated in aged apoE-TR mice (Fig. 5c). Altogether, these results suggest that both aging and apoE4 are associated with downregulation of Wnt/β-catenin signaling in AD.

Downregulation of *WNT1* and *PORCN* and inhibition of Wnt/β-catenin signaling in human iPSC-derived astrocytes carrying *APOE4*

Human induced pluripotent stem cell (iPSC) technology has shown great promises in pathological analyses of neurodegenerative diseases [66]. ApoE in the brain is mainly produced by astrocytes and to a lesser extent from microglia and other brain cells [67]. Therefore, the effects of *APOE* genotype on *WNT1*, *PORCN* and *RSPO2* expression were further evaluated in isogenic human iPSC-derived astrocytes carrying *APOE* ε4/ε4 (Par-E4/E4) or *APOE* ε3/ε3 (Iso-E3/E3). While *RSPO2* expression was undetectable by quantitative qPCR, *WNT1* expression was dramatically decreased in *APOE4* astrocytes when compared to *APOE3* astrocytes (Fig. 6a). There was a slight reduction in *PORCN*

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expression, and its protein level was greatly downregulated in *APOE4* astrocytes when compared to *APOE3* astrocytes (Fig. 6b–6d), indicating that PORCN is downregulated at both the transcriptional and post-transcriptional levels. Importantly, the level of β-catenin was significantly deceased in *APOE4* astrocytes (Fig. 6e). Thus, downregulation of Wnt1 and PORCN could be associated with the inhibition of Wnt/β-catenin signaling in iPSC-derived astrocytes carrying *APOE4*.

Conclusion

By examining the expression of Wnts, Rspos and Wnt acylation/diacylation enzymes in temporal cortex of human postmortem brain samples, 5xFAD mice and apoE-TR mice, and iPSC-derived astrocytes carrying *APOE4* or *APOE3*, the current study demonstrates that the expression of Wnt1, Wnt6, PORCN and Rspo2 is dysregulated in apoE4- and aging- dependent manners in AD, which is associated with the inhibition of Wnt/β-catenin signaling in the AD brain. Specifically, downregulation of *WNT1*, *PORCN* and *RSPO2* expression could directly result in the inhibition of Wnt/β-catenin signaling, while upregulation of non-canonical *WNT6* could indirectly suppress Wnt/β-catenin signaling. Our findings support the notion that restoring Wnt/β-catenin signaling is an attractive therapeutic strategy for disease-modifying treatment of AD [8]. Notably, Wnt1-mediated Wnt/β-catenin signaling is specifically required for midbrain dopaminergic neuron progenitor cell specification, proliferation and neurogenesis [68–71], and Wnt1 can enhance microglial integrity and prevent microglia loss during Aβ-induced early and late apoptotic injury [72]. In this study, we have demonstrated that *Wnt1, PORCN, Rspo2* and *Wnt6* are dysregulated at the transcriptional levels in human AD brains, it will be important to study the changes of Wnt1, PORCN, Rspo2 and Wnt6 at protein levels in the future. Moreover, the pathological significance of dysregulation of Wnt1, PORCN, Rspo2 and Wnt6 as well in AD warrants further investigations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Availability of Data and Material

All data generated during this study are included in this article or are available on reasonable request from the corresponding author.

