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Using genetics to enable Alzheimer's disease prevention studies

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Abstract

Curing Alzheimer's disease (AD) remains an elusive goal and may prove to be impossible due to the very nature of the disease. While modulating disease progression is an attractive target and will alleviate the burden of the most severe disease stages, this strategy will not reduce disease prevalence. Preventing or, as will be described, delaying the onset of cognitive impairment and AD will provide the greatest benefit to individuals and society by pushing the onset of disease into later ages. Because of the highly variable age of disease onset, AD prevention studies that do not stratify participants by age-dependent disease risk will be operationally challenging – large in size and of long duration. We present a composite genetic biomarker to stratify disease risk that facilitates clinical studies in high risk people. In addition, we discuss the rationale for the use of pioglitazone to delay disease onset in high risk people.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that progresses over an extended time course, with much of the neuropathological damage occurring silently, without overt clinical symptoms. The disease gradually robs people of their independence and dignity and it is, inescapably, fatal. The vast majority of AD cases, and the majority of all dementia, is due to late-onset, sporadic disease rather than the early-onset form that is linked to completely penetrant mutations in the genes encoding amyloid precursor protein (*APP*), its processing enzymes presenilin 1 (*PS1*) or presenilin 2 (*PS2*), or via duplication of *APP*¹.

There is a conflict of interest

Dr. Roses is CEO and owner of Zinfandel Pharmaceuticals, Inc which is in an alliance with Takeda Pharmaceuticals to test the efficacy of pioglitazone in a pharmacogenetically-stratified delay of onset clinical trial. Dr. Roses also has an appointment at Duke University Medical Center. Drs. Crenshaw, Saunders, Lutz, Gottschalk and Grossman are paid consultants for Zinfandel Pharmaceuticals. Dr. Burns is a Zinfandel Pharmaceuticals employee and Dr. Brannan is employed by Takeda Pharmaceuticals International, Inc.

Early onset AD is rare, accounting for <1% of all AD patients. By contrast, late onset AD, which is the topic of this article, is the most common form of dementia and typically affects individuals in or after the 7th decade of life. Although genetics is not determining for the development of late onset AD, it does account for up to 80% of personal risk for eventually developing the disease sometime after the age of ~65 years². One in 8 people in the United States who are over age 65 are now living with AD, and with more people living to older ages the incidence of AD will continue to climb³. In the absence of an effective therapy that at least delays disease onset, but preferably both delays onset and slows progression, it has been projected that 9–16 million Americans will be living with this disease by 2050, with almost half living with advanced disease^{3, 4}, and worldwide 106 million people will be afflicted with this fatal disease⁵. The financial costs associated with AD propagate beyond the direct cost of caring for patients to include lost productivity and substantial emotional capital expended by care-givers. It is estimated that US expenditures on AD and other dementias will be \$200 billion in 2012. According to one model, this cost will increase more than 5 fold by 2050³. In addition, the healthcare systems in the US will have to undergo structural and organizational changes at the State and Federal levels to accommodate the burgeoning numbers of individuals requiring long-term care in facilities or at home.

In recognition of the growing burden of AD, national plans to address this challenge have been advanced in a number of countries including, as of 2011, the United States. The necessity for a national plan was detailed in a 2007 publication, “Developing a National Alzheimer’s Strategy Equal to the Epidemic”⁶. In 2009, the Alzheimer’s Study Group released its report, “A National Alzheimer’s Strategic Plan: The Report of the Alzheimer’s Study Group” (http://www.alz.org/documents/national/report_ASG_alzplan.pdf). An important pillar of the Alzheimer’s Solution Project proposed by the Study Group was an initiative to develop the means to delay or prevent AD. In January 2011, President Obama signed the National Alzheimer’s Project Act into law (<http://www.gpo.gov/fdsys/pkg/PLAW-111publ375/pdf/PLAW-111publ375.pdf>;⁷), and one of the chief aims of this legislation is to “accelerate the development of treatments that would prevent, halt, or reverse the course of Alzheimer’s disease.”

