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Rapamycin as a preventive intervention for Alzheimer's disease in APOE4 carriers: targeting brain metabolic and vascular restoration

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Alzheimer's disease (AD) is the most common form of dementia, affecting over 50 million people worldwide. This figure is projected to nearly double every 20 years, reaching 82 million by 2030 and 152 million by 2050 (Alzheimer's Disease International). The apolipoprotein ε4 (APOE4) allele is the strongest genetic risk factor for late-onset AD (after age 65). Apolipoprotein E, a lipid transporter, exists in three variants: ε2, ε3, and ε4. APOE ε2 (APOE2) is protective against AD, APOE ε3 (APOE3) is neutral, while APOE4 significantly increases the risk. Individuals with one copy of APOE4 have a 4-fold greater risk of developing AD, and those with two copies face an 8-fold risk compared to non-carriers. Even in cognitively normal individuals, APOE4 carriers exhibit brain metabolic and vascular deficits decades before amyloid-beta (Aβ) plaques and neurofibrillary tau tangles emerge—the hallmark pathologies of AD (Reiman et al., 2001, 2005; Thambisetty et al., 2010). Notably, studies have demonstrated reduced glucose uptake, or hypometabolism, in brain regions vulnerable to AD in asymptomatic middle-aged APOE4 carriers, long before clinical symptoms arise (Reiman et al., 2001, 2005).

Early intervention to preserve brain metabolic function may be crucial in preventing the onset of AD or slowing its progression in APOE4 carriers. A recent study by Sanganahalli et al. (2024) demonstrated that Rapamycin, an anti-aging intervention, restored neuronal mitochondrial function and synaptic activity in young asymptomatic APOE4 mice. This study utilized an advanced APOE4 mouse model (the E4FAD mice) and compared the results with APOE3 mice (the E3FAD mice). Healthy young E4FAD and E3FAD mice were fed Rapamycin 16 weeks prior to the development of Aβ plaques and cognitive impairments. To assess mitochondrial oxidative metabolism and neurotransmission rates, they employed a novel *in vivo* proton-observed carbon-edited (^{1}H-[^{13}C]) magnetic resonance spectroscopy technique alongside *ex vivo* mitochondrial respiration measurements using the Seahorse platform.

The *in vivo* proton-observed carbon-edited data enabled the calculation of total glutamate–glutamine neurotransmitter cycling (V_{cycle}) and total neuronal TCA cycle ($V_{TCA,N}$), as shown in **Figure 1A**. Neuronal glucose oxidation ($CMR_{glc(ox),N}$) was derived as half of $V_{TCA,N}$. The analysis revealed that Rapamycin significant increases in V_{cycle} fluxes in both E3FAD (E3FAD-Rapa) and E4FAD (E4FAD-Rapa) mice compared to their respective controls (**Figure 1B**). However, only the E4FAD-Rapa group exhibited a significant increase in $V_{TCA,N}$ compared to its controls (**Figure 1C**). To facilitate cross-comparisons, they used the ratio of V_{cycle} to $CMR_{glc(ox),N}$ as an index. A notable increase in this ratio was found in the E4FAD-Rapa group compared to the E4FAD control (E4FAD-Ctrl) group (**Figure 1D**). Their results highlight that Rapamycin enhances synaptic and mitochondrial activities in young, healthy E4FAD mice. This is particularly important because brain metabolic deficits are now recognized as playing a more critical role in driving severe cognitive impairment to severe stages than Aβ and tau levels (Hammond et al., 2020). Therefore, the findings imply that Rapamycin may reduce AD risk for APOE4 carriers and slow disease progression by preserving brain metabolism.