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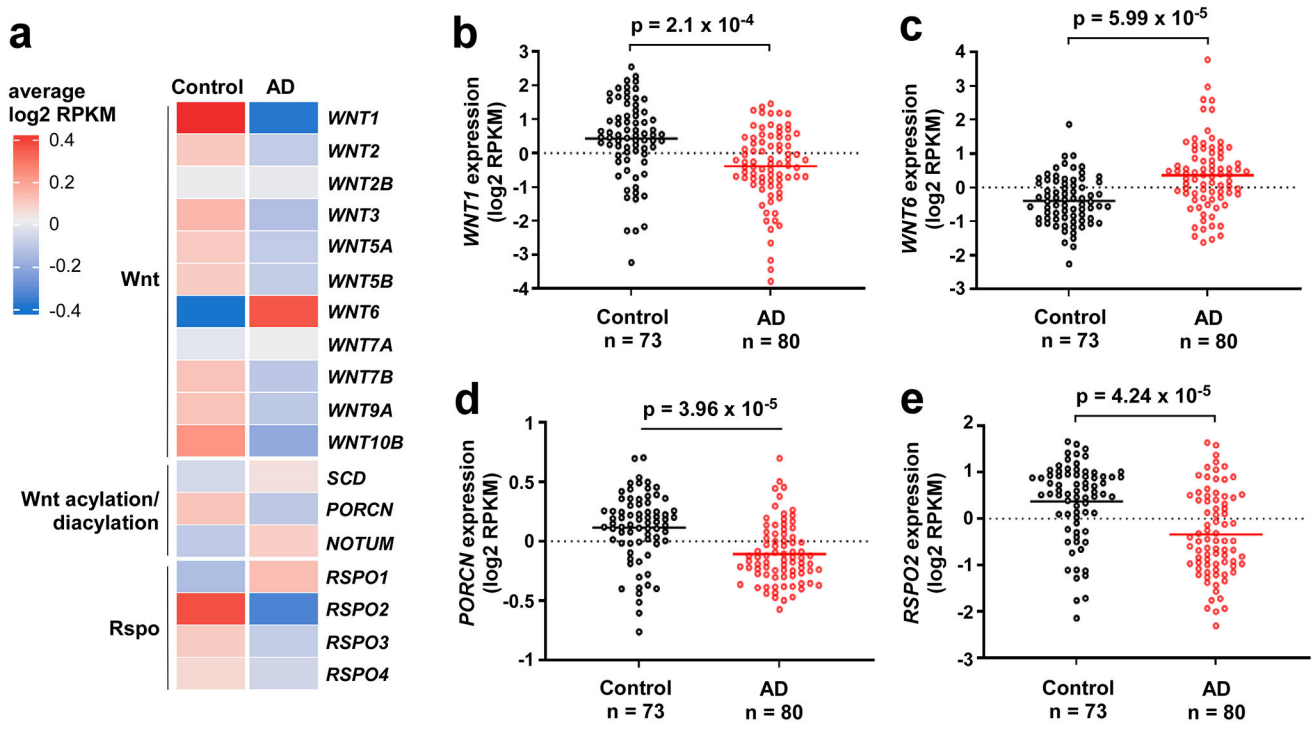

Fig. 1. Dysregulation of *WNT1, WNT6, PORCN* and *RSPO2* expression in human AD brains.

The expression levels of *WNTs*, *RSPOs*, and genes of Wnt acylation/diacylation enzymes were extracted from the temporal cortex RNA sequencing data of the Mayo Clinic human postmortem brain samples. (**a**) The heat map of differentially expressed mRNAs of *WNT*s, *RSPO*s, and genes of Wnt acylation/diacylation enzymes in AD brains and controls. (**b-e**) Comparison of *WNT1* (**b**), *WNT6* (**c**), *PORCN* (**d**) and *RSPO2* (**e**) expression between AD brains and controls. Gene expression levels between groups were compared using Analyses of Variance models (ANOVA) as described in the section of Materials and methods.

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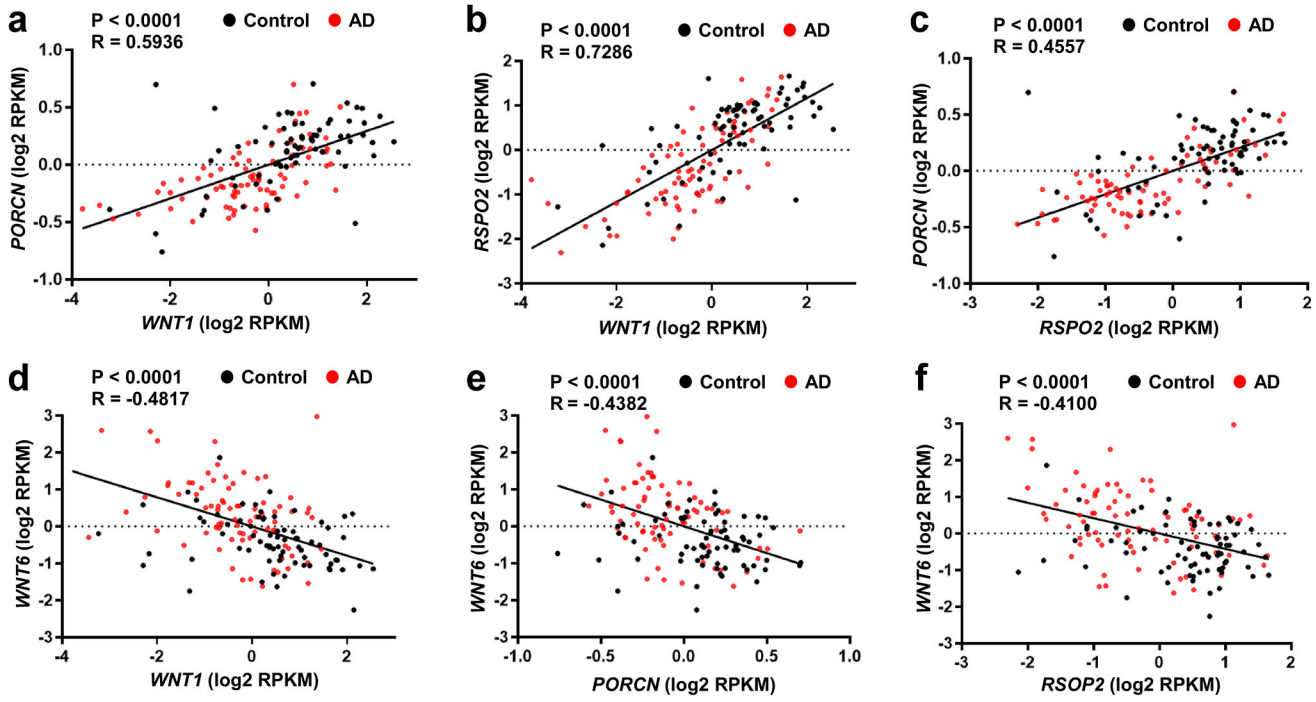