Physicians currently have few therapies to offer AD patients or individuals with predementia cognitive impairment, and those treatments that are available do not alter progression of AD, although they do provide slight or moderate improvement in cognition and/or function and behavior for some patients^{8–11}. These drugs are the acetylcholinesterase inhibitors, galantamine, rivastigmine, and donepezil, and the N-methyl D-aspartate receptor antagonist, memantine. Pharmaceutical companies are actively pursuing therapies that modify the progression of diagnosed AD, but the development landscape is littered with disappointing failures and unconfirmed successes in small studies¹². These failures have led experts in the field to suggest that, once entrenched, disease progression may be inexorable and a better strategy may be to attempt to intercede prior to the onset of clinical symptoms, before development of mild cognitive impairment (MCI). To prove that a therapy “prevents” AD, subjects would have to be followed to the end of their lifetimes, this can never be proven in the context of a limited-term clinical trial. Therefore “delay of onset” of MCI due to AD¹³ is a more realistic goal although extension studies could be designed to confirm delay of AD

dementia or prevention of AD. Delaying AD onset by as little as 5 years is expected to provide significant societal and individual benefit^{4, 14}.

A number of large, multi-center, placebo-controlled, double-blind, randomized AD primary prevention studies have been conducted, enrolling cognitively normal subjects and measuring the development of all-cause dementia or probable AD (Table 1). The Alzheimer's Disease Anti-inflammatory Prevention Trial (ADAPT) investigated naproxen and celecoxib¹⁵. ADAPT was halted early because of the potential for non-steroidal anti-inflammatory agents to induce cardiovascular harm; however an analysis of the available data indicated that, rather than preventing AD, celecoxib and naproxen increased the hazard ratio for incident AD or all-cause dementia¹⁶. The Women's Health Initiative Memory Studies (WHIMS) investigated estrogen with and without progestin^{17, 18}. While the estrogen plus progestin study of WHIMS was also halted early because of the potential for health risks, the combination treatment or estrogen alone increased the risk for dementia or MCI incidence in the population tested¹⁹. The Ginkgo Evaluation of Memory (GEM) study investigated ginkgo biloba extract but this treatment did not have a significant effect relative to placebo on any of the cognitive or functional endpoints tested²⁰. GuidAge also tested ginkgo biloba extract²¹ and, like the GEM study, did not show evidence that the treatment lowered the incidence of dementia relative to placebo. These failures could have been due to targeting the wrong disease pathway, choosing a drug that didn't hit the disease pathway as expected, or because the target population or the risk enrichment scheme was not optimal for a disease prevention trial. There is evidence that the enrichment strategies did not perform as expected, with lower event rates than anticipated. For example, in ADAPT the observed event rate in the placebo group is less than half that which was expected with the enrichment strategy²². Similarly, it was determined that the major limitation of the GuidAge study was that the incidence of dementia was lower than planned for, resulting in limited statistical power to detect a treatment effect²³.

Most of the cited prevention studies employed some form of disease risk enrichment because the variability in age of disease onset and rate of decline in cognition in the predementia phase, and the relatively low annual rate of conversion to disease in the general population, would otherwise necessitate very large subject numbers and extended time-lines. Employing an enrichment strategy increases study power and reduces the number of trial participants and trial duration²⁴. For example, some studies increased the odds for incident cases of AD by enrolling individuals of advanced age, those who had a family history of disease, or had an existing memory complaint (Table 1). These strategies are ineffective for predicting risk at the level of the individual and in some cases subjects are likely to have already stepped into the AD continuum^{13, 25}. There is a pressing need for a robust, simple, and qualified²⁶ prognostic enrichment strategy that will identify subjects at increased personal risk of developing mild cognitive impairment due to AD (MCI due to AD); that is, to identify people who are at increased risk of developing the earliest symptoms of probable AD but do not already have the disease¹³. By increasing the number of events (diagnosed cases of MCI due to AD), using a prognostic biomarker will increase study power within a clinical trial.