Brain metabolism is closely linked to vascular function, and findings from Sanganahalli et al. align with previous reports demonstrating Rapamycin's ability to improve brain vascular function and reduce Aβ deposits in the brain, particularly in the context of cerebral amyloid angiopathy. These improvements are critical for preventing microvascular disruptions associated with AD. A study by Lin et al. in 2013 highlighted Rapamycin's role as a nitric oxide-dependent vasodilator, essential for restoring cerebral blood flow in AD mouse models and reducing cerebral amyloid angiopathy-related Aβ accumulation linked to vascular damage. Further, Lin et al. in 2020 demonstrated that in E4FAD mice, Rapamycin restored cerebral blood flow (especially in females), enhanced blood-brain barrier activity for Aβ transport, stabilized neurotransmitter levels, preserved neuronal integrity, reduced free fatty acid levels, improved spatial memory, and decreased Aβ retention (Lin et al., 2020). These findings underscore Rapamycin's potential as a preventive therapy by targeting both brain vascular and metabolic pathways in cognitively normal APOE4 carriers, offering a promising strategy to reduce the risk of AD progression.

Rapamycin, a macrocyclic lactone, was first discovered in 1975. It was isolated from the bacterium *Streptomyces hygroscopicus* in soil samples collected on Easter Island. Initially investigated as an antifungal agent, Rapamycin gained US Food and Drug Administration approval in 1999 as an immunosuppressant to prevent organ transplant rejection (marketed as Sirolimus or Rapalog). By the early 1990s, research identified Rapamycin as an inhibitor of the mechanistic target of rapamycin (mTOR), a critical nutrient sensor and regulator of cellular growth, proliferation, and survival in eukaryotic cells (reviewed by Richardson et al., 2015).

The mTOR pathway is central to maintaining cellular growth, autophagy, and metabolic balance in neurons—processes vital for brain health. In AD, dysregulated mTOR signaling is linked to impaired autophagy and the accumulation of toxic proteins, including Aβ plaques and hyperphosphorylated Tau, which drive neurodegeneration. Additionally, excessive mTOR activation is associated with heightened oxidative stress, mitochondrial dysfunction, and neuroinflammation, all of which exacerbate cognitive decline in AD (Davoody et al., 2024).

Beyond its role as an mTOR inhibitor, rapamycin exerts diverse effects on immune modulation and cellular homeostasis through both mTOR-dependent and independent pathways. It modulates immune responses by influencing T-cell activation and differentiation, promoting immune tolerance through enhanced regulatory mechanisms. Furthermore, rapamycin induces autophagy, a critical cellular process for degrading and recycling damaged proteins and organelles. By activating autophagy, rapamycin may reduce Tau pathology and Aβ accumulation—key drivers of AD progression. This autophagic activation underpins rapamycin's neuroprotective properties, offering the potential for alleviating AD pathology by clearing neurotoxic aggregates (Davoody et al., 2024).

Since rapamycin is already US Food and Drug Administration-approved, recent studies have focused on examining the safety and effectiveness of low-dose or intermittent dosing regimens in older adults. Unlike the high doses (e.g., 5 mg/day) traditionally used as an immunosuppressant in organ transplantation, preliminary findings suggest that lower doses (e.g., 1 mg/day or 6 mg/week) may safely improve organ function and alleviate age-related conditions. Notably, some research suggests that low or intermittent doses of mTOR inhibitors can enhance immune function and reduce the risk of infections in older adults, without exhibiting immunosuppressive effects and with minimal side effects (Mannick and Lamming, 2023).

The availability of direct-to-consumer genetic testing has made it easier for individuals to access their APOE status. Narasimhan et al. (2024) highlight the critical role of APOE4 in AD progression and discuss emerging APOE-targeted therapies that could transform clinical care. They emphasize the need to integrate APOE genotype testing into routine practice to enable personalized treatment strategies for AD. However, they also caution that knowledge of genetic risk, without actionable interventions, may increase stress and anxiety in individuals. Recent preclinical studies by Sanganahalli et al. (2024) and Lin et al. (2020) propose promising preventive strategies for middle-aged, presymptomatic APOE4 carriers. Notably, since 2017, over 1500 healthy APOE4 carriers have reportedly pursued off-label rapamycin (Sirolimus) therapy (Rapamycin Therapy), fueling discussions about the potential of early intervention to mitigate AD risk in this high-risk population.

