Biomarker-guided drug therapy: personalized medicine for treating Alzheimer's disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with significant memory decline and cognitive impairment. AD is characterized by two classical neuropathological hallmarks, namely the amyloid-beta (Aβ) plaques and neurofibril tangles. Currently, there are no disease-modifying treatments available for AD, except for a couple of the US Food and Drug Administration (FDA)-approved drugs to improve cognitive function by blocking N-methyl-D-aspartate receptors or cholinesterase activity (Panza et al., 2019). While these drugs offer some symptomatic relief against AD, they do little to halt the progression of the disease. For over two decades, the amyloid cascade hypothesis of AD has been the central focus for the development of biomarkers and disease-modifying therapeutic strategies, supported by strong genetic, biochemical and histopathological evidence. Unfortunately, over 15 years of clinical failure with several classes of anti-Aβ drugs that affect the formation, aggregation and clearance of Aβ have made the research community rethink the strategies to develop appropriate treatments for AD (Panza et al., 2019). AD is characterized by a vast heterogeneity in its pathophysiology that is influenced by several risk factors such as aging, lifestyle, and genetic and environmental changes. The complex etiology of the disease, coupled with the failure of past clinical interventions directed at a "fit-for-all" therapy, demands a change in therapeutic strategies for an effective and more favourable outcome against AD. There is thus, a need for the development of tailored/targeted therapy for specific AD subpopulations that share distinct genetic, molecular or pathological properties. In this regard, our perspective discusses three potential molecular biomarkers, namely monoacylglycerol lipase (Mgll), apolipoprotein E4 (APOE4) and the phosphatidylinositol 3-kinase (PIK3)/protein kinase (AKT)/glycogen synthase kinase-3β (GSK-3β) signaling pathway, as prime candidates for targeted therapy.

Mgll as a biomarker for metformin-targeted therapy against AD: We provided the first evidence that metformin, an FDA-approved drug, when given systemically, can enhance adult neurogenesis and improve spatial memory in normal adult mice by targeting a signaling-directed epigenetic pathway involving an atypical protein kinase C (aPKC)-mediated histone acetyltransferase phosphorylation of CREB-binding protein (CBP) at Ser436 site, about 8 years ago. This study raised an interesting question regarding the efficacy of metformin in treating AD-associated memory decline. Since diabetes or hyperinsulinemia is a potential risk factor for AD and metformin is an anti-diabetic drug, multiple retrospective and prospective human studies have been conducted to test the effects of metformin on AD-associated memory decline. However, these studies have yielded controversial results. While some epidemiological studies suggest that metformin improves AD-associated cognition, others have conversely found that long-term use of metformin may instead cause symptoms of dementia (Moore et al., 2013; Koenig et al., 2017). Additionally, using different AD animal models, researchers have further shown the opposite effects of metformin on AD pathology (Barini et al., 2016; Farr et al., 2019). Together, these studies suggest that metformin treatment may only be beneficial to a subpopulation of AD patients. Thus, it is imperative to probe molecular targets that metformin directly acts on to improve memory as well as understand how the same molecular pathway may be perturbed in AD and contribute to its pathogenesis. In this regard, following our early work showing that metformin enhances neurogenesis and spatial memory through the aPKC-CBP pathway, we further revealed that the aPKC-CBP pathway is also activated in an age-dependent manner to maintain homeostatic neurogenesis and spatial memory during the aging process. In our recent publication, we found that 3xTg mice, a murine AD model, exhibited an age-dependent impairment of the aPKC-CBP pathway characterized by perturbed homeostatic neurogenesis and spatial memory (Syal et al., 2020). Importantly, using our transgenic mouse model CbpS436A, where the aPKC-CBP pathway is deficient, together with RNA-seq analysis, we identified that monoacylglycerol lipase (Mgll) gene expression is directly repressed upon activation of the aPKC-CBP pathway with metformin treatment. Coincidently, we also found that Mgll levels were abnormally upregulated in 3xTg mice during the aging process and that metformin was able to reactivate the impaired aPKC-CBP pathway to repress Mgll expression, thus rescuing both hippocampal neuronal differentiation and spatial memory deficits in 3xTg mice (Syal, et al., 2020). In this regard, Mgll is a perfect candidate biomarker to identify prospective patients in the early stages of AD that are best suited to receive metformin as a treatment against the disease. Since the aging process in the context of neural stem cell function starts early in young adults, aging-related molecular changes are often triggered to maintain homeostatic neurogenesis for the formation of new memory throughout adulthood. However, a perturbation of these molecular changes during pathological aging, resulting in impaired neurogenesis and memory decline, may represent early signs/biomarkers of AD, which can be used for screening for targeted therapy in the early stages of the disease. Incidentally, other animal-based studies have suggested that Mgll is a promising therapeutic target to ameliorate AD-associated neuropathology and memory decline (Chen et al., 2012). However, despite the promising therapeutic potential of Mgll against AD, there are no FDA-approved drugs that target Mgll other than a couple of Mgll inhibitors currently in Phase II clinical trials. By elucidating how Mgll is (mis)regulated in the context of aging and AD, our research provides a strong rationale to develop a clinical protocol where abnormal Mgll levels in AD patients could be used to identify potential metformin-responsive patients for targeted therapy (Syal et al., 2020).