A biomarker to enable primary prevention – evolution of understanding

We are approaching the 20th anniversary of the publication of a series of papers describing the association between *APOE* and risk of development of AD and age of onset (AOO) of the disease²⁷. Everyone possesses two copies of the *APOE* gene, one on each of their two copies of chromosome 19. Two non-synonymous single nucleotide polymorphisms (SNPs) in *APOE* give rise to three possible alleles, *APOE* ϵ 2, ϵ 3, and ϵ 4, each of which codes for a different protein isoform. *APOE* ϵ 3 is by far the most common allele in every human population. *APOE* ϵ 4 is associated with increased risk and earlier disease onset relative to *APOE* ϵ 3; *APOE* ϵ 2 is associated with reduced risk of AD and later disease onset relative to *APOE* ϵ 3. Although incompletely penetrant, homozygous ϵ 4 carriers (*APOE* ϵ 4/4, approximately 2% of Caucasians) have the highest risk of developing AD. In one study of autopsy-confirmed AD, the *APOE* ϵ 4/4 subjects (<2% of Caucasians) had ~20 (95% CI, 10.4–41) times greater odds of developing AD than *APOE* ϵ 3/3 subjects (the neutral reference genotype). *APOE* ϵ 3/4 (~20% Caucasians) and *APOE* ϵ 2/4 (~2% Caucasians) subjects had 5.5 (95% CI, 4.2–7.2) and 3.2 (95% CI, 1.7–6.2) times greater odds, respectively, of developing the disease^{28, 29}. Although only advanced age is a more significant risk factor than the *APOE* ϵ 4 allele, the ϵ 4 allele is potentially informative of the genetic risk for developing AD for only a quarter of Caucasians (*i.e.* for *APOE* ϵ 4/4, *APOE* ϵ 3/4, and *APOE* ϵ 2/4). The relative infrequency of *APOE* ϵ 4 in any population limits its usefulness as a marker for the stratification of population-based clinical studies and *APOE* ϵ 4 carriage, alone, does not provide sufficient sensitivity, selectivity or predictive power to be used as a diagnostic or prognostic tool for AD³⁰. Simply put, *APOE* ϵ 4 can't address risk for those non- ϵ 4 carriers who are at risk of developing AD.

Another genetic locus in the vicinity of *APOE* is also associated with age of development of AD and stratifies AOO of cognitive impairment³¹. The second risk locus is a polymorphic, deoxythymidine (polyT) tract, rs10524523 (523), located in intron 6 of the *TOMM40* gene. This gene encodes the channel subunit of the outer mitochondrial membrane protein import complex. *TOMM40* is not only adjacent to *APOE* on chromosome 19 but the two genes reside within an extended region of linkage disequilibrium (LD). The discovery of 523 hinged upon the structure of a phylogenetic tree developed from the DNA sequences of a 10 kilobase genomic region encompassing parts of *TOMM40* and *APOE*. This detailed mapping technique described the co-inheritance of specific lengths of the polyT tract, 523, with different alleles of *APOE*³¹. The different tract lengths were categorized as three alleles, short (S, <19 T residues; L, >19 T but < 29 T residues; VL, > 29 T residues). The linkages that are most common in Caucasians are as follows; *APOE* ϵ 4 is almost always linked to 523 L; *APOE* ϵ 3 and *APOE* ϵ 2 may be linked to either a 523 S or 523 VL allele. The key observation was that an excess of disease cases mapped onto one of the two major branches of the phylogenetic tree. This branch was home to all of the *APOE* ϵ 4-containing DNA sequences (haplotypes) and a subset of the *APOE* ϵ 3-containing haplotypes. The distinguishing feature of the *APOE* ϵ 3 haplotypes that segregated to the higher risk branch was that the *APOE* ϵ 3 was linked to a 523 VL allele. By contrast, those *APOE* ϵ 3 haplotypes that were in the lower risk branch almost always contained 523 S alleles. With relatively few exceptions (only ~2% of ϵ 4 haplotypes), ϵ 4 was connected to an L allele. Therefore, the *APOE* ϵ 4 and the 523 L alleles reliably report the presence of the other in Caucasians. Note

that the 523 allele frequencies vary across different ethnic groups (Figure 1 and ³²), as do *APOE* allele frequencies. In addition, the linkage between *APOE* ϵ 4 and the 523 L allele is not as invariant in other groups as it is for Caucasians. Genotyping samples of African-American and Ghanaian subjects revealed that *APOE* ϵ 4 is inherited with L alleles approximately 50% of the time, compared to 98% in Caucasians, and is otherwise inherited with S alleles^{32, 33}. This may provide an explanation for the variable genetic association of *APOE* ϵ 4 with African and African American study populations over the years³⁴⁻³⁷.

The distribution of *APOE* ϵ 3 across the phylogenetic tree suggested that the heterogeneity in AOO of AD in individuals who carry *APOE* ϵ 3 might be explained by linkage to alternative 523 alleles (S or VL). The hypothesis was first assessed with a simple association test in a group of *APOE* ϵ 3/4 (*i.e.* 523 VL/L and S/L) subjects. The average age of disease onset in this small sample differed according to 523 genotype, with VL/L subjects developing disease ~ 7 years earlier than S/L subjects (70 versus 77 years, respectively)³¹. Prior to the discovery of the association between 523 and age of AD onset, the average age of disease onset for *APOE* ϵ 3/4 subjects was estimated to be 75 years³⁸, but there was a great deal of variation in the actual age of diagnosis. The relationship between all 523 genotypes and AOO has now been explored in a larger cohort (Figure 2).