It is important to note that the response to rapamycin varies significantly by APOE genotype. Studies by Sanganahalli et al. (2024) and Lin et al. (2020) revealed that mice carrying the human APOE ε3 allele (E3FAD mice), a neutral AD risk factor, responded differently to rapamycin compared to E4FAD mice. While rapamycin enhanced mitochondrial oxidative metabolism and excitatory neurotransmission in E4FAD mice, it promoted glycolysis and inhibitory neurotransmission in E3FAD mice, without improvements in cerebral blood flow or Aβ clearance. These genotype-specific effects highlight the necessity of personalized therapeutic approaches, emphasizing the importance of incorporating genetic profiling into clinical trials and treatment strategies.

Taken together, emerging research continues to emphasize the pivotal role of APOE4 in AD and the significant potential of early interventions aimed at correcting metabolic dysfunction in APOE4 carriers. The study by Sanganahalli et al. (2024) demonstrates that rapamycin can restore mitochondrial function (**Figure 1E**, circle 1) and synaptic activity (**Figure 1E**, circle 2) in asymptomatic APOE4 mice before the onset of AD-related pathology. These findings align with growing evidence that deficits in glucose metabolism are closely tied to cognitive decline in AD, underscoring the importance of metabolic preservation as a preventive strategy. Beyond its metabolic effects, rapamycin has shown vascular and neuroprotective benefits in preclinical models, including improved brain vascular function (**Figure 1E**, circle 3), reduced Aβ deposition (**Figure 1E**, circle 4), and enhanced cognitive performance (**Figure 1E**, circle 5), particularly in APOE4 carriers (Lin et al., 2020).

Given its US Food and Drug Administration approval and established safety profile at lower or intermittent dosing regimens, rapamycin represents a compelling candidate for preventive AD therapy (**Figure 1E**). Tailoring its use based on APOE genotype and metabolic profile could optimize its efficacy, ensuring that treatment is personalized to the specific needs and risks of each patient. Future therapeutic approaches could greatly benefit from combining rapamycin with other targeted treatments, such as anti-Aβ antibodies, tau modulators, or anti-inflammatory agents, to tackle the multifaceted nature of AD and enhance therapeutic outcomes (Dyck, 2018). Advances in genetic testing, biomarker discovery, and therapeutic innovation are paving the way for a precision medicine approach to AD prevention and treatment. Rapamycin's dual capacity to address metabolic and neurovascular protection makes it a key component in this evolving therapeutic landscape, particularly for individuals with heightened genetic risk such as APOE4 carriers. By integrating these advancements, the field is poised to deliver more effective and personalized solutions for combating AD.