APOE4 as a candidate for targeted therapy against AD: The presence of the E4 allele of APOE4 is the most prevalent genetic risk factor for AD. APOE4 is expressed by more than half of AD patients and is thus an important potential therapeutic target against AD. While apolipoprotein E (APOE) also occurs in two other polymorphic forms, APOE2 and APOE3, the carriers of APOE4 are more likely to develop AD. Further, it has been reported that APOE4 carriers present AD-associated cognitive changes much earlier in a dose-dependent effect. In contrast, APOE2 carriers exhibit a resistant effect relative to APOE3 and APOE4 carriers. Thus, while APOE4 protein appears to be susceptible, APOE2 may be resistant against AD (Safieh et al., 2019). Researchers have identified multiple ways in which APOE4 contributes to AD neuropathology. Not only does APOE4 trigger Aβ accumulation and increase tauopathy, but it also speeds up the development of AD-like cerebral glucose hypo-metabolism decades before the onset of the clinical features of AD. Additionally, APOE4 is known to enhance and prolong the neuroinflammatory response by stimulating the activation of microglia and the levels of proinflammatory cytokines (Safieh et al., 2019). However, it must be noted that several other factors can influence the risk of AD development in conjugation with or even independent of the APOE4 carrier status. Some of these factors include lifestyle, pharmacogenomics, AD comorbidities such as diabetes, and other biological and behavioral factors. Interestingly, studies have shown that other AD risk factors like hyperlipidemia may affect carriers of APOE4 differently than APOE4 non-carriers (Berkowitz et al., 2018). Therefore, any therapeutic strategies targeting APOE4 must take these other factors into account to ensure effectiveness.

Several targeted therapeutic strategies against APOE4 are currently being developed (Safieh et al., 2019). One such approach is anti-APOE4 immunotherapy involving the use of antibodies to target and neutralize APOE4 protein. Another strategy is the development of small molecules to block APOE4 domain interactions to counteract its pathological effects. Since APOE4 is susceptible to degradation by neuronal proteases that yield neurotoxic fragments, the identification of these proteases and the development of inhibitors against them will also help against APOE4 toxicity. In addition, since APOE4 lowers the levels of APOE2 receptors, one possible therapeutic approach could be to increase the expression of APOE2 receptors to promote its "protective" effect versus the "toxic" effect of APOE4. In general, the landscape of human APOE4-targeted therapies is currently bare, and advanced animal-based studies are needed to provide a strong rationale to translate these findings from the laboratory to the clinic (Safieh et al., 2019).

Finally, the recent development of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) gene-editing technology will make *in vivo* editing of APOE4 possible. However, it is important to note that the CRISPR

technique is still in its infancy and that new data regarding possible off-target effects and mosaicism, where not all copies of the target gene are edited, continue to be reported (Safieh et al., 2019).