The Kaplan-Meier curves of Figure 2 present a retrospective stratification, by *TOMM40* genotype, of the ages of onset of MCI for subjects, all Caucasian, that were followed in the Joseph & Kathleen Bryan Alzheimer's Disease Research Center (Bryan ADRC) at Duke University. For this cohort, cognitive changes were monitored over time using a battery of neuropsychological tests to determine the age of onset of cognitive impairment and probable AD dementia. This is an important point – the subjects were followed prospectively to catch the earliest clinical symptoms of the disease process, and ascertainment variability was minimized because all subjects were assessed at one research center using validated tests and standardized practices and definitions of cognitive status.

Each curve shows the proportion of subjects of each 523 genotype that remains unaffected by MCI at each age. The *APOE* genotype that corresponds to each 523 genotype is also shown on the graph. The L/L curve in Figure 2 corresponds closely to *APOE* ϵ 4/4 since 98% of all *APOE* ϵ 4 alleles are linked to L in Caucasians. In the Bryan ADRC cohort, 50% of the L/L subjects were diagnosed with probable AD by age 76. For the *APOE* ϵ 3/4 subjects, stratification by 523 genotype gives two, separable, age-of-onset distributions, corresponding to VL/L and S/L. For these subjects, it is the 532 allele (S or VL) linked to the *APOE* ϵ 3 that is driving the difference in AOO. Instead of one curve for *APOE* ϵ 3 homozygotes, three distinct curves can be constructed, for S/S, S/VL and VL/VL. Differences in AOO for these subjects are completely independent of carriage of *APOE* ϵ 4. As with *APOE* ϵ 3, *APOE* ϵ 2 may be linked to an S or a VL allele. Like previous reports, carriage of at least one allele of *APOE* ϵ 2 typically results in later AOO³⁹, except when it is paired with an *APOE* ϵ 4 (*i.e.* also an L allele) in the genotype. Since *APOE* ϵ 2 is rare, it is difficult to test the combined effect of carriage of ϵ 2 and the 523 alleles. The cohort used to develop the AOO distributions in Figure 2 contained only 78 *APOE* ϵ 2 carriers. Of these, only 5 subjects developed cognitive impairment (shown in Figure 2 as diamonds (*APOE* ϵ 2/3) and open circles (*APOE* ϵ 2/4)).

Close inspection of the AOO curves suggests that VL has a different effect depending on whether it is in the context of *APOE* $\epsilon 3/4$ or *APOE* $\epsilon 3/3$. In the first case, VL is associated with earlier AOO, whereas in the latter case it is associated with later AOO. This conundrum is partially addressed by functional experiments in people who are still in the normal cognition range, which suggest that carriage of VL by *APOE* $\epsilon 3$ homozygotes adversely affects cognition and brain volume prior to the development of cognitive impairment. Johnson et al. demonstrated that cognitively normal, late middle aged, *APOE* $\epsilon 3/3$ subjects who were homozygous for the VL allele performed *worse* on a word retrieval task, a test of episodic memory, than their S/S counterparts⁴⁰. Furthermore, in a magnetic resonance imaging study of a sample of these *APOE* $\epsilon 3/3$ subjects, there was an inverse relationship between number of VL alleles and grey matter volume in two regions of the brain that are affected early in the progression of AD, the ventral posterior cingulate and medial ventral precuneus⁴⁰. Caselli *et al.*, analyzed the episodic memory performances of cognitively normal people using the Rey Auditory Verbal Learning Test (Rey AVLTL)⁴¹. Carriage of at least one *APOE* $\epsilon 4$ and no S allele (i.e. *APOE* $\epsilon 3/4$ VL/L) was associated with accelerated memory decline after age 60 relative to *APOE* $\epsilon 4$ carriers with at least one S allele, though the relative contributions of the *APOE* and *TOMM40* loci could not be distinguished. Within the *APOE* $\epsilon 3/3$ subjects, those who were homozygous for the VL allele demonstrated a reduced test-retest effect (cognitively normal subjects typically perform better on retest because of practice) on the Rey AVLTL whereas S/S carriers had normal test-retest measures. This suggested that the VL allele had a deleterious effect with respect to being able to recall the test and thus improve performance. The difference between S/S and VL/VL carriers was particularly evident before age 60. A third study also found an *APOE*-independent effect of 523 on cognitive performance in cognitively-normal, older adults aged 64-93 years⁴². In this cohort, the S/S carriers within the *APOE* $\epsilon 3/3$ sub-group performed better than S/VL heterozygotes on tests of episodic memory, attention, and executive function. We speculate, based upon this functional data, that the VL/VL genotype (in *APOE* $\epsilon 3$ homozygotes) may be associated with presymptomatic AD processes that are most evident at younger ages when subtle signs of cognitive dysfunction are not masked by later pathology and that, in the presence of *APOE* $\epsilon 4$ (i.e. in *APOE* $\epsilon 3/4$ subjects), this early VL effect is exacerbated.