Fig. 2. Correlations among *PORCN, RSPO2, WNT1* and *WNT6* expression in human AD and controls brains.

The expression levels of *PORCN, RSPO2, WNT1* and *WNT6* were obtained from the RNA sequencing data of the temporal cortex human postmortem brain samples. The associations among *PORCN*, *RSPO2*, *WNT1* and *WNT6* expression were analyzed in human 80 AD brains and 73 controls by linear regression with the GraphPad Prism 9 software. The values of R and p were listed.

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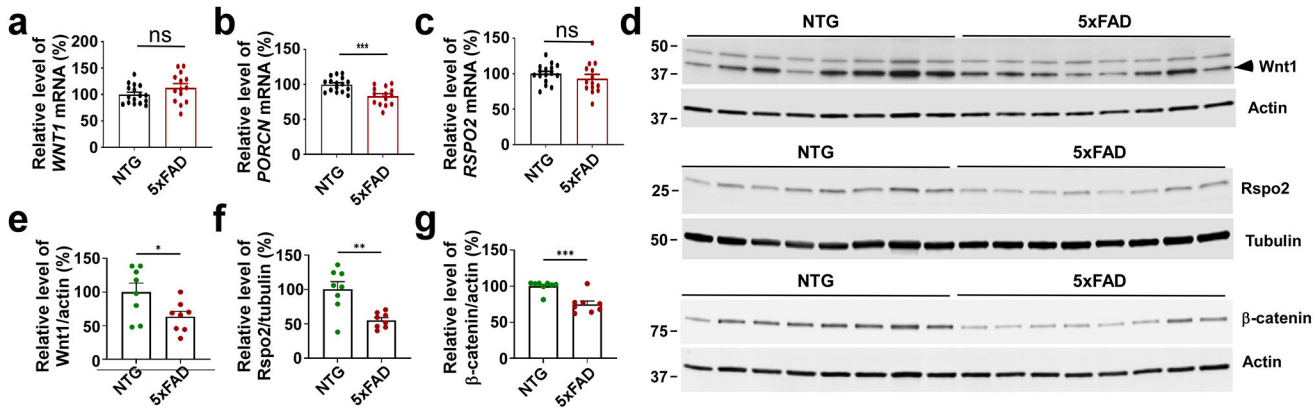

Fig. 3. Downregulation of Wnt/β-catenin signaling in the 5xFAD amyloid model mice.

(**a-c**) The mRNA levels of *WnT1*, *PORCN* and *RSPO2* in the cortex of 5xFAD mice (6 male and 8 female) and non-transgenic (NTG) littermate controls (8 male and 8 female) were examined by quantitative qPCR. (**d-g**) The protein levels of β-catenin, Wnt1 and Rspo2 in the cortex of 5xFAD mice (4 male and 4 female) and non-transgenic (NTG) littermate controls (4 male and 4 female) were examined by Western blotting and normalized with actin or tubulin level. The allow points the position of the 41 kDa band of Wnt1. Unpaired t-test. ns, no significance; *, P < 0.05; **, p < 0.01; *** p < 0.001.