PI3K/AKT/GSK3β signaling as biomarkers for targeted therapy against AD: GSK-3β activity has been well-known to play a critical role in the abnormal hyper-phosphorylation of Tau protein, thus contributing to AD-associated neurofibrillary tangle development (Lloren-Martin et al., 2014). GSK-3β is rendered inactive when phosphorylated at Ser9 by active PI3K/AKT signaling (Kitagishi et al., 2014). However, when the PI3K/AKT signaling pathway is dysfunctional, such as due to insulin resistance, it increases GSK-3β activity and leads to Tau hyperphosphorylation, predisposing to AD. This regulation of the PI3K/AKT/GSK-3β pathway appears to be crucial for AD pathogenesis (Kitagishi et al., 2014). Thus, targeted therapy to reduce GSK-3β activity has become a promising approach to treat AD (Lloren-Martin et al., 2014). Lithium, a mood stabilizer used in patients suffering from mood disorders, is now known to both, directly and indirectly, inhibit GSK-3β activity. Many studies have tested the effects of lithium on various AD animal models. As with metformin treatment, the administration of lithium yielded controversial results in different AD animal models. While lithium administration successfully reduced the neuropathology and cognitive deficits in rats that received intra-hippocampal injections of Aβ, in rats overexpressing GSK-3β, as well as, in several murine models overexpressing human amyloid precursor protein, it failed to show promise in other murine models of AD (Lloren-Martin et al., 2014). These outcomes further emphasize that a "fit-for-all" therapy will most likely not work against AD. Thus, lithium treatment may only be beneficial against AD-associated cognitive deficits and neuropathology in patients exhibiting abnormal GSK-3β activity in their brains. To this end, positron emission tomography imaging using GSK-3β-specific neuro-radiotracers may be used to identify patients exhibiting abnormal GSK-3β activity to select candidates for effective lithium treatment. While no GSK-3β-specific neuro-radiotracer has been approved for use in humans yet, one compound has shown great promise in the primate brain (Liang et al., 2016). In addition, phospho-GSK-3β (Ser9) and total GSK-3β ELISA kits have been developed for commercial use to assess human samples. This could be used to screen AD sub-populations for effective lithium treatment as well.

In summary, although several well-known biomarkers, such as neurofilament light chain and amyloid precursor protein, for AD neuropathology have been well-studied over 30 years, disease-modified treatments for AD are still lacking, raising the questions for amyloid cascade hypothesis as the principal cause of AD and its clinical phenotype. Now, increasing knowledge of specific AD pathophysiological mechanisms opens a new avenue and holds great promise for the development of future biomarker-guided targeted therapies (**Figure 1**). As evidenced in oncology medicine, biomarker-based diagnostics can accurately and reliably identify patients precisely and early in the disease process. The availability of these biomarker-based diagnostics for AD is thus, expected to shift the therapeutic strategies away from the traditional "one-size-fits-all" approach to "magic bullet" drugs to develop biomarker-guided targeted therapies. However, more work needs to be done to study differences in biomarkers collected from different sources, such as cerebrospinal fluid, blood, saliva, and urine.