Developing a risk algorithm for clinical trial enrichment

Together the *APOE* and 523 genotypes are prognostic of age-dependent risk for most Caucasians, and employing knowledge of the two genotypes together provides a better means, relative to those that were used previously, to enrich a clinical trial with at-risk subjects.

Based on the data presented, a 'risk algorithm', composed of *APOE* and *TOMM40* genotypes, and age at randomization, has been developed. This algorithm is not a diagnostic, but it is a means for enriching a clinical trial with subjects who have higher risk of developing MCI due to AD during the course of a study of approximately 5-year duration. A straightforward classification scheme that constitutes the risk algorithm is summarized in Table 2. For the age range in the trial (68–83 years), the low risk stratum will include *APOE* $\epsilon 2/2$ and *APOE* $\epsilon 2/3$ subjects, and a proportion of *APOE* $\epsilon 3/3$ subjects. All VL/VL subjects will also be classified as low risk, as Figure 2 demonstrates that these subjects are at

relatively low risk of developing cognitive impairment in this age range. The rare *APOE* $\epsilon 2/4$ subjects will be designated as high risk for the purpose of the trial. *TOMM40* 523 L/L subjects and those with VL/L will also be classified as high risk. There are three common genotypes with risk that changes as a function of age at randomization. The age at which these subjects are classified as high risk is the point on each of the curves where the slope increases. Based on the Bryan ADRC sample, for which we have the most reliable AOO data, S/L, one of the two *APOE* $\epsilon 3/4$ groups, becomes high risk beginning at age 74 years; S/S subjects and S/VL (sub-groups of *APOE* $\epsilon 3/3$) enter the high risk category at ages 77 and 76, respectively.

We have designed a pharmacogenetically-enriched, double-blind, delay-of-disease-onset clinical trial of cognitively-normal subjects aged 68–83, inclusive, using the enrichment scheme (see Figure 3). The subjects in this study will be classified as having high or low risk for development of cognitive symptoms over the subsequent 5 years, which we predict will be the duration of the event-driven study, according to the risk algorithm we just described. High risk subjects will be randomized to active therapy or placebo; low risk subjects will be treated with placebo only. The low risk subjects will not be exposed to active treatment because these subjects may not receive benefit from an intervention and the number of subjects required to measure a significant difference between active and placebo treatment in this group, given the expected low event rate, would be very large.

The risk algorithm presented in Table 2, was developed using data from Caucasian subjects. Before a similar risk algorithm can be developed for non-Caucasians, we need to acquire data on the interaction between *APOE* and *TOMM40* genotypes and disease risk in different races⁴³.

Co-development of a prognostic test and therapeutic drug – the test

Should the treatment prove to be efficacious, the risk algorithm must be qualified in order to support prescribing by physicians. This is accomplished in this co-development program. To validate a prognostic biomarker for patient care, more is needed than demonstration of an association between a biomarker and a phenotype in a cross-sectional or retrospective study. Any prognostic biomarker must be validated in a well-powered, prospective study that clearly establishes the relationship between the biomarker and the clinical or disease outcome²⁶ The risk algorithm described here will be validated during the conduct of a phase 3, delay-of-onset study. Upon completion of the trial, the positive and negative predictive values for the risk algorithm will be calculated by comparing the high risk, placebo-treated group with the low risk group (all treated with placebo). Knowledge of these test performance metrics is necessary for the qualification of the risk stratification algorithm for use as a prognostic biomarker in the clinic.

The risk algorithm is intended to fill an important gap in AD clinical research that currently hinders the development of therapeutics to delay or prevent the onset of pathological cognitive decline. The gap is the inability to identify individuals who are at greater risk of developing cognitive impairment, within a defined time-frame, before clinical symptoms are manifest.