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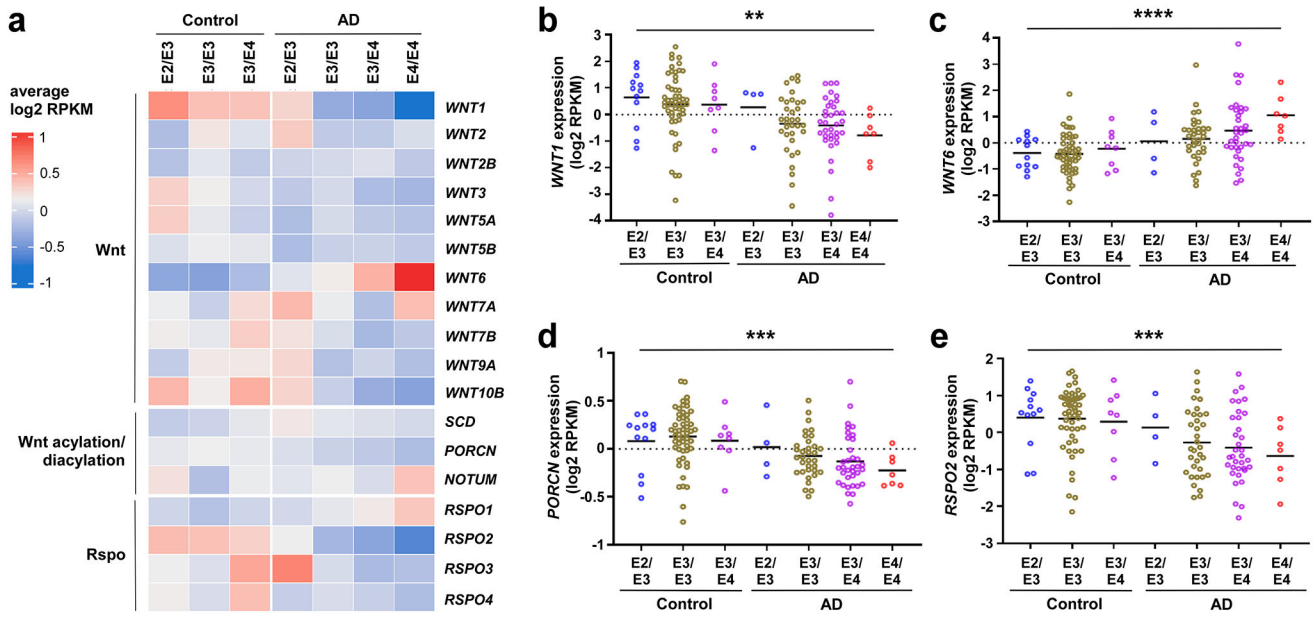

Fig. 4. The expression of *WNT1, WNT6, PORCN* and *RSPO2* displays an *APOE* genotype-dependent manner in human AD brains.

The expression levels of *WNT1*, *WNT6*, *PORCN* and *RSPO2* were generated from the RNA sequencing data of the temporal cortex human postmortem brain samples. Eighty AD samples were grouped by *APOE* genotype into *APOE2*/*APOE3* (n = 4), *APOE3*/*APOE3* (n = 34), *APOE3*/*APOE4* (n = 35) and *APOE4*/*APOE4* (n = 7). Seventy-three control samples were grouped into *APOE2*/*APOE3* (n = 12), *APOE3*/*APOE3* (n = 53), *APOE3*/*APOE4* (n = 8). (**a**) The heat map of differentially expressed mRNAs of *WNTs*, *RSPOs* and genes of Wnt acylation/diacylation enzymes and in AD brains and controls sub-grouped by apoE isoforms. (**b-e**) Comparison of *WNT1* (**b**), *WNT6* (**c**), *PORCN* (**d**) and *RSPO2* (**e**) expression among AD brains and controls with different *APOE* genotypes. One-way ANOVA tests. Difference among means of 7 groups: **, $p < 0.01$; ***, $p < 0.001$, ****, $p < 0.0001$.