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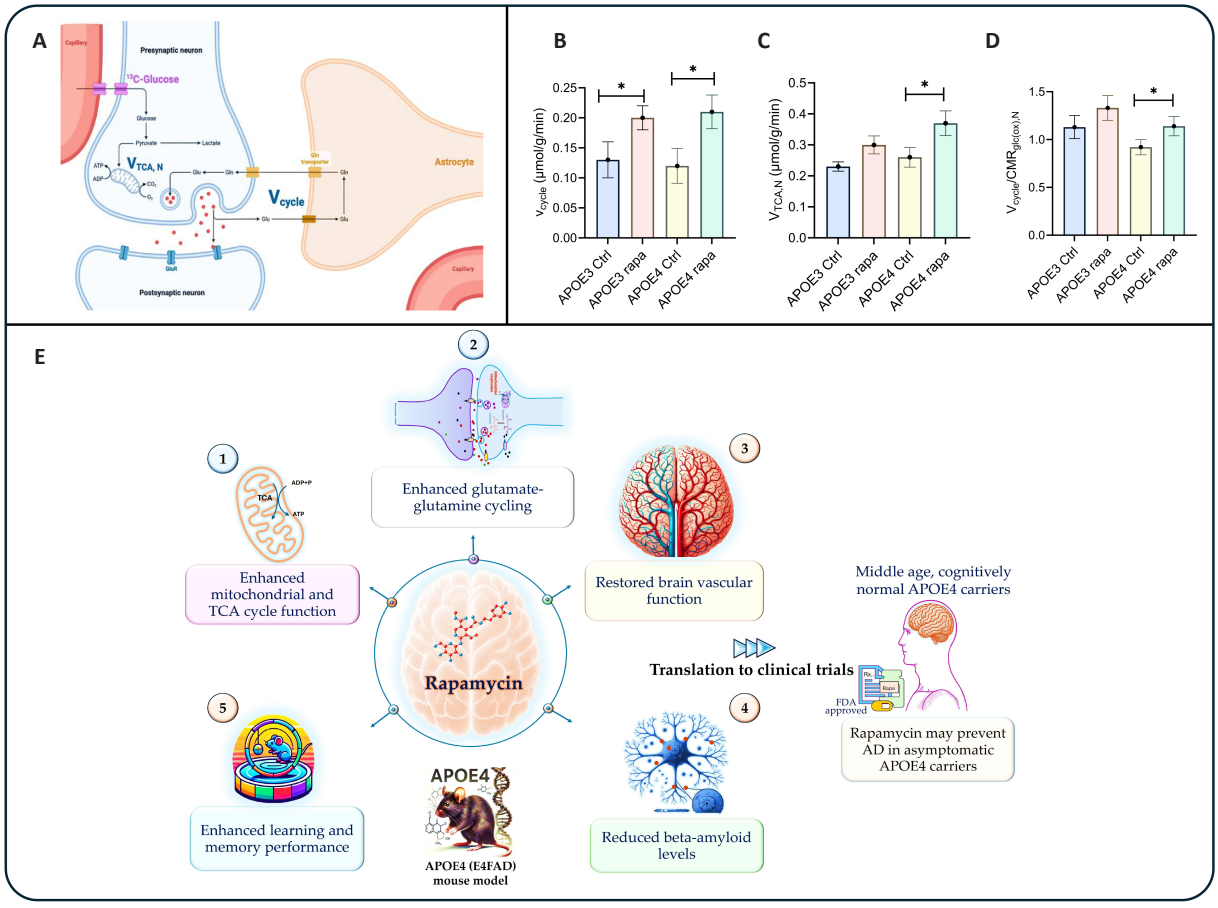

Figure 1 | **Potential of rapamycin in promoting brain metabolic function and further translation to clinical trials in Alzheimer's disease.**
(A) Schematic representation illustrating resting glutamate-glutamine neurotransmitter cycling (V_{cycle}) and neuronal TCA cycle ($V_{TCA,N}$). (B) Rapamycin significantly increased glutamate-glutamine neurotransmitter cycling (V_{cycle}) in both E3FAD and E4FAD mice. (C) Neuronal TCA cycle ($V_{TCA,N}$) was unchanged in E3FAD mice but significantly increased in E4FAD mice. (D) Rapamycin significantly increased the $V_{cycle}/CMR_{glc(ox),N}$ ratio in E4FAD, but not E3FAD. *P < 0.05; the error bars represent the standard deviation. (E) Summary of the proposed benefits of rapamycin treatment in APOE4 mouse models of Alzheimer's disease. Rapamycin enhances (1) mitochondrial function, (2) improves glutamate-glutamine cycling, (3) restores brain vascular function, (4) reduces beta-amyloid levels, and (5) enhances cognitive performance. Translation to clinical trials is suggested for middle-aged, cognitively normal APOE4 carriers to evaluate rapamycin's preventive potential against Alzheimer's disease. ##Circles (1–2) were reported in Sanganahalli et al. (2024) and (3–5) were reported in Lin et al. (2020). Created with GraphPad Prism and Microsoft PowerPoint. AD: Alzheimer's disease; ADP + P: adenosine diphosphate plus phosphate; APOE4: apolipoprotein E4; ATP: adenosine triphosphate; CMRglc(ox),N: cerebral metabolic rate of glucose oxidation in neurons; Ctrl: control; E3FAD: APOE3 familial Alzheimer's disease mouse model; E4FAD: APOE4 familial Alzheimer's disease mouse model; FDA: U.S. Food and Drug Administration; IHC: immunohistochemistry; rapa: rapamycin; TCA: tricarboxylic acid; Vcycle: glutamate-glutamine neurotransmitter cycling rate; VTCA,N: neuronal TCA cycle rate.

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