Recent guidelines from National Institute on Aging-Alzheimer's Association workgroups recommend that early symptomatic AD, termed MCI due to AD, can be diagnosed using core clinical criteria that are based in part upon neuropsychological tests and measures of subtle functional changes that will be used in the clinical trial described here¹³. This set of recommendations also discusses use of an imaging biomarker or a biomarker expressed in cerebrospinal fluid (CSF) for research purposes¹³. The delay-of-onset trial is designed as a primary prevention trial that uses recommended clinical criteria – operationalized using a battery of neuropsychological tests, informant ratings and measures capturing change in function, neurological evaluation, exclusion of medical causation, and judgment of a clinician – to *select* subjects clinically unaffected by the disease process and also to *evaluate* subjects during the course of the study. The trial will not select subjects using imaging or biochemical biomarkers, although imaging may be used to rule out other possible causes of cognitive symptoms. However, the Phase 3 study described here may provide the time course for development of clinically-assessed MCI due to AD against which expressed biomarkers, like CSF beta-amyloid or tau protein, can be evaluated. At present, these markers are not validated by regulatory agencies. It is anticipated that by the time the Phase 3 study completes, one or more expressed markers may be analytically validated at which point it would be instructive to retrospectively assess the biomarkers in the clinical trial population. It should also be noted that while this trial has been powered according to the anticipated enrichment provided by the risk algorithm for Caucasian subjects, and the endpoints will be qualified only in Caucasians, there is a need to generalize the prognostic biomarker to other populations. To this end, the trial will enroll non-Caucasians and, in parallel, independent studies of the relationship between *APOE-TOMM40* haplotypes and development of MCI due to AD in non-Caucasian cohorts with accurate age of onset information will be conducted.

Co-development of a prognostic test and therapeutic drug – the drug

In addition to prospectively qualifying the risk algorithm as a companion, prognostic biomarker, the clinical trial will simultaneously test a low dose of pioglitazone (PIO), to delay the onset of mild cognitive symptoms of the AD type (*i.e.* MCI due to AD)¹³. This co-development clinical study design was first discussed during a US Food and Drug Administration (FDA) Voluntary eXploratory Data Submission process, and is now the basis of Investigational New Drug and Investigational Device Exemption submissions to the FDA and the European Medicines Agency (pending at the time of publishing) sponsored by a Zinfandel Pharmaceuticals-Takeda Pharmaceuticals alliance. The clinical trial is expected to launch in 2013.

The risk algorithm will be qualified for use as a prognostic biomarker by assessing its performance in the placebo-treated groups for predicting low and high risk subjects. The efficacy of PIO to delay the onset of MCI due to AD will be tested by comparing the incidence of MCI due to AD in the high risk, PIO-treated group versus the high risk, placebo-treated group. While this study will test PIO, the study design is suitable for testing efficacy of any therapeutic with a sound rationale for delaying the onset of MCI due to AD. Enrichment of the clinical trial with subjects at high risk for developing MCI due to AD will increase statistical power to detect a treatment effect in this risk group. PIO is currently

marketed under the trade name Actos® for the treatment of type 2 diabetes mellitus (T2DM). In the delay of MCI due to AD study, PIO will be administered at a dose strength that is significantly lower than strengths marketed for treatment of type 2 diabetes mellitus (*i.e.* 15, 30, or 45 mg daily). We are currently evaluating *in vivo* changes in neural activity in response to repeated dosing to establish the minimal potentially efficacious dose to delay disease onset. A preliminary rat study showed that as a result of daily administration of very low doses of PIO (0.04 mg/kg – 0.32 mg/kg, resulting in serum exposure roughly equivalent to a daily human dose of 1.5 – 12 mg) elicited rapid changes in neural activity across the brain⁴⁴. In this experiment, the pharmacodynamic marker for neural activity was resting state functional connectivity in awake rats as measured by blood-oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI)⁴⁵. fMRI experiments to test the effect of PIO on human brain activity and to establish dosage are currently underway. Unlike the rat study, these experiments will report on brain activity during tasks that challenge specific cognitive functions, *e.g.* episodic memory, that are of particular interest in the earliest stages of cognitive impairment and in Alzheimer's dementia⁴⁶.