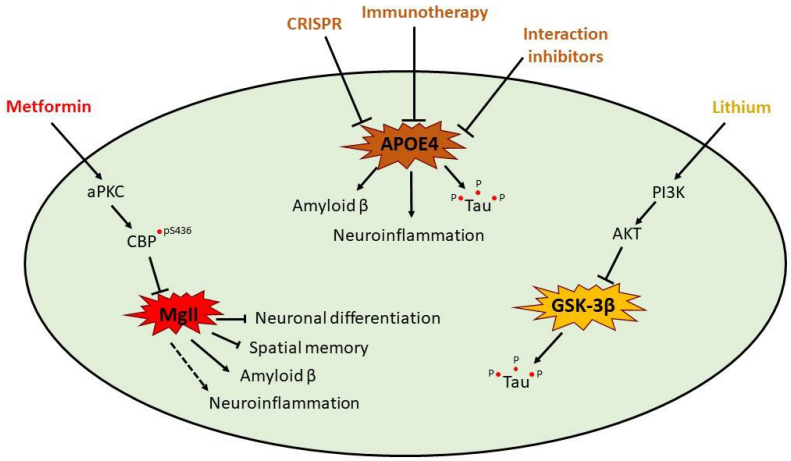

Figure 1 | **Schematic of pathways related to biomarkers-guided drug therapies against AD.** Mgll upregulation is a biomarker to select metformin as a targeted drug for AD treatment (Syal, et al., 2020). Genetic detection of APOE4 is a biomarker to select for immunotherapy and interaction inhibitors that block APOE4 activity to treat AD (Safieh et al., 2019). Dysregulated GSK3β activity is a biomarker to use lithium to treat AD (Kitagishi et al., 2014; Lloren-Martin et al., 2014). AD: Alzheimer's disease; aPKC: atypical protein kinase C; APOE4: apolipoprotein E4; CBP: CREB-binding protein; GSK-3β: glycogen synthase kinase-3β; Mgll: monoacylglycerol lipase.

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References

Barini E, Antico O, Zhao Y, Asta F, Tucci V, Catelani T, Marotta R, Xu H, Gasparini L (2016) Metformin promotes tau aggregation and exacerbates abnormal behavior in a mouse model of tauopathy. Mol Neurodegener 1:16.
Berkowitz CL, Mosconi L, Rahman A, Scheyer O, Hristov H, Isaacson RS (2018) Clinical application of APOE in Alzheimer's prevention: a precision medicine approach. J Prev Alzheimers Dis 5:245-252.
Chen R, Zhang J, Wu Y, Wang D, Feng G, Tang YP, Teng Z, Chen C (2012) Monoacylglycerol lipase is a therapeutic target for Alzheimer's disease. Cell Rep 2:1329-1339.
Farr SA, Roesler E, Niehoff ML, Roby DA, McKee A, Morley JE (2019) Metformin improves learning and memory in the SAMP8 mouse model of Alzheimer's disease. J Alzheimers Dis 68:1699-1710.
Kitagishi Y, Nakanishi A, Ogura Y, Matsuda S (2014) Dietary regulation of PI3K/AKT/GSK-3β pathway in Alzheimer's disease. Alzheimers Res Ther 6:35.
Koenig AM, Mechanic-Hamilton D, Xie SX, Combs MF, Cappola AR, Xie L, Detre JA, Wolk DA, Arnold SE (2017) Effects of the insulin sensitizer metformin in Alzheimer disease: pilot data from a randomized placebo-controlled crossover study. Alzheimer Dis Assoc Disord 31:107-113.
Liang SH, Chen JM, Normandin MD, Chang JS, Chang GC, Taylor CK, Trapa P, Plummer MS, Para KS, Conn EL, Lopresti-Morrow L, Lanyon LF, Cook JM, Richter KE, Nolan CE, Schachter JB, Janat F, Che Y, Shanmugasundaram V, Lefker BA, et al. (2016) Discovery of a highly selective glycogen synthase kinase-3 inhibitor (PF-04802367) that modulates tau phosphorylation in the brain: translation for PET neuroimaging. Angew Chem Int Ed Engl 55:9601-9605.
Llorens-Martín M, Jurado J, Hernández F, Avila J (2014) GSK-3β, a pivotal kinase in Alzheimer disease. Front Mol Neurosci 7:46.
Moore EM, Mander AG, Ames D, Kotowicz MA, Carne RP, Brodaty H, Woodward M, Boundy K, Ellis KA, Bush AI, Faux NG, Martins R, Szoeke C, Rowe C, Watters DA; AIBL Investigators (2013) Increased risk of cognitive impairment in patients with diabetes is associated with metformin. Diabetes Care 36:2981-2987.
Panza F, Lozupone M, Logroscino G, Imbimbo BP (2019) A critical appraisal of amyloid-β-targeting therapies for Alzheimer disease. Nat Rev Neurol 15:73-88.
Safieh M, Korczyn AD, Michaelson DM (2019) ApoE4: an emerging therapeutic target for Alzheimer's disease. BMC Med 17:64.
Syal C, Kosaraju J, Hamilton L, Aumont A, Chu A, Sarma SN, Thomas J, Seegobin M, Dilworth FJ, He L, Wondisford FE, Zimmermann R, Parent M, Fernandes K, Wang J (2020) Dysregulated expression of monoacylglycerol lipase is a marker for anti-diabetic drug metformin-targeted therapy to correct impaired neurogenesis and spatial memory in Alzheimer's disease. Theranostics 10:6337-6360.

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