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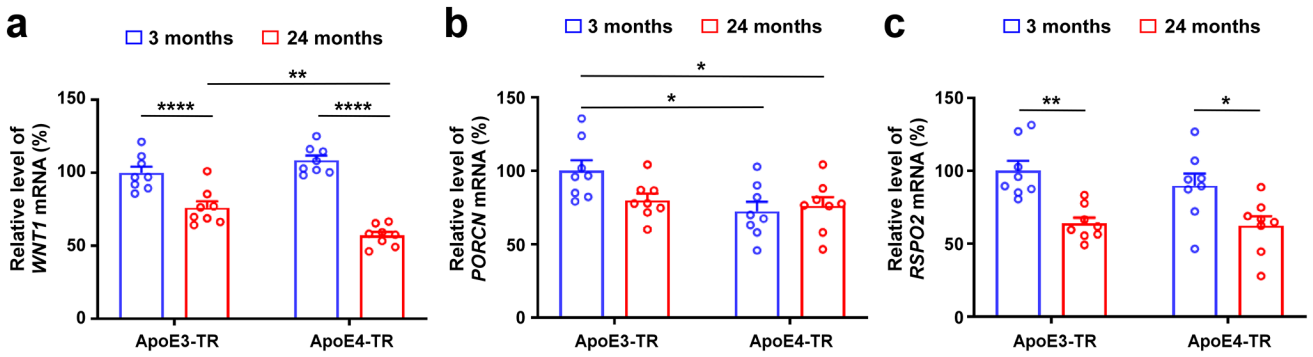

Fig. 5. Downregulation of *WNT1*, *PORCN* and *RSPO2* in apoE-TR mice.

The expression of *WNT1*, *PORCN* and *RSPO2* was analyzed by qPCR in the cortex of female apoE-TR mice at the age of 3 and 24 months. Two-way ANOVA tests. * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$. N = 8 mice per group.

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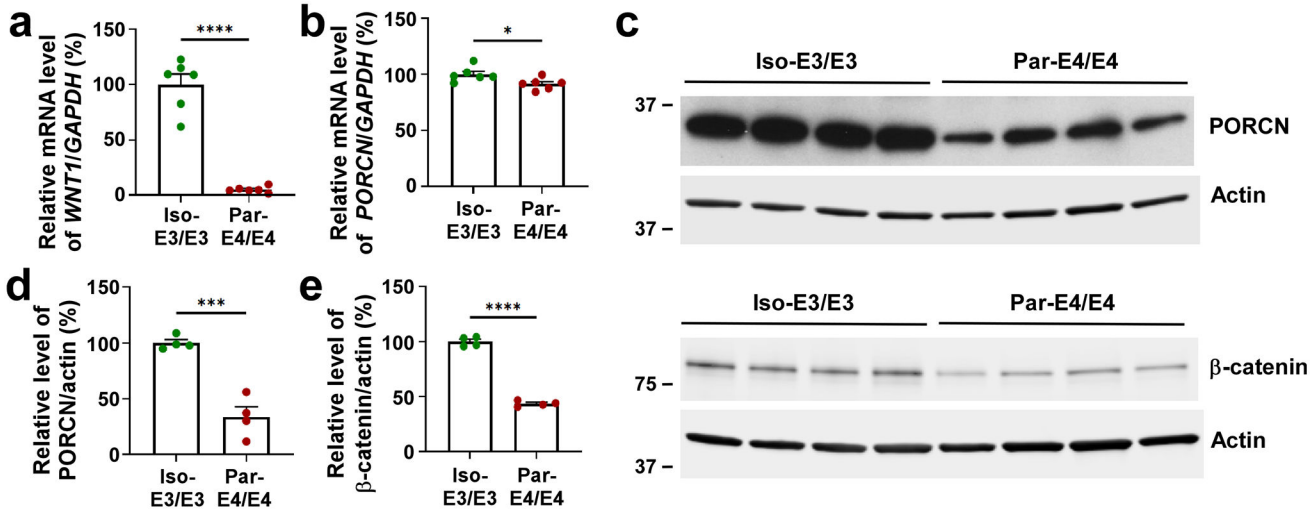

Fig. 6. Isogenic conversion of *APOE4* to *APOE3* results in upregulation of Wnt/β-catenin signaling in iPSC-derived astrocytes.

(**a, b**) The expression of *WNT1* and *PORCN* was analyzed by qPCR in isogenic iPSC-derived astrocytes carrying *APOE* ε4/ε4 (Par-E4/E4) or *APOE* ε3/ε3 (Iso-E3/E3) (n = 6 clones). (**c-e**) The protein levels of PORCN and β-catenin were examined by Western blotting (n = 4 clones) in isogenic iPSC-derived astrocytes. Unpaired t-test. ns, no significance; ***, $p < 0.001$; **** $p < 0.0001$.