The choice of PIO for the delay-of-onset trial was based on several factors; not least among them was the safety of the drug which is demonstrated by the extensive human use history in T2DM. Moreover, there is a large body of evidence demonstrating that peroxisome proliferator-activated receptor γ (PPAR γ) agonists, including PIO, prevent or reverse AD pathology in a number of pre-clinical studies and modulated symptoms in small human AD treatment (not delay-of-onset or prevention) trials. The detailed mechanism underlying the salutary effects of PPAR γ agonists on AD-related pathophysiologies has not been determined. However, the PPAR γ receptor is widely distributed throughout the brain in all cell types and supports neurogenesis and the normal functions of neurons, microglial cells, and astrocytes. Activation by PPAR γ agonists in AD ameliorates a number of pathophysiological processes, including mitochondrial dysfunction, neuroinflammation and increased amyloid burden^{47, 48}. Transgenic mouse models of familial AD and rat models of aging have also provided important insights. PPAR γ agonists improved learning and/or memory in every transgenic mouse study where this was evaluated, and also caused decreased A β levels and amyloid burden, reduced astrocytic and astroglial activation and, when tested, reduced oxidative stress^{49,50-55}. In addition, Strum *et al.* demonstrated that orally administered ROSI penetrated the blood brain barrier and induced neuronal mitochondrial biogenesis in mice⁵⁶.

Several small human trials have examined the effectiveness of a PPAR γ agonist for the treatment (not the delay) of probable AD or mild cognitive impairment. These small studies indicated that ROSI or PIO could improve or stabilize cognition, cerebral blood flow, and plasma A β 40/A β 42⁵⁷⁻⁶¹.

Three phase 3 studies examined the efficacy of ROSI-XR (rosiglitazone-extended release tablets) for AD. In two of these studies, REFLECT-2 and -3, ROSI-XR was adjunctive to donepezil or any acetylcholinesterase inhibitor, respectively, for 48 weeks⁶². In REFLECT-1, ROSI-XR was tested as monotherapy for 24 weeks⁶³. None of the studies provided statistically significant evidence for efficacy of ROSI for treatment of frank AD. However, these studies revealed positive trends on some cognitive tests which suggest that PPAR γ

agonists warrant further study for AD treatment. It should also be noted that the phase 2b study of ROSI⁵⁹ was limited to Caucasian subjects whereas the phase 3 studies were not.

The primary prevention, or delay-of-onset, trial described here is an important and necessary experiment. It expands our understanding of how to co-develop medicines and companion prognostic (broadly designated as “diagnostic”) tests, how to ethically and practically conduct primary prevention studies through the use of a prognostic enrichment strategy, it may prove the effectiveness of a safe drug for a new indication and, most importantly, if successful this product of this trial will significantly reduce the future burden of AD.

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Figure 1. Frequencies of *TOMM40* 523 polyT lengths in different groups

PolyT length is in number of T residues. The Caucasian samples (n=463) in panel A were obtained from the Wisconsin Registry for Alzheimer's Prevention⁶⁴. The Ghanaian samples (B, n=41) were from elderly, non-demented subjects. The Far Eastern samples (C-E, n=60 in each case) were from young, healthy subjects. 523 allele lengths were determined by sequencing. Blue bars, 523 alleles connected to *APOE* ε4; red bars, 523 alleles linked to *APOE* ε3 (or *APOE* ε2); green bars indicate when the linkage between 523 and an *APOE* allele could not be unambiguously assigned knowing only the genotypes at the two loci.

Figure 2. Age of onset of cognitive impairment as a function of *TOMM40* 523 genotype

The Bryan ADRC, Memory, Health and Aging cohort⁶⁵ (n=508, 106 conversion events) was followed prospectively at the Bryan ADRC at Duke University. Cognitive status was determined using standard neuropsychological tests^{66, 67}. Age at which cognitive impairment occurred was retrospectively stratified by *TOMM40* genotype and Kaplan-Meier curves were constructed. *TOMM40* genotype was determined using sequencing-based genotyping method (Polymorphic DNA Technologies, Inc). *TOMM40* genotypes and the corresponding *APOE* genotypes are indicated on the figure. The red line corresponds to *APOE* ε4/4; the two green lines correspond to *APOE* ε3/4, and the three blue lines correspond to *APOE* ε3/3. Note that, within this cohort, there are 78 individuals who carry an *APOE* ε2 allele, but only 5 convert to cognitive impairment during the study (VL/L (*APOE* ε2/4), n=1; S/L (*APOE* ε2/4), n=2; VL/VL (*APOE* ε3/3), n=2). These individuals are indicated as points on the appropriate *TOMM40* genotype curve; open circles and closed diamonds indicate the age of symptom onset for the *APOE* ε2/4 and *APOE* ε2/3 cases, respectively. Number of subjects per 523 genotype and (number converted to case status): L/L, n=23(11); VL/L, n=54(24); S/L, n=72(23); S/S, n=100(20); S/VL, n=138(22); VL/VL, n=51(6).

Figure 3. Pharmacogenetically-enriched, primary prevention clinical trial design

Subject registries are established at a number of international sites that have resources for identifying an epidemiological population of the appropriate age range; competencies for neuropsychological testing are developed at each site. At the start of the trial, entry and exclusion criteria are reviewed for each subject and eligible subjects undergo a battery of neuropsychological tests. Those subjects with normal cognition (age-normed) proceed into the prevention study, are genotyped and segregated to the high and low risk strata of the study according to the risk algorithm (Table 1). Low risk subjects are treated with placebo and high risk subjects are randomized to placebo or PIO.

Table 1

Comparison of previous AD primary prevention studies

The listed studies represent the intervention, primary prevention trials that were of large size, were multi-center, placebo-controlled, double-blind and randomized. A number of smaller prevention studies have been undertaken, but these are not summarized here.

Study (Location)	Age	Eligibility	Study size	Enrichment strategy	Planned (actual) study duration in years	Intervention	Primary endpoint [criteria]	Results
GEM (USA)	75	Normal cognition or MCI	3,069 (2,587 cognitively normal & 483 MCI)	Very old age	5 (6.1)	Ginkgo biloba extract	Dementia (all-cause) incidence [DSM-IV and CDR]	No difference between groups
ADAPT (USA)	70	Normal cognition	2,128	Alzheimer-like dementia in a first degree relative	7 (1.5)	Naproxen or celecoxib	AD incidence [DSM-IV, NINCDS-ADRA]	No difference between groups (trial terminated early)
WHIMS (USA)	65-79	Normal cognition	4,532	Postmenopausal women	8.5 (5.6)	Estrogen and progestin	Dementia incidence [DSM-IV]	Greater risk of dementia in treatment versus placebo arms (trial terminated early)
WHIMS (USA)	65-79	Normal cognition	2,947	Postmenopausal women	5	Estrogen	Dementia incidence [DSM-IV]	Greater risk of dementia in treatment versus placebo arms (trial terminated early)
GuidAge (France)	70	Self-reported memory complaint	2,854	Spontaneous memory complaint to the general practitioner	5	Ginkgo biloba extract	AD incidence [DSM-IV, NINCDS-ADRA, NINDS-AIREN]	No difference between groups. Positive result on a pre-specified secondary endpoint (news release, IPSEN Jun10)

Abbreviations: DSM-IV, Diagnostic and Statistical Manual of Mental Disorders 4th edition; CDR, Clinical Dementia Rating; MCI, mild cognitive impairment; NINCDS-ADRA, National Institute of Neurological Disorders and Communicative Disorders, Alzheimer's Disease and Related Disorders; NINDS-AIREN, National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l'Enseignement en Neurosciences; GEM, Ginkgo Evaluation of Memory study; ADAPT, Alzheimer's Disease Anti-inflammatory Prevention Trial; WHIMS, Women's Health Initiative Memory Study

Table 2**Risk stratification scheme**

APOE $\epsilon 2$ carriers are assigned to the high or the low risk strata according to *APOE* genotype. Risk is assessed for all other subjects according to *TOMM40* genotype. *APOE* $\epsilon 2/2$, *APOE* $\epsilon 2/3$ and VL/VL subjects are considered to be at low risk. Certain genotypes are considered high risk in the context of the targeted age range for the delay-of-onset clinical trial. Subjects with any of three 523 genotypes, S/L, S/S, and S/VL will be placed in the high risk category if he or she enters the trial at or older than the age indicated. 523 genotype frequencies are from the Cache County Study of Memory cohort (n=2,042)⁶⁸. *APOE* genotype frequencies are for Caucasian controls from Farrer et al. (n=6,262)⁴³.

523 or <i>APOE</i> genotype	Genotype frequency	Risk for the Study
For <i>APOE</i> $\epsilon 2$ carriers		
<i>APOE</i> $\epsilon 2/2$	1%	All low risk
<i>APOE</i> $\epsilon 2/3$	13%	All low risk
<i>APOE</i> $\epsilon 2/4$	3%	All high risk
For non- <i>APOE</i> $\epsilon 2$ carriers		
523 L/L	2%	All high risk
523 L/VL	13%	All high risk
523 S/L	14%	74 is high risk
523 S/S	17%	77 is high risk
523 S/VL	36%	76 is high risk
523 VL/VL	18%	All